AREA-WIDE CONTROL OF INSECT PESTS

Area-Wide Control of Insect Pests

From Research to Field Implementation

Edited by

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Preface

The world population is still growing at an alarming rate, requiring ever increasing productivity and less waste in agriculture to cope with the increasing demands to satisfy food security for all humans. Alleviation of poverty is in many countries hampered by a myriad of insect pests that cause enormous economic losses to agricultural commodities, both at the pre- and postharvest stages. Initially, most of these insect pests were controlled to a varying degree by the use of broad-spectrum insecticides. However, the indiscriminate use of these chemicals as a control tactic is no longer sustainable in view of increased development of resistance, pollution of soils and surface water, residues in food and the environment, representing risks to human health and biodiversity, etc. As a consequence, demands have been voiced at least since "Silent Spring" in 1962 for control tactics and approaches that are not only efficient, but also sustainable and friendlier to the environment.

Integrated pest management (IPM) has been accepted since the 1960's and 70s as a viable pest management strategy that aims at integrating control tactics to maintain damage levels below a certain economic threshold level whilst also protecting the environment by thriving to limit the use of pesticides. Classical IPM is however a localized approach, with the objective of protecting crops or livestock that is largely under the control of each farmer, with little collaboration or any coordinating structure. Control is exercised only in the areas of economic interest, often resulting in the main or residual pest population pockets remaining in the surrounding areas that have no economic value. These constitute permanent sources from where the commercial areas under control are re-invaded.

A quite different, more efficient and sustainable approach is the integration of control tactics against an entire pest population, i.e. area-wide integrated pest management (AW-IPM) or total population management. The AW-IPM is a coordinated, sustainable and preventive approach that targets pest populations in all areas, including non-commercial urban settings, non-cultivated and wild host areas.

The coordination required among farmers and all other stakeholders for an area-wide approach, makes AW-IPM programmes complex, management intensive, requiring long-term commitment and funding. Although they result in more sustainable control of insect pests, there is by no means a guarantee for success. This new textbook on area-wide control of insect pests collates a series of selected papers that attempts to address various fundamental components of AW-IPM, e.g. the importance of relevant problem-solving research, the need for essential baseline data, the significance of adequate tools for appropriate control strategies, and the value of pilot trials, etc. Of special interest are the numerous papers on pilot and operational programmes that pay special attention to practical problems encountered during programme implementation.

The book is a compilation of 66 papers that are authored by experts from more than 30 countries. Each paper was peer-reviewed by at least one, in most case two or more independent, outside experts and edited for the English language by Dr James Dargie, former Director of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. We both thank the many reviewers and Jim whose meticulous work and suggestions improved many of the papers. In addition, the editors subjected each paper to an in-depth technical quality control process. As a result, we trust that the technical quality of the papers is optimal, the information provided accurate, up-to-date and of a high international standard. This process of peer-review, editing and formatting has taken considerable time and we appreciate the patience of the authors.

The Editors
June 2007

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Introductory Remarks

Since area-wide integrated pest management (AW-IPM) programmes almost always are social enterprises each with a diverse set of stakeholders reliant on advanced technology, they tend to be complex to implement, especially in terms of management. Therefore, the appearance of this book, "Area-wide control of insect pests: from research to field implementation" is most timely, and it will be invaluable for informing concerned scientists, leaders of private firms, commodity organizations and public agencies, bankers, legislators, students and those interested in placing management of major insect pest problems on a sustainable and environmentally acceptable footing.

Although the need to develop and implement more effective strategies of combating pests and pathogens has always been dire, the urgency of this challenge has increased sharply for two reasons. The first reason is the rapid increase in the world population, which more than doubled from roughly 2.5 billion to 6 billion people in the second half of the 20th century; and the second reason is that the rapid globalization of travel and trade in agricultural and other products has dramatically increased the spread of pests, pathogens and other invasive harmful organisms.

Many of the economically most damaging pests are invasive alien species that have escaped the constraints, which keep their populations in check in their regions of origin. In North America roughly one-half of the major pests originated abroad, and this seems also to be true in other continents. Major exotic pests and pathogens – many adapted for wide dispersal and high rates of reproduction – are becoming established with increasing frequencies on all continents and on many ecologically sensitive islands. Therefore, to facilitate the expansion of international agricultural trade while minimizing the further spread of some major pests, commodities of which they are hosts are increasingly produced for export in pest free areas or in areas of low pest prevalence that obtained their favourable phytosanitary status through AW-IPM approaches.

Currently, for the most part, the control of many highly mobile and very destructive insect pests is still carried out by individual producers who rely heavily on the use of broad-spectrum insecticides. Although other control technologies are often incorporated into the producer's IPM system, these technologies, too, are usually applied by producers independently of other producers, and without due consideration of surrounding host and non-host areas. Such an uncoordinated farm-by-farm IPM pattern provides opportunities for the pest population to build up and to establish damaging infestations.

Consequently, on most farms insect pest populations increase to damaging levels each year, and the farmer is forced to apply fast-acting insecticides as a rescue treatment. This defeats the primary goal of the IPM system, which is to take maximum advantage of naturally occurring biological control agents. Similarly in combating pests and pathogens of concern to human and animal well-being, less than thorough treatment of the entire population fails to provide durable relief. Thus the key concept of the AW-IPM strategy is to address the whole pest population including all places of refuge or foci of infestation from which recruits could come to re-establish damaging densities of the pest population in areas of concern.

The area-wide approach is not new, but originated several thousand years ago. In the Roman Empire it was recognized that some services carried out area-wide were more efficient and cheaper than when left to the action of individual citizens. As such, garbage was diligently removed from some cities, clean water was brought from distant sources and public baths were provided. The sudden appearance in 1347 of Black Death, a bacterial disease transmitted by the flea, *Xenopsylla cheopis* (Rothschild), led to the invention of quarantine to contain the epidemic and to stamp it out. Beginning in the late 1920s,

when catastrophic locust plagues were widespread in Africa and southwest Asia, continent-wide campaigns have been organized to protect against highly devastating locust species; and these campaigns, now led by FAO's Locust Group, employ sophisticated technologies. During the past one-half century the area-wide application of the sterile insect technique in combination with other technologies against an array of major insect pests has served to focus the attention of scientists and administrators on ways of applying the area-wide approach in the combat against many other pests and diseases.

The principles of AW-IPM are addressed in this book's introductory chapter. The chapter argues that each fundamental component of classical IPM, be it a cultural, biological or chemical control tactic, applied against an entire insect population (total population management) will lead in most cases to more sustainable pest control as compared to a localized farm-by-farm approach. Some fundamental management and strategic challenges of AW-IPM programmes are likewise addressed, including the make or break environmental and economic issues.

Successful AW-IPM programmes require basic research and preparatory activities including methods development, feasibility studies, pilot trials and a regulatory framework. These aspects are dealt with in subsequent sections. Section 2 covers and illuminates several important basic research areas including genetics, transgenesis, genetic sexing, cryobiology, physiology, insect symbionts and mating behaviour strategies.

Ecological heterogeneity at within field, within farm, and broader spatial scales profoundly affects the population dynamics of pests and their natural enemies and other aspects of their ecology. Methods of systems analysis, mathematical modelling and a number of geo-spatial technologies (geographic information systems and global positioning system) have been adapted to cope with the spatio-temporal complexity in AW-IPM programmes and have contributed greatly to increased effectiveness and efficiency of programme activities. These and other methods development tools are described in section 3.

Feasibility studies addressing economic, social and technical considerations are required prior to any major and costly field programme. A science-based analysis of these considerations will enable a judgment to be made as to whether the various control tactics can be applied on an area-wide basis and whether the envisioned control strategy is the most appropriate for the particular pest situation. Section 4 provides examples of how such elucidating studies have been conducted for different pest situations.

AW-IPM programmes are dependent on the synergistic collaboration of many stakeholders. They require the entry onto private properties. They can affect the movement of goods, and they can also impact or inconvenience the non-farming community. Thus AW-IPM requires that a sensitive and effective regulatory framework be developed by the relevant national and international regulatory agencies. Commercialization of part or even entire AW-IPM programmes, a complex and sometimes contentious issue, holds the promise of properly capitalizing such programmes, introducing efficiencies and tackling pest problems that government cannot afford to address. These regulatory and privatization issues are discussed in section 5.

Pilot field programmes are often carried out following a feasibility study with a favourable outcome. Such programmes are needed to evaluate and fine-tune various control tactics and field methodologies to increase their effectiveness and efficiency. Pilot programmes can vary in size and scope as is described in the chapters in section 6.

Section 7 describes operational AW-IPM programmes against key pests such as the boll weevil, several lepidopteran pests, the bont tick, termites, mosquitoes, fruit flies, etc. Several of the chapters emphasize the technical and managerial difficulties encountered during the implementation of eradication, suppression, containment or preventive AW-IPM programmes and attempt to extract important lessons.

As a concluding chapter (section 8) a critical review is provided of AW-IPM programmes in terms of their successes and failures, and key factors are identified which must be addressed in order to improve the chance of success.

The chapters in this text book originate from papers and selected posters presented at the 2nd FAO/IAEA International conference on area-wide control of insect pests. To complete the book, several invited chapters have been included. This book is an invaluable compendium of reports on operational AW-IPM programmes. It will help to further develop the theory, technology and practice of such programmes. Graduate students will learn much about the history, accomplishments, problems and the great potential of the area-wide strategy. Entrepreneurs and policy-makers will gain in-depth perspective on aspects of commercialization.

I am honored greatly to have been asked to write these introductory comments in this text book devoted to the area-wide management of insect pests. The prodigious progress in AW-IPM made in recent decades confirms that the area-wide strategy has a far greater potential than any other approach to achieve sustainable management of many major insect pests. Truly we are now at the beginning of an era of decidedly improved and sustainable insect pest management.

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Section 1

Setting the Scene

Area-Wide Integrated Pest Management (AW-IPM): Principles, Practice and Prospects

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ABSTRACT Integrated pest management (IPM) has remained the dominant paradigm of pest control for the last 50 years. IPM has been endorsed by essentially all the multilateral environmental agreements that have transformed the global policy framework of natural resource management, agriculture, and trade. The integration of a number of different control tactics into IPM systems can be done in ways that greatly facilitate the achievement of the goals either of field-by-field pest management, or of area-wide (AW) pest management, which is the management of the total pest population within a delimited area. For several decades IPM and AW pest control have been seen as competing paradigms with different objectives and approaches. Yet, the two "schools" have gradually converged, and it is now generally acknowledged that the synthesis, AW-IPM, neither targets only eradication, nor relies only on single control tactics, and that many successful AW programmes combine a centrally managed top-down approach with a strong grass-roots bottom-up approach, and that some are managed in a fully bottom-up manner. AW-IPM is increasingly accepted especially for mobile pests where management at a larger scale is more effective and preferable to the uncoordinated field-by-field approach. For some livestock pests, vectors of human diseases, and pests of crops with a high economic value and low pest tolerance, there are compelling economic incentives for participating in AW control. Nevertheless issues of free riders, public participation and financing of public goods, all play a significant role in AW-IPM implementation. These social and managerial issues have, in several cases, severely hampered the positive outcome of AW programmes; and this emphasises the need for attention not only to ecological, environmental, and economic aspects, but also to the social and management dimensions. Because globalization of trade and tourism are accompanied by the increased movement of invasive alien pest species, AW programmes against major agricultural pests are often being conducted in urban and suburban areas. Especially in such circumstances, factors likely to shift attitudes from apathy to outrage, need to be identified in the programme planning stage and mitigated. This paper reviews the evolution and implementation of the AW-IPM concept and documents its process of development from basic research, through methods development, feasibility studies, commercialization and regulation, to pilot studies and operational programmes.

KEY WORDS area-wide IPM, field-by-field IPM, suppression, eradication, feasibility studies, pilot programmes, operational programmes, commercialization, regulation, public good, free rider, public participation

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1. Introduction

If major advances are to be made in coping with most of the major arthropod pest problems, then the tactics and strategies for managing such insects, ticks and mites must change. They must change from the current, limited scale, reactive, broad-spectrum measures to preventive measures that are target-pest specific and rigidly applied on an area-wide basis (Knipling 1992).

Around 850 million people remain malnourished (FAO 2006) in spite of significant progress over the last four decades towards food security in several regions of the world, and numerous positive developments in the area of food and agriculture. In 1996, the World Food Summit held in Rome, Italy addressed this persistent crisis by launching the very ambitious goal, later incorporated into the Millennium Development Goals, of halving the number of hungry people by 2015 (FAO 1996). However, the Food and Agriculture Organization of the United Nations (FAO) has analysed the world food insecurity situation and indicated that, unfortunately, progress is insufficient to meet the Summit's target (FAO 2006). Furthermore, the world population is expanding by ca 75-80 million people each year, and most likely will rise to about 9000 million by the year 2050, and thus require food production levels at least 50% higher than those in 2000 (Alexandratos 1999). At the same time agricultural research and extension budgets and aid to agriculture are shrinking, natural resources are degrading, and the growth of the world's agricultural production is continuing to slow down (Bautista and Valdés 1993, Braun et al. 1993, Alexandratos 1999).

This continued rapid growth of the world population, which is causing the biggest surge in demand for food in history, including demands for significantly more animal proteins (Delgado et al. 1999) and biofuels, stands in stark contrast to the shrinking *per capita* land area available for agriculture. This discrepancy cannot be addressed by horizontal agricultural expansion, i.e. by an increase in cultivated surface area, but requires development and promotion of more intensive cropping and livestock systems on existing farmland (Borlaug 1997). Access to new technologies and the knowledge to use or adapt them locally will be vital, coupled with less wastage and improved penetration into national and global markets. As expansion of arable land will only play a minor role in some regions and attaining substantial increases in crop yields will become increasingly difficult in others, the focus will have to shift towards a more efficient use of agricultural resources (Trewavas 2001). There could hardly be a less efficient use of resources than to invest land, water, fertilizer, seeds, labour, and energy to produce agricultural commodities, only to have the investment partially or totally destroyed by insects or other pests. Preharvest losses in developing countries are estimated at more than one third of attainable crop production, while postharvest losses add at least another 10-20%. Insects, followed by pathogens and weeds, cause the largest portion of these losses (FAO 1975, Oerke et al. 1995, Yudelman et al. 1998, Thomas 1999).

Insects have proven to be among the most formidable adversaries of mankind. Since they appeared on the scene some 390 million years ago, they have diversified into several million species that have adapted to almost all available ecosystems. This large diversity has allowed them to effectively compete with mankind since the introduction of agriculture over the last ten millennia. The increase in agricultural production of the past decades has not been accompanied by a comparable reduction in overall losses inflicted by insect pests: on the contrary, agricultural intensification has increased both yields and vulnerability to pests (Yudelman et al. 1998). Furthermore our mobile society is redistributing species around the globe at an unprecedented pace with major consequences for agriculture and ecosystems (FAO 2001). Undoubtedly, insects will continue to challenge mankind, and their resourcefulness is forcing a review of established ways of dealing with these pests and stimulating the development of new innovative control tactics (Klassen 2005). Investing in improved pest management should therefore be an integral

component of national strategies to raise productivity and to assure future global food security.

The sudden availability of very effective and persistent synthetic organic insecticides immediately after the Second World War marked the onset of chemically-based warfare against most insect pests. Before that, insect pests had to be kept at bay by making use of various natural control factors. The importance of the new synthetic chemicals to control vectors of major diseases and their contributions to the green revolution cannot be questioned (Oerke et al. 1995). Chemical control has offered an effective and economical way to deal with a multitude of arthropod problems, to quell outbreaks, to decimate vectors of parasitic and infectious diseases of humans and livestock, to suppress noxious insect developing in dung on rangelands where domestic animals are produced, to suppress mites in honey bee hives, and to control termites and numerous household pests in urban ecosystems, etc. Pesticides have offered the pesticide user the freedom and flexibility to control pests on his property at any time without regard for the opinions and actions of his neighbours. Without chemical pest control, global agricultural productivity would have been less, food prices much higher and the available food of lower quality (Knipling 1979). The ready accessibility of these "off-the-shelf", relatively cheap and often subsidized chemicals was undoubtedly one of the main reasons the uncoordinated control of key insect pests on a field-by-field basis became so widely and firmly established during the last 60 years.

The drawbacks of the widespread use of these broad-spectrum insecticides were, however, recognized early (Carson 1962). They pollute soils and water, are a hazard to many non-target and beneficial insects, lead to outbreaks of secondary pests, cause acute and chronic poisoning of farmers, accumulate and biomagnify in the food chain and represent serious concerns to human health (Repetto and Baliga 1996). The general public's increased awareness and demand for more environment-friendly pest control tactics, the swift development of pesticide resistance, and the need for ever new, more complex and much costlier products, gave rise to the concept of integrated pest control (IPC) (Stern et al. 1959, FAO 1966), later called integrated pest management (IPM) (Bottrell 1979). In the 1960-70s, stimulated by this need for reduced and more selective use of insecticides, IPM became gradually accepted as a viable and sustainable pest management strategy that incorporates some of the traditional practices of the pre-insecticide era (such as field sanitation, biological control and use of pest and disease resistant livestock breeds and crop varieties) together with more selective synthetic organic pesticides to maintain pest population levels below an economic threshold. IPC-IPM has remained the dominant paradigm of pest control for the last 50 years (Kogan 1998).

2. Integrated Pest Management (IPM): a Reaction Against the Abuse of Insecticides

IPM offers a strategic approach to solving pest problems in an ecosystem context while guarding human health and the environment (Brader 1979). It has been endorsed by essentially all the multilateral environmental agreements that, since the United Nations Conference on Environment and Development in Rio de Janeiro in 1992, have transformed the global policy framework of natural resource management, agriculture, and trade. More than half the world's human population lives in countries that are guided by national IPM policies, and that account for most of the world's staple crop production. IPM is increasingly practiced in agroecosystems featuring perennial tree crops, annual field crops, crops, in protected cultivation, ornamental crops, rangelands, intensive pastures, roadways, recreational parks, forests, dairies, barnyards, and urban ecosystems. Small-scale family farms in tropical and temperate zones as well as multinational corporate food producing and processing firms

apply IPM. IPM systems range over a continuum, from those still dependent to a considerable degree on the use of pesticides all the way to those called biointensive that rarely require chemical treatments (Vandeman et al. 1994). Along this continuum, reliance on pesticide treatment-oriented interventions decreases and reliance on biological and cultural practices increases and requires an increasing number of available methods in managing pests (Benbrook et al. 1996).

2.1. FAO's Code of Conduct on the Distribution and Use of Pesticides

The International Code of Conduct on the Distribution and Use of Pesticides (revised from the original 1985 version) was adopted by the 123rd session of the FAO Council in November 2002. The Code embodies a modern approach, leading to sound management of pesticides with a focus on risk reduction, protection of human and environmental health, and support for sustainable agricultural development by selectively using pesticides as a component of various IPM strategies (FAO 2003a). Thus, the Code designed standards of conduct to promote IPM, including the integrated management of public health vectors.

The Code defines IPM as:

> *...the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption in agroecosystems and encourages natural pest control mechanisms (FAO 2003a).*

The Code has been approved and adopted by 185 FAO member governments, endorsed by non-governmental organizations and by the pesticide industry. Therefore the definition of IPM in the Code carries a degree of accessible authority that many other published definitions of IPM do not (Bajwa and Kogan 1996). In its article on pesticide management, the Code calls for support to alternatives to conventional pesticides:

> *Governments, with the support of relevant international and regional organizations, should encourage and promote research on, and the development of, alternatives posing fewer risks: biological control agents and techniques, non-chemical pesticides and pesticides that are, as far as possible or desirable, target-specific, that degrade into innocuous constituent parts or metabolites after use and are of low risk to humans and the environment.*

The Code reflects evolving responses to changing conditions with emphasis on protecting the integrity of agroecosystems, encouraging natural pest control mechanisms, and reducing risks to human health and the environment. In the articles of the Code on pesticide management, the wider range of stakeholders, and the emphasis on promoting increased participation of farmers, women's groups, and others reflect recent experiences with successful IPM (van den Berg 2004).

2.2. Selected Successes of IPM

IPM has had a varied history, and simply mixing different management tactics does not constitute IPM (Ehler and Bottrell 2000). Successful IPM is "knowledge intensive" and has never been successful without basic research on ecosystems, particularly to comprehend the food webs or communities of species through which energy flows in agroecosystems (Barfield and Swisher 1994, Wood 2002). Understanding why a pest becomes a pest (Lewis et al. 1997) comes from a fundamental understanding of the pest's life history and the ecology of crop-pest-natural enemy interactions, and rarely from a revolutionarily new control tactic (Kogan 1998, Thomas 1999).

The rice brown planthopper *Nilaparvata lugens* (Stål) was the key insect pest of the Asian green revolution in rice. While single tactics of insecticides and vertical host-plant resistance largely dominated research and pest control application, a series of studies in the mid 1970s elucidated the mechanism of out-

breaks of secondary pests due to the overuse of subsidized insecticides. This was shown through analyses of multi-species rice field agroecosystems that concentrated on the rice crop, the planthoppers, their predators, and parasitoids. The results, made available by FAO, were then successfully applied in the mid 1980s through national IPM policies and programmes in the Philippines, Indonesia, India, Vietnam, China, and other major rice-producing countries (DeBach and Rosen 1991, Kogan 1998, Bartlett 2005). As a result of these policies, over USD 250 million per year in governmental insecticide subsidies were eliminated.

Subsequently Settle et al. (1996) showed how the rice field's aquatic food web, driven by decomposition of rice roots, rice straw, and other organic matter from previous seasons, through dozens of aquatic arthropods, produced sufficient numbers of predators, to protect the rice crop from the seedling stage to maturity. Rice field dwelling arthropod species that do not feed on rice still serve as important food sources in building up populations of natural enemies of rice pests. The species richness of natural freshwater ecosystems (streams, ponds, rivers, and lakes) permits irrigated rice agroecosystems to draw from their larger natural species pools to quickly fill in the essential guild structure of the cultivated aquatic rice agroecosystem, buffering it from immigrant pest populations. In addition, Ives and Settle (1997) challenged the conventional wisdom on synchronous rice planting, showing that in the presence of natural enemies asynchronous rice planting results in the lowest overall pest densities, since early arriving generalist predators decimate incipient infestations of pests and suppress their populations to a greater extent than is accomplished by killing large numbers later.

3. Field-by-Field and Area-Wide Pest Management Approaches

3.1. Field-by-Field Pest Management

Insect pest control measures can be applied either field-by-field (Fig. 1) or on an AW basis (Fig. 2), the latter addressing the total pest population within a delimited area. The simplest and most widely used strategy has been field-by-field management, which addresses only small fractions of a pest population at any given time. It allows individual crop and livestock producers, households, and businesses, to control pests independently, without investing effort in coordination, without having to obtain the consent or collaboration of other stakeholders and most importantly without taking into account the pest individuals that frequently migrate into the treated area from infestations in the untreated surroundings. Insects, themselves, are mobile but, can also be transported passively with wind, on animal hosts, or in infested commodities traded locally or internationally. This mobility severely compromises the effectiveness of uncoordinated farm-by-farm, orchard-by-orchard, or herd-by-herd control efforts, and results in the frequent need for curative or therapeutic back-up measures (Lewis et al. 1997) and the eventual overreliance on them. However, field-by-field pest control does not demand long-term commitment to an organized effort and its funding requirements, but relies on remedial interventions triggered when a pest population reaches a certain threshold. Field-by-field pest control is, therefore, largely a reactive approach to protect humans, animals, crops, forests, houses, wooden structures, etc., rather than a preventive pest population management approach (Pedgley 1993, Abeku 2007).

As a result of the complexity of many agroecosystems, as well as the site-specific nature of a majority of pest problems, predetermined thresholds often become operationally intractable and in some pest situations, the threshold is zero tolerance (Ehler and Bottrell 2000). Thus a field-by-field IPM approach is often insufficient, particularly when pests are quite mobile. Furthermore, the cost of generating the large amount of ecological information needed to develop and implement functional IPM systems for such local situations cannot be afforded by most devel-

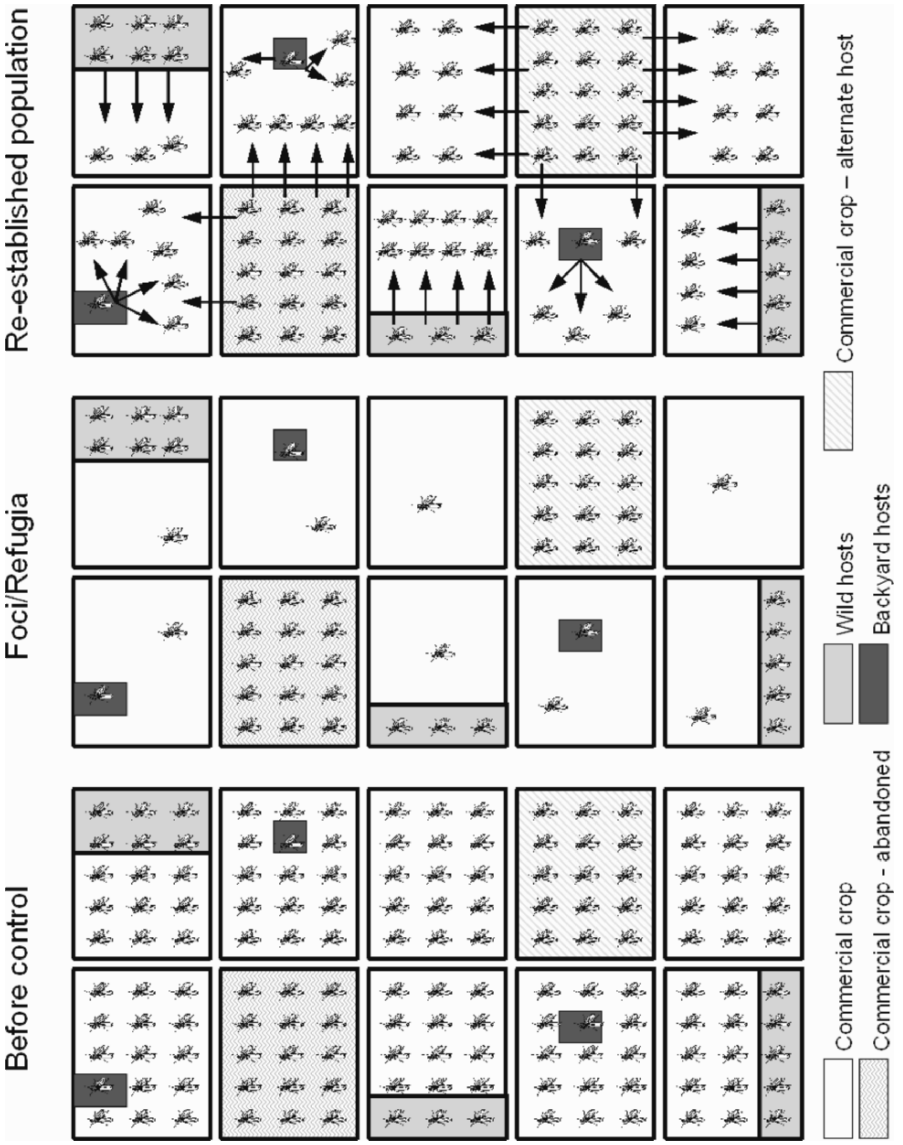

Figure 1. *Graphic display of field-by-field IPM, where the pest is suppressed below an economic threshold level in areas of commercial interest, but often not in abandoned crops, alternate hosts, backyard hosts or on wild hosts. As a result, significant untreated refugia of the pest remain from which recruits re-establish damaging densities of the pest population.*

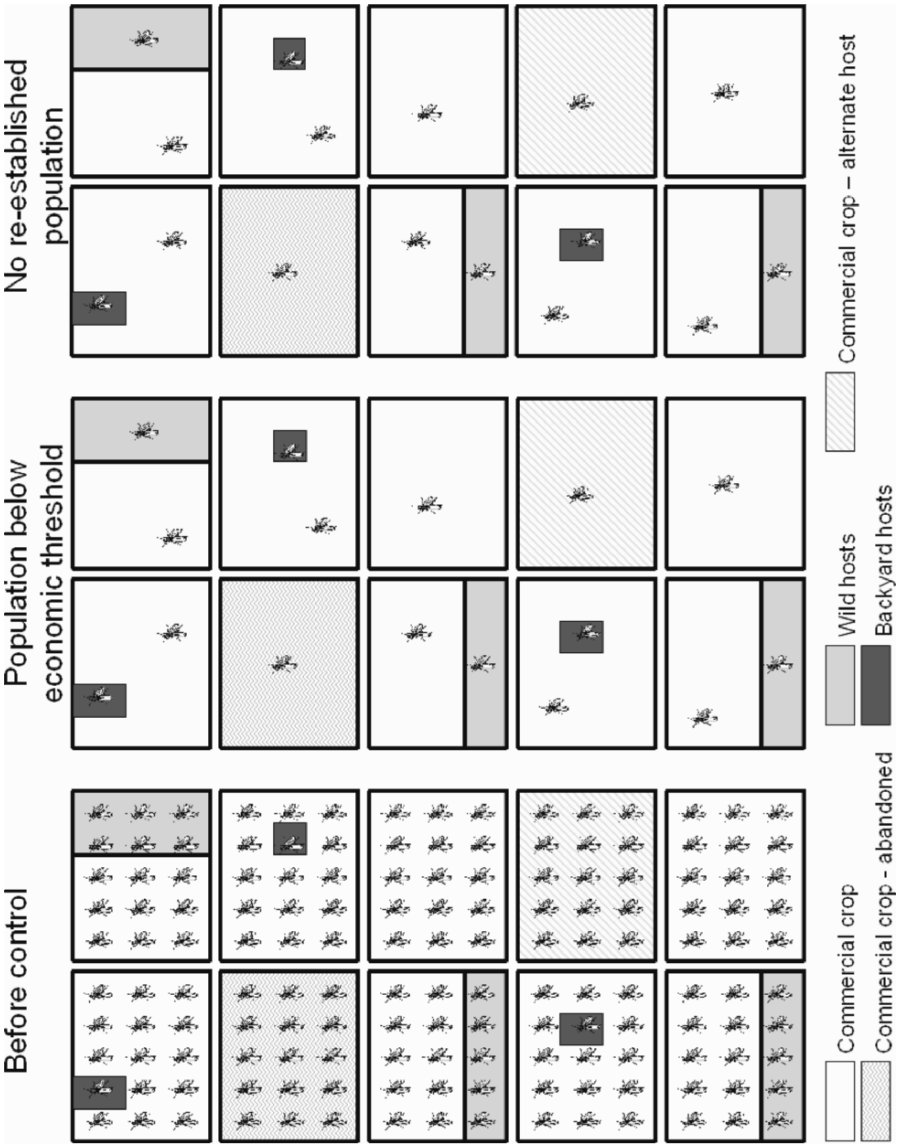

Figure 2. *Graphic display of AW-IPM, where the pest is suppressed below an economic threshold level in all areas, including abandoned crops, alternate hosts, backyard hosts or on wild hosts. As a result, no significant untreated refugia of the pest remain from which recruits can re-establish damaging densities of the pest population.*

oping countries (Morse and Buhler 1997).

3.2. The Principle of Total Population Management

Knipling (1960, 1972) used simple population models to show that small fractions of an insect pest population left uncontrolled can rapidly nullify the benefits of strongly suppressing the main pest population in a large area. One of these models compares the dynamics of an hypothetical insect pest population that has a fivefold natural rate of increase per year in an area, and where each year 99 percent of the population is destroyed on 90% of the host resources (but no control is conducted on the remaining 10%) with an area where only 90% of the population is destroyed on 100% of the total host resources (Table 1). The model shows that because no control was exercised on 10% of the host resources, 100 times more pests were produced in the first scenario (where 99% kill was achieved each generation in the large treated fraction) than in the second scenario where no refugia were left but the total population was subjected to only 90% kill in each generation.

From this Knipling (1972, 1979) deduced the basic principle of total population control:

> *Uniform suppressive pressure applied against the total population of the pest over a period of generations will achieve greater suppression than a higher level of control on most, but not all, of the population each generation*.

These refugia are permanent and prolific sources of immigrants and therefore represent a constant economic threat, requiring applications in cultivated areas of significant amounts of insecticides year after year, even in situations where major pests are managed under an IPM approach. This failure to address the total pest population in an area often compromises the basic goal of IPM, i.e. the reduction of insecticide use. In contrast the central paradigm of AW control, variously also called landscape, large-scale, preventive or total population management (Pedgley 1993, Ekbom et al. 2000, Carrière et al. 2001, Smith et al. 2006), recognizes the need to address the existence of all foci/refugia from which recruits can invade suppressed or cleared areas (Byers and Castle 2005, Klassen 2005).

Total population management is also required to reduce the probability of the development of insecticide resistance, or of the emergence of strains of a pest capable of over-

Table 1. *Relative number of insects developing each generation in two hypothetical population management systems. Each of the two populations has an initial population size of one million insects and a fivefold increase in reproducing insects is assumed with each generation (after Knipling 1979).*

Generation	Number of insects in an area with				
	99 percentage control on 90 percentage of the target population			90 percentage control on 100 percentage of the target population	
	treated area (90%)		untreated area (10%)	treated area (100%)	
1	900 000	9000[1]	100 000	1000 000	100 000[1]
2	45 000	450	500 000	500 000	50 000
3	2250	22	2 500 000	250 000	25 000
4	110		12 500 000	125 000	
Total insects in 4th generation:		12 500 110		125 000	

[1]number of insects surviving treatment

coming host resistance. In particular, AW pest management strategies, guided by the effective use of geographic information systems (GIS) technology, can be applied to achieve effective resistance management (Carrière et al. 2001, Sexson and Wyman 2005, Wu, this volume). Nevertheless, there is a potential danger in AW population suppression, since continuous and thorough suppression applied on an AW basis will select genes in the pest's gene pool that enable the pest to overcome the survival threatening control agent. For example, in the absence of refugia, as part of a resistance management programme, repeated AW applications of an insecticide will select for resistance to that insecticide, repeated AW use of baits will select for avoidance behaviour, and repeated AW planting of the same resistant crop variety will select genes for overcoming the crop's host resistance.

3.3. Origins of Area-Wide (AW) Approaches

The AW approach is not new but evolved centuries ago. Reduced cost, increased effectiveness and greater physical protection were generally the underlying reasons for the development of AW services. In earlier times, essential items such as the water supply, fuel, lighting, and sewage disposal were provided on an individual basis and each extended family satisfied these needs independently of its neighbours. This was an uncoordinated approach with the result that these individual services often proved to be not only unreliable, but also inefficient and could only be obtained at great individual expense. The desire for more effective and efficient delivery of these essential services transformed them from "individual" to "area-wide" and led eventually to the creation of public or private companies that supplied clean water and electricity, and that took responsibility for the collection of the garbage and disposal of sewage. Likewise, originally each head of the clan took responsibility for the physical protection of his kin. Soon feudal societies developed in which some individuals and later institutions specialized to provide protection for much larger groups. Eventually, police forces, state and national armies emerged that were able to deal with larger-scale military threats. The AW approach for these services made them more effective and less costly because the providers could use technically more advanced methods that were not available to the individual (Lindquist 2000). Today, AW services (e.g. mail service, retailing, ambulance, fire protection, public health, high-speed transport, telephone, internet services, etc.) are much more widespread than ever before.

3.4. Area-Wide Approaches Applied to Insect Pest Control

Applied to insect pests, AW approaches have their ancient roots in coping with vector-borne diseases and locust plagues (Klassen 2000, 2005). In the 14th century, the systematic use of quarantines in some European city-states contained bubonic plague transmitted by the oriental rat flea *Xenopsylla cheopsis* (Rothschild) and this approach was gradually adopted throughout Europe. The late 19th century included AW approaches such as the development of classical biological control and the use of pest-resistant plants (for example the grafting of all European grapes on phylloxera-resistant American rootstocks in the 1870s).

A campaign to eradicate the invasive gypsy moth *Lymantria dispar* (L.) from 1890 to 1901 in Massachusetts mainly using a modestly efficient phytotoxin, was quite successful initially but had to be abandoned after public opposition to the spraying. Since then, the objective of this AW campaign has reverted to limiting or retarding the spread of this pest (Sharov et al. 2002).

In 1906, a massive effort in the southern USA was initiated to eradicate two *Boophilus* cattle tick species that transmit cattle tick fever (Klassen 1989). A strategy of starving the ticks by making most pastures cattle free, combined with an arsenic-dipping programme and restricting cattle movement to tick-free counties was rigorously implemented for 37 years. In 1943, the ticks had been eliminated

from the USA at a total cost that was equivalent to the yearly losses before the programme was initiated. To date, the southern USA has remained free of these ticks, as the result of an effective quarantine programme.

In 1911 the Government of Portugal decided to eradicate the tsetse fly *Glossina palpalis palpalis* Robineau-Desvoidy and trypanosomosis from the Island of Principe off the west coast of equatorial Africa. The suppressive system consisted of sticky black cloths worn by workers, treatment of people with the trypanocidal arsenical, atoxyl, clearing of vegetation, corralling of domestic livestock and eradication of feral pigs. The campaign was concluded in 1914 (McKelvey 1973): it was the first successful tsetse eradication programme.

After the World Health Assembly urged the World Health Organization (WHO) to organize the eradication of malaria in 1955, enormous progress was made in the following 15 years, and malaria was declared eradicated in 37 countries (Spielman et al. 1993). Social pressure to devolve more control to the local level and the banning of DDT resulted in the disintegration of the programme. To date, more than 400 million malaria cases are reported annually worldwide and more than one million people, mainly children in Africa, succumb to the disease every year (Marshall 2000).

In the 1950s and 1960s, a significant AW campaign was implemented against the introduced Khapra beetle *Trogoderma granarium* Everts which had become established in the south-western USA and northern Mexico. A major quarantine and treatment effort, which involved the fumigation of all warehouses, seed storage facilities, ships and other transport facilities, was initiated cooperatively by all states in these regions. Funding for this AW programme was shared between the federal and state governments and the private sector. By 1966 all known populations of the pest had been eradicated. Another introduction of this pest was eliminated between 1980 and 1983 in the north-eastern USA (Klassen 1989).

In 1973, the exotic cassava mealybug *Phenacoccus manihoti* Matile-Ferero was observed attacking cassava around Kinshasa (Democratic Republic of Congo, formerly Zaire) and Brazzaville (Republic of Congo) and in subsequent years it dispersed throughout sub-Saharan Africa, causing starvation of more than 200 million people (Neuenschwander et al. 1988). The International Center for Tropical Agriculture, Cali, Colombia, identified a suitable parasitoid *Epidinocarsis lopezi* DeSantis in Paraguay, which was brought to Africa by the International Institute for Tropical Agriculture in Ibadan, Nigeria. The parasitoid was successfully mass-reared and released in 38 African countries. This is an outstanding accomplishment in classical biological control, and it continues to keep the mealybug at bay.

Cereal aphids have become a major threat to production of sorghum, wheat and barley in central-western North America following the emergence of virulent biotypes of the green bug *Schizaphis graminum* (Rondani) during the 1960s, the establishment of the Russian wheat aphid *Diuraphis noxia* (Kurdjumov) in 1986, and the subsequent emergence of new biotypes of the latter species. These pests have been causing annual losses in grain production in excess of USD 250 million, and have caused large and unsustainable increases in the use of insecticides on these low profit margin crops. Therefore, a very large classical biological control programme involving the introduction of parasitoids from Eurasia was inititated (Brewer and Elliott 2004). However, an analysis of the wheat agroecosystem has revealed the presence and economic impact of many species of indigenous natural enemies, and it has been difficult to demonstrate any contribution from imported exotic species. As in the above mentioned problem of the brown planthopper on rice, understanding the community structure of the agroecosystem, initially intended as a side product of the classical biological control campaign, turned out to be the crucial foundation of the AW-IPM programme. Many other examples of successful or unsuccessful AW programmes to suppress

or eradicate insect pest populations are listed in Klassen (1989, 2005).

3.5. Public Participation, Public Good, and Free Riders

As discussed above and by Vreysen et al. (this volume), AW programmes are not always successful. Social concern over methods, too many free riders, or insufficient compliance by all stakeholders have in several cases severely hampered success (Klassen 2000), emphasizing the need for attention not only to ecological, environmental, and economic, but also to social and managerial dimensions.

3.5.1. Public Participation

Public participation or "ownership" by the general public of AW programmes is crucial for their success. Yet often such support is weak, and special public information efforts are needed to convince the stakeholders of the wider benefits. Mumford (2000) summarizes this aspect well:

> *The main area in which problems lie with area-wide pest control appears to be in the mechanisms for public participation. Reports over many years cite technical, economic and environmental success with the concept, but there is still indifference, reluctance and antagonism.*
>
> *... these attitudes may be the result of a lack of opportunity for involvement and ownerships of programmes, which may be seen as being imposed from above/outside, managed by technocrats or otherwise not arising from or meeting the needs of the people directly concerned. None of these issues should be insurmountable, but it is worth noting that area-wide pest management is an activity in which social participation and attention to the "reasonable person" is as important as technical proficiency.*

Outrage factors pose a great threat to AW programmes. Once a large segment of the public has become outraged, it is almost impossible to lead them back to an attitude of trust and support (Sandman 1987). People in significant numbers have expressed outrage against AW programmes conducted for the benefit of agriculture or public health in urban settings, and such instances are likely to increase because of the recent surge in frequency of establishment of invasive alien pests near international airports, seaports and elsewhere in metropolitan areas. Protesters in urban communities fearful of mandatory pesticide applications attempted to halt the campaigns to eradicate the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in the Los Angeles Basin by the State of California and the United States Department of Agriculture (USDA) during the 1980s (Lorraine and Chambers 1989). During the recent campaign to eradicate citrus canker from Florida (1995-2006), protesters in urban communities in south-eastern Florida, outraged by the entry of inspectors and workers into backyards of homes without search warrants and the destruction of apparently healthy citrus trees, delayed programme implementation from 2001 to 2004 by challenging its legality in court. In 2004 three hurricanes spread the canker bacterium from infected trees left untouched during the three-year litigation so widely that the programme was judged to be not feasible, and it was terminated in 2006 after the expenditure of ca USD 875 million (Bouffard 2006). In India a WHO programme to eradicate *Aedes aegypti* (L.), the vector of yellow fever and dengue, was terminated during the 1970s because journalists incited irrational fear of the sterilized male mosquitoes (Nature 1975). Clearly potential outrage factors need to be identified during the planning stages of AW programmes, and a well funded public information programme must be launched at the outset of each programme. Public information specialists must be trained to be up front, open and honest about all issues which may be perceived negatively especially by urban stakeholders.

3.5.2. Public Good

Public health, a clean environment, ecosystem services, roads, public education, a wholesome food supply, etc., have all been labelled as public goods (Johnson 2005). The application of IPM in agriculture and human health gives rise to positive externalities for the common or public good, resulting in benefits such

as reduced risks to humans and the environment. In contrast a negative externality arises when a farmer, through injudicious pesticide use, pollutes the environment and harms his neighbours. In the case of mobile pests, damage is a function of the total pest population in the entire area. Pest control by any farmer results, therefore, in pest suppression and less damage for the neighbours as well, although only the one farmer incurs the cost of the control. This spill-over benefit for the other farmers in the vicinity is a positive externality, which unfortunately encourages free riders and is often not conducive for a collaborative programme against the pest within a community or at larger scales (Yu and Leung 2006).

3.5.3. Free Riders

Free riders are individuals choosing to benefit from a public good or a positive externality without contributing to the costs of producing the benefits (Johnson 2005), and they represent a problem for IPM implementation against mobile pests. Farmer associations or "community IPM", effectively promoted by FAO through IPM farmer field schools in developing countries (van den Berg 2004), can help address this problem. Actually community IPM is more than pest management and offers an entry point to improve the farming system as a whole, developing the enhanced management skills necessary for sustainable environment-friendly agricultural and rural development (Dilts 2001). Nevertheless, the cultural and socio-economic background of stakeholders significantly affects the collaboration rate in community-based projects (Smith et al. 2006, Stonehouse et al. 2007).

Non-collaborators represent a major weakness when operating programmes at a larger scale (for example regional public health vaccination campaigns, or global rinderpest or polio eradication). A few free riders or "refuseniks" can negate many positive impacts of AW programmes. Since externalities may affect a large number of stakeholders, obtaining full participation and dealing with sceptical, negligent, or even antagonistic third parties is an unavoidable and difficult challenge. In addition, merely because a good is said to be public, such as an unpolluted or disease-free environment, does not automatically imply that all people value it equally. Nevertheless, often a public good cannot be provided, or provided to an adequate extent, without strong support for a mechanism of collective action.

3.5.4. Subsidies, Government Provision, and Legal Authority

State subsidies, or complete government provision, and/or regulation or even legal authority for enforcement by authorities can at least partially overcome this type of market failure. All of these solutions represent an "involuntary" provision through taxation to partially or fully fund the public good. With benefits at least as large for society as for growers and other IPM practitioners who carry the financial burden of implementation, a strong case has been made for financial incentives as a stimulus to entice more participation and expansion of IPM adoption (Brewer et al. 2004). Since benefits to society of AW interventions tend to be unusually large, the allocation of public funds to many of such programmes in public health and agriculture appears to be strongly justified, and indeed, many AW pest control programmes are partially funded by the state (Bassi et al., this volume). The establishment and enforcement of legal rules by authority (Klassen 2000), for example, the removal of mosquito breeding sites, the implementation of quarantines, or the removal of wild hosts in the surroundings of crops (Kovaleski and Mumford, this volume) is greatly facilitated by establishing such "official" AW pest management activities.

3.6. Linking Area-Wide Approaches with IPM (AW-IPM)

The concept of AW pest control, developed by USDA under the direction of E. F. Knipling, has been closely identified with the programme to eradicate the New World screwworm *Cochliomyia hominivorax* (Coquerel),

which started in Florida in 1957 and reached Panama in 2001 (Klassen and Curtis 2005). During these same decades many academic institutions developed and promoted the IPM paradigm. For several decades IPM and AW pest control were seen as competing paradigms with different objectives and approaches (Perkins 1982), and each competing against the other with its own core organizational base, i.e., state universities (IPM) versus federal research and regulatory agencies (AW).

3.6.1. Suppression versus Eradication

While under IPM suppression, pests can be tolerated at certain levels as part of healthy agroecosystems in which all components have a functioning role, IPM practitioners perceived AW pest control as targeting only eradication, an end point to which some had strong philosophical objections. Practitioners of AW control were accused of only wanting to eradicate pest populations based entirely on the initial promise of synthetic insecticides and that they were unwilling to manage and otherwise learn to live with pests. However in reality, Knipling (1966, 1969, 1972) had early on included suppression in AW control of key pests, although the possibility of eradication of selected populations of major pest species, if practical and economically and environmentally advantageous, was preferable (Kogan 1998). For example, since about 1960 Knipling worked toward the eradication of boll weevil *Anthonomus grandis* Boheman in the USA, but in this he was effectively opposed for decades by IPM practitioners. Eradication of this key invasive pest on cotton, which was finally initiated in the 1980s and is now nearing completion (El-Lissy and Grefenstette, this volume), was foreseen to significantly and permanently reduce insecticide use and provide major environmental and economic benefits. It was also foreseen that removal of the pest would cause much cotton production to shift from the central part of the cotton belt to the south-eastern USA, where in the absence of this pest cotton production is the most profitable. Currently none of the several ongoing AW control programmes are intended to eradicate the target plant pest or pest complex; instead they are designed to manage selected pests across an expansive geographic landscape (Pedgley 1993, Coop et al. 2000, Hendrichs et al. 2005, Sexson and Wyman 2005, Carrière et al. 2006, Abeku 2007).

In recent years organizations concerned with the preservation of biodiversity and conservation of natural ecosystems have become alarmed that their investments in conservation are at serious risk of being undone by invasive alien species (Sklad et al. 2003). Moreover, conservation scientists have found eradication to be essential for the restoration of certain natural ecosystems badly damaged by invasive species, and attitudes toward eradication are being revisited (Myers et al. 2000, Clout and Veitch 2002, Simberloff 2002, Pérez Sandi Cuen and Zimmermann 2005).

3.6.2. Area-Wide Integration

While the integration of compatible control tactics was seen as the cornerstone of the IPM concept, adherents of the IPM school did not perceive such integration to be integral to AW pest control systems, even though most AW suppression or eradication campaigns employed simultaneously several control tactics to achieve their goal. However, the integration of all available weapons is a fundamental requirement for the success of both field-by-field and AW-IPM. For example the New World screwworm eradication programme relied not only on the release of sterile insects, but integrated their use into a system including quarantines to prevent spread and reinvasion, closely scheduled examination of all livestock, collection and identification of specimens from wounds, diligent treatment of all wounds, navels and other sites of oviposition of the parasite, scheduling of cultural practices related to livestock management such as branding, castration and dehorning only when the parasite was least prevalent, and extensive public information activities to obtain transparency and secure the collaboration of all stakeholders. Clearly, the New World screwworm eradication programme

targeted both the adult and immature stages of the parasite. Indeed Knipling (1979) proposed integrating control tactics that address immature stages (for example natural enemies) with those that target the adult stage (sterile insects, mating disruption), or those that are very effective against high population densities (use of bio-pesticides and baits) with those that are effective against low population densities (mating disruption systems, sterile insects, parasitoids, etc.).

3.6.3. Top-Down Approach

While for IPM a bottom-up approach at the farmer and community level was the operational mode, AW control was seen as needing a top-down approach, centrally managed by an organization, and with a mandatory component to insure full participation of stakeholders within a region. Even though AW programmes are usually, but not always, centrally managed, they cannot afford to ignore the concerns of farmers and communities. Indeed AW programmes cannot succeed unless they secure the active enthusiastic participation of all stakeholders, especially that of farmers and rural communities to achieve their goals. And where such a programme involves urban communities, it is even more important to assure that the urban people understand the importance of the programme and are willing to tolerate and contribute to its costs.

While not all AW-IPM programmes include mandatory components to insure stakeholder participation, a regulatory framework undoubtedly facilitates programme effectiveness. Some regulations established by regulatory authorities with stakeholder input are critical to success of certain AW programmes. These may include mandatory crop destruction by a certain date to prevent overwintering of the pest, mandatory seeding or planting dates to provide for suicidal emergence of the pest, access to private property by inspectors, etc. Since such regulations may be difficult to enforce, it is important that at the outset a referendum is held to determine if at least two-thirds of the stakeholders would willingly comply. On the other hand special regulations are not needed to assure the success of many AW programmes, and in such instances it would be most unwise to impose them.

3.6.4. What is Area-Wide?

The term area-wide, coined for total population management is widely entrenched, even though it can be misleading (and has been at least partially responsible for misunderstanding the concept), since the concept deals primarily with a total population in a delimited area, the influence of migration/dispersal on its dynamics, and its ecological relationships within its ecosystem. Including the distribution of the pest population in space and availability in time appears to be the logical next step in the ongoing evolution of IPM from originally managing a single pest in a field, to multiple pests in a field, to a larger scale that includes multiple fields and multiple crops. Actually large geographic areas are not a prerequisite for the AW approach, because addressing pest populations within a closed greenhouse, for example, also involves managing them at the population level, where their temporal dynamics and spatial distribution are known (Casey et al. 2007).

3.6.5. Merging of IPM and AW Control

Over time, as described above, the two schools have gradually converged and the differences between them have turned out to be less critical than originally perceived (Coppedge 1994, Kogan 1994, 1995, Parry 1995, Kogan 1998, Coop et al. 2000, Tan 2000, Faust 2001, Yu and Leung 2006). AW-IPM is a very broad and flexible concept and is increasingly accepted for those situations of mobile pests where management at a larger scale is advantageous to maximize the AW, not necessarily local, efficacy of management tactics (Cronin et al. 1999). At the same time grower education, for example through the FAO-organized IPM farmer field schools, is also leading to some concerted action to scale up the IPM movement at the community level (Dilts 2001, Pontius et al. 2002). Rice farmers are gradually moving from field-by-field to

AW-IPM implementation by coordinating at the village, subdistrict, and in some cases even district levels (Matteson 2000).

The interaction of AW application of tactics and coordination of activities among stakeholders is also not straightforward. While synchronous AW application of control measures is generally more efficient to preclude pest population refugia (Byers and Castle 2005), in other situations AW spatial asynchrony is deliberately adopted over broad geographic regions. For example, spatial asynchrony of control measures has a greater effective suppressive effect on the total pest population in the rice agroecosystem, because it favours earlier migration of natural enemies into the rice fields (Ives and Settle 1997).

3.6.6. Situations Advantageous for Area-Wide Control

Economics undoubtedly plays a major role in the initial grower decision to participate in AW-IPM (Sexson and Wyman 2005), and deteriorating market conditions may cause the grower to neglect or even abandon a crop in a field or an orchard. Farmers who cultivate crops with a high economic value and low pest tolerance risk suffer greater losses than farmers who cultivate crops with a low economic value and high pest tolerance (Yu and Leung 2006). In the latter situation there are fewer incentives for farmers to cooperate through an AW approach, whereas in the first case the economic advantages of participation are much greater (Stonehouse et al. 2007). This is particularly so for crops such as vegetables and fruit, or for some livestock or human diseases, where the acceptable thresholds are so low that the presence of even a few pest or vector individuals often triggers the need for remedial applications.

Using a mathematical model, Yu and Leung (2006) derived several favourable and unfavourable conditions for implementing AW-IPM. In their view, AW-IPM is more likely to succeed where the number of farmers is small, and the cultivated crops are similar (low farm heterogeneity). The stability of the cooperation among the farmers is enhanced by short detection times and high discount rates. The model likewise demonstrates that a one-off suppression of the pest under the leadership of a third party facilitates the cooperation of heterogeneous groups of farmers in AW-IPM.

4. Area-Wide Control of Insect Pests: from Research to Field Implementation

Operational AW-IPM programmes are complex, long-term and proceed from basic research, through methods development, feasibility studies, commercialization and regulation, to field pilot studies, which could eventually culminate into an operational programme. The various components of this development process are briefly documented and discussed.

4.1. Basic Research

AW-IPM is a science-based activity and, hence, relies on knowledge generated in basic science to provide new tools and technologies. The link between basic science and AW-IPM programmes is often seen as tenuous, as much time and applied research is required to turn a basic invention into a product for use in a field programme. One example of this process has been the basic genetic research conducted over ten years that culminated in the development of genetic sexing strains for the Mediterranean fruit fly. Genetic sexing has revolutionized the use of the sterile insect technique against this pest. Unfortunately this technology cannot be transferred directly to other species, although it could be created in many pest species with somewhat less effort than was required in the Mediterranean fruit fly. Moreover, genetic transformation of insect pests may lead to the development of generic sexing systems. Many of the proposed uses of genetic transformation aim to replace or complement existing technologies, and, therefore, do not represent completely new concepts. As such, genetic sexing, insect marking, and sterilization are all proposed as

targets for genetic transformation. In a few pest species all these goals have been achieved at the laboratory scale, but the larger challenge of scaling up and validating these systems at meaningful levels for AW-IPM programmes remains to be done.

Genetic transformation was first developed to study gene function in *Drosophila melanogaster* Meigen and then roughly ten years of effort were required to simulate this accomplishment in the first major pest species, the Mediterranean fruit fly. Now, it is possible, through the development of better vector systems and transformation markers, to genetically engineer most pest species, including several dipteran and lepidopteran pests (Atkinson, this volume). Lepidoptera have a different sex determination system than Diptera and this difference could be exploited to produce a genetic sexing strain with non-transgenic males for release (Marec et al., this volume). The identification of specific chromosome markers (Makee and Tafesh, this volume) will aid this approach.

Each AW-IPM programme requires a regulatory framework tailored to its specific needs. However none of the currently existing regulatory frameworks provides for the deployment of any genetically transformed product. This issue was discussed in Rome at a meeting on risk assessment, which provided some guidance on deploying transgenic arthropods (IAEA 2006). Currently, the North American Plant Protection Organization (NAPPO) is developing a regional standard (RSPM) to facilitate the importation and confined release of transgenic arthropods (NAPPO 2007). Strategies to facilitate the acceptance of deploying transgenic insects in AW-IPM programmes are being developed (Handler et al., this volume).

Characterization of pest populations to be targeted by an AW-IPM programme is an essential first step in mounting an AW-IPM programme. To this end advances in DNA analysis are providing increasingly sophisticated tools to assess the genetic variability of different field populations of a pest species including the degree of isolation between them (Torres et al., this volume). However, these studies do not provide information on the presence or absence of behavioural mating barriers between various field populations or between released sterile insects and target populations. The existence of behavioural barriers to mating must be determined through the conduct of mating compatibility studies; and these should be conducted under semi-natural conditions (Cayol et al. 2002, Vera et al. 2006).

The quality of performance in the field of insects released in AW-IPM programmes is of paramount importance. Therefore, great diligence is exercised in the mass-rearing and handling of predators and parasitoids in augmentative biocontrol programmes and of sterile males released in SIT programmes. Thus it is important to note that dietary, hormonal, and semiochemical treatment of sterile males prior to release (Teal et al., this volume) can greatly increase their effectiveness. Similar conclusions were reached with respect to predators reared for release in augmentative biocontrol programmes (Thompson 1999).

Likewise, new techniques that allow the storage of insects for varying lengths of time and at various stages could be very beneficial to AW-IPM programmes. Maintenance of certain strains of the pest insect that have particular characteristics is labour intensive, expensive and tends to expand as new strains are developed. Strains also need to be maintained over long periods of time even though they may only be used for limited periods. In addition, storage of insects can alleviate potential hazards of the rearing process such as strain deterioration, disease outbreaks, labour unrest, or mechanical failure. Cryopreservation is considered to be a possible solution, and recent advances have made this technology available for some pest insects such as transgenic New World screwworm (Leopold, this volume).

Population suppression in AW-IPM programmes can be achieved by reducing the reproductive potential of the target population using either natural or artificially induced sterility. Four decades ago, cytoplasmic

incompatibility was used to demonstrate population suppression in isolated populations of *Culex pipiens* L. (Laven 1967), and since then scientific knowledge about its causative agent *Wolbachia pipientis* Hertig has dramatically increased (Werren 1997). *Wolbachia* is a bacterial symbiont known to be widespread in many arthropod species, including some populations of tsetse, where its presence does not seem to cause cytoplasmic incompatibility. Other symbionts play a role in insect fitness and a better understanding of the symbiont-host interaction can have important benefits for mass-rearing and field deployment of sterile insects. Tsetse flies carry obligate symbionts that synthesize essential amino acids and vitamins that are not present in their diet of vertebrate blood (Aksoy and Weiss, this volume), and it may be possible to develop tsetse strains refractory to the trypanosome parasite, which would remove a potential health hazard in a sterile release programme. For other insect pests, such as the Mediterranean fruit fly, *Wolbachia* is naturally absent, but strains have now been artificially infected to generate cytoplasmic incompatibility (Bourtzis, this volume).

New developments in science and technical innovations are the lifeblood of evolving AW-IPM programmes. Technology stagnation leads to lost opportunities, non-competitive strategies, lowered programme morale and funding fatigue. However, scientific and technological advances need to be adapted and tailored to meet important needs of specific AW-IPM programmes. It is therefore no coincidence that the most successful AW-IPM programmes are associated with a distinct but separate entity responsible for research, and/or methods development and improvement.

4.2. Modelling and Methods Development

Knowing where pest populations are in time and space is indispensable information needed to effectively plan, implement and evaluate AW-IPM programmes. Mathematical models and spatial analytical tools (remote sensing (RS), GIS and global positioning systems (GPS)) are increasingly used (FAO/IAEA 2006, Abeku 2007) to provide guidance for decision making in AW-IPM programmes, for risk mapping and assessing the potential of alternative tactics and for identifying synergistic interactions between their components.

Simple, easy to understand mathematical models were used by E. F. Knipling to demonstrate the essential theoretical underpinning of AW pest suppression and management using a wide range of control tactics (Knipling 1966, 1979). Since then, a number of more sophisticated models have been developed that examine factors such as residual fertility, competitive ability, mating patterns, immigration, etc. (Barclay 2005). In AW-IPM programmes which include the release of insects, optimal release strategies constitute a key component, which has serious economic implications and which often influences success or failure. Release strategies are influenced by the critical overflooding ratio, i.e. the number of sterile insects that have to be released to induce a downward trend in the target population, and the dispersal potential of the released insects. New population models that deal with inherited sterility in Lepidoptera demonstrate the benefits of releasing sterile insects in as close proximity as possible to the wild insects, and if the spatial distribution of the target population is not known, many small releases regularly spaced are more likely to achieve the goals of the programme than large, less frequent and widely spaced releases (Kean et al., this volume). Recent one-dimensional random walk models of the dispersal of West African riverine tsetse show the importance of habitat topography on dispersal and the effects of using either complete or partial release-recapture data sets in the model (Bouyer et al., this volume).

Many models have been developed to address the issue of risk. They are well advanced for several major insect vectors of disease. Although the first mathematical "model" describing the role played by an insect vector in disease epidemiology was published almost one century ago by Sir

Ronald Ross (Ross 1911), the use of these spatial tools has not been widespread, but that situation is rapidly changing. Although the quantity and quality of geographical data input in these spatial models has dramatically increased, ground-truthing and correlating entomological data with climatic, biological and topographical variables is still lacking for many ecological situations. Models can be also be used as a forecasting tool to aid farmers in initiating control actions, and these can be based on climate (e.g. the model for myiasis risk in the UK (Wall and Pitts, this volume)), or on insect density (e.g. AW-IPM in commercial wheat storage (Flinn et al., this volume)).

The term autodissemination is used to describe the release of beneficial organisms for the additional purpose of carrying other control agents into a field population. Electrostatic powders have been developed which adhere to insect cuticle. These powders could be used to coat the insect cuticle with bioactive entities such as pheromones or microorganisms. Preliminary data indicate that this mechanism has potential for use in a "lure-and-kill" strategy against fruit flies, or for use in mating disruption in Lepidoptera (autoconfusion). The latter system could be made more cost-effective by replacing the sterile male moths by "cheaper" sterile insects (e.g. sterilized Mediterranean fruit flies) in those environments where they could survive (Howse et al., this volume). More detailed and extensive studies are needed to validate the potential of this approach.

An independent applied research or methods development group to solve problems and fine-tune technologies is an important component of operational AW-IPM programmes. For example, significant progress has been made in improving the accuracy and quality of release technologies for sterile Mediterranean fruit flies in terms of (1) aircraft navigation based on GPS-guided systems that also control and monitor fly release rate, climatic conditions, aircraft altitude, speed, track, etc., and (2) use of cryogenics for chilled adult release technology in aircraft. These two sets of improvements have enhanced both the distribution of the released insects and their quality, respectively (Tween and Rendón, this volume).

4.3. Feasibility Studies

The next critical step in the decision-making process towards the development of an AW-IPM programme is a feasibility study. Feasibility studies have components ranging from the collection of entomological (Vreysen 2005), and other baseline data, benefit/cost analyses (FAO/IAEA 2007), the consideration of ways to eliminate or mitigate factors with outrage-inducing potential, and securing government and other stakeholder commitment to an assessment of available and required resources and infrastructure. Limited pilot field tests to evaluate and validate new technologies could likewise be included in this phase as they are small, both in scale and in cost, and do not have the primary goal of population control.

Often major outbreaks of pest populations are difficult to combat, as they require timely detection, rapid mobilization of resources, the development and implementation of a plan of action, and the erection of the necessary political and regulatory framework in which to operate. Locust problems in Africa and Asia exemplify these difficulties, and after centuries of struggle against these plagues, a very large number of countries over huge areas of land are still vulnerable to depredation by locusts. It is recognized that the solution to the problem lies in a preventive or pro-active approach where control techniques are applied to prevent the development of damaging swarms (van Huis, this volume). In spite of the availability of better bio-pesticides, more sophisticated forecasting and early warning systems, focusing only on the recession areas will be far from easy in view of the vastness of the regions involved and the lack of basic infrastructure in many affected countries. The control of the enormous outbreaks of forest pests, such as the mountain pine beetle *Dendroctonus ponderosae* Hopkins in

North America, face similar difficulties, although in this case more resources are available (Carrol, this volume).

Development of complex integrated approaches required for multiple pest agro-ecosystems, such as those in cotton and rice, is difficult, but much can be achieved by designing or selecting cultural practices that take crop phenology and the life-history traits of the pests into account (Zhu et al., this volume, Mukhitdinov, this volume). Synchronous or asynchronous planting or harvesting dates, crop rotation and the use of alternative hosts can be very effective when applied in a systematic fashion. In such multipest systems the conservation of natural enemies is of great significance (Stewart et al. 2007), while species-specific control tactics such as mating disruption or sterile insects may not have a role.

The elimination of the tsetse fly *Glossina austeni* Newstead population from Unguja Island, Zanzibar, United Republic of Tanzania, raised expectations for ambitious tsetse eradication projects in many parts of mainland Africa. This development has been encouraged and supported by the African Union's Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC 2000), which has secured substantial financial support of the African Development Bank. The transition from an isolated relatively small island to much larger target areas with different tsetse species, topographies and socio-economic dimensions clearly entailed the need for extensive feasibility studies in order to ensure future success. The two most advanced programmes are located at the latitudinal limits of the tsetse distribution in Africa, i.e. KwaZulu-Natal, South Africa in the south (Kappmeier et al., this volume) and the Southern Rift Valley, Ethiopia in the north (Alemu et al., this volume). Feasibility studies in both these areas have indicated the logistical limitations of conventional community-based tsetse suppression methods for such extensive areas, such as insecticide-impregnated targets, traps or the use of insecticides as a pour-on formulation on livestock. AW suppression using the sequential aerosol technique (SAT) guided by a GPS-based navigation system, which has been applied with great success against the savannah tsetse species *Glossina morsitans centralis* Machado in Botswana (Kigori et al. 2006), will be needed. Although this programme indicated no significant negative long-term effects on biodiversity and non-target species, the presence of national parks in target areas will require appropriate environmental impact studies and full and timely consultation with all the relevant stakeholders.

The mosquito vector of malaria *Anopheles arabiensis* Patton is complicit in many deaths, much illness and economic stagnation; and the feasibility of eradicating or strongly suppressing it at its natural limits along the Nile in Northern State, Sudan is being assessed (Malcolm et al., this volume). In this study extensive larval surveys and other GIS-aided baseline data are being collected systematically (Cox, this volume). These data will serve as the foundation of future population suppression or eradication campaigns.

Many highly destructive invasive species are Lepidoptera, which pose a greater threat to agricultural production worldwide than any other Order of arthropods. The false codling moth *Thaumatotibia leucotreta* (Meyrick) and the cactus moth *Cactoblastis cactorum* Berg have been targeted for extensive feasibility studies for AW-IPM programmes. The false codling moth is a major pest of many crops including citrus, cotton, maize, etc., and it has acquired resistance to many insecticides. At present, this dangerous pest is still restricted to the African continent. The development of an AW-IPM programme against the false codling moth in South Africa, that includes the use of sterile insects, could also be of great benefit in the event of an outbreak of this major pest on other continents (Carpenter et al., this volume). The cactus moth was originally a textbook example of an effective biological control agent when it was imported from its native range in Argentina to control introduced *Opuntia* spp. cactus species in Australia. Subsequently the cactus moth was

introduced for the same purpose on Nevis in the Caribbean, and by 1989 it had spread to neighbouring islands and to southern Florida (Pérez Sandi Cuen and Zimmermann 2005). This invasive species quickly dispersed northward in Florida to Alabama and is threatening south-western USA's and Mexico's fragile ecosystems that are based on the many species of indigenous cacti (K. Bloem et al., this volume). The transboundary nature of many invasive pests requires international co-operation. Thus a bi-national Mexico-USA programme has been initiated to stop the westward spread of the cactus moth (Hernández et al., this volume).

The importance of comprehensive baseline data collection, benefit/cost analyses and other assessments as part of detailed feasibility studies cannot be overstated. Such sound information is essential not only for identifying the most appropriate strategy and technology, and for designing the technical aspects of a programme, but also for fund raising and obtaining the commitment of all stakeholders. In some instances large-scale AW-programmes have suffered because they were mounted before all the essential information had been collected (Vreysen et al., this volume).

4.4. Commercialization and Regulation

Regulation and commercialization of new technologies and services can be seen as two sides of the same coin in that commercialization can only flourish within a strong regulatory framework and commercial interests are sometimes pivotal in the push to develop regulatory frameworks. Regulatory frameworks are also instrumental in facilitating international trade in agricultural products. Moreover AW-IPM programmes combined with systems approaches that mitigate the risk of introducing exotic pests provide very powerful tools when incorporated into these frameworks (Griffin 2000). Safeguarding against invasive alien species is most effective when pest free products are shipped from the exporting country to the port of entry of the importing country. The World Trade Organization recognizes the International Plant Protection Convention (IPPC) as the international standard setting body for phytosanitary issues, and an increasing number of IPPC's International Standards for Phytosanitary Measures (ISPMs) recognize the power of AW-IPM programmes in establishing low-prevalence and pest free areas (Devorshak, this volume). The postharvest use of ionizing radiation as a component of a systems approach to phytosanitation has been approved and articulated in ISPM-18 (FAO 2003b). Generic doses of ionizing radiation required to destroy many groups of insect pest are being developed (Follett, this volume).

Many components of AW-IPM programmes are very amenable to commercialization, e.g. pheromones, bio-pesticides, attractants, aircraft services, traps, field monitoring, etc., since all of these technologies and services are already being provided by commercial firms to various IPM programs. They can be purchased and applied only when needed by individual farmers, but also by associations of farmers, organizations or governments. Living organisms such as parasitoids, sterile insects and other beneficial organisms present more of a challenge for commercialization. As in every type of endeavour, commercialization of any new process will involve the development of a business plan which takes into account current practices, protection of intellectual property, markets, benefit/cost analysis, etc. (Quinlan et al., this volume). Based on this type of analysis there is now a thriving industry producing and marketing biological control agents (Evans 2004). Interestingly, it has proven much more difficult to privatize sterile insect production even though there appears to be a substantial market. This is related to the sterile insects themselves and how they have to be used, and to questions of competition between public and private rearing facilities (Bassi et al., this volume). Historically most mass-rearing facilities in SIT programmes have been government funded and government operated, and this situation still prevails widely. The govern-

ment-operated mass-rearing programmes, to some extent, constitute production subsidies that distort the market and make it very difficult for the private sector to compete. Despite all the difficulties, real or imagined, there are now at least three commercial organizations at various stages of producing or selling sterile insects for operational programmes (Barnes, this volume, Bassi et al., this volume). Quality assurance will be a key issue in the sustainability of a commercial enterprise selling sterile insects. For fruit flies, a quality control manual (FAO/IAEA/USDA 2003) is available, which is currently used by producers and consumers alike. In order to command respect for the high quality of their product, some production facilities have acquired ISO certification (López-Rueda et al. 2004, Cáceres et al. 2007).

4.5. Pilot Programmes

Pilot programmes assess the effectiveness of the different control technologies and of the overall system on a scale that is relevant to decision making for any future operational AW-IPM programme. Pilot programmes also identify weaknesses in programme management, public acceptance, and public education needs. For example, pilot trials of AW approaches against pests of annual crops, which integrated cultural practices, have proven very effective in identifying weaknesses pertaining to the above-mentioned technical, managerial and social dimensions (Abel et al., this volume) or for such major pests as fire ants (Vander Meer et al., this volume).

Invasive species are an increasing problem in terms of agriculture, natural ecosystems and human health. In Europe the introduced mosquito *Aedes albopictus* (Skűse), a vector of dengue and yellow fever, is causing much concern (Bellini et al., this volume). The red palm weevil *Rhynchophorus ferrugineus* Olivier (Krishnakumar and Maheswari, this volume), a devastating pest of palm trees, is spreading at alarming rates in many parts of the Mediterranean and the Middle East. Initial pilot trials have given encouraging indications of the potential of an AW-IPM approach to control these two pests in areas with limited outbreaks. However, in each of these cases, the control system requires further development.

A number of pilot mass-rearing facilities are being constructed, but these enterprises are faced with the urgent needs to train staff and to validate technologies on a larger scale before investing in industrial-type facilities (M'Saad-Guerfali et al., this volume). Multipurpose rearing facilities, which can simultaneously serve the needs of different programmes, have long been considered to have advantages over facilities custom-built for one species. For some pest species, e.g. fruit flies, this can be readily accomplished. For example the fruit fly programme in Thailand rears and sterilizes *Bactrocera correcta* Bezzi and *Bactrocera dorsalis* Hendel in the same facility (Orankanok et al., this volume). In Brazil, where mass-rearing of fruit flies recently began, a facility is planned that will also rear parasitoids of fruit flies and possibly a moth species (Malavasi et al., this volume).

Pilot trials are essentially an upscaling and refocusing of R and D, using the data made available from the feasibility studies to expand activities into field situations. They are a step of fundamental importance in progressing carefully towards the establishment of operational AW-IPM programmes.

4.6. Operational AW-IPM Programmes

The sets of tactics and strategies that have been fully tested and validated in pilot trials can be developed into full-scale operational programmes. However, in contrast to pilot trials, operational programmes leave very little margin for error in view of the large commitments of resources, their dependence on the strong support of rural and urban stakeholders, and their dependence on a helpful regulatory and political climate. It will always be very challenging to modify or to introduce a major new technology in a suc-

cessful programme. Therefore, most successful AW-IPM programmes operate without an integral R and D component; instead they usually have an associated methods development programme, which develops and validates new technical improvements.

As indicated above, AW-IPM programmes have many facets pertaining to economics and food security, the environment and sustainability, transboundary and trade problems, and either population suppression or eradication, as well as sociological and political components (Pimentel, this volume). This philosophy has been effectively marshalled in the development of AW-IPM for the major pests of cotton (Henneberry, this volume, Wu, this volume). This very important fibre crop is grown on four continents and consumes up to 25% of all insecticides used in agriculture. In combination with the more traditional pest control approaches, the development and marketing of *Bt*-cotton has had a major impact on the economics of cotton production, on reducing insecticide use, and on farmers' ability to manage the suite of cotton pests. This type of biotechnological development can greatly facilitate the adoption of AW-IPM programmes; but it does require that susceptibility to the *Bt*-toxin is maintained in the pest population through the use of refugia planted either with cotton lacking the *Bt*-toxin or another crop that is highly susceptible to the species of Lepidoptera that attack cotton.

Probably the largest ongoing AW programme is the one focused on eradicating the boll weevil from the USA. This programme, based on some effective pilot trials in Virginia and North Carolina, started in 1983 and has since eradicated this pest from about 5.3 million hectares of cotton. This remarkable achievement is based on the systematic use of effective monitoring and detection tools together with relatively few control techniques. The goal, to rid the USA of the pest by 2008, appears achievable, and is resulting in very significant reductions of insecticide use on cotton (El-Lissy and Grefenstette, this volume).

The traditional approach of controlling the Formosan subterranean termite *Coptotermes formosanus* Shiraki in the French Quarter of New Orleans aimed to protect individual buildings by applying highly toxic liquids around each one, independent of actions to protect neighbouring structures. This was highly ineffective as it hardly affected the size of the termite population in the area. A radical change in the control strategy was taken in 1998 when the programme adopted an AW-IPM approach that aimed at termite population suppression with the aid of advanced termite detection technologies and the use of other termite suppression technologies (Lax et al., this volume). The application of termite suppression control tactics on a population level, rather than focusing on the protection of individual buildings, has drastically increased the effectiveness of termite control (Smith et al. 2006).

Although lepidopteran pests are among the most destructive pests of agriculture, few AW-IPM programmes have been mounted against them. The overwhelming majority of lepidopteran pests are still controlled on a field-by-field basis. One of the exceptions is the programme against the codling moth *Cydia pomonella* (L.) in British Columbia, Canada. There, the coding moth is being effectively managed with an AW programme, which has integrated the release of sterile moths with insecticide applications, sanitation procedures and mating disruption in certain areas. Originally this programme was designed to eradicate the codling moth from the fruit producing interior valleys of British Columbia, but in the absence of the political will needed to establish quarantines to maintain a pest free area, and coupled with excellent success in eliminating almost all damage from the pest, the goal of the programme has been changed to on-going population suppression (Bloem et al., this volume). In spite of internal political and funding problems, the programme is a major success and is supported strongly by the growers and the local urban communities. A number of other successful AW codling moth programmes are mainly based on the use of mating disruption (Coop et al. 2000). Another

programme in Brazil targets the eradication of isolated populations of codling moth in four urban settings in the southern part of the country (Kovaleski and Mumford, this volume). Other operational AW-IPM programmes against lepidopteran pests include the successful elimination of the invasive Australian painted apple moth *Teia anartoides* Walker in New Zealand (Suckling et al., this volume), and the effective prevention of establishment of the pink bollworm *Pectinophora gossypiella* (Saunders) migrants in the non-infested cotton-producing central valley of California, after this invasive pest had become established in the early 1960s in northwestern Mexico and southern California (Henneberry, this volume).

Ongoing operational AW-IPM programmes reflect the emphasis given to the total population suppression approach in the different geographical areas of the world. The Americas have actively embraced AW-IPM largely to facilitate expansion of agricultural trade. This applies especially to fruit fly pests. The success of these programmes has not been due only to the efficiency of the technologies used, but, also, to effective management structures and aggressive support by producers, exporters, and regulatory and political authorities, who are convinced of the value of the AW approach. This is attested by the many operational AW-IPM programmes against fruit fly pests in Central America and Mexico (Reyes et al., this volume, Montoya et al., this volume), South America (Gonzalez and Troncoso, this volume, Guillén and Sánchez, this volume), the USA (Mau et al., this volume) and Australia (Jessup et al., this volume). Most of these programmes, only some of which integrate sterile insects, are long established, and have brought great benefits to the agricultural community. They are sustainable, expanding and technically innovative. In contrast, and with some recent exceptions, such as the citrus industry in southern Spain, Europe has not shown much enthusiasm to adopt the AW approach despite Europe's pressing need to improve the protection of its environment and to make its agriculture more competitive.

Success in an AW programme, however, cannot be taken for granted. For example the eradication programme for the tropical bont tick *Amblyomma variegatum* F., vector of heartwater fever and the cause of dermatophilus, in the Caribbean (Pegram et al., this volume) has not proceeded according to plan. Started in 1994, the programme relied on a strategy of using periodically scheduled pour-on acaricide applications by the farmers, coupled with an intensive public relations campaign. Initially the programme was successful and the parasite was removed rapidly from Puerto Rico, where cattle are managed in fenced pastures. It proceeded more slowly on most other islands, since a majority of the livestock owners are landless, and they attend to their free-roaming animals only when time permits. Nevertheless, various islands were certified provisionally tick free by 2003, but some of them have been re-infested and the tick has continued spreading to additional islands. The setbacks in the programme seem related to the inability to diligently treat free-roaming unmanaged livestock on a fixed schedule, funding shortfalls and unwillingness of donors to delegate management of funds to the programme, the arrival in the Caribbean region of the cattle egret *Bubulcus ibis* L. which can carry the tick on flights between islands, etc. It is possible that in the future the programme may change its focus from eradication to suppression, and that the tropical bont tick will continue to spread within the greater Caribbean region and to the Americas.

Most operational AW-IPM programmes, especially those integrating the release of insects, are complex and management intensive. Success depends on continuous and positive interactions between all essential stakeholders. Aside from a series of managerial prerequisites, a number of technical requirements need to be in place before success can be obtained. A common denominator of all successful AW-IPM programmes, irrespective of the combination of tactics used, or the degree of centralized coordination, is an effec-

tive management structure together with partnerships appropriate to the geographic scale and transboundary nature of the target pest populations. Vreysen et al. (this volume) provide a realistic overview of the constraints, misconceptions, pitfalls and opportunities associated with AW-IPM programmes; and this chapter is highly recommended reading for political leaders, managers and institutions contemplating AW-IPM programmes.

5. Conclusions

Interest in AW-IPM programmes and the number implemented have grown substantially in the last ten or 15 years, yet they are still the exception rather than the rule in the many circumstances where they would be appropriate and highly advantageous. However, social, political, and economic factors must come together with science before an AW suppression or eradication programme can be developed and implemented (Faust 2001). Furthermore, the arduous journey from research results to feasibility studies, to pilot testing, to eventual successful field operations requires close collaboration between researchers, extension specialists, community leaders, and the agriculture, natural resources and public health communities.

Technical challenges include (1) determining the key insect pest species, (2) defining the geographic areas, (3) obtaining the extensive biological and ecological baseline data needed to understand the target pest population dynamics, ecological relationships and distributions in space and in time, (4) determining gene flow between reproducing pest individuals in the target population and neighbouring populations, (5) developing and integrating appropriate and compatible control tactics (Thomas 1999), (6) ensuring that they are applicable for AW use (some tools that are effective at the local level, for example mass-trapping, become logistically impractical at a larger scale), and (7) developing strategies for effective implementation (modified after Lindquist 2001).

Managerial challenges include (1) obtaining the commitment of all private and public stakeholders to support, participate in and finance the AW programme, (2) carrying out appropriate feasibility studies, (3) identifying factors likely to induce outrage and ways of mitigating them, (4) developing a professional business plan for the programme, (5) establishing an effective and dedicated organization with full time staff to coordinate and implement the programme, (6) implementing a training programme; (7) establishing communication mechanisms among all rural and urban stakeholders, (8) establishing a system of programme evaluation, and (9) obtaining research support for the programme (modified after Lindquist 2001).

The future prospects for AW-IPM are encouraging, although this approach may not be applicable to all mobile pests or to all agricultural or human health situations. AW-IPM tends to be best suited for crops of high economic value, for some key livestock pests, and major human disease vectors. AW-IPM tends to be most easily implemented in rural areas where the number of farmers is small, the agroecological heterogeneity is low, and where the management of few mobile key pests at larger scales is more effective and shows clear advantages over the uncoordinated field-by-field approach. However much thought and political support needs to be given to combating invasive alien pests of agriculture in urban settings, where they tend to establish initially as a consequence of trade, tourism and smuggling (FAO 2005). It is absolutely essential to proactively win, and then retain, the goodwill of urban dwellers who will be directly affected by programme operations.

Economics plays a major role in grower decisions to participate in AW-IPM programmes. The potential for economic growth can motivate growers and communities to become educated in the AW approach. Such local leaders may take concerted action to achieve coordination of pest control actions at the village, subdistrict, and district levels, and, thereby, scale up IPM from independent field-by-field efforts to AW implementation.

Therefore, adoption of an AW approach can be a gradual process, a continuum starting at the field and community levels, and if social, political, and economic factors are favourable, eventually moving to larger geographical scales. Increasingly, the goal of AW-IPM programmes is pest population suppression with eradication held in abeyance for very special situations related to facilitate international trade, or to eliminate populations of major disease vectors, or recent introductions of very destructive invasive pests. Eradication is the strategy of choice, provided it is achievable and decisively advantageous environmentally and economically (Fraser et al. 2005).

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Area-Wide Pest Management: Environmental, Economic, and Food Issues

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ABSTRACT Insect pests destroy approximately 14% of all potential food production despite the yearly application of more than 3000 million kilograms of pesticides. This contributes to rising human malnutrition which in 2004 was estimated by the World Health Organization to have reached 3700 million - the largest number in history. Several major insect pests of crops and livestock are effectively controlled using area-wide pest management practices. As an example, the New World screwworm fly *Cochliomyia hominivorax* (Coquerel) that attacks livestock, especially cattle, was successfully eradicated by releasing radiation-sterilized screwworm flies over large areas. Area-wide insecticide treatments in the USA have also proved effective in the control of the boll weevil, while timed crop-planting over wide areas enables crops like wheat to evade major pests and has also been proven highly successful against rice pests in the USA and Asia. Yet, when the basic ecology of the insect pests and crops are ignored, major crop losses can occur, as illustrated by the manipulation of corn production in the USA. Damages caused by invading insect pests that attack established crop, forest, and natural ecosystems continue to be challenges to pest management specialists. Approximately 40% of the insect and mite pests of crops grown in the USA are introduced species and they cause about USD 100 000 million in damage and control costs each year. The most recent introductions include the long-horned beetle *Anoplophora glabripennis* (Motschulsky) and the emerald ash borer *Agrilus planipennis* Fairmaire that were both accidentally introduced from Asia. Area-wide strategies to control these destructive forest pests are being implemented.

KEY WORDS insecticides, invasive species, economics, area-wide programmes, wheat, cotton, New World screwworm

1. Introduction

Meeting the food supply needs of an ever-increasing world population is both critical and at the same time stressing the natural resources needed for crop production. This paper examines the extent of crop and livestock damage caused by insect pests and how these losses affect human food supplies. It also reviews successful area-wide pest control programmes and assesses the impacts of invasive insect pest species on control projects needed to reduce crop losses.

2. Status of Food Security

According to the World Health Organization (WHO), more than 3700 million people were malnourished in 2004 (WHO 2004). This is the largest number and proportion of malnourished people ever reported! In assessing malnutrition, WHO includes deficiencies of calories, protein, iron, iodine, and shortages of vitamins A, B, C, and D in its evaluation (Sommer and West 1996, Tomashek et al. 2001). The current world hunger and shortages of nutrients for so many people alerts us to the growing insecurity of world food supplies and the vulnerability of human health and productivity.

The report of the Food and Agriculture Organization of the United Nations (FAO), confirms that available food per capita has been declining since 1984, based on available

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cereal grains (FAO 2003). This is alarming because cereal grains make up about 80% of the world's food supply (Pimentel and Pimentel 1996). Although grain yields per hectare in both developed and developing countries are increasing, the rate of increase is slowing. For example, according to the United States Department of Agriculture (USDA), grain yields in the USA increased by about 3% per year between 1950 and 1980, but since then the annual rate of increase for corn and other major grains has been only about 1% (USDA 1980, 2004). Meanwhile, according to the Population Reference Bureau (PRB), the human population has increased to 6500 million and continues to expand by around 70 million per year, placing ever-increasing demands on agricultural production (FAO 2003, PRB 2004). Many countries have populations that are expanding at a rate of about 1.3%. Others like China, with a population of 1300 million and a government policy of permitting only one child per couple, has a population that is increasing at a rate of 0.6% or eight million per year (PRB 2004). The US population also is growing rapidly. It currently stands at nearly 300 million, having doubled during the past 60 years, and based on a current growth rate of about 1.1%, it is projected to double to 600 million in less than 70 years (USCB 2004), i.e. it is growing at a per capita rate that is nearly twice that of China (PRB 2004).

3. Food Losses to Pests

Worldwide there are an estimated 70 000 pest species destroying agricultural crops and livestock. While approximately 10 000 of these are insects and mites, 50 000 are plant pathogens and 10 000 species are weeds; less than 10% of the total identified pest species are generally considered as major pests.

The species present vary both geographically and with each crop type. Approximately 99% of the crops grown in a country are introduced plant species (Pimentel et al. 2000). Frequently the insect and mite species are specific to a particular region and many have moved from feeding on native vegetation to feeding on crops that were introduced into the region (Hokkanen and Pimentel 1989). Indeed, usually insects moving to feed on a crop are new associations with their host plants and some become major pests (Pimentel 1988).

Despite the annual investment of USD 35 000 million for the application of three million metric tons of pesticides (Table 1), plus the use of various biological and other non-chemical controls worldwide, more than 40% of world crop production valued at USD 750 000 million is destroyed by pests (Oerke et al. 1994). Considering all pests, insects cause an estimated 14% of crop losses, plant pathogens 13%, and weeds 13%. In total, the value of such losses is estimated to be USD 300 000 million per year.

In the USA, the proportion of annual crop production lost by pests is estimated to be similar to world pest losses or about 37% (13% to insects, 12% to plant pathogens, and 12% to weeds) (Pimentel et al. 1991). Since total crop production in the USA is valued at about USD 160 000 million/year (USDA 2004), pests are destroying an estimated USD 60 000 million/year in this country despite all efforts to control them with pesticides plus a wide array of non-chemical controls. Currently, the USA invests about USD 8000 million in pesticide applications which saves about USD 30 000 million per year in crops while the use of non-chemical controls like natural enemies also helps to save crops valued at an estimated USD 30 000 million per year (Pimentel 1997). In general, there is a USD three to four return per USD invested in pest control (Pimentel 1997).

The share of crops lost to insects in the USA has nearly doubled from 7% in 1945 to 13% at present (Pimentel et al. 1993), despite a more than ten-fold increase in both the amount and selective toxicity of synthetic insecticides applied. This is mainly because various changes have occurred in agricultural production technology. However, there have also been significant improvements in terms of increased target selectivity and decreased

residue levels.

Without pesticides and non-chemical controls, the losses of crops due to pests would be even more severe than occurs at present. Oerke et al. (1994) estimated that without human-directed pest controls, world crop losses would increase to 70% and to USD 525 000 million annually, reducing world food supplies and increasing malnutrition dramatically (WHO 2004).

Added to the damage that pests inflict during the growing season are the substantial losses that occur during the lengthy time many food crops are stored prior to their use. Worldwide, an estimated additional 25% of harvested crops are lost to other pests during the postharvest period. This means that, in total, pests are causing a 52% loss of all crops despite all pest control technologies used.

Table 1. *Estimated annual pesticide use from 1995 to 2005*[1].

Country/region	Pesticide use (10^6 metric tons)
United States	0.5
Canada	0.2
Europe	1.0
Other, developed	0.5
Asia, developing	0.3
China	0.2
Latin America	0.2
Africa	0.1
Total	3.0

[1]Source: D. Pimentel, unpublished

4. Area-Wide Integrated Pest Management (AW-IPM) Programmes

Depending on the specifics of geographic region, the crop and animal pest problem, and the technologies available, some pests can be more effectively controlled by area-wide control strategies than by specific farm-by-farm control programmes.

4.1. New World Screwworm and the Sterile Insect Technique

The United States Animal and Plant Health Inspection Service (APHIS) estimates that the New World screwworm *Cochliomyia hominivorax* (Coquerel) caused more than USD 750 million in damage to livestock in the USA each year (APHIS 2004). In addition to cattle, other livestock, whitetail deer and other wildlife, are susceptible to New World screwworm infestations. The female New World screwworm lays its eggs in wounds, and the larvae bore deeply into the wound on warm-blooded animals and feed on the living tissue. In addition, facultative larvae of other fly species which feed on dead tissue are frequently present in the wounds along with the New World screwworm larvae, intensifying the damage (Spradbery 2002). Eventually the infected animal dies prematurely.

In 1957, an area-wide integrated pest management (AW-IPM) programme was initiated in Florida, by integrating the release of insects sterilized using ionizing radiation with population reduction methods such as insecticidal wound treatment (Meyer 1994, Spradbery 2002), and in due course this programme unfolded in phases to eradicate the New World screwworm from the USA in 1982, then Mexico in 2001 and finally all of Central America and Panama in 2004 (Wyss 2000, APHIS 2004). For the programmes in the USA and Mexico, up to 500 million sterile screwworm flies could be produced per week in a rearing facility at Tuxtla Gutiérrez, Chiapas, Mexico, and then released in the designated areas.

Another successful New World screwworm eradication programme was implemented in the Libyan Arab Jamahiriya in 1990-1992 (FAO 1992, Lindquist et al. 1993) by the Government of Libya and FAO. This dangerous pest had become established for the first time outside of the Western Hemisphere in an area of 26 500 square kilometres along the Mediterranean Sea with several million head of sheep and numerous camels. The pest was discovered attacking large mammals in the

Tripoli Zoo as well as humans, many of whom were treated in hospitals. Apparently the pest had been imported with a consignment of sheep air-freighted from a country in South America. The infestation was contained by establishing quarantine checkpoints along all routes leading out of the infested area, treating all wounds of animals every two to three weeks with an insecticide, and then releasing sterile flies. Forty million sterile flies were flown on a weekly basis from the rearing facility in Mexico and released from small aircraft over a total of 41 000 square kilometres in Libya and Tunisia (Lindquist et al. 1993).

4.2. Synchronized Crop Rotations and Conservation of Natural Enemies for Control of Rice Pests

In the past, rice was normally grown all year-round in Indonesia, and during 1970-1980 there was a gradual build-up of pest populations, especially of the brown plant hopper *Nilaparvata lugens* (Stål). Rice yields declined despite the heavy use of insecticides that started in 1980 (Oka 1991, 1995) because these chemicals destroyed beneficial parasites and predators that helped control the brown plant hopper. By 1985 uncontrollable outbreaks of the pest were common, rice yields fell dramatically, and as many as 80 000 hectares of rice had to be abandoned (Oka 1988, 1991, 1997, Resosudarmo 2001, Phanthong and Patterson 2005). The loss of rice in just a two-year period totalled USD 1500 million (Oka 1991, 1995).

Eventually, a control programme for Indonesian rice was developed. The first step was to ban 57 of the 64 pesticides in use for Indonesian crops (Oka 1991, Poapungsakorn et al. 1997, Resosudermo 2001). Also, extension agents were trained to identify and protect beneficial parasites and predators, the overall goal being to treat with insecticides only when necessary since generally brown plant hoppers are effectively controlled by indigenous spiders and other predators (Heinrichs 1991, Oka 1991). Moreover, since insecticides have a greater impact on predators than on the pest, brown plant hopper populations are able to resurge after being sprayed. In the past farmers induced resurgence of plant hopper populations by beginning to spray 40 days after transplanting the rice. However, cage studies showed that smallholders who delayed spraying until 65 days after transplanting saved two insecticide applications and realized a yield increase worth USD 588/hectare (Reichelderfer et al. 1984).

Along with the insecticide management programme for the brown plant hopper, an innovative rice culture programme was implemented. Instead of growing rice year-round, production was restricted to a specified nine-month period of the year, leaving three months when no rice was produced. This three-month gap resulted in brown plant hopper populations declining to extremely low levels before the new rice crop was planted again (Oka 1991, Matteson 2000, van den Berg et al. 2004). This strategy also enabled beneficial predator and parasite populations to increase and help reduce the number of brown plant hoppers. As the numbers of the plant hoppers decreased, the amount of insecticide applied also declined. Equally important, insecticide resistance in the brown plant hopper population also declined. Thus, if and when the brown plant hopper populations reached outbreak levels, insecticides were more effective.

Within five years, total pesticide use fell by 65% and rice yields increased by 12% (Oka 1991, 1995, Resosudermo 2001). These changes in rice production practices in Indonesia based on the ecology of a major rice pest were successfully adapted to an AW-IPM programme.

4.3. Boll Weevil Area-Wide Eradication

For decades, the boll weevil caused more than USD 350 million each year in damages and control costs to cotton crops in the USA (Chenault 2005). However, an area-wide eradication programme was started in 1978 with joint funding from USDA/APHIS (30%) and

cotton growers (70%). This programme has proven highly successful (ACRPC 2005, El-Lissy, this volume).

The programme, using annual spraying with malathion and pheromone traps to delimit infestations started in a large region covering about 1.1 million hectares of Virginia, North Carolina, South Carolina, Georgia, and Alabama. Insecticides were applied late in the growing season against weevils still reproducing and those entering diapause, with as many as 15 insecticide applications per year being made against dense persistent populations. Planting by all growers was synchronized and delayed, short-season varieties were grown, harvested as soon as possible, and stalks were destroyed immediately after harvest. Eradication was usually accomplished by the end of the third growing season (Dickerson et al. 2001). About nine years were required to complete this segment of boll weevil eradication (NCC 2005). The second area to be treated included California, Arizona, and New Mexico where the treatment continued from 1983 until 1987 to ensure eradication. The third area treated included the large cotton areas in Alabama and Tennessee and treatment lasted from 1993 until 1994 when the boll weevil was eliminated. The final programme started in 1996 in Mississippi, Arkansas, Oklahoma, Missouri, and Texas (NCC 2005), and is continuing to date. Texas is requiring a major effort because the area is extremely large (5.8 million hectares) and grows more cotton than any other state. Once this project is completed, a barrier will be established and maintained between Mexico and Texas. Concerted efforts will have to continue to deal with any infestation caused by boll weevils getting carried by wind or otherwise into parts of the vast cotton-growing regions of the USA.

Several factors contributed to the successful area-wide control of the boll weevil. Systematic area-wide treatment prevented the possibility of isolated populations of boll weevils surviving in the areas under eradication. Furthermore, malathion is highly effective against the boll weevil and the pest did not evolve resistance to the pesticide. This lack of resistance in the boll weevil contrasts to the housefly *Musca domestica* L. and several other species of insects that have evolved high levels of resistance to insecticides within about eight generations (Pimentel and Bellotti 1976).

4.4. Hessian Fly Control

Wheat was brought to the USA in the late 1600s and is now grown on 30 million hectares. The Hessian fly *Mayetiola destructor* (Say) was first found on Long Island, New York, in 1799, probably having been inadvertently introduced on straw by troops engaged in the American War of Independence (Metcalf et al. 1962). Soon the fly established itself and became a major wheat pest (Pauly 2002, Davis et al. 2004).

In the USA, wheat production includes spring and autumn plantings. Similarly, there are autumn and spring generations of the Hessian fly. Winter wheat, which is planted in September, coincides with the late August emergence of the Hessian fly. The emergence of the Hessian fly is triggered by rains coming in mid to late August. One of the effective controls is an area-wide "fly-free date" which is determined by extension entomologists in each major wheat-growing region (Foster and Hein 1998, PSU 2005).

After the flies emerge and die, the farmers then plant their winter wheat. The prime challenge is to plant the wheat early enough that the wheat germinates and starts to grow before the growing season ends due to the increasingly cold temperatures.

Another generation of the Hessian fly emerges in spring. Again extension entomologists observe the emergence of the fly, usually about April, and a designated fly-free date is established for farmers to plant spring wheat. Again timing is crucial, as late planting in the spring may reduce yields (Foster and Hein 1998).

A second strategy is to plant Hessian fly-resistant varieties of wheat (Gallun et al. 1975, ACES 2005). Because Hessian flies

develop resistance to the resistant wheat varieties in four or five generations, a new genotype of resistant wheat must be planted in the region every five years.

Although several parasitic wasps attack the Hessian fly and help reduce its numbers, they cannot be relied on to provide effective control. Also, the extensive use of insecticides is not recommended because they are costly.

Numerous other area-wide environmental controls are available for farmers to implement, including burying all wheat stubble after harvesting and destroying any volunteer plants that grow. However, the most economical and effective controls for the Hessian fly are establishing area-wide "fly-free dates" combined with the planting of Hessian fly-resistant wheat varieties.

4.5. Wheat Aphid Control

The Russian wheat aphid *Diuraphis noxia* (Kurdj.) and the green bug *Schizaphis graminum* (Rodani) are major pests in the wheat-growing region of Oklahoma in the USA (Wright 2005). The Russian wheat aphid invaded Texas in 1986, spread rapidly across the Great Plains, and proved difficult to suppress. The green bug, native to Europe, was first reported in Virginia in 1882 and because of its capacity to disperse and its great prolificacy it has become a key pest of wheat, sorghum and barley. Both species have a considerable capacity to develop new biotypes that can overcome resistant cultivars of wheat (Porter et al. 1997, Burd et al. 1998).

To help control these pests, the USDA designated a six-state area in the region around Oklahoma for their area-wide control. First, an effective educational programme was started to provide farmers with detailed information concerning the ecology of the pests. The control programme includes the planting of aphid-resistant varieties and applying insecticides only when treatment is required.

Both types of area-wide control have been successful in preventing Russian wheat aphid and green bug populations from reaching the high levels of infestations that cause major economic losses to the wheat crop.

4.6. Area-Wide Control of Corn Pests: Past and Present

Not all pest control projects (even those including area-wide management) are successful, one example being with corn in the USA when several changes occurred in corn production following the passage of the 1950 Farm Bill by the United States Congress.

The 1950 Farm Bill legislation provided commodity price support for corn, wheat, peanuts, cotton, and several other crops (NAS 1989). However, the bill stipulated that only a single crop could be grown if the farmer was to receive commodity price support, forcing many farmers to raise only one crop and abandon crop rotations. This change in corn production was followed by increased insect, plant pathogen, and weed problems for corn and other crops, plus greater use of pesticides and fertilizers (NAS 1989).

In 1945, no pesticides were used in corn production (Pimentel et al. 1991, Pimentel 1997). Today, corn receives significantly more insecticide and more herbicide than any other crop grown in the USA (USDA 2004). Specifically, corn production now uses a thousand-fold more insecticide than in 1945, while at the same time crop losses to insects have increased nearly four-fold from 3.5 to 12% today (Pimentel 1997). The main reason for the increased crop losses due to insects is that now only half of the corn area is grown in rotation, the other half being grown as corn-on-corn (USDA 2004). This continuous corn production has increased insect pest problems, primarily the corn rootworm complex (northern corn rootworm *Diabrotica barberi* Smith and Lawrence, western corn rootworm *Diabrotica virgifera* LeConte, Mexican rootworm *Diabrotica virgifera zea* Krysan and Smith, and southern corn rootworm *Diabrotica undecimpunctata howardi* (Barber)), as well as other insect pests, all of which require insecticide treatments. The corn rootworm complex is among the most

economically and environmentally damaging insect pest problem of corn production systems in the USA. Annually, crop losses and control costs attributed to the corn rootworm complex approach USD one billion (Gray 1999, Tollefson and Levine 1999) and ten million hectares of corn are treated with soil insecticides to protect the crop from larval feeding damage.

The changes in agricultural technology of corn production fostered by the 1950 Farm Bill have had numerous other negative impacts including: increased insecticide and herbicide use causing chemical pollution of ground and surface waters; increased soil erosion and soil infertility; increased use of nitrogen fertilizer caused in part by leaching in rapid water run-off from the corn fields and resulting in increased pollution of waterways as far downstream as the Gulf of Mexico; increased dependence on fossil energy; and increased water, air, and soil pollution from animal wastes as a result of livestock once produced on grain-farms being transferred to large livestock feeding units.

The rotation of corn with soybeans, wheat, and other non-corn crops reduces several insect pests of corn, including: the corn rootworm complex, corn root aphid *Anuraphis maidiradicis* (Forbes), corn leaf aphid *Rhopalosiphum maidis* (Fitch), and fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Wright 2005). Corn rotation with other non-host crops also helps reduce plant pathogens and weed pest pressure. With reduced pest problems because of crop rotations, corn yields increased about 8%; which compares favourably with the ineffective results of recommended insecticide treatments (Pimentel et al. 1993).

Populations of the European corn borer *Ostrinia nubilalis* (Hübner) are not reduced significantly by rotations because this species can fly long distances. When the European corn borer arrived in New England in 1917 from southern Europe, it caused complete crop loss in early-planted sweet corn. Between 1948 and 1958, this invasive species became widely established west of the Mississippi River and caused enormous losses of corn (Metcalf et al. 1962). A major effort led by the USDA failed to eradicate the pest (Klassen 1989), while the numerous species of natural enemies of the pest introduced from abroad have provided only marginal control (Bradley 1952). However, the assiduous effort to develop resistant hybrid corn has gradually but decisively reduced the destructiveness of this pest (Brindley and Dicke 1963, Brindley et al. 1975, Gallun et al. 1975). Currently only about 10% of the corn area is treated with insecticides for the corn borer. One difficulty in treating for the corn borer is that the treatment has to be perfectly timed to kill the larvae just after they hatch and before they bore into the corn. Once inside the corn, the young larvae are not susceptible to insecticide treatments.

Recent changes in corn rootworm population behaviour are now severely hindering the utility of traditional corn rootworm management approaches. These include increased incidence of extended diapause in northern corn rootworm populations (eggs remain dormant for an entire year), insecticide resistance in Nebraska western corn rootworm populations, and western corn rootworm adaptation to crop rotation strategies in Illinois, Indiana, and Ohio. As a result, there is a need to develop an area-wide approach to protect corn production across the country.

In order to determine whether rootworm populations could be strongly suppressed and the use of soil insecticides reduced by using the adult rootworm attractant, cucurbitacin, in an aerially applied bait, a pilot area-wide programme was conducted from 1997 through 2001 by the USDA in cooperation with five Land Grant universities in corn-producing states (Chandler 1998, 2003, Parimi et al. 2003, Chandler 2005). Individual fields were aerially sprayed with the baited insecticides when the number of corn rootworms captured in yellow sticky traps reached a set threshold. Suppressing adult rootworms minimized the number of eggs laid and resulted in fewer larvae avail-

able to damage corn roots in the following growing season.

5. Challenges of Insect Invaders

Worldwide, the movement of exotic insects, mite species and plant species from one ecosystem to another is a continuing problem for all agriculturists dealing with pest control (Pimentel et al. 2000). Borders are becoming increasingly irrelevant in the context of international travel and trade, and this facilitates the movement of invasive species (National Plant Board 1999). Technological advances in transportation in recent decades also actually facilitate both the survival and successful colonization of invasive species. The volume of air cargo of perishable agricultural commodities such as cut flowers, fruits and vegetables as well as the rate of arrival of damaging species at ports of entry in the USA is doubling every five to six years (Zadig 1999, Klassen et al. 2002). Frank and McCoy (1992) found an average rate of establishment of exotic arthropods species in Florida of 14.2 per year, and Thomas (2000) listed 150 exotic arthropod species that had been established in Florida from 1986 through 2000.

As noted by Evans (2004) there is growing concern with regard to the level of resources that countries now have to put aside to address this growing problem. For example, the average annual spending of APHIS on its emergency programmes for the period 1989-2002 rose exponentially from about USD 6.4 million in 1989 to USD 334.8 million in 2001, which is not sustainable. Also, new technologies needed to combat invasive species cannot be developed with sufficient rapidity to meet this challenge.

The damage caused by invading insect pests that attack established crop, forest, and natural ecosystems is enormous (Pimentel 2002). For example, approximately 40% of insect and mite pests of crops in the USA are introduced species. Yearly they cause about USD 10 000 million in crop damage and control costs. The most recent introductions include the long-horned beetle *Anoplophora glabripennis* (Motschulsky) and the emerald ash borer *Agrilus planipennis* Fairmaire that were both accidentally introduced from Asia. The long-horned beetle is now destroying maple trees, while the emerald ash borer is killing ash trees in the same region (Hoebeke 2004). Area-wide strategies to control these destructive pests are being implemented because they are major threats to valuable tree species in the North American forest ecosystems.

Although port-of-entry inspection is important, it must be greatly augmented with a risk-based management strategy that requires mitigation of pest risk at origin, i.e., where the commodity to be imported is being produced. Risk mitigation conducted at origin assures that clean products arrive at the port of entry (McDonell 2004). An important approach to offshore mitigation is the creation of pest free areas. Indeed countries, which export raw agricultural commodities, can effectively remove the threat of exotic pests to the importing country by creating and maintaining pest free areas of production (Malavasi et al. 1994). According to the FAO, a pest free area is:

> *An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (FAO 2005b).*

There are two officially recognized situations where a pest free area can be applied: (1) large geographic areas, such as the entire country of Chile that is certified free of fruit flies of economic importance and where this condition is officially maintained (FAO 1996), and (2) pest free places of production or production sites (a defined portion of a place of production) in which a specific pest does not occur and in which this condition is officially maintained. In contrast with the pest free area, in this case, the condition is maintained for a defined period and the area is managed as a separate unit (FAO 1999). To facilitate this approach the Secretariat of the International Plant Protection Convention has developed International Standards for the establishment

and maintenance of pest free areas.

Requirements to establish pest free fields of crop production include a sensitive detection programme, suppression of the quarantine-significant pest to non-detectable levels, strict control of the fields, and safeguards to prevent infestation during packing and transit to the port of export (Riherd 1993, Malavasi et al. 1994). Florida is able to export grapefruit to Japan by creating pest free grapefruit groves in about 22 counties. Regulatory experts from Japan inspect the entire process of production, packing and transit. By 1980, Chile had succeeded in eliminating the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and since then Chilean fruits in huge volumes have entered the markets in the USA without the need for any quarantine treatments. Likewise the Mexican States of Baja California, Chihuahua and Sonora have been freed of all economically-important species of fruit flies, so that citrus, stone fruits, apples and vegetables are being exported from these states without any postharvest treatment.

6. Conclusions

Balancing the production of an adequate food supply against the basic needs of humans to sustain their nutritional needs will become more difficult in the coming decades. Fortunately the development of successful pest control operations is improving, especially those based on area-wide interventions. Yet, with all control projects, the basic ecology of the pests, the role of biological control and the safe use of insecticides must be major factors in the development of all pest management programmes. Insect control tactics applied on an area-wide basis have a number of advantages (Klassen 2005), including the use of approaches that may prevent or retard the development of insecticide resistance. In addition, area-wide approaches offer the potential to take advantage of methods friendly to the environment (SIT, parasitoids, semiochemicals, mating inhibitors, etc.) which cannot be applied on a farm-by-farm basis and which all contribute to the reduction of the use of broad-spectrum insecticides.

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Section 2

Basic Research

Engineering Insects for the Sterile Insect Technique

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ABSTRACT The mass-release of sterile insects (the sterile insect technique (SIT)) is a highly effective component of area-wide methods of pest control with no environmental impact. The SIT relies on the sterilization of large numbers of insects, usually by irradiation. The SIT has been used successfully against several pest insects. However, modern biotechnology could potentially provide several improvements. These include: (1) improving the identification of released individuals, (2) removing the need for radiation-sterilization, (3) reducing the hazard posed by non-irradiated accidental releases from the mass-rearing facility, and (4) providing automated sex-separation prior to release (genetic sexing). None of these are necessarily unattainable by classical methods. However, the use of recombinant DNA methods may allow these benefits to be obtained in a shorter period and to be transferred more readily from one species to another than are the products of classical genetics. The potential of these methods, and the progress towards realizing this potential, is discussed.

KEY WORDS genetic sexing, transformation, genetic sterility, marking, stability, fitness

1. Introduction

The sterile insect technique (SIT) has been used successfully against several insect species (Lindquist et al. 1992, Krafsur 1998, Tan 2000, Wyss 2000, Koyama et al. 2004, and other papers in this volume). However, modern biotechnology could potentially provide several improvements in the operation or security of a pest control programme integrating the SIT (Heinrich and Scott 2000, Thomas et al. 2000, Alphey 2002, Handler 2002, Benedict and Robinson 2003, Horn and Wimmer 2003, Gould and Schliekelman 2004). These include: (1) improving the identification of released individuals by providing a genetic marker allowing easy discrimination between wild-type and released insects, (2) removing the need for radiation-sterilization by providing some sort of "genetic sterilization", (3) reducing the hazard posed by non-irradiated accidental releases from the mass-rearing facility by arranging that the insects need an artificially-provided condition, for example a dietary supplement, in order to survive or reproduce, and (4) providing automated sex separation prior to release to eliminate females from the released population (genetic sexing). None of these is necessarily unattainable by classical methods. However, the use of recombinant DNA methods may allow these benefits to be obtained in a shorter period and to be transferred more readily from one pest species to another than are the products of classical genetics.

2. Genetic Markers

It is essential to be able to detect the presence of wild insects, even in the presence of overwhelming numbers of released sterile insects. This requires that the released insects be marked in some way to distinguish them from wild insects. This has been done by adding a

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dye to their food, or dusting the pupae or adult insects with a fluorescent dye (Hagler and Jackson 2001). Provision of a suitable genetic marker in the strain would obviate the need for such a dye. This would reduce the amount of handling required, and the possibilities for human error. A candidate marker, *Sergeant* (*Sr2*) has recently been described for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), which has a dominant effect on adult cuticle pigmentation (Niyazi et al. 2005). This mutation is also a recessive lethal. This would normally prevent the production of a true-breeding strain, but this problem can be avoided in this case due to the unusual genetics of Mediterranean fruit fly genetic sexing strains constructed at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria (Robinson 2002, Niyazi et al. 2005).

An alternative would be to provide a dominant marker by transgenesis, for example a gene expressing a fluorescent protein. Such systems have been widely used in *Drosophila* and pest insects, including several tephritids, mosquitoes and moths (Berghammer et al. 1999, Catteruccia et al. 2000, Peloquin et al. 2000, Pinkerton et al. 2000, Tamura et al. 2000, Handler and Harrell 2001, Horn et al. 2002, Perera et al. 2002, Allen et al. 2004a). In contrast to classical mutagenesis, dominant markers can readily be generated by this method and these markers are not associated with recessive lethality. It is clear that such markers can be provided in most species of interest for the SIT. Two recent papers (Catteruccia et al. 2003, Irvin et al. 2004) have shown that specific strains carrying such markers showed a severe reduction in fitness relative to the untransformed progenitor strain. However, in these cases the loss of fitness appears to have been due to inbreeding depression; when this was avoided, no such loss of fitness was observed (Allen et al. 2004b, Moreira et al. 2004).

An additional possibility would be to label sperm. It would be desirable to be able to determine whether a trapped female had mated a wild or a sterile male before she was captured. Different programmatic responses might be desirable for each of these possibilities, so accurate diagnosis is important. However, a quicker and more convenient option would be simply to look at the sperm, if a suitable fluorescent marker could be provided. Such marked sperm have been produced in *Drosophila* and used to monitor sperm storage and competition in laboratory experiments (Civetta 1999). This has also recently been achieved in the mosquito *Anopheles stephensi* Liston (Catteruccia et al. 2005), using a β2-tubulin promoter from *Anopheles gambiae* Giles to give expression in the male germ-line. These authors further demonstrated that automated sex separation could be achieved using the sex-limited expression of a fluorescent protein in the larval testis and a Complex Object Parametric Analyser and Sorter (COPAS) – a sorter based on fluorescence.

3. Genetic Sterilization

Ionizing radiation damages insects and can thereby have a significant negative impact on their subsequent performance (Shelly et al. 1994, Lance et al. 2000, Barry et al. 2003, Kraaijeveld and Chapman 2004), and therefore on the cost and effectiveness of the sterile insect release, although the exact magnitude of this effect is still controversial (Robinson et al. 2004). Irradiated male Mediterranean fruit flies compete less well for mates, and are less effective at inducing female refractoriness to remating (Kraaijeveld and Chapman 2004). In some cases, irradiated insects also have a reduced lifespan after release relative to wild insects. This further reduces their effectiveness. However this reduction in lifespan is probably not entirely due to radiation, other possible contributory factors being damage due to handling and distribution, and the conditions and genetic pressures of mass-rearing.

Radiation works in this context by inducing random dominant lethal mutations in the gametes of irradiated insects. Progeny from such gametes therefore die, typically early in

embryogenesis. It should be possible, in principle, to remove the need for irradiation by using engineered dominant lethals instead (Fryxell and Miller 1995, Alphey 2002, Alphey and Andreasen 2002). Strains with the necessary properties were constructed in *Drosophila* some years ago (Heinrich and Scott 2000, Thomas et al. 2000, Horn and Wimmer 2003). Much more recently, the construction of potentially suitable strains of Mediterranean fruit fly has been described (Gong et al. 2005). These, the first such strains described for any pest insect, contain a novel, single-component, repressible, dominant, lethal gene which gives up to 99.8% mortality in heterozygotes not provided with dietary tetracycline. Penetrance of lethality and effect on adult male lifespan varied, as expected, from one insertion line to another – this is the well-known phenomenon of position effect. However, the best line showed 99.8% lethality in the absence of tetracycline, no effect on adult male lifespan even in the absence of tetracycline, and no detectable negative effect on male mating competitiveness. The effect on female refractoriness to remating was not measured.

Along with the major benefit of eliminating the requirement for irradiation, and thereby the expense, security issues, and damage to the insects associated with this, there are some potential drawbacks with this approach, and with the particular strains described by Gong and colleagues (2005). Mass-release of genetically engineered insects, even of this particularly benign type, will face regulatory hurdles that have not been imposed on the use of irradiation and the products of classical genetics. The present strains require the addition of tetracycline, or a suitable analogue, to the larval diet. Such antibiotics are not normally used in Mediterranean fruit fly mass-rearing, but chlortetracycline is a component of the standard diet used for mass-rearing pink bollworm. It is also a component of the liquid diets being developed for Mediterranean fruit fly mass-rearing by the United States Department of Agriculture's Agricultural Research Service (USDA-ARS), so the use of tetracyclines seems not to be an insuperable problem. Another issue is the use of transposons as gene vectors. Consideration of the mechanisms and rates of horizontal gene transfer, and of its consequences in this context, clearly lead the writer to conclude that this is not a serious issue for non-autonomous transposons that contain no sequences likely to give a selective advantage to the recipient, and no functional selfish DNA or gene-drive systems.

However, some commentators believe otherwise, and this will doubtless be one of several issues that regulatory authorities will need to consider. In any case, this is not an insuperable issue, as systems are being developed to stabilize transposable elements after insertion (Handler et al. 2004, Dafa'alla et al. 2006, Handler, this volume). The strains described by Gong et al. (2005) have a larval lethal phase, rather than the (preferred) embryonic lethality caused by irradiation. This could be a problem for some bisexual releases; in such cases efforts would need to be made to develop a transgene system with an earlier lethal phase (Horn and Wimmer 2003), or a genetic sexing system.

Perhaps the only non-trivial drawback of repressible lethals as a replacement for radiation sterilization is the question of resistance management. Though heritable resistance to the SIT is possible, for example by assortative mating, in which the wild females preferentially mate wild males rather than sterile males, in the 50-year history of the SIT there has been very little evidence of this (but see Koyama et al. 2004 for one clear example). The use of engineered repressible lethals would open up another possible form of resistance, namely "biochemical" resistance to the lethal effector molecule of the engineered system. This is essentially impossible for radiation-based SIT, as the random nature of the radiation damage means that every sperm is damaged in a different way, and it seems inconceivable that the wild population could evolve a mechanism to overcome this random damage. Whether such resistance could arise in practice, and over what

timescale, is entirely a matter of speculation at present. This possibility of resistance is not a new issue, and applies equally to the other major pest control methods of chemical pesticides and genetically engineered insecticidal crops. In each case, the evolution and spread of resistance can be managed or mitigated by various methods, including "stacking" effector molecules, etc. These methods can also be applied to the use of engineered repressible lethals.

Although only published so far for the Mediterranean fruit fly, similar systems are under development in the author's laboratory for several other pest species. In this context, it is important to note that the constructs of Gong et al. (2005) contained no Mediterranean fruit fly DNA, and might therefore be expected to work in a range of species with little or no modification.

4. Reduced Escape Hazard

Insect pest control programmes with an SIT component mass-rear the pest insect on a massive scale. Until these insects have been irradiated, any escape would be unwelcome, and potentially catastrophic. In fact, SIT applications have an excellent safety record in this respect, but non-irradiated releases have indeed occasionally happened, for example of New World screwworm in Mexico and Panama in 2003 (del Valle 2003). However, all accidental outbreaks were quickly eliminated by the release of additional sterile insects. Natural disasters (e.g. hurricanes or earthquakes), accidents or sabotage could also have very severe consequences. The use of genetic sexing strains for Mediterranean fruit fly SIT, such as the strains based on the *temperature sensitive lethal* (*tsl*) mutation mitigates these consequences as colony females are temperature sensitive. However, the engineered repressible lethal strains described above have the potential to provide a more satisfactory solution. These insects, or their progeny, will die unless they are artificially provided with an antidote to the lethal genetic system. This neatly overcomes the escape hazard, as such strains can neither establish in the wild on their own, nor form viable hybrids with any pre-existing wild pest population.

For this application, one would envision introducing an engineered repressible lethal genetic system into a strain currently used for the SIT, either a sexing strain or wild type, depending on what is available in the species of interest. It would also be simple to arrange that the engineered lethal system is associated with a useful genetic marker. This strain would then be used exactly as at present, but with the advantages of the genetic marker and of a dramatically reduced escape hazard.

The question of resistance, discussed above, does not apply to this approach as radiation would still be the primary basis of sterility, with the engineered system being a backup. This principle could still allow the use of a reduced radiation dose, relying on the engineered system to kill the few insects that would otherwise have been produced.

5. Genetic Sexing

For many insects, it would be highly preferable to eliminate females from the release population. This is for several reasons: (1) the females of some species are damaging while the males are not, (2) released males may court co-released sterile females, if present, rather than seeking out wild females, (3) the presence of females may require the use of a higher radiation dose than would be optimal for males, and (4) even if the females are merely neutral to the programme, they consume diet and add to distribution costs. Highly effective genetic sexing strains have been produced for various species, based on translocation of a dominant selectable marker to the Y chromosome (Whitten 1969, Whitten and Foster 1975, Seawright et al. 1978, Robinson 1989, Hendrichs et al. 1995, Franz et al. 1997, Robinson et al. 1999, Robinson 2002). Unfortunately, these chromosome aberrations that are an integral part of selection systems tend to reduce the rearing productivity of the flies that carry them. In Mediterranean fruit flies, these aberrations are also unstable (Franz et al. 1994, Kerremans

and Franz, 1995) but adequate strain quality can be maintained during large scale mass-rearing through the use of a filter rearing system (Fisher and Cáceres 2000). Furthermore, each of these sexing strains must be developed anew for each new species – genetic tools developed in one species by classical mutagenesis cannot be transferred to another species.

Recombinant DNA methods offer the prospect of simpler systems for genetic sexing, in which a repressible or inducible female-specific lethal is used. Such constructs could be introduced into an otherwise wild-type genetic background. Changing from permissive to repressive conditions in the last generation of mass-rearing would provide a single-sex population for release. For this purpose, the repressive condition could be high or low temperature (as for the present *tsl* strains), presence or absence of a dietary chemical, or any other convenient environmental parameter that can be tied to a change in gene expression or function. Such systems have been demonstrated in *Drosophila* (Heinrich and Scott 2000, Thomas et al. 2000), in Mediterranean fruit fly (Fu et al. 2007), and work is in progress to develop them for pest insects.

The *tsl*-based genetic sexing strains (e.g. VIENNA 8) developed at the FAO/IAEA Agriculture and Biotechnology Laboratory for the Mediterranean fruit fly, are extremely effective, despite some modest drawbacks. The greatest advantage of transgene-based sexing systems, relative to current practice, may therefore be seen in other pest insects, for which no such system is presently available. Development of a sexing system would have significant benefits for several species, including the New World screwworm *Cochliomyia hominivorax* (Coquerel), various fruit flies (e.g. the Mexican fruit fly *Anastrepha ludens* (Loew)), and anopheline mosquitoes, and has also been advocated for some moth species (Marec et al. 2005).

6. Disadvantages of Recombinant DNA Methods

Apart from the specific issues discussed above (use of tetracycline, possibility of resistance to a dominant lethal-based genetic sterilization method), two more general issues have been raised as possible limitations to the use of recombinant DNA methods for the purposes described above. These are fitness and stability.

6.1. Fitness

Two recent papers (Catteruccia et al. 2003, Irvin et al. 2004) have appeared to imply that all transgenic mosquitoes will have severely reduced fitness or mating competitiveness, compromising any genetic control strategy based on such insects. In fact these studies used a small number of highly inbred lines, and much of the fitness cost may be attributed to this inbreeding. Furthermore, even if these lines were shown to have low fitness, it does not follow that this will be true for all transgenic strains. A more recent study that avoided inbreeding found no significant deleterious effect in insects expressing a synthetic peptide as well as a fluorescent marker protein (Moreira et al. 2004). This is consistent with a much larger study of transgenic *Drosophila*, which found only modest effects on fitness in many strains (Lyman et al. 1996).

6.2. Stability

This relates to the use of non-autonomous transposable elements as transformation vectors. There is some debate over the stability and environmental safety of current transformation technology, which is based on the use of these transposons. The writer does not agree that the use of large non-autonomous transposons automatically results in an unacceptable risk. This is particularly clear when, as in the applications described above, the transposon contains no components that might confer a selectable advantage on a recipient, i.e. no insecticide or antibiotic resistance genes. Compared to the use of genetically-modified crops that (1) generally incorporate antibiotic resistance, herbicide tolerance and/or insect toxicity (from *Bacillus*

thuringiensis (de Barjac)), (2) liberate pollen into the environment, and (3) can hybridize with wild relatives far more readily (e.g. canola), the issue does not seem daunting. Furthermore, technical progress in this area may overcome or bypass this difficulty. There are several published methods, or obvious variants thereof, that would do this, though demonstrated only in *Drosophila* so far (Rong and Golic 2000, Groth et al. 2004, Handler et al. 2004, Horn and Handler 2005, Oberstein et al. 2005, Wimmer 2005), apart from the phage ϕC31 system, which was also shown to work efficiently in a mosquito (Nimmo et al. 2006). More generally, no strain or genetic construct is truly stable. All are subject to random mutation. This will lead, at a very low frequency of perhaps 10^{-7}-10^{-8} per insect generation, to the production of defective versions of the transgenic strain, particularly ones that have lost the intended function. The presence of such defective versions arising in the release generation should be entirely inconsequential, as they would be vastly outnumbered by correctly functioning insects. If the original transgene system was mildly deleterious, revertants arising within the breeding population of the mass-rearing facility might have a modest selective advantage over the original transgenic strain. In this case the defective version would tend to spread through the breeding population, which would be highly undesirable. However, this is not a new problem. The current translocation-based Mediterranean fruit fly sexing strains are also unstable. Since the males are semi-sterile and the females weakened by the recessive mutations they carry, breakdown products, or wild-type chromosomes introduced into the facility from outside, will spread rapidly through the mass-rearing population. This problem was overcome by introducing an elegant filter rearing system, in which a relatively small population is carefully maintained and monitored to ensure its genetic quality (Fisher and Cáceres 2000). Samples from this colony are taken, expanded for several generations and then released; no insects from this much larger population are used for subsequent breeding. This system is likely to be required for large-scale rearing of any non wild-type strain, whether produced by classical or molecular genetics. It should be more than capable of handling the level of instability that might be expected from a typical transgenic strain, although actual mass-rearing will be required to address this experimentally.

7. Combining the Benefits of Recombinant DNA Methods

The various improvements to the SIT discussed above could be provided independently, but it would be more efficient to combine them. For example, while there may be several ways to construct a genetic sexing strain, the use of a repressible system, where the repressor is not found in the natural environment of the insect, would have the additional advantage that escaped females, or female progeny, would die in the wild. The escape hazard would thereby be greatly reduced, which would not typically be true of inducible lethal systems, although they might give strains that were perfectly adequate for the purpose of genetic sexing. Furthermore, such a strain might also be used to remove the need for irradiation. Elimination of all female progeny will generally be as effective as killing all the progeny. Indeed it may be significantly better, particularly if several unlinked female-lethal constructs are combined in the same strain (Schliekelman and Gould 2000, Thomas et al. 2000).

It therefore seems likely that it will be possible in the near future to combine all of the above advantages – genetic marker, genetic sterilization, reduced escape hazard, and genetic sexing – into a single compact construct or genetic system. The construction of an effective non-sex-specific lethal system in Mediterranean fruit fly (Gong et al. 2005) is a major step towards this goal.

8. Conclusions

Recombinant DNA methods show great potential for improving SIT. Each of the

improvements discussed above, incorporated singly or in combination into a field programme, could provide substantial benefits (Simmons et al., this volume), and future advances in molecular biology and genetics are likely to allow further improvements as yet unheralded. Strains with sufficient potential to warrant incorporation into a field programme are already available, though some field-testing is urgently required to confirm this potential. The first few strains may well not perform in the field exactly as they do in the laboratory, and some modification of both strains and testing methods may be required. Even if the strains themselves are found not to need any further refinement, some adjustments may be necessary in the mass-rearing methods to incorporate these new genetic strains, as was required during the change to the current Mediterranean fruit fly sexing strains.

A more serious limitation to progress is the lack of a well-developed regulatory process to oversee the introduction of transgenic strains into field use. It is essential that the field use of recombinant DNA methods is subjected to appropriate expert assessment. Developments in the laboratory have not yet been matched by parallel developments in the regulatory process. However, some progress has been made, particularly in the USA. It is essential that countries that have an interest in the SIT urgently clarify and update their regulatory procedures, so that the potential of this new approach can be realized in the field.

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The *hobo*, *Hermes* and *Herves* Transposable Elements of Insects

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ABSTRACT Transposable elements remain the only way to introduce genes into insects so that they are stably inherited through successive generations. Progress in the transformation of non-drosophilid insect species, such as mosquitoes and true fruit flies, has resulted mainly from the identification and utilization of new insect transposable elements. The *hAT* superfamily of transposable elements consists of members from plants, fungi and animals and includes the active insect transposable elements *hobo*, *Hermes* and *Herves* from *Drosophila melanogaster* (Meigen), *Musca domestica* (L.) and *Anopheles gambiae* Giles, respectively. These three elements offer a unique system for study since they are all active yet show, at some levels, significant sequence divergence. The central premise of the authors' research is that the success with which transposable elements can be used as genetic tools in insects, particularly in field applications, is dependent on knowledge of how they work in the cell nucleus. To this end a structure:function analysis of these three transposable elements has been undertaken, as well as, in the case of *Herves*, an analysis of its distribution in field populations of *A. gambiae* in regions of Africa. Also discussed are the possible roles that host factors may play in *Hermes* and *Herves* element transposition and the implication that these might have for the use of transposable elements in genetic control programmes. Attempts to generate and test hyperactive forms of the *Hermes* element transposase are also discussed.

KEY WORDS transposable elements, transformation, *Hermes*, *Herves*, *hobo*, *hAT* elements, host factors

1. Genetics and Insect Pest Control

The use of ionizing radiation to induce mutations, many of which produce chromosomal rearrangements, has provided a ready source of variation that has led to some of the pioneering developments in animal genetics. It also led to an appreciation that chromosomal rearrangements such as translocations could be employed in the control of insect pest species. Whitten (1985) provides an illustrative examination of this subject and cites Serebrovsky's 1940 manuscript as the moment at which the use of chromosomal translocations to control the populations of pest species was first canvassed. In a subsequent manuscript, Serebrovsky (1941) described further elaborate genetic systems such as genetic sexing strategies and further translocation-based systems that became bulwarks of some of the approaches to genetic control that are used today. Later, and quite independently in the USA, Bushland and Hopkins (1951) responding to a suggestion by Knipling, and later Knipling (1955) proposed the use of ionizing radiation to sterilize insect pests. The subsequent deployment of the sterile insect technique (SIT) for the elimination of New World screwworm *Cochliomyia*

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hominivorax (Coquerel) populations over several decades and the many attempts to repeat this successful paradigm in other pest species is well documented (Dyck et al. 2005).

The principle of the SIT proposed by Knipling, Bushland and Hopkins, and the methods of pest insect control proposed by Serebrovsky achieve their goals of pest insect control through very different approaches. As Whitten (1985) explained, there is no underlying genetic theory to the SIT, rather large numbers of insects are exposed to sufficient levels of ionizing radiation to render them sterile and they are then released into the field, once again in large numbers. The increase in genetic load leads to population suppression and even elimination. The types of mutations and chromosomal damage induced in the gametes of these insects are irrelevant; all that matters is that it occurs at a frequency that meets the quality control standards of the released insects. The methods described by Serebrovsky (1941) are rooted in genetic theory. The types of induced chromosomal rearrangements, their stability over time and their mode of transmission through generations in the laboratory and in the field, form the basis of the genetic control programme. In Serebrovsky's approach, knowledge of cytogenetics was critical.

In the 60 years since these pioneers proposed these approaches to genetic control, genetics has undergone perhaps three revolutions. One was the discovery of the structure of DNA, the second was the development of recombinant DNA techniques, and the third is the birth, still in progress, of genomics. All of these have impacted human welfare and all will have similar effects on how the future genetic control of pest insects is achieved. In the SIT today, convergence of Bushland's, Hopkins', Knipling's and Serebrovsky's strategies are seen in genetic sexing strains developed at the FAO/IAEA Agriculture and Biotechnology Laboratory, Austria for use in area-wide integrated pest management (AW-IPM) programmes using the SIT for the control of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). These strains contain a sex-linked chromosomal translocation which joins the male-determining Y chromosome to a locus located near the translocation breakpoint that contains the wild-type allele of a temperature sensitive lethal allele present on the 5th chromosome. Females are homozygous for the lethal alleles and are killed as embryos through incubating eggs at the restrictive temperature.

2. Insect Transgenesis

The development of *Drosophila melanogaster* Meigen genetic transformation by Rubin and Spradling (1982) led to the proposal to use this technology in pest insects to efficiently generate new genotypes. This new technology would augment existing approaches to AW-IPM and would also lead to new approaches to insect genetic control in general. In programmes using the SIT, the traditional and still current method used to generate new genetic sexing strains is chemical and/or radiation mutagenesis followed by breeding. If the desired strain requires a translocation then both the labour and time required to generate this strain can be very large. Furthermore, once obtained, the strain may not exhibit the performance characteristics required for effective use of SIT within the overall programme. It may be difficult to rear in large numbers and recombination may separate the temperature sensitive locus from the male-determining region of the Y chromosome at a frequency not commensurate with continual and repeated mass-rearing.

Insect transformation is achieved through the use of transposable elements. The attraction of transposable element-mediated transgenesis to those wishing to use genetics in pest control is that new mutants can be quickly and easily constructed provided that a transposable element can be found that introduces genes into the target insect species. Indeed, an insect heterozygous for the new mutation can be made in one generation and homozygotes pure breeding for this allele within a further generation. These can then be tested for their suitability for use in pro-

grammes integrating the SIT. A further advantage of transposable element-mediated transformation is that the change to the insect genome is precise and small and therefore can be completely characterized by molecular biological techniques. This is in contrast to the classical sexing strains, which contain chromosomal rearrangements with changes in linkage groups and recombination frequencies of hundreds, if not thousands of genes associated with the translocation. The product of the genetic engineering approach is thus very well defined, yet it is the manner in which the new genotype is made, and the fact that it typically contains new DNA from other organisms, that attracts the ire of critics of this technology. That transposable elements – jumping DNA – are naturally occurring, and can move between species, is also a point of some concern.

The history of finding transposable elements that could transform insect species outside of the family Drosophilidae is beyond the scope of this article and has been more than adequately covered elsewhere (Handler 2000). To summarize a ten-year history from 1985 to 1995: the *P* element so successfully used as a gene vector in *D. melanogaster* was incapable of performing the same feat in other insect species at any reasonable frequency. This led to a search for alternate transposable elements that had host ranges, which included pest insect species. These elements were identified primarily on their mobility in genetic assays although bioinformatics analysis of whole genomes now provides an alternate means by which functional transposable elements can be identified. The elements identified through the basis of their mobility are the *Minos* element from *Drosophila hydei* Sturtevant (Franz and Savakis 1991), the *Mos1* (*mariner*) element from *Drosophila mauritiana* Tsacas & David (Medhora et al. 1988), the *Hermes* element from the house fly *Musca domestica* (L.) (Warren et al. 1994), the *piggyBac* element from *Trichoplusia ni* (Hübner) (Cary et al. 1989), and the bacterial *Tn5* element. The *Herves* element from *Anopheles gambiae* Giles was identified through a bioinformatic analysis of the whole genome sequence of this species (Arensburger et al. 2005). The discovery by Savakis and his colleagues in 1995 that the *Minos* element could repeatedly be used to stably transform *C. capitata* was a breakthrough in showing that transformation of pest insects could be achieved (Loukeris et al. 1995). James soon showed that repeated transformation of the mosquito *Aedes aegypti* (L.) could be achieved through using the *Hermes* or *Mos1* elements (Coates et al. 1998, Jasinskiene et al. 1998). Currently, *piggyBac* enjoys the most use amongst non-drosophilid insect geneticists. It has a wide host range, appears to have little if any requirement for host factors, and displays a distribution amongst arthropods that suggests it has been active through recent insect evolution.

3. Transposable Elements

The aforementioned transposable elements are classified as class II elements because they transpose via a DNA intermediate. They have a simple structure consisting of two terminal inverted repeat sequences located at either end of the transposable element and a gene encoding a transposase located within the element (Fig. 1). In bacteria, other genes, for example those encoding antibiotic resistance are also present on the element. Class II transposable elements transpose through the recognition of DNA sequences within and internal to the terminal inverted repeat sequences by the transposase, followed by excision of the element and its integration to a second site elsewhere in the genome to create target site duplications at the site of insertion. If no copy of the element remains at the original donor site, transposition is non-replicative since the copy number of the element within the genome does not increase. If a copy of the element remains at the donor site then transposition is replicative since the copy number of the element increases with each transposition.

The process of insect transformation is dependent on the characteristics of the transposable element used. It follows that under-

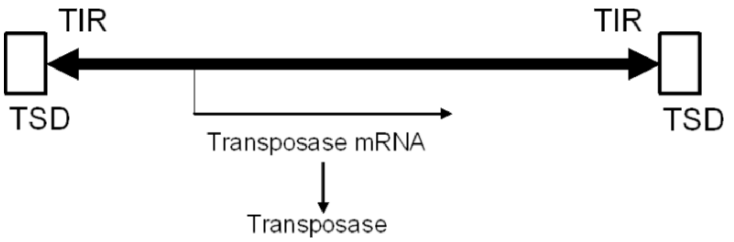

Figure 1. *The general structure of class II transposable elements. (TIR: terminal inverted repeat sequences, TSD: target site duplications).*

standing the molecular basis of transposition for each of these elements will lead to a better understanding of how they will behave when introduced into new host species, such as an insect pest that is the target of a programme involving the SIT or a vector of disease in which the aim to is to use the mobility properties of the transposable element to quickly move a beneficial transgene through the vector population. Indeed, it is often the fact that transposable elements are used to generate transgenic insects that concerns opponents of this technology. Concerns about the ability of these elements to cross species barriers and to even lead to the extinction of non-target species are raised as reasons why this technology should not be applied to pest insects. These are coupled with the origin of the element itself (which typically comes from another species), and of the transgene and genetic marker it carries also being from another species. Yet it is foreseeable through the development of pest insect genome projects that the transgene, genetic marker and even the element itself may come from the target pest insect itself, so the issue of foreign DNA would be rendered mute. In such a case, it is likely that concerns about the mobility properties of the transposable element itself would remain as a possible barrier to the application of transgenic-based technology in AW-IPM programmes.

The central tenant of the authors' research is that an understanding of transposable element behaviour comes from examining, in detail, the biochemical mechanism of transposition and how this might be modified when the element is introduced into a new host species. Answers to pertinent questions of regulation, copy number, host range and stability are inevitable outcomes of this research. These can then be used to realistically address two main and opposite prerequisites of transposable elements used in AW-IPM programmes:

(1) For programmes using the SIT: the requirement that the element be stably inserted at desired genomic sites that allow desired levels of expression of the transgene. If the purpose of the programme is to genetically tag every insect with a marker gene or sequence, then it is critical that every insect raised and released contains this transgene in the identical genomic location and that expression levels be constant from insect to insect, generation upon generation. This requires the transposable element containing the transgene to be genetically stable at a frequency at least equal to the spontaneous mutation frequency of the insect. If the purpose of the programme is the development of genetic sexing strains in which the female sex is eliminated before mass-release, the same requirements exist for stability of the element and consistency of expression.

(2) For programmes requiring the spread of a beneficial gene through an insect population: the requirement is for this gene to also remain inserted within the transposable element, but for this element to be highly mobile so that the transgene, and the beneficial effects arising from its expression are spread through the pest population as rapidly as possible.

These are competing performance characteristics for a transposable element. Both require the element to remain intact, yet one demands genomic stability while the other requires high rates of mobility. It stands to reason that it is unrealistic to expect that an unmodified transposable element introduced into either the same, or a different insect species could attain both. It is reasonable to propose that hyperactive forms of an element can be obtained since there is selection against increase in transposable element copy number in natural systems (Pasyukova et al. 2004). A hyperactive element would damage its host through mutagenesis and so jeopardize its own survival. Consequently there is selective pressure on the element to reduce its transposition rate to the point where it has little effect on the host, yet retains its own ability to propagate through the generations.

4. Functional Insect Members of the *hAT* Element Superfamily of Transposable Elements

The four eukaryote transposable elements used to transform non-drosophilid species represent four different families of class II elements. Transposable elements are assigned to these families based on differences in their DNA sequence, differences in the amino acid sequence of their transposases (which will determine their confirmation as active enzymes), and the resulting differences in the way they excise from, and integrate into, DNA. The *hAT* element super family is widespread across eukaryotes with members found in humans, fish, insects, plants and fungi. Some members of this family exhibit wide host ranges. The *Ac* element of maize is mobile in other plants while the *Hermes* element of the housefly is mobile in other insect species. The insect members of this family, *hobo*, *Hermes* and *Herves* are particularly interesting since all are active elements and are mobile in non-host insect species. Moreover *Herves* is distantly related to *hobo* and *Hermes* and is more closely related to *hAT* elements found in plants.

Hermes has been used to genetically transform five insect species, including mosquitoes and *C. capitata*, while *Herves*, a recently discovered element from the mosquito *An. gambiae*, is interesting due to both its ability to transform *Drosophila* and the fact that it is native to a mosquito species that is one of the key targets of planned genetic control strategies (Arensburger et al. 2005). *Hermes* behaves differently in the germ-line and somatic nuclei of mosquitoes (O'Brochta et al. 2003). In the former it integrates via a transposase-dependent mechanism that leads to integration not only of the element but also of flanking plasmid DNA. In the latter it integrates through canonical cut-and-paste transposition with no flanking DNA participating in the integration. In higher flies, this mode of integration is seen in both germ-line and somatic nuclei suggesting perhaps that host factors present in the germ-line nuclei of mosquitoes may influence the transposition mechanism.

These three *hAT* elements present a unique opportunity to examine transposition of related, but different elements *in vivo* in insects as well as *in vitro*. The authors' focus has been on the *Hermes* element since, of the three, it displays the widest host range and so has been developed as a gene vector in insects.

4.1. The Hermes *Element*

Hermes is 2749 base pairs in length and has 17 base pairs imperfect terminal inverted repeat sequences (Warren et al. 1994). It encodes a transpose 612 amino acids in length. Like other *hAT* elements, *Hermes* creates eight base pairs target site duplications at the integration site and these sites have a loose consensus sequence of 5' GTnnnnAC 3'. *Hermes* was isolated from the housefly through its ability to recognize and excise the related *hobo* element when *hobo* was present in plasmids injected into developing housefly eggs in the absence of *hobo* transposase (Atkinson et al. 1993). The *Hermes* element was isolated from housefly genomic DNA

using degenerate primers designed to conserve regions between the *Ac*, *Tam3* and *hobo* element transposase-encoding sequences.

Hermes can transpose in 12 insect species (Atkinson et al. 2001). Transposition is measured through an interplasmid transposition assay performed in developing embryos or in cell culture. Since the majority of nuclei in embryos are somatic, these assays inevitably measure mobility in somatic nuclei. Upon integration into the germ-line nuclei in mosquitoes, *Hermes* becomes relatively immobile (O'Brochta et al. 2003). Transformations of *Ae. aegypti* and *D. melanogaster* with autonomous *Hermes* elements show that subsequent germ-line transpositions do not occur at detectable frequencies (O'Brochta et al. 2003). In contrast, in the same individuals, somatic transpositions of autonomous *Hermes* elements occur quite frequently. These data suggest that a system repressing *Hermes* transposition in the genome may be operating in both *Drosophila* and mosquitoes.

Hermes transposase has been expressed in both transformed *Drosophila* S2 cells and in *Escherichia coli* (Migula) Castellani and Chalmers. Purification of polyhistidine-tagged *Hermes* transposase from *E. coli* has enabled the biochemistry of *Hermes* transposition to be investigated (Zhou et al. 2004). This is a compelling question since *hAT* elements are a distinct superfamily of transposable elements. *Hermes* excises from the donor site through the creation of double-strand breaks. These are staggered with the break in the top strand occurring one base pair into flanking DNA and the break in the bottom strand occurring at the junction between *Hermes* and flanking donor DNA. Unlike other transposable elements, hairpin intermediates are formed on the flanking donor DNA rather than on the transposable element ends. This supports genetic data previously obtained from the *Tam3*, *Ac* and *hobo* elements showing that repair of empty donor sites following transposable element excision occurred through templated addition of new DNA based on flanking sequences (Coen et al. 1986, Atkinson et al. 1993, Weil and Kunz 2000). This mechanism of excision prior to integration is peculiar to *hAT* elements and links them with the process of V(D)J recombination which is responsible, in large part, for the diversity displayed by the vertebrate acquired immune system. This relationship, which is also reflected in the secondary structures of the *hAT* element transposases and the RAG1 subunit of the enzyme that mediates the recombination of the immunoglobulin and T-cell receptor genes supports the hypothesis that V(D)J recombination and transposable elements, specifically *hAT* elements, are extant forms of an ancient recombination system, opening up the possibility of using either of these systems as a model for the other. For example, insights into aberrant recombination of V(D)J regions with other regions of the genome might be explored through an examination of *hAT* element behaviour in more tractable experimental systems.

ClustalW alignments of the secondary structures of *hAT* element transposases show that they contain four domains: a *BED* domain containing the nuclear localization signal of the transposase, a second, perhaps discrete DNA binding domain, a catalytic domain, and an insertion domain (Zhou et al. 2004). The catalytic domain is shared with the *Tn5* transposase, the RAG1 subunit and the HIV integrase. *Hermes*, *hobo* and *Herves*, together with the other *hAT* elements, contain the dichlordiphenyl-dichlorethylen (DDE) catalytic triad of acidic amino acids found in the RAG1 subunit and the retroviral integrases. Mutations of each of these to the non-polar amino acid alanine show that each mutation renders the *Hermes* transposase incapable of strand breakage and joining that are part of the mechanism of excision and integration (Zhou et al. 2004). These are first strand cleavage in which a nick is introduced into the strand of DNA one nucleotide upstream from the *Hermes* terminal inverted repeat sequences and second strand transfer in which the 3' OH group on the second strand is joined to the target DNA. These data suggest that this catalytic triad participates in the key chemical steps of *Hermes* transposition consistent with their role in the retroviral integrases.

Secondary structure alignments reveal that, for all these recombinases, the three members of this triad are located on an RNaseH-like fold, which consists of several ß sheets flanked on either side by alpha helices. Unlike HIV integrase and the RAG1 subunit, the third member of the catalytic triad in the *Hermes* transposase is a large linear distance from the central aspartate, being separated from it by the large insertion domain. *Tn5* and RAG1 contain a much smaller insertion domain separating the second and third members of the catalytic triad. The crystal structure of the *Hermes* transposase has been resolved and shows that, despite the linear distance of the final glutamate from the second aspartate, the insertion domain serves to bring this glutamate back to the active site so that it is spatially close to the other two members of the catalytic triad (Hickman et al. 2005). Interestingly, approximately one half of the insertion domain contains the least conserved region amongst the insect *hAT* transposases. This might mean that this region contributes little to function of the transposase, or it may indicate that each region defines a variation of function unique to each transposase and its corresponding element.

The C end of the catalytic domain is highly conserved amongst the *hAT* transposases and, to a lesser extent, between the *hAT* transposase and *Tn5* transposase and the retroviral integrases. It contains the final member of the catalytic triad as well as amino acids that are required, in part, for oligomerization of the *Hermes* transposase. The region most responsible for this particular oligomerization is located on an a helix between amino acids 551 and 562 located only ten residues from the glutamate at 572 that forms part of the catalytic triad (Michel et al. 2003). The fact that this region of the catalytic domain forms part of the catalytic site and yet is also involved in forming weak bonds with adjacent *Hermes* monomers suggests that it has a dual function in the transposase.

Other motifs conserved between these recombinases include a small motif containing an argine at position 318 and a tryptophan at position 319 which might be involved in the flipping out of adjacent nucleotides which is required for hairpin formation and a CxxH motif located downstream from the second catalytic residue at 248 (Zhou et al. 2004).

The identification of conserved regions and specific residues of the *Hermes* transposase, together with the regions of the *Hermes* element with which they interact will be critical in determining how *Hermes* might be modified in order to obtain hypo- or hypermobility. Specific amino acids can be altered and the effects of these changes on mobility of the element examined *in vitro* and *in vivo* in insects. Expression of the *Hermes* transposase both in *E. coli* and in yeast opens up the possibility of identifying randomly generated mutations that might elevate activity in these model organisms. Identification of the rate limiting steps in transposition, through understanding the chemistry of transposition, will aid in the identification of *Hermes* mutants that would have activity optimized either for strain stability (hypomobility) or for the rapid spread of the element through an insect population (hypermobility). Several aspects of *Hermes* biochemistry would be predicted to be rate limiting steps of transposition amenable to manipulation. These are the binding strength of the transposase for its target sites located towards the ends of the element and the catalytic activity of the transposase. Increasing the binding strength may increase the rate of transposition by increasing the likelihood of strand breakage and transfer occurring following binding of the enzyme. This could be achieved through either modifying those regions of the transposase proposed to bind to the element (domains I and II) or by changing the nucleotide sequence of the binding site. To this end, there is preliminary evidence indicating where these binding sites are located in the *Hermes* element (T. Laver, R. Hice and P. Atkinson, unpublished). Mutations which directly affect the catalytic properties of the transposase may act to increase the frequency of first strand cleavage and perhaps more closely couple this with second strand transfer. Implicit with this, is

the rate at which the target DNA is acquired by the transposase, and whether donor and target DNAs are acquired more or less simultaneously or whether target capture occurs only after the flanking donor DNA has dissociated from the transposase. Interestingly, recent data from the related RAG1 enzyme suggests that target DNA capture occurs only after DNA flanking the donor breakpoint has been released (Matthews et al. 2004).

Hypomobility of an element once integrated into a target genome could be achieved though removing the binding site of the transposase. *FRT* (FLP recombinase recognition target) sites located either side of the binding site could, in the presence of the FLP recombinase, recombine to delete this site. The element would be rendered immobile, even should a source of transposase be subsequently introduced into the strain. The strategy of modifying the phenotype of *Hermes* to suit area-wide control strategies can be achieved through understanding how the element undergoes transposition and will be applicable to other transposable elements, such as *piggyBac*, which are also used to genetically engineer pest insects.

4.2. The hobo *Element*

The outcome of studies with *Hermes* can be extrapolated to the *hobo* element of *D. melanogaster* and the more distantly related *Herves* element of *An. gambiae*. *Hobo* was the first of the insect *hAT* elements to be identified and is responsible for a dysgenic system in *Drosophila* (Yannopoulos et al. 1987). It has yet to be determined whether *hobo* is truly incapable of being used as a transformation vector in non-drosophilid species. While early attempts were unsuccessful, these experiments suffered from the lack of a robust genetic marker such as the green fluorescent protein. Interplasmid transposition of *hobo* occurs in developing embryos of Australian sheep blowfly (*Lucilia cuprina* Wiedemann) and Queensland fruit fly (*Bactrocera tryoni* (Froggatt)), suggesting that transposition occurs in at least the somatic nuclei of these species (O'Brochta et al. 1994).

The *hobo* transposase is 53% identical to the *Hermes* transposase and differs from it in having an extra 49 amino acids at the amino terminus of the protein. Removal of this terminus results in a truncated protein that is more active than the full-length protein, perhaps suggesting that, as for the *Ac* transposase, this amino end suppresses transposase activity (Y.-J.Kim and P. Atkinson, unpublished). *Hobo* presents a unique opportunity for studying insect *hAT* elements *in vivo*. Unlike the *Hermes* element and the *Herves* element (discussed below) in which all natural host individuals examined so far contain these elements, *D. melanogaster* consists of H and E strains, the former containing *hobo* elements while the later lacks them. As for the *P* element, a reason for the versatility of the *hobo* element in *Drosophila* is that E strains are used as transformation recipients leading to efficient transformation. The same applies for *Hermes* and *Herves* mediated transformation of *Drosophila*. Understanding the regulatory mechanisms that act post-integration of an element is important in determining the dynamics of these elements in these species and *Drosophila* provides an ideal model for these studies.

4.3. The Herves *Element*

The *Herves* element is from the mosquito *An. gambiae* and was identified using a bioinformatics screen of the recently completed genome project of this organism (Arensburger et al. 2005). *Herves* is an active transposable element and can be used to transform *Drosophila* at frequencies approaching those of the *P*, *Hermes* and *piggyBac* elements. That *Herves* is active and native to *An. gambiae*, the principal vector of malaria in sub-Saharan Africa makes it an interesting element for study since it might be developed into an endogenous gene vector in this important species.

The *Herves* transposase is 21% identical to the *Hermes* transposase. The *Herves* terminal inverted repeat sequences are 11 base pairs

long and differ in 2/11 positions to those of the *hobo* element, and in 2/11 positions to those of the *Hermes* element. *Herves* is a distantly related element to these other insect *hAT* elements and it remains to be determined if there are subtle differences in its mechanism of excision and integration relative to these elements. Such differences would not be expected to be seen in first strand cleavage or second strand transfer, rather they might be manifested in target site selection or in the nucleotides of the *Herves* element bound to the transposase.

Herves is most likely active in field populations of *An. gambiae*. Analysis of populations collected from Furvela along the southern coast of Mozambique revealed that *Herves* was present in *An. gambiae sensu stricto* and in two other species of the *An. gambiae* complex, *Anopheles merus* Donitz and *Anopheles arabiensis* Patton (Arensburger et al. 2005, O'Brochta et al. 2006). Copy numbers varied from 4.5 to 7.3 elements per genome and the high degree of insertion-site polymorphism within populations is consistent with this element being recently active.

5. Conclusions

Insect transformation, and technologies that arise from it, are both dependent on the behaviour of transposable elements. Transposable element behaviour is, in turn, regulated by the properties of the element itself and by host factors within the genome. The *hAT* superfamily of transposable elements contains three active members from at least three species of Diptera and so provides multiple opportunities to examine the relationship between transposable element structure and function. Examination of *Hermes* illustrates that changes made to the element and its transposase can be tested both *in vitro* and *in vivo* and may lead to the generation of new versions of *Hermes* that possess performance characteristics required for use in insect control programmes.

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Improving the Ecological Safety of Transgenic Insects for Field Release: New Vectors for Stability and Genomic Targeting

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ABSTRACT Genetically transformed insect pests provide significant opportunities to create strains to improve the sterile insect technique (SIT) and new strategies based on conditional lethality. A major concern for programmes that rely on the release of transgenic insects is the stability of the transgene, and maintenance of consistent expression of genes of interest within the transgene. Transgene instability could influence the integrity of the transformant strain upon which the effectiveness of the biological control programme depends. Loss or intragenomic transgene movement could result in strain attributes important to the programme being lost or diminished, and the mass-release of such insects could significantly exacerbate the insect pest problem. Instability resulting in intragenomic movement may also be a prelude to intergenomic transgene movement between species resulting in ecological risks. This is a minor concern for short-term releases where transgenic insects should not survive in the environment beyond one or two generations, but transgene movement may occur into infectious agents during mass-rearing, and the potential for movement after release is a possibility for programmes using many millions of insects. Random genomic insertion is also problematic for transgenic strain development due to genomic position effects that influence transgene expression, and insertional mutations that negatively affect host fitness and viability. New types of vectors are described that allow post-integration immobilization by deleting terminal vector sequences required for transposition, and genomic targeting by a recombinase-mediated cassette exchange strategy.

KEY WORDS biological control, transposable elements, transgenic strains, sterile insect technique, conditional lethality, insect transformation, vector stabilization, vector targeting, recombinase-mediated cassette exchange

1. Introduction

In recent years the development of transgenic insect strains has advanced rapidly, with more than 20 species within four orders of insects being genetically transformed (Handler 2001). The ability to efficiently introduce recombinant DNA into insect host genomes provides significant opportunities to study the genetic basis of insect biology in a wide range of species, in ways previously limited to model insect systems such as *Drosophila*. Gene transfer also provides the opportunity to create transgenic strains that may be used directly to control the population size or behaviour of agriculturally and medically important insects. Transgenic strains may be created to improve existing biological control strategies, such as the sterile insect technique (SIT), or to provide the means for new strategies for biological control based on conditional lethality (Alphey 2002, Handler 2002a). For beneficial insects, their vigour and reproductive capacity may be enhanced, in addition to their ability to produce and process proteins. In some cases, such as vectors of dis-

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ease, instead of suppressing the population of pest insects, they may be transformed into inhospitable hosts for the parasites or pathogens that they normally transmit (James 2005).

The significant advances in basic and applied studies that genetically transformed insects may provide must, however, be viewed in light of several limitations that are inherent to the gene transfer vector systems used to integrate transgenes into the host genome (Handler 2004). All of the heritable germ-line transformations in insects have been achieved with vectors derived from a type of mobile DNA known as transposable elements, which include the elements *Hermes*, *mariner*, *Minos*, and *piggyBac*. While these elements provide advantages over other types of vectors and transformation strategies, a major consideration is their potential for remobilization which can compromise the stability of the transgenic host strain, and thus adversely affect programme effectiveness. While the transposase enzyme required for transposon movement is typically eliminated after integration, the undetected or unintended presence of the transposase or related enzymes within the host can result in vector remobilization. While generally not considered to be a problem for small-scale laboratory studies, the rearing and release of many millions of insects for biological control programmes increases the probability that such rare events may occur. Other caveats relate to the generally random nature of transposon integration into host genomes. Localized genomic effects on gene expression can result in variable transgene expression depending upon the integration site. Vector integrations into coding regions or important regulatory regions can result in mutations having deleterious effects on the transformed host's fitness and viability. Thus random integrations make comparative gene expression studies problematic, and greatly reduce the efficiency of creating optimal strains for applied use.

To address these limitations on transgenic insects, new vectors have been developed that can be stabilized subsequent to genomic integration, and for which defined integration target sites may be created within the genome. These, and similar strategies by other laboratories should provide a new generation of vectors that both increase the efficiency of transgenic strain development, and at the same time, increase the effectiveness of transgenic strains and their ecological safety.

2. Transgenic Insects for Biological Control

Genetically transformed insect strains have great potential for improving existing biological control programmes for pest species such as those integrating the SIT, or to develop new control strategies based on the conditional regulation of genes that encode lethal products (Handler 2002a). For beneficial insects, the potential exists to develop transgenic strains having enhanced immune systems, increased longevity and reproductive capacity, or heightened response to odorant cues elicited by prey insects.

Vectors of disease may also be eliminated by biological control methods, or potentially, allowed to exist but made refractory to the parasites and pathogens that they normally transmit so that they no longer threaten human or animal health (James 2005). Several of these strategies are discussed in more detail in this volume (Aksoy et al., Alphey et al., Bourtzis et al., this volume), but briefly, lethality induced by transgenic techniques can improve the SIT by allowing more efficient genetic sexing by causing lethality specifically in females, or for male sterilization by specifically eliminating tissues required for fertility (Handler 2002a). Alternatively, genes involved in sexual differentiation, or sex determination, may be manipulated so that the sexual phenotype is disrupted or reversed (Handler 1992, Pane et al. 2005). Most straightforwardly, transgenic strains marked with green fluorescent protein (GFP) or red fluorescent protein (DsRed), initially used to select transformants, may also be used to unambiguously identify insects in the field after release (Horn et al. 2002). This in itself

would be a major advance over using fluorescent powders for marking (Hagler and Jackson 2001). Novel strategies for biological control have also been proposed whereby the offspring of mass-released insects either die or are sterile (Heinrich and Scott 2000, Thomas et al. 2000, Horn and Wimmer 2003). A variety of mutant and normal genes affecting cell viability are potentially useful for these strategies, including genes involved in programmed cell death (White et al. 1994), genes encoding toxin subunits such as diphtheria (Kalb et al. 1993), or mutations that cause a normal gene product, such as *DTS-5* (Saville and Belote 1993) or *Notch*^{60g11} (Fryxell and Miller 1994), to become toxic at either high or low temperature, respectively.

A critical component of these strategies is the regulated expression of the lethal product, both to maintain breeding populations in facilities and to target the lethal phenotype to a particular tissue or stage in development. This can be achieved by making transgene activity conditional to a particular temperature range, chemical treatment, or by the interbreeding of specific genotypes. Such methods have already been tested in *Drosophila*, and include the use of temperature sensitive lethal alleles or the use of ectopic transcriptional regulators such as the *Gal4/UAS* system (Brand et al. 1994) or the *Tet-off/on* systems (Bello et al. 1998) that respond to dietary tetracycline.

3. Transposon Vectors and Insect Transformation

To better understand the limitations and risks, as well as the advantages, associated with genetically transformed insects, it is helpful to understand the methods and mechanisms used to create them. All of the heritable transformations of insect germ-lines have utilized Class II transposable elements as vectors that transpose by a DNA-mediated "cut-and-paste" process (Atkinson et al. 2001). These mobile genetic elements are typically one to three kilobases in length and have terminal sequences that are inverted repeats of one another. The terminal inverted repeats are usually 30 base pairs or less, but some are several hundred base pairs and some terminal regions also have subterminal inverted repeat sequences. The terminal inverted repeats and adjacent DNA are excised and reinserted together into a new DNA insertion site as part of the transposition process. In between the terminal inverted repeats is a gene for a transposase enzyme that binds to the terminal sequences to catalyse both the "cut-and-paste" processes. In this way, most transposons are self-contained autonomous elements that may require other host-encoded nuclear proteins. Importantly, while the terminal inverted repeats and transposase gene are usually linked as a *cis*-acting unit, the terminal inverted repeats and intervening DNA can be mobilized by an unlinked transposase gene acting in *trans*. This feature has allowed the development of defective non-autonomous vector plasmids that only include the terminal inverted repeats, marker genes, and genes of interest with its transposase gene either mutated or deleted. These vectors can then only be mobilized by a separate source of transposase helper, provided by a plasmid-encoded gene lacking terminal inverted repeats, or the transposase RNA or protein. When co-injected into preblastoderm embryos, the transposase catalyses integration of the vector, but does not integrate itself in the absence of terminal inverted repeats, and is eventually diluted with cell division. Once the transposase is lost, vector integrations into the germ-line chromosomes should remain stable.

All insect transformations to date have utilized transposon vectors, though the first two vectors originally discovered and used in *Drosophila melanogaster* Meigen, *P* (Rubin and Spradling 1982) and *hobo* (Blackman et al. 1989), have not been found to be effective in other species (Handler 2001). Other functional elements that have been used in non-drosophilids depended on the fortuitous discovery of new transposons, or the directed search for *hobo*-related elements in non-drosophilids. These include the *Minos* element discovered in *Drosophila hydei* Sturtevant (Franz and Savakis 1991), which is

closely related to *Tc* elements from nematodes, and the *Mos1 mariner* element from *Drosophila mauritiana* Tsacas & David (Medhora et al. 1988). Elements from the *hobo*, *Ac*, *Tam3* (*hAT*) family include *Hermes*, discovered in the house fly *Musca domestica* L. (Warren et al. 1994) and *Herves*, recently discovered in *Anopheles gambiae* Giles (Arensburger et al. 2005). *Hermes* is widely functional, but quite importantly, it has been shown to functionally interact with *hobo* (Sundararajan et al. 1999), providing some of the strongest experimental evidence to support the need for methods to stabilize transgene integrations.

The most widely used transposon vector to date is the *piggyBac* element discovered in a baculovirus passed through a cell line of the cabbage looper *Trichoplusia ni* (Hübner) (Fraser et al. 1983, Cary et al. 1989). A *piggyBac* vector was first used to transform several tephritid species, and use of a lepidopteran transposon in dipteran species portended the broad functionality of this element, which has been proven by its use in nearly 20 species within four orders of insects (Handler 2002b). Molecular analysis of *Bactrocera dorsalis* Hendel transformants, however, indicated the potential for cross-mobilization in this species since Southern hybridizations showed genomic sequences in the host strain that were closely related to *piggyBac* (Handler and McCombs 2000). This was confirmed by polymerase chain reaction (PCR) analysis, and further studies now show that *piggyBac* elements, having greater than 95% nucleotide identity, exist throughout the *B. dorsalis* complex and several other closely related species (G. Zimowska and A. Handler, unpublished).

The finding of a moth transposon in dipteran species suggested that *piggyBac* might also exist in other moths, and this has been confirmed by Southern hybridization and sequence analysis of elements isolated by PCR (G. Zimowska and A. Handler, unpublished). These species include *Helicoverpa zea* (Boddie), *Helicoverpa armigera* (Hübner), and *Spodoptera frugiperda* (J. E. Smith), in addition to new elements discovered in *T. ni*. This is a strong indication that *piggyBac* has been horizontally transmitted between distantly related species, and for this to occur, functional elements must exist in these species or associated organisms. As with *Hermes*, these findings raise the concern for potential remobilization and instability of transgenes vectored by the respective transposons. While the existing data raise most concern for *Hermes* and *piggyBac*, both the *Minos* (Avancini et al. 1996) and *mariner* (Robertson and MacCleod 1993) elements also exist in broad, potentially functionally compatible, families of elements, and unless proven otherwise the concerns for transposon vector stability must be extended to these, and possibly all future transposon vectors as well.

4. Methods to Stabilize Transposon Vectors

While genetically transformed insects present a wide array of possibilities to create strains with attributes that can greatly improve existing biological control methods and the development of new strategies for control, the effective use of such strains will depend on the reliable expression of the integrated genes of interest, as well as maintenance of strain fitness and viability under mass-rearing protocols. It is also critical that the transgene vector is stably integrated to maintain strain integrity and to prevent possible interspecies movement of the transgene into unintended hosts, which is a major concern for ecological safety. Current knowledge of known transposons, and especially those used for insect transformation, makes these concerns of primary importance.

The major contributing factor to vector instability is most likely the presence of the same transposon, or a functionally related system, in the host genome or in an associated infectious or symbiotic organism within the host. The former possibility can be tested by direct structural tests using DNA hybridization or PCR analyses to detect the same element or a related element with a high degree of sequence identity. If a related system is

functionally conserved but lacking sufficient structural identity for easy detection, as would be the case for *hobo* and *Hermes*, functional assays may be performed. Indeed, transposition and excision assays that show mobility in the host in the absence of an exogenous source of transposase (i.e. by injecting only donor and target plasmids for transposition, or indicator plasmid for excision) provide the most straightforward test for cross-mobilization (Atkinson et al. 1993). However, these assays are typically performed in cell lines and embryos, and are probably not sensitive enough to detect mobility catalysed by a non-host source of transposase, and especially from coexisting organisms that proliferate post-embryogenesis. Given these caveats, it is highly unlikely that the complete potential for transgene vector remobilization can be definitively and unambiguously assessed, leaving open the possibility, regardless of how minimal, that transgenic insertions will not remain stable. This creates a significant point of concern for the ecological risk assessment that will be required for a transgenic release certification. Indeed, addressing the issues of potential transgene instability and interspecies movement was a primary concern in response to the environmental assessment for the release of a transgenic pink bollworm *Pectinophora gossypiella* (Saunders), solicited by United States Department of Agriculture's Animal and Plant Health Inspection Service-Plant Protection and Quarantine (USDA-APHIS-PPQ). The inability to adequately address these issues had led us and others to consider development of a new generation of vectors that can be immobilized, with respect to transposase activity, after initial genomic integration has been achieved.

4.1. Vector Immobilization

Immobilization or stabilization of a transposon vector is most straightforwardly achieved by deleting or rearranging DNA within the vector that is required for transposition. This includes the terminal inverted repeats and possibly additional adjacent DNA. For most transposons this includes up to 100 base pairs of terminal sequence (though longer for elements such as *Minos* whose terminal inverted repeats themselves are 255 base pairs). Deletion or rearrangement of these sequences is most simply achieved by introducing short sequences that specifically recombine with one another in the presence of an appropriate enzyme. Two examples of these systems are the *FRT*/FLP recombinase system from the two micron plasmid of yeast (Andrews et al. 1985) and the bacteriophage Cre-*loxP* system (Siegal and Hartl 1996). A functional *FRT* recombination site consists of two 13 base pair inverted repeats separated by an eight base pair spacer that specifically recombine with one another in the presence of FLP recombinase. Depending upon their location and orientation, *FRT* recombination can result in chromosomal rearrangements or the targeting of a plasmid carrying an FRT to a genomic *FRT* site (Rong and Golic 2000). Recombination of *FRT*s in direct orientation results in the deletion of the intervening DNA, while *FRT*s in the opposite orientation results in inversions. It should thus be possible to position FRTs in vectors to create rearrangements within a vector, or between two independent vectors after their genomic insertion by injection of plasmid-encoded FLP recombinase (Handler 2004). While theoretically attractive, use of recombination systems is not simple. Placement of recombination sites within the vector may be difficult without negatively affecting its ability to integrate initially (due to disruption of the terminal sequence). Rearrangement between independent vectors is more plausible, but requires the vectors to be linked closely on the same chromosome with recombination sites in an indirect orientation, to avoid lethal deletions resulting from directly oriented sites. With recombination sites in indirect orientation, an *FRT* inversion between distantly integrated vectors has been achieved in *Drosophila* resulting in stabilization of both vectors since the inversion reconstitutes chimeric vectors, each having a terminal sequence of the other

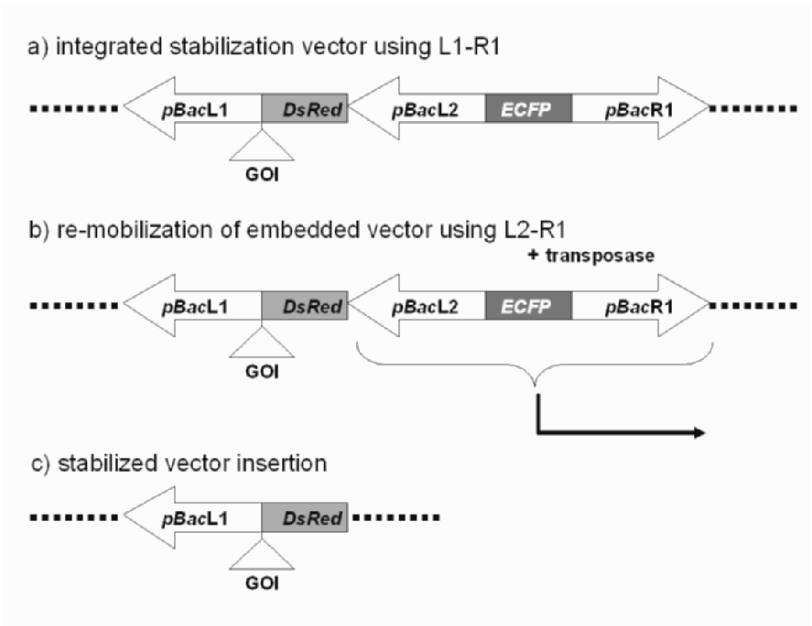

Figure 1. *Transgene stabilization by terminal sequence deletion of the pBac{L1-PUbDsRed1-L2-3xP3-ECFP-R1} vector. The diagram (not to scale) shows relative positions of the pBacL1, pBacL2 and pBacR1* piggyBac *terminal sequences, the PUbDsRed1 and 3xP3-ECFP markers, and an insertion site for genes of interest. Transposase is provided by either mating to a piggyBac jumpstarter strain, or by injection of a* piggyBac *helper plasmid. Integration of the entire stabilization vector is determined by the presence of both DsRed and ECFP markers, and terminal sequence deletion is determined by remobilization of the L2-3xP3-ECFP-R1 embedded vector resulting in loss of the ECFP phenotype. The genomic stabilized transgenes include the pBacL1 terminus, the DsRed marker, and any inserted gene of interest.*

(E. Wimmer, personal communication).

This approach has the added advantage of creating a balancer chromosome (within the inversion) in which normal recombination is suppressed in heterozygotes. This is a very encouraging result using an elegant approach to achieve vector stabilization, but its success in *Drosophila* will be difficult to repeat in other insects where inserting linked vectors, and mapping and determining vector orientation is much more difficult. This will be ameliorated to some extent in insects whose genome has been sequenced. In order to simplify and extend the use of vector stabilization to many species, another approach has been taken that results in terminal sequence deletion without the use of recombination sites.

4.2. Vector Stabilization by Terminal Sequence Deletion

To stabilize transposon vectors subsequent to genomic integration, a method to delete a terminal vector sequence required for mobility was first tested in *Drosophila* by introducing an internal tandem duplication of the other terminal sequence, with independent fluorescent protein markers placed between each set of termini (Handler et al. 2004) as shown in Fig. 1. Specifically, the *piggyBac* vector, pBac{L1-PUbDsRed1-L2-3xP3-ECFP-R1}, was created by placing a duplicated 5' terminal *piggyBac*

sequence (pBacL2) internal to the flanking 5' (pBacL1) and 3' (pBacR1) termini, with independent markers placed between each set of termini. Genes of interest to be stably integrated would be placed between the duplicated termini. Transformation with this vector can result in two types of integration: either the shorter embedded L2-3xP3-ECFP-R1 sequence may integrate by itself, or the entire L1-PUbDsRed1-L2-3xP3-ECFP-R1 vector may integrate.

In general, shorter vectors transpose more efficiently than longer vectors, and indeed, transformation with this vector resulted in seven lines with only the embedded L2-3xP3-ECFP-R1 vector, and one line with the entire L1-PUbDsRed1-L2-3xP3-ECFP-R1 vector. However, after mating the L1-PUbDsRed1-L2-3xP3-ECFP-R1 strain to a *piggyBac* transposase jumpstarter strain (having a chromosomal source of the functional transposase gene), the L2-3xP3-ECFP-R1 vector was remobilized resulting in progeny having only the L1-PUbDsRed1 transgene sequence genomically integrated. In the absence of the R1 3' *piggyBac* terminus, it was expected that the remaining genomically integrated sequence would remain stable with respect to remobilization by a source of transposase. This was tested by mating the stabilized line to the jumpstarter strain, which showed that no remobilization occurred (by loss of phenotype) in more than 7000 progeny assayed. This compared to about 5% remobilization rate in the original L1-PUbDsRed1-L2-3xP3-ECFP-R1 vector. This showed that the transgene was stabilized owing to the loss of the 3' *piggyBac* terminus. A similar stabilization vector has been integrated into the Caribbean fruit fly *Anastrepha suspensa* (Loew) and the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) with the embedded vectors remobilized by injection of transposase helper plasmid. The testing for stability in resulting progeny of these species is in progress.

5. Vector Targeting

A major difficulty in creating optimal transgenic strains for biological control is decreased fitness and viability due to vector integrations disrupting vital gene functions, and diminished or altered transgene expression due to genomic position effects. Both drawbacks can be minimized by having transgene integrations limited to defined genomic target sites known to be devoid of vital DNA and subject to minimal position effects. To target a plasmid donor vector to a specific genomic locus an FLP recombinase-mediated cassette exchange (RMCE) system (see Baer and Bode 2001) was modified for use in insects, and tested in *Drosophila* (Horn and Handler 2005). A recombinase-mediated cassette exchange system is based upon double recombination between small recombination sites (such as *FRT* or *loxP*) within a genomic target site, and a plasmid donor sequence as shown in Fig. 2.

A *linotte* homing sequence from *Drosophila* was added since such sequences placed within a plasmid vector are known to target the same endogenous genomic sequences, and it was reasoned that this might enhance recombination between the donor plasmid and the genomic target site. Target site strains were created by transformation with a *piggyBac* vector (pBac{3xP3-FRT-ECFP-linotte-FRT3}) having two heterospecific *FRT* recombination sites (*FRT* and *FRT3*) surrounding an enhanced cyan fluorescent protein (ECFP) marker coding region and the linotte homing sequence. Transformant lines from embryos having an integrated target vector were then injected with a donor vector plasmid (pSL-FRT-EYFP-linotte-FRT3) having corresponding *FRT/FRT3* sites surrounding an enhanced yellow fluorescent protein (EYFP) marker coding region and *linotte* sequences. Recombination between the target and donor *FRT/FRT3* sites was mediated by co-injection of an FLP recombinase helper plasmid (pKhsp82-FLP).

Targeting of the genomic acceptor site by recombination with the donor plasmid was determined in the progeny of the injected embryos, with recombinants identified at a frequency of about 23% by screening for conversion of the enhanced cyan fluorescent protein to the enhanced yellow fluorescent pro-

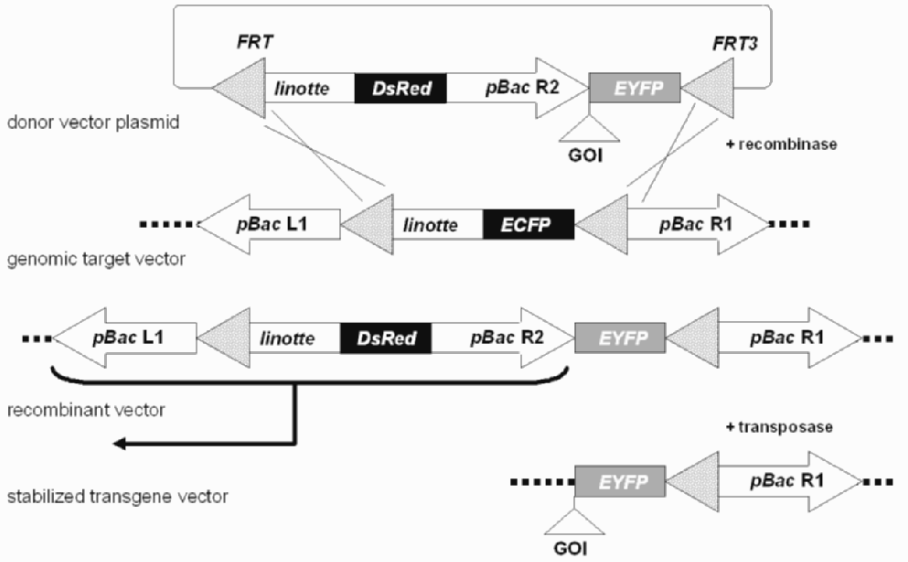

Figure 2. *Genomic targeting by recombinase-mediated cassette exchange (RMCE) and subsequent target site vector stabilization by terminal sequence deletion. The genomic target vector, pBac{3xP3-FRT-ECFP-linotte-FRT3}, is first integrated into a host genome by transposase-mediated germ-line transformation. It is then targeted by recombinase-mediated cassette exchange by co-injection of the donor vector plasmid {pSL-FRT-EYFP-linotte-FRT3}, and the FLP recombinase helper plasmid, pKhsp82-FLP, into target strain embryos (see text for details of plasmid constructions). Recombinants are identified by the exchange of the enhanced cyan fluorescent protein (ECFP) marker in the target strain for the DsRed and enhanced yellow fluorescent protein (EYFP) markers introduced by* FRT/FRT3 *double-recombination with the donor vector. The genomic target site is subsequently stabilized by transposase-mediated remobilization of the pBacL1 terminus from the target vector and a pBacR2 terminus from the donor vector (Fig. 1), which is determined by loss of the DsRed phenotype. The genomic stabilized transgenes from this strategy include the pBacR1 terminus, the enhanced yellow fluorescent protein marker, and any genes of interest (GOI) inserted into the donor vector.*

tein eye fluorescence marker phenotype. However, in addition to cassette exchange products from double reciprocal *FRT* and *FRT3* crossovers, integration products from single *FRT* crossovers were also identified, but these could be discriminated by separable fluorescent markers (e.g. a DsRed marker placed outside the *FRT*s in the donor plasmid; not shown in Fig. 2). To stabilize targeted insertions, a new recombinase-mediated cassette exchange donor vector had a *piggyBac* 5'-terminus incorporated to allow post-integration deletion of the *piggyBac* 3'-terminus as described above. New transgene vectors such as these, that allow genomic targeting and post-integration stabilization, should significantly improve the efficient creation and safety of insects intended for field release.

6. Conclusions

The use of transgenic insect strains to improve the biological control of insect pests has enormous potential for success, but the development and release of such transgenic strains must be approached with a very high level of caution. While the actual risk of transgene remobilization may be very small, the large

number of insects used for field release programmes, the inability to retrieve these insects once released, and the known potential for remobilization of defective vectors certainly heightens the real and perceived concerns for transgenic release. Methods for vector stabilization after initial genomic integration by recombinase-mediated cassette exchange as described here, or by intervector recombination being developed by other laboratories, should eliminate the major cause of vector instability resulting from the unintended presence of transposase. In addition, vectors that allow genomic targeting should not only increase the efficiency of creating transgenic strains for biological control, but should also enhance the ability to compare and monitor transgene expression and stability between strains. This should improve the evaluation of transgenic strains for ecological safety as part of risk assessment protocols, and strain effectiveness during development and implementation. Thus, new vectors that allow both genomic targeting and subsequent stabilization should provide a significant advance in the development of transgenic insect strains for biological control.

7. Acknowledgements

Grateful appreciation is extended to those who provided unpublished data for this article and to the USDA-Cooperative State Research, Education, and Extension Service-National Research Initiative-Competitive Grants Program (A. M. Handler) and Fonds der Chemischen Industrie (C. Horn) for providing support.

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Development of an Embryonic Lethality System in Mediterranean Fruit Fly *Ceratitis capitata*

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ABSTRACT The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is one of the world´s most destructive insect pests, costing farmers billions of dollars annually. Improved biological strategies are needed to increase the efficacy of area-wide integrated pest management (AW-IPM) programmes. Transgenic methodology could enhance and widen the applicability of the sterile insect technique (SIT) as a component of AW-IPM programmes and a transgenic approach to sterilize insects with an embryonic lethal transgene combination instead of conventional radiation was successfully tested in *Drosophila melanogaster* Meigen. This system is currently being transferred to *C. capitata*, in order to test its feasibility in this species and compare its effectiveness to radiation sterilization. Therefore two strategies are being followed: (1) direct transfer of the constructs used in *D. melanogaster* and assessment of their functionality in *C. capitata*, and (2) isolation of genes active during early embryonic development of *C. capitata* for use in an embryonic lethality system with endogenous components. If proven functional and effective in *C. capitata*, such a system might be transferable to other insect pests.

KEY WORDS *Ceratitis capitata*, cellularization, cDNA-subtraction, conditional embryonic lethality, sterile insect technique (SIT), insect transgenesis

1. Introduction

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is one of the worlds' most important pests of fruits and vegetables, attacking more than 260 different fruits, vegetables and nuts. The direct damage caused by feeding larvae and the quarantine status of this insect have major impacts on many agricultural economies. Biological approaches to insect pest management offer alternatives to insecticidal control. The sterile insect technique (SIT) is a powerful component of area-wide integrated pest management (AW-IPM) programmes to suppress or eliminate populations of economically important pest species by the mass-release of radiation-sterilized insects (Knipling 1955). However, the use of radiation for sterilizing insects does have some adverse effects on their competitiveness which in turn reduces the efficiency of the technique (Cayol et al. 1999, Calkins and Parker 2005).

Recently a transgene-based embryonic sterility system was successfully established in *Drosophila melanogaster* Meigen (Horn

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 85–93.

and Wimmer 2003) and this system may provide an alternative to the use of radiation in AW-IPM programmes integrating the SIT. The aim of the studies reported here was to establish and evaluate such a system in *C. capitata*.

2. Transgene-Based Sterility System in *Drosophila melanogaster*

A novel transgenic approach was developed to induce sterility without interfering with gametogenesis or with other larval and adult stages of the insect life cycle. Sterility is based on the transmission of a transgene combination that causes dominant embryo-specific lethality in subsequent progeny. This dominant lethality is suppressible by additives in the larval diet, thereby enabling rearing of such strains. This should allow the generation of competitive sterile insects that can transfer competitive sperm (Horn and Wimmer 2003), carrying the transgene, to wild females. The embryos produced by the females will carry the dominant transgene and, in the absence of the additives, the embryos will die. For the effector gene causing organismal lethality, a hyperactive allele of the pro-apoptotic gene *head involution defective* (*hid*) was chosen, which induces cell death when expressed ectopically (Grether et al. 1995). To avoid down regulation of HID by developmental signalling pathways, the phosphoacceptor-site mutant allele hid^{Ala5} (Bergmann et al. 1998) was used. To limit the effect of the transgenes to the embryonic stage, enhancer-promoters of genes that are expressed at high levels but are specific to the cellularization stage were used. In *D. melanogaster* the genes *serendipity α (sry α)* and *nullo* encode structural components of the microfilament network that are specifically required for blastoderm cellularization (Ibnsouda et al. 1993, Postner and Wieschaus 1994). To establish conditional embryonic lethality, a suppressible binary expression system based on the tetracycline controlled transactivator tTA (Gossen and Bujard 1992) was employed. By adding tetracycline to the larval diet the transgene activity can be suppressed. In *D. melanogaster*, hid^{Ala5} specifically causes embryonic lethality when driven by tTA under the control of the enhancer-promoter from a cellularization gene, and can be suppressed by tetracycline provided maternally to the egg (Horn and Wimmer 2003). Due to the inhibition of the tTA-DNA binding by tetracycline, the tTA protein functions as a switch to discriminate restrictive from permissive conditions. Under restrictive conditions (without tetracycline) 99.9% of *D. melanogaster* embryos that inherited one copy of the transgene combination were killed. Under permissive conditions (with tetracycline), lethality was suppressed which allowed the continuous generation of large numbers of transgenic insects (Fig. 1). Strains homozygous for the transgene combination can be propagated on tetracycline-containing food. Males from these *D. melanogaster* strains are competitive in laboratory mating assays and transmit the transgene combination, which causes dominant embryonic lethality in offspring. Thus the transgene-based suppressible embryo-specific lethality system may enable competitive sterile insects to be produced without irradiation and is therefore of interest for improving conventional SIT and widening its applicability.

3. Transfer of the Transgene Embryonic Lethality System to *Ceratitis capitata*

3.1. Direct Transfer of the Drosophila*-Used Transgenes to* Ceratitis capitata

For fast and easy transfer of the embryo-specific lethality system from the model organism to the pest species, direct use of the *D. melanogaster* transformation constructs in *C. capitata* was pursued (Horn and Wimmer 2003). This involved taking the driver construct pBac{3xP3-EYFP;>>s1-tTA>>} (Horn and Wimmer 2003) and digesting it with *Bgl*II. The fragment, which contains the *tTA* gene under control of the -276:+45 *sry α* promoter region, was inserted into the *Bgl*II site of the transformation vector pB[PUb-

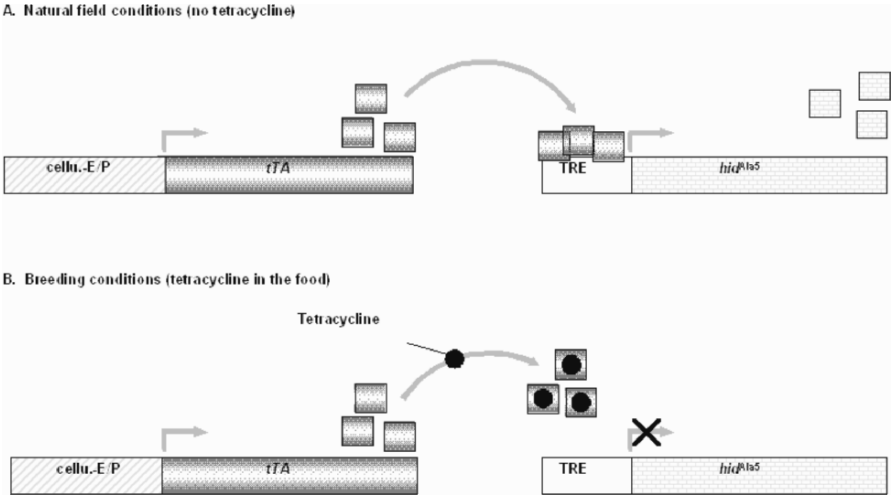

Figure 1. *Binary expression system for conditional embryonic lethality. Enhancer-promoters of the cellularization genes (cellu.-E/P)* sry α *or* nullo *were selected, which mediate gene expression exclusively during early embryogenesis. The tetracycline controlled transactivator (tTA) is based on a bacterial-viral fusion protein and mediates gene expression by binding to a* tTA-response element (TRE)*. The main advantage of this system is that targeted gene expression can be controlled by the food supplement tetracycline. (a) Under natural field conditions there is no tetracycline and the tTA proteins bind to the* TRE *leading to the expression of* hid^{Ala5}*, which causes lethality. Induction of lethality is limited to the early cellularization stage of embryogenesis because the genes* sry α *and* nullo*, are exclusively expressed at the cellularization stage. (b) Under laboratory rearing conditions the larval diet contains tetracycline, which binds to tTA. Tetracycline-bound tTA cannot bind to the* TRE *thereby suppressing* hid^{Ala5} *expression and allowing all progeny to survive.*

DsRed1] (Handler and Harrell 2001). For germ-line transformation this construct was injected together with a helper plasmid into the posterior of early *C. capitata* embryos, resulting in four transgenic lines. These are currently being analysed for transgene-mediated *tTA* expression.

The results will determine: (1) whether the complex interaction between enhancers and promoters of stage-specifically expressed genes (Blackwood and Kadonaga 1998) is the same or different between *D. melanogaster* and *C. capitata,* and (2) whether a *D. melanogaster* promoter can act as an adequate alternative to an endogenous *C. capitata* promoter to enable high expression rates to be obtained.

3.2. Embryonic Cellularization Genes from Ceratitis capitata

3.2.1. Searching for sry α *and* nullo *by Degenerate Polymerase Chain Reaction (PCR)*

According to fossil records, the phylogenetic distance between *Drosophila* spp. and *Ceratitis* spp. can be estimated to be around 100 million years (Naumann 1994). Since the cellularization genes *sry α* and *nullo* are fast evolving even within the Drosophilidae (Ibnsouda et al. 1993, Hunter et al. 2002), it might be challenging for the *D. melanogaster* constructs to function in a more distantly related species such as *C. capitata*. Therefore, we started to isolate cellularization genes *sry α* and *nullo* in *C. capitata*.

To obtain specific DNA sequences of *C. capitata* homologues of the genes *nullo* and *sry α*, mRNA from 0-48 hour embryo collections was isolated and translated into double-stranded cDNA for later PCRs. Degenerate primers were designed on the basis of amino acid sequence comparisons between known drosophilid *nullo* and *sry α* proteins (Ibnsouda et al. 1998, Hunter et al. 2002) under the following conditions: primer length between 18-29 base pairs and a maximum of 64 permutations. Using the cDNA collection and the degenerate primers, gradient PCRs were carried out (annealing temperature: gradient from 39°C to 50°C) with all suitable primer combinations. If possible, nested PCRs were performed after the primary gradient PCRs for a more selective amplification and a reduction of background. As a control, gradient PCRs were carried out with only one of the degenerate primers to check whether these already lead to non-specific amplifications. The DNA fragments of possibly interesting bands were cut out of an agarose gel, purified (QiaEX II Gel Extraction Kit, Qiagen, Hilden), ligated into the vector pCRII (TA Cloning Kit Dual Promoter (pCRII), Invitrogen) and transformed. The DNA clones were sequenced and analysed by "basic local alignment search tool" (BLAST) algorithms (Altschul et al. 1997) as well as *in situ* hybridizations to whole mount *C. capitata* embryos.

Unfortunately none of the BLAST hits matched the *nullo* or *sry α* genes from drosophilids. Also none of the *in situ* hybridizations with probes from sequences with no BLAST hits gave expression patterns comparable to *D. melanogaster nullo* or *sry α*. Thus the cellularization-specific genes could not be obtained by this degenerate PCR approach based on sequence similarities to drosophilid genes. One reason for this might be the fast evolution of developmental genes in drosophilids (Schmid and Tautz 1997).

3.2.2. Cellularization in Ceratitis capitata

Because *nullo* and *sry α* homologues from *C. capitata* could not be isolated by PCR with degenerate primers, blastoderm-specifically expressed genes were isolated in an independent experiment. For this purpose, we first determined the time window of cellularization in *C. capitata* and this knowledge was used to select differentially expressed genes by cDNA subtractions (3.2.3.).

To determine the time window of cellularization, embryos were fixed at one hour intervals after oviposition followed by immunofluorescence staining of cell membranes and nuclei. For comparison, the same staining was done on *D. melanogaster* whose embryonic development lasts 22 hours at 25°C and whose cellularization takes place between 2 hours 10 minutes and 2 hours 40 minutes after oviposition. In contrast to *D. melanogaster*, embryonic development in *C. capitata* takes 48 hours and cellularization takes place later and for a longer period from nine to 12 hours after oviposition (Fig. 2). In *C. capitata*, the typical elongation of the nuclei could not be observed during the slow phase of cellularization as described for *D. melanogaster* (Lecuit and Wieschaus 2000).

3.2.3. Enrichment of Cellularization-Specific Gene Transcripts by cDNA-Subtraction

In *D. melanogaster* cellularization genes are highly and exclusively expressed during the superficial cleavage of insect embryos (Postner and Wieschaus 1994, Lecuit and Wieschaus 2002). Thus isolating one or more of these genes and particularly their promoters in *C. capitata* would allow a *C. capitata*-specific embryonic lethality system to be generated. With knowledge of the cellularization time window (3.2.2.) a stage-specific screening was performed. Since strongly expressed genes, which exist during all stages of embryogenesis, would prevent a successful and effective cDNA screen for cellularization-specific genes, a cDNA subtraction approach was used (Diatchenko et al. 1996) for the selective isolation of genes, which are specifically expressed during *C. capitata* cellularization.

Using the cDNA transcripts isolated from the cellularization stage those cDNA transcripts, which are also present in other embry-

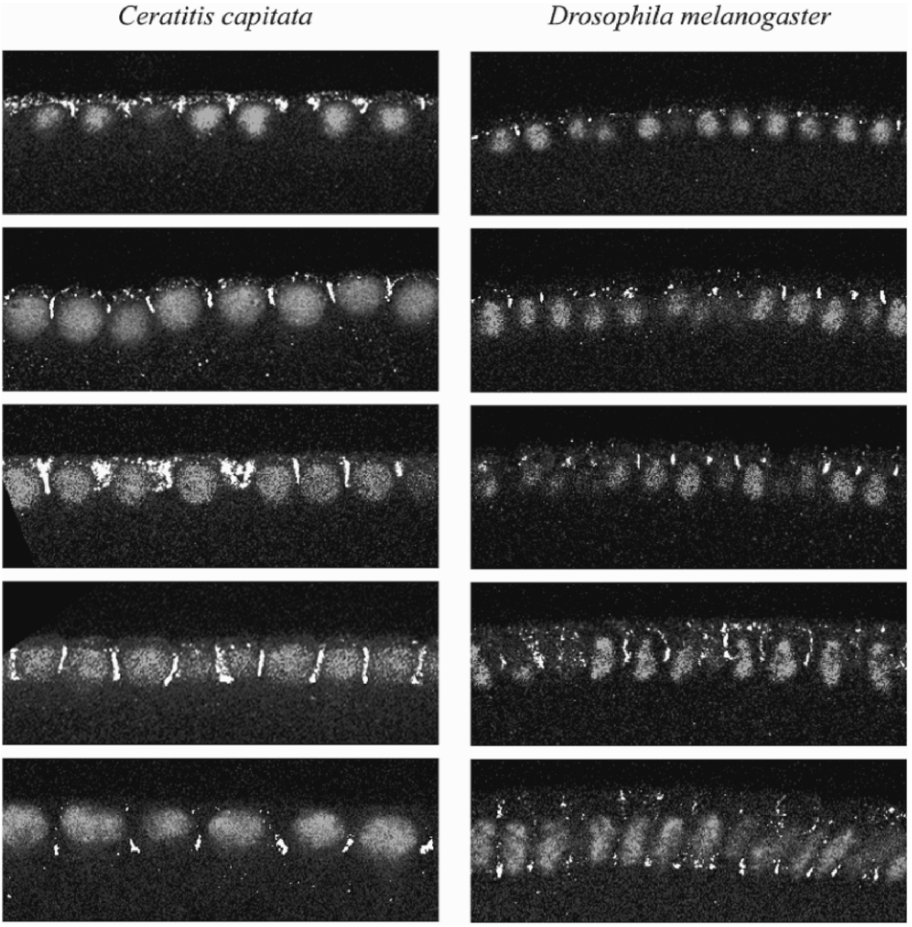

Figure 2. *Superficial cleavage during insect development. Comparison of the cellularization of (left side)* Ceratitis capitata *and (right side)* Drosophila melanogaster *embryos. The immunofluorescence staining with primary armadillo antibody and secondary Alexa488-marked antibody shows the invagination of the cell membrane (bright white stripes between the large gray nuclei). Nuclei are stained with propidiumiodide (large gray balls). In* C. capitata*, cellularization takes places between nine hours (upper, left panel) and 12 hours (lower, left panel) after oviposition. In* D. melanogaster*, cellularization takes places between two hours and ten minutes (upper, right panel) and two hours and 40 minutes (lower, right panel) after oviposition. Panels between represent intermediate stages of cellularization in chronological (vertical) order.*

onic developmental stages, were subtracted. Two subtractions were carried out: (1) a [0-6 hours + 15-21 hours] double-stranded cDNA collection from a double-stranded cDNA collection of cellularization stages (9-12 hours), and (2) a [0-6 hours + 15-48 hours] double-stranded cDNA collection from a double-stranded cDNA collection of a widened "cellularization" time window (7.30-12.30 hours).

The second subtraction was performed because only 4% of the isolated genes in the first subtraction were identified as cellulariza-

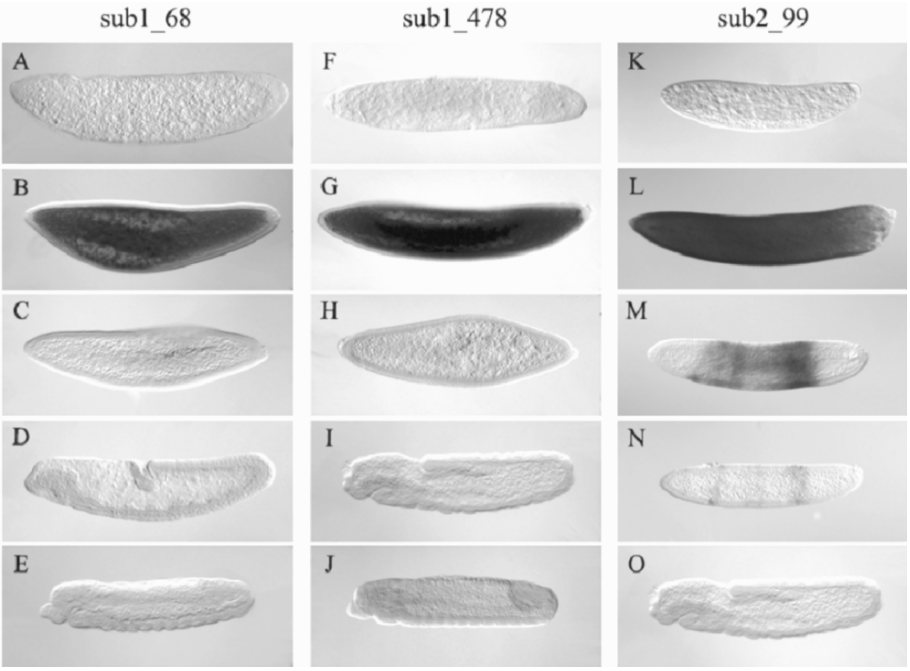

Figure 3. *Cellularization-specific gene expression patterns 1.* Ceratitis capitata *gene sequences* ***sub1_68*** *and* ***sub1_478.*** *(a) and (f) preblastodermal embryo without expression, (b) and (g) strong expression at the onset of cellularization, (c) and (h) very weak expression at the end of the cellularization, (d) and (i) no expression during gastrulation, (e) and (j) no expression during after germ band retraction.* ***Sub2_99*** *(k) preblastodermal embryo without expression, (l) strong expression at the onset of cellularization, (m) strong expression restricted to the centre of the embryo and lacking at the anterior and posterior pole during the slow phase of cellularization, (n) weak expression reduced to three stripes in the centre of the embryo at the fast phase of cellularization, and (o) no expression during and after germ band elongation.*

tion specific by *in situ* hybridizations and BLAST searches. In addition many housekeeping genes had been amplified. The second subtraction conditions were improved by using a widened cellularization time window to make sure that cellularization sequences, produced at earlier time points and also responsible for cellularization, could be identified. Furthermore, the subtracted cDNA pool was expanded to 48 hours to improve the exclusion of non-differentially expressed genes. This increased the efficiency to ~12%. PCR products from these subtractions were agarose gel purified, ligated and transformed into the vector pCRII (for details see 3.2.1). Transformants were pre-selected by restriction enzyme digest patterns and their plasmids then isolated and sequenced. The DNA clones obtained were analysed by *in situ* hybridizations to whole mount *C. capitata* embryos.

From 720 transformants (subtraction 1 (sub1): 550; subtraction 2 (sub2): 170), putative identical clones were identified by enzyme restrictions and 106 probably different clones sequenced (sub1: 45; sub2: 61). Six of the 106 clones were expressed exclusively during cellularization of *C. capitata* (sub1: two; sub2: four; Figs. 3 and 4). Additional three clones were highly expressed during cellularization, but their expression was not

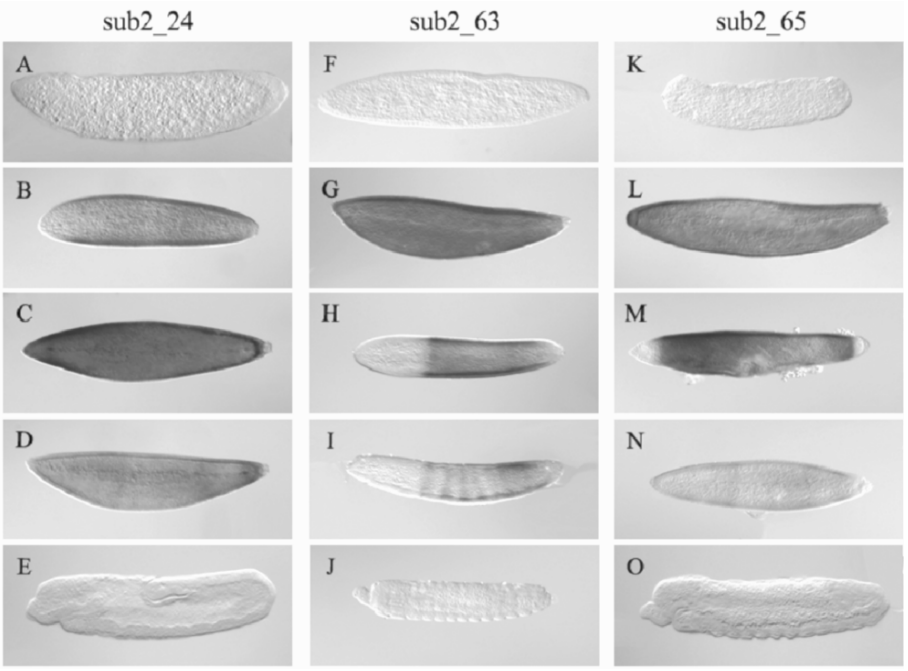

Figure 4. *Cellularization-specific gene expression patterns 2.* Ceratitis capitata *gene sequences* ***sub2_24****. (a) preblastodermal embryo without expression, (b) strong expression at the onset of cellularization, (c) very strong expression during the slow phase of cellularization, (d) reduced expression during the fast phase of cellularization, (e) no expression during germ band retraction.* ***sub2_63*** *(f) preblastodermal embryo without expression, (g) strong expression at the onset of cellularization, (h) strong expression exclusive of the posterior and anterior pole in the slow phase of cellularization, (i) weak expression ending up in stripes exclusive of the posterior and anterior pole in the fast phase of cellularization, (j) no expression after germ band retraction.* ***sub2_65 (k)*** *preblastodermal embryo without expression, (l) strong expression at the onset of cellularization in the whole embryo, (m) very strong expression exclusive of the anterior and posterior pole during the slow phase of cellularization, (n) weak expression reduced to broad stripes at the fast phase of cellularization, and (o) no expression after germ band retraction.*

restricted to this stage (data not shown).

4. Conclusions

A transgene-based embryonic lethality system established in *D. melanogaster* is being evaluated following injection of the driver construct of the binary expression system into *C. capitata* embryos. It will be interesting to determine whether the *sry α* promoter from *D. melanogaster* is also active in *C. capitata* as well as to what extent and in which stages it leads to expression.

To search for cellularization-specific genes in *C. capitata* by cDNA subtraction, the cellularization time window of *C. capitata* embryogenesis was first determined. Six cellularization-specifically expressed candidate genes were isolated by the cDNA subtraction screen. Current work involves searching for the promoter/enhancer regions of these genes by inverse PCR of genomic DNA for use in a

C. capitata specific transgene-based embryonic lethality system.

Once the promoters/enhancers are available, a transgenic sterility system for *C. capitata* will be constructed and its fitness and competitiveness compared to flies sterilized by radiation.

5. Methods

Secondary antibodies (Jackson Immunoresearch) were obtained commercially. The anti-Armadillo antibody (mAb N2 7A1) (Peifer et al. 1994) was obtained from the Developmental Studies Hybridoma Bank (University of Iowa). Antibody stainings were performed as described by MacDonald and Struhl (1986). For the cDNA subtraction the Clontech PCR-Select cDNA Subtraction Kit (BD Biosciences, Heidelberg) was used. The RNA probes for *in situ* hybridization were made with DIG-RNA-labelling Kit (Roche, Mannheim) and hybridizations were performed as described in Davis et al. (2001).

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New Sexing Strains for Mediterranean Fruit Fly *Ceratitis capitata*: Transforming Females into Males

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ABSTRACT Sex determination mechanisms, differing in their modality, are widely represented in all the various animal taxa, even at the intraspecific level. Within the highly diversified class Insecta, Drosophila has been used to unravel the molecular and genetic mechanistic interactions that are involved in sex determination. Indeed, the molecularly characterized genes of the *Drosophila* sex determination hierarchy X:A > *Sxl* > *tra* > *dsx* have been fruitful starting points in the cloning of homologous genes from other insect species. This genetic cascade seems to control sex determination in all *Drosophila* species. Sex determination in the tephritid Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), which diverged from *Drosophila* 90-100 million years ago, contrasts to that found in *Drosophila*. A different primary signal, a Y-linked male-determining factor (M), still to be molecularly identified, dictates maleness whereas in *Drosophila*, the primary signal is the X:A (X chromosome:autosome) balance. However, the *Drosophila* sex-determining pathway, apart from the X:A > *Sxl* initial regulatory segment, is functionally conserved in *C. capitata*. The *tra* gene (*Cctra*) of *C. capitata*, as in *Drosophila*, is the master gene for femaleness through its regulation of the *dsx* gene and it is dispensable for maleness. In contrast to *Drosophila* however, where *tra* is a subordinate target of *Sxl*, *Cctra* seems to initiate an autoregulatory mechanism in XX embryos that provides continuous *tra* female-specific function and acts as a cellular memory maintaining the female pathway. Indeed, a transient interference with *Cctra* expression in XX embryos by RNA interference (RNAi) treatment can cause complete sexual transformation of both germ-line and soma in adult flies, resulting in fertile XX pseudomales. The development of new transgenic sexing strains of *C. capitata* able to produce male-only progeny following heat-shock treatments is now feasible and a concrete possibility. Evolutionary considerations strongly suggest that this biotechnological strategy to produce male-only progeny could be developed for many other Tephritidae and other dipteran species where the sterile insect technique (SIT) is employed within the framework of area-wide integrated pest management programmes.

KEY WORDS Mediterranean fruit fly, *Ceratitis capitata*, Tephritidae, SIT, sexing, sex determination, RNA interference, transgenic, biotechnology, biological control

1. Introduction

Sex determination systems show variability among animal species, including those that are closely related (Marin and Baker 1998, Saccone et al. 2002). The most common, and presumably the most primitive system, is based on a male-determining factor linked to the heteromorphic Y chromosome or one of the homomorphic chromosomes (e.g., Calliphoridae, Culicidae, Chironomidae, Muscidae, Tephritidae). *Drosophila melanogaster* (Meigen) uses a complex genetic mechanism of X chromosome and autosome

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counting, called the X:A ratio, an otherwise quite rare sex-determining system (Cline and Meyer 1996). Sex determination in the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) contrasts to that found in *D. melanogaster* (Fig. 1a). The two species belong to the Acalyptratae group and are 90-100 million years phylogenetically distant (Beverley and Wilson 1984). The identification of XXX viable and fertile females and XXY fertile males in a wild Mediterranean fruit fly population led to the discovery that the Y chromosome determines the male sex (Lifschitz and Cladera 1989). The analysis of aneuploid offspring generated by Y:autosome translocations predicted the existence of a male-determining factor (M) in the long arm of chromosome Y (Robinson et al. 1999). Using a series of Y chromosome deletions, Willhoeft and Franz (1996) mapped the M factor more precisely to a region located in the first third of the long arm close to the centromere and representing about 15% of the whole Y chromosome. Furthermore, this study demonstrated that the remaining 85% of the Y chromosome contained no material required either for sex determination or for the development of the testes.

The genetic cascade regulating sexual development in *D. melanogaster* is well understood at the molecular level (Cline 1993, Cline and Meyer 1996). The primary signal is polygenic and is determined by the ratio of X chromosomes to sets of autosomes (X:A ratio). When this ratio is 1.0 (XX:AA) in females, the *Sex-lethal* gene (*Sxl*) is activated, while with a ratio of 0.5 (X:AA) in males, *Sxl* remains inactive. *Sxl* now acts as the key on/off switch that controls all aspects of somatic sexual dimorphism via a short cascade of subordinate regulatory genes (Nagoshi et al. 1988). When the gene is active, it dictates female development; when it is inactive, male development follows. Once the gene is activated in females, its products initiate a positive autoregulatory mechanism that guarantees the continuous production of *Sxl* protein, thus forming a cell memory of the sex and maintaining the cells on the female pathway throughout development (Bell et al. 1991). In males, however, where *Sxl* is not activated, the gene will remain functionally off. *Sxl* produces sex-specific mRNAs by alternative splicing: the female-specific mRNAs encode full-length functional *Sxl* protein, while the male-specific ones have an additional stop-containing exon and encode a truncated non-functional *Sxl* peptide. The on/off state of *Sxl* activity is set early during embryogenesis by a complex combination of transcriptional and post-transcriptional gene regulation (Bell et al. 1991, Keyes et al. 1992). The initial activation of *Sxl* in XX embryos relies on the use of an alternative XX embryo-specific promoter (P_e) that responds to the genes signaling the X:A ratio (Parkhurst et al. 1990). *Sxl* pre-mRNAs produced from P_e are spliced in a female-specific mode by the spliceosome, independently of additional trans-acting factors, such as the *Sxl* protein itself (Horabin and Schedl 1996, Zhu et al. 1997). The RNA-binding *Sxl* proteins translated from these early mRNAs then initiate the autoregulatory loop by directing the female-specific processing of the pre-mRNAs produced from the late constitutive *Sxl* promoter. The late pre-mRNAs, in contrast to the early *Sxl* pre-mRNAs, can be spliced in the female-specific mode only in the presence of *Sxl* protein. To execute the correct developmental programme, *Sxl* transmits the determined state to *transformer* (*tra*) (Boggs et al. 1997), the next gene in the cascade. At this level, *Sxl* regulates the choice between two alternative 3' splice sites in the pre-mRNA of *tra* (Inoue et al. 1990, Valcárcel et al. 1993). In the absence of the *Sxl* protein, the more proximal site is used resulting in a *tra* mRNA that encodes a truncated and probably inactive protein. When the *Sxl* protein is present, it will bind to the *tra* pre-mRNA enforcing the use of the distal 3' splice site and hence the production of an mRNA with a full-length open reading frame (Sosnowski et al. 1989). The state of activity of *tra* is then transmitted to *doublesex* (*dsx*) (Burtis and Baker 1989), the last component of the sex determination genetic pathway. In females, the *tra* protein, together with the con-

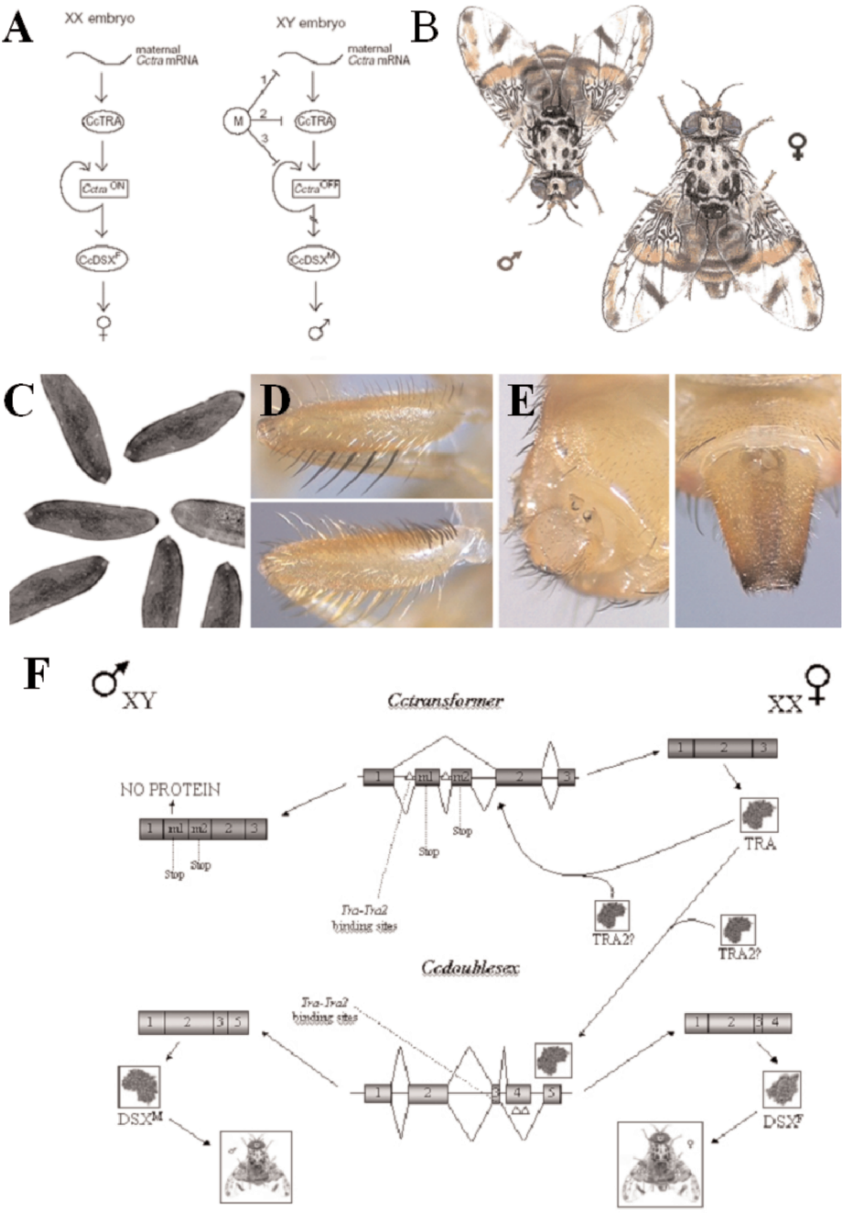

Figure 1. *Diagram showing (a) the model of sex determination in* Ceratitis capitata *(Pane et al. 2002), (b) adult male and female Mediterranean fruit flies, (c) distribution of SXL protein in* Ceratitis *syncytial blastoderm embryos (Saccone et al. 1998). XX and XY embryos exhibit strong nuclear staining in all cells, (d) sexual dimorphism of the bristles present on the forlegs respectively of females (upper) and males (lower), (e) sexual dimorphism of the external reproductive apparatus in males (left) and females (right), and (f) molecular pathway for sex determination in* Ceratitis capitata.

stitutively expressed *tra*-2 protein, binds to *dsx* pre-mRNA directing its female-specific splicing, such that a mature mRNA encoding dsx^F protein is generated (Hoshijima et al. 1991, Tian and Maniatis 1993). In males, the absence of the *tra* protein causes male-specific splicing and the production of dsx^M protein. The dsx^F and dsx^M proteins are transcription factors that regulate the activity of sex-specific differentiation genes (Burtis and Baker 1989).

A parallel comparative molecular study has been undertaken to identify potential sex determination genes in *C. capitata* by using *Drosophila* genes as probes for cDNA and genomic libraries and *Sxl*, *tra* and *dsx* homologous genes have been isolated (Furia et al. 1993, Saccone et al. 1996, 1998, Pane et al. 2002). In contrast to *D. melanogaster*, the *Sxl* homologue in *C. capitata*, *CcSxl*, expresses the same mRNAs and protein isoforms in both XX and XY insects irrespective of the primary sex-determining signal (Saccone 1997, Saccone et al. 1998) (Fig. 1c). In addition, experiments with two inducible transgenes demonstrated that the corresponding *Sxl* isoforms, although highly conserved (80% amino acid identity), have surprisingly no significant sex-transforming effects when expressed in *D. melanogaster* (Saccone et al. 1998). These findings suggest that *Sxl* acquired its master regulatory role in *D. melanogaster* during evolution of the Acalyptratae group, most probably after phylogenetic divergence of the genus *Drosophila* from other genera of this group (Saccone et al. 1998). In contrast, *C. capitata tra* (*Cctra*) and *dsx* (*Ccdsx*) genes produce sex-specific transcripts by alternative splicing as in *D. melanogaster* and these data suggested their functional conservation as key sex-determining regulators (Saccone et al. 1996, Pane et al. 2002). RNA interference (RNAi) against *Cctra* confirmed its key role in controlling female sex determination in this species, as in *Drosophila*, and interestingly led to the production of male-only progeny composed of abnormal XX (karyotypic females, phenotypic males) and normal XY flies (Pane et al. 2002). Recently, other surprising results have been obtained when the very weakly conserved $Cctra^F$ protein (12% amino acid identity when compared to $Dmtra^F$ protein), expressed in *D. melanogaster* transgenic flies was able to rescue a *tra* null mutation (Pane et al. 2005). Also $Ccdsx^M$ protein (50% amino acid identity when compared to $Dmdsx^M$ protein), when expressed in *D. melanogaster* transgenic flies caused partial masculinization of both soma and germ line (Salvemini 2004).

2. Autoregulation of the Female-Determining *transformer* Gene

In contrast to the *Drosophila* homologue, the *Cctra* gene is able to autoregulate in females, promoting its own female-specific splicing (Pane et al. 2002). The female-specific transcripts, but not the male ones, encode functional $Cctra^F$ protein that presumably migrates in the cell nucleus to bind the *Cctra* pre-mRNA and promote the exclusion of male-specific exons (Fig. 1F). Considering that the male-specific exons contain stop codons, the male-specific *Cctra* transcripts show premature termination of the translation when used by the ribosomes and hence no *Cctra* functional protein is produced in XY embryos during all stages of development. Pane et al. (2002) have proposed a *C. capitata* sex-determination model in which XX and XY embryos have a maternal $Cctra^F$ protein or a maternal *Cctra* mRNA that promotes by default a female-specific splicing of the *Cctra* zygotic pre-mRNA, once the gene is transcriptionally active (Fig. 1A). However, the presence of the Y chromosome and hence of the M factor, blocks this initial regulatory event only in XY embryos. In XX embryos, early production of zygotic female-specific *Cctra* transcripts leads to the translation of newly formed $Cctra^F$ protein that migrates to the nuclei and promotes again female-specific *Cctra* splicing, setting a positive feedback loop. In XY embryos the M factor, directly or undirectly, "impairs" the maternal $Cctra^F$ protein feminizing activity, leading to male-spe-

cific splicing of *Cctra* pre-mRNA. As no functional zygotic $Cctra^F$ protein is produced in XY embryos, the splicing pattern of *Cctra* is irreversibly established in a male-specific mode, shutting down the gene for the remainder of fly development. The presence/absence of the $Cctra^F$ protein respectively in XX and XY flies causes the production either of $Ccdsx^F$ protein or $Ccdsx^M$ protein, by modifying the sex-specific splicing of the downstream *Ccdsx* gene (Pane et al. 2002) (Fig. 1f). These two isoforms seem to act as alternative transcriptional factors promoting either female or male sexual differentiation of *C. capitata*, as in *D. melanogaster* (Fig. 1b, d, e).

A transient interference with *Cctra* expression during embryogenesis by RNAi can cause complete sexual transformation of both germ-line and soma in adult flies, resulting in a fertile male XX phenotype (Pane et al. 2002). The male pathway seems to result when *Cctra* autoregulation is prevented and instead splice variants with truncated open reading frames are produced. Under normal conditions, this repression is achieved by the M factor. It is proposed that the RNAi against *Cctra* artificially imitates the repression caused normally by the M factor only in XY embryos (Fig. 1a). Thus, as in *Drosophila*, *Cctra* acts as a genetic switch between female (when functionally on) and male (when functionally off) development. As described above, the male-specific short peptides encoded by the alternatively spliced male-specific transcripts seem to be non-functional, at least during the early embryonic stages, because the RNAi has no evident effects on the development of XY males.

It can be inferred from these results that early application of RNAi transiently eliminates *Cctra* mRNAs and therefore prevents continued production of the *tra* protein. Once *tra* pre-mRNA production is resumed at a later stage in development, the unproductive male mode of *tra* splicing is launched because of the absence of functional *tra* protein. Likewise, absence of the *tra* protein causes its direct target *dsx* to be spliced in the male mode. These results are compatible with the likelihood that *Cctra* sustains the productive mode of its splicing by an autoregulatory feedback loop and mediates female differentiation, at least in part, by the control of its target gene, *dsx*. The initiation of the autoregulatory loop in XX embryos could be based on maternal *Cctra* mRNAs (or proteins) that have been detected in unfertilized eggs by RT-PCR experiments (Pane et al. 2002). These mRNAs are spliced in the female mode and hence could provide a source of *tra* protein activity that allows female-specific splicing of zygotic *Cctra* pre-mRNA.

Hence the *Ceratitis capitata transformer* gene has two key regulatory functions: one that is homologous to the *Drosophila transformer* function, promoting female-specific splicing of the *Ceratitis doublesex* pre-mRNA (female-determining function) and a novel second function necessary to promote and maintain the female-specific splicing of its own pre-mRNA (sex determination memory function). In this respect the *Ceratitis tra* gene autoregulatory function is analogous to the one exterted by the *Sxl* gene in *Drosophila* to maintain female sex determination during all development (Pane et al. 2002). For these reasons we prefer to refer to the *Cctra* gene as the *Ceratitis auto-tra* gene, because this is the first autoregulating version of the *Drosophila transformer* gene. Novel *transformer* homologous genes will be isolated in future in other insect species: those *tra* genes, lacking the ability to autoregulate but involved in female sex determination should be considered homologues of the *Drosophila tra* gene, while the other *tra* genes able also to autoregulate should be considered homologues of the *Ceratitis auto-tra* gene, and possibly named referring to the *Ceratitis* gene.

3. Manipulation of the *transformer* Gene to Produce Sexing Strains

The discovery that injected double-stranded (ds) RNA can induce post-transcriptional gene silencing in many distantly related species, including the free-living nematode

Caenorhabditis elegans (Maupas) and *D. melanogaster*, has given geneticists a powerful tool to investigate the function of unknown genes and to manipulate the expression of known genes without the need for mutants. However, although dsRNA injected into pre-blastoderm *D. melanogaster* embryos is a strong inhibitor of the corresponding gene's function during early development, it does not produce a very robust effect at later stages (Kennerdell and Carthew 1998, Misquitta and Paterson 1999). To overcome this limitation, efforts were made to develop a system in which the dsRNA is produced *in vivo*, by targeted transcription of an inverted repeat transgene (Fortier and Belote 2000, Giordano et al. 2002). For example, an expressed inverted repeat of a portion of the *Drosophila* sex differentiation gene, *tra-2*, driven by a *GAL4*-dependent promoter, genetically represses the endogenous wild-type *tra-2* function, producing a dominant loss-of-function mutant phenotype and leading to a masculinization of *D. melanogaster* XX female flies (Fortier and Belote 2000).

A transgene was constructed that was able to transcribe, under the heat-shock promoter, an inverted repeat corresponding to the *Cctra* sequence. This construct was inserted into a transposon vector and transgenic lines obtained. Masculinization of XX individuals following a pulse heat-shock treatment of the embryos was expected. Indeed a transient endogenous production of double-strand RNA specific for the *Cctra* gene should induce a transient destruction of the corresponding maternal *Cctra* mRNAs in both XX and XY embryos. The transient lack of this early protein, artificially induced by RNAi, is expected to cause the collapse of the *Cctra* autoregulatory positive loop in XX embryos, as mediated by the M factor in XY embryos. However, only one out of six transgenic lines produced male-only progeny (95% males and 5% intersexes), following a temperature change from 20°C to 25°C during all development.

4. Conclusions

The major experimental problems to be addressed in future to develop an efficient sexing system based on *in vivo* RNAi using transgenesis are: (1) the sensitivity of the transgene to the position of integration, (2) the leakiness of the *Drosophila hsp70* promoter in transcribing dsRNAs in the absence of heat-shock, (3) the extent to which the fertility of the transgenic lines is possibly affected by basal levels of *Cctra* dsRNA molecules (in the absence of heat-shock), (4) the extent and penetration of masculinization induced by the transgene in XX individuals, (5) the relative competitiveness of XX transgenic males in matings with wild-type females, compared with wild-type males, (6) the optimal heat shock conditions required to induce masculinization, and (7) the environmental impact and the possible risks related to the mass-release of sterile transgenic males. Future work will focus on these questions and provide the basic knowledge needed to transfer this technology from the laboratory to the field.

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Developing Transgenic Sexing Strains for the Release of Non-Transgenic Sterile Male Codling Moths *Cydia pomonella*

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ABSTRACT Sterile insect releases for management of lepidopteran pest populations are based on bisexual releases. Male-only releases of codling moth *Cydia pomonella* (L.) have never been tested in the field, due to the lack of efficient ways to separate males from females or produce only males. Recently, a new approach for the development of genetic sexing strains in Lepidoptera has been proposed. It is based on the construction of transgenic females carrying a dominant conditional lethal mutation (DCLM) in the female-determining W chromosome. Such a transgenic sexing strain would be propagated under permissive conditions. Under restrictive conditions, the W-linked DCLM would kill all females while allowing mass-rearing and release of radiation-sterilized, but non-transgenic males. This paper describes the principle of the proposed transgenic approach and discusses its benefits, environmental safety, and other potential concerns. The aim is to develop transgenic sexing strains in the codling moth. Appropriate molecular tools for codling moth transgenesis are already available and germ-line transformation in this species has been successfully accomplished. Significant progress has also been made in codling moth cytogenetics. In particular, data on the codling moth sex chromosomes and their identification, obtained using advanced molecular cytogenetic methods, will facilitate the development of genetic sexing strains in this pest.

KEY WORDS codling moth, transgenesis, W chromosome, genetic sexing, cytology, dominant conditional lethal mutation

1. Introduction

In Lepidoptera, area-wide programmes integrating the sterile insect technique (SIT) have been successfully implemented against two species: containment of the pink bollworm *Pectinophora gossypiella* (Saunders) in the USA (Staten et al. 1993), and suppression of the codling moth *Cydia pomonella* (L.) in Canada (Dyck et al. 1993, Bloem and Bloem 2000, S. Bloem et al., this volume). The use of inherited sterility against various lepidopteran pests is also a very promising approach (Carpenter and Gross 1993, Makee and Saour 1997, Bloem et al. 2001, Carpenter et al. 2001, Nguyen Thi and Nguyen Thanh 2001, Seth and Sharma 2001, Carpenter et al. 2005). The success of these programmes and the potential use of inherited sterility have encouraged research activities aimed at improving its efficiency and applicability worldwide.

Current programmes using SIT and/or inherited sterility for the population control of lepidopteran pests rely on bisexual releases, but there are reasons to believe that the release of sterile males only would bring significant improvement to these technologies (Marec et al. 2005). On the other hand, recent results involving field-cage experiments with the

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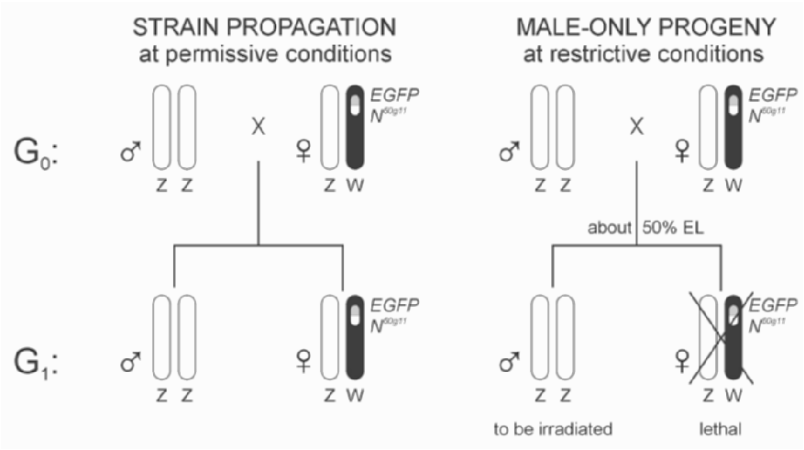

Figure 1. *Theoretical scheme to generate a genetic sexing strain based on the use of transgenic females that have an insert in their W chromosome containing the enhanced green fluorescent protein marker gene* (EGFP) *and a conditional dominant lethal mutation of the* Drosophila Notch *gene* (N^{60g11})*. If kept under restrictive conditions (low temperature), the strain produces only males that can be irradiated and released (G_0 and G_1: generations, Z and W: sex chromosomes, EL: embryonic lethality (Modified after Marec et al. (2005)).*

cactus moth *Cactoblastis cactorum* (Berg), suggest that sterile females have a positive impact on population suppression (Hight et al. 2005). However, caution is required with the interpretation of these data as field-cage tests cannot reflect all factors involved in field programmes. This has been well demonstrated in programmes using the SIT against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). Although field-cage tests were inconclusive, the use of genetic sexing strains, enabling mass-production and release of males only, has resulted in enormous economic benefits by decreasing release costs and increasing the efficiency of the sterile males in comparison with bisexual releases (Robinson 2002, Rendon et al. 2004). Obviously, large-scale field tests are needed to compare the efficiency of male-only with bisexual releases for the control of lepidopteran pests.

A convenient genetic sexing system to produce male-only progeny in key lepidopteran pests is not yet available. Sophisticated genetic sexing strains such as those constructed in the Mediterranean fruit fly (reviewed by Robinson 2002), cannot be developed in any lepidopteran pest because their sex chromosome system is different to that in fruit flies. In moths and butterflies, the chromosome mechanism of sex determination is of the WZ/ZZ type. Females are the heterogametic sex with a pair of WZ sex chromosomes, while homogametic males have a ZZ pair (Traut 1999). This means that sexing strains constructed on a similar principle as in the Mediterranean fruit fly would eliminate male but not the female progeny.

Recently, a new approach for the development of genetic sexing strains in Lepidoptera has been proposed (Marec et al. 2005), based on the construction of transgenic females carrying a dominant conditional lethal gene in the female-determining W chromosome. The main advantage of this approach is that the males are not transgenic and, therefore, could be released as in a normal area-wide integrated pest management (AW-IPM) programme using the SIT.

Based on this principle, it is intended to develop transgenic sexing strains in the

codling moth *C. pomonella*. A detailed analysis of the codling moth karyotype with a particular focus on the identification and characterization of sex chromosomes is available (Fukova et al. 2004, 2005), and successful and stable germ-line transformation has also been achieved (Ferguson et al. 2004). The principle of the transgenic approach and the molecular tools available for codling moth transgenesis are described below and data on codling moth cytogenetics are summarized. Also included is a discussion as how the sex chromosomes could be used to develop transgenic sexing strains.

2. Mendelian Approaches to Genetic Sexing in Lepidoptera

In the silkworm *Bombyx mori* (L.), several mutant strains have been constructed, in which wild-type alleles of autosomal marker genes coding for visible traits such as egg colour, larval phenotype or cocoon colour, are translocated onto the W chromosome while their recessive mutant alleles remain located on autosomes. The visible traits then exhibit sex-limited inheritance. This makes possible the separation of males and females according to the sex-specific phenotype during embryonic, larval, and pupal development, respectively (Nagaraju 1996). However, in lepidopteran pests convenient selectable marker genes are not available (Robinson 1971).

An alternative approach has been to use female killing systems based on balanced lethal strains as proposed by Strunnikov (1975) for the silkworm *B. mori*. To date, balanced lethal strains have been developed in two species, the silkworm and the Mediterranean flour moth *Ephestia kuehniella* Zeller (reviewed by Marec et al. 2005). Briefly, the balanced lethal-2 strain of the latter species produces males, which are heterozygous, in *trans*, for two sex-linked recessive lethal mutations. Sexing is achieved when balanced lethal-2 males are outcrossed to wild-type females. Such crosses produce almost exclusively male progeny, while the female progeny die during embryogenesis because they are hemizygous for one of the lethal mutations (Marec 1991, Marec et al. 1999).

This scheme has several drawbacks. Firstly, the development of balanced lethal strains is laborious and difficult due to lack of the sex-linked markers needed to detect and effectively rear insects with lethal mutations and the T(W;Z) translocations required for the construction of balanced lethal strains (Marec 1991). Secondly, the maintenance of two different colonies, a wild-type strain and a balanced lethal strain (Fig. 3 in Marec et al. 1999), and routine checking of the balanced lethal strain to prevent colony breakdown due to genetic recombination or contamination, represent serious obstacles for a mass-rearing facility. Finally, an additional sex separation is indispensable before crossing balanced lethal males with wild-type females to produce male-only progeny for irradiation and release. Considering these disadvantages, this technology is not applicable for mass-rearing.

3. Principle of the Transgenic Approach to Genetic Sexing

Theoretically, a dominant conditional lethal mutation (DCLM) located in the female-determining W chromosome would be a simple and efficient selective mechanism for the development of genetic sexing strains in Lepidoptera, easily applicable in a mass-rearing facility. The DCLM would be inherited exclusively by mutant females, whereas males would be wild-type. The strain would be propagated under permissive conditions, and the female progeny eliminated under restrictive conditions. However, it would be very difficult to induce a DCLM in the W chromosome since it is largely heterochromatic and probably genetically inert in most lepidopteran species (Traut 1999). Therefore, it would be better to try to identify a DCLM in the gene-rich Z chromosome (or in an autosome) and then translocate it onto the W chromosome (Fig. 2 in Marec et al. 2005). Unfortunately, no DCLM has been reported to date in any lepidopteran species and a labour-intensive

and long-term study would be required to obtain a desirable mutation in a particular pest.

In order to circumvent this problem, Marec et al. (2005) proposed that genetic sexing in Lepidoptera, based on W-linked DCLMs, can be accomplished through the use of transgenesis. Recently, successful and stable germ-line transformation has been achieved in several lepidopteran species using the transposable element *piggyBac* (Peloquin et al. 2000, Tamura et al. 2000).

The key requirement under this proposal is the insertion of a DCLM of a known gene, which is conserved in insects and expressed during embryogenesis, into the W chromosome (Fig. 1). For this purpose, a mutant allele of the *Notch* gene, N^{60g11}, has been chosen. The N^{60g11} allele was originally isolated in *Drosophila melanogaster* (Meigen). It encodes a truncated form of the *Notch* protein, which causes the death of heterozygous *Drosophila* embryos kept below 20°C (Fryxell and Miller 1995). Thus, N^{60g11} is dominant, sensitive to cold temperature and seems to be well suited for genetic sexing. A plasmid construct required for germ-line transgenesis is already available in the laboratory of L. Neven. It contains the *piggyBac* transposon with N^{60g11} in tandem with the enhanced green fluorescent protein (EGFP) marker gene from the jelly fish *Aequorea aequorea* (Forskal), under the *B. mori Actin A3* promoter (Fig. 3 in Marec et al. 2005).

A transgenic strain would be reared at temperatures above 20°C in order to maintain the large production colony. For male production, eggs would be held at a temperature below 20°C for a specified length of time to kill female embryos. The eggs would then be returned to permissive conditions, where only male larvae would hatch. This step could be easily checked because no larva expressing the enhanced green fluorescent protein (i.e. transgenic female larva) should hatch. The male-only progeny would be kept at normal temperature until adulthood. After completing their development the adult males would be irradiated and released (Fig. 2).

The transgenic approach has several advantages: (1) the sexing process is simple and can be carried out easily on large numbers of eggs, (2) the process does not require any additional technology in a mass-rearing facility, (3) any escaped females would die if temperatures dropped below 20°C, (4) the released males would not be transgenic, and (5) any genetic background can be introduced through mating males with the transgenic females. As the males are not transgenic, they would not be negatively affected by pleiotropic effects of the transgene used for the elimination of females and they should not trigger any regulatory concerns related to their release.

Nevertheless, there is a concern about this proposed transgenic approach related to the possibility of inserting a transgene into the largely heterochromatic W chromosome as the inserted transgene could be silenced by neighbouring heterochromatin. In addition, there is no evidence that the transgene will be expressed and exhibit the desired temperature lethality in female embryos. However, these are testable research questions that can be addressed.

4. Genetic Transformation in the Codling Moth

The groundwork for the creation of stably transformed codling moth has been established in the laboratory of L. Neven, where the first successful germ-line transformation has been achieved in this species (Ferguson et al. 2004). The molecular tools available for the development of transgenic sexing strains in the codling moth and the current progress are summarized as follows: (1) codling moth eggs are suitable for DNA injection as they attach firmly to the collecting device and have a transparent chorion, (2) the *piggyBac* transposon has been identified as an effective transposable element to insert foreign DNA into moth embryos, (3) the enhanced green fluorescent protein was found to be a useful marker for identifying transformants; however, it is not ideal due to excessive autofluorescence of

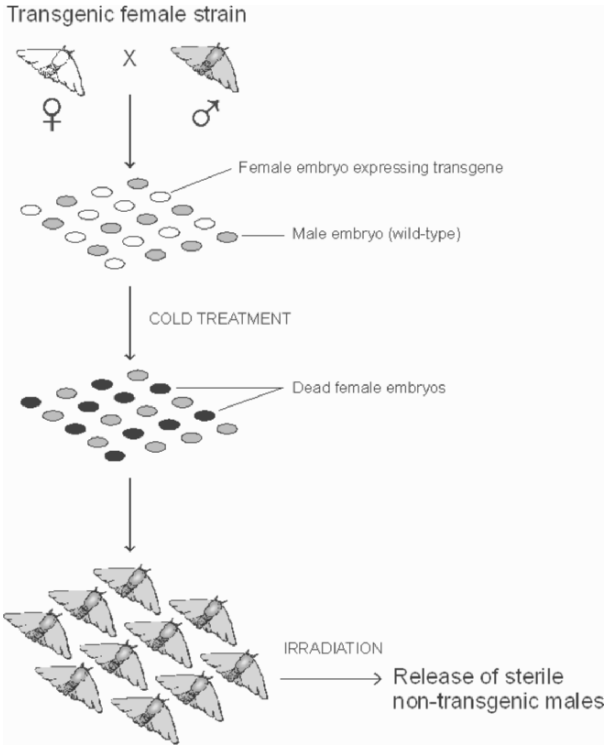

Figure 2. *Transgenic genetic sexing approach to a non-transgenic release of sterile male Lepidoptera (for details, see text).*

the codling moth in the range used to visualize the enhanced green fluorescent protein. Therefore, it would be worthwhile to replace the enhanced green fluorescent protein with another fluorescent marker such as DsRed2 (Horn et al. 2002), (4) the *B. mori Actin A3* promoter was found to function in the codling moth; however, it sometimes exhibited a chimeric fluorescence pattern and therefore, it would be better to replace it with the artificial *3xP3* promoter that supports the enhanced green fluorescent protein expression specifically in the eyes of all life stages (Thomas et al. 2002), (5) an appropriate lethal gene, the dominant cold-sensitive allele N^{60g11}, has been identified. A plasmid construct containing the *piggyBac* transposon with N^{60g11} in tandem with the enhanced green fluorescent protein marker gene under the *Actin A3* promoter is available, and (6) eggs from transgenic lines heterozygous for the enhanced green fluorescent protein and N^{60g11} were exposed to 12°C for two days and 50% of the embryos died and none of the survivors, either larvae or adults, expressed the enhanced green fluorescent protein. The wild-type and enhanced green fluorescent protein controls showed a normal egg hatch and development. This indicates that those embryos carrying the N^{60g11} transgene died, confirming the lethality of this gene at low temperatures (L. Neven, unpublished).

Based on the above it can be concluded that the development of transgenic genetic sexing strains in the codling moth according to the proposed scheme is technically feasible.

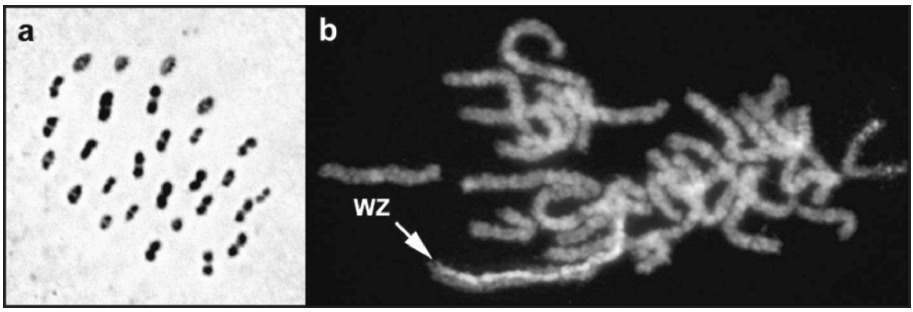

Figure 3. *Photograph showing codling moth chromosomes, (a) squashed spermatocyte, stained with lactic acetic orcein, showing meiotic metaphase I with n=28 bivalents and (b) fluorescent image of spread pachytene oocyte stained with genomic in situ hybridization (GISH) and counterstained with 4',6-diamidino-2-phenylindole (DAPI). The WZ bivalent (arrow) is recognized by prominent hybridization of the Cy-3 labelled, female-derived probe to the W chromosome.*

However, there is still the need to determine experimentally the overall behaviour of the transgene.

5. Codling Moth Cytogenetics

Until recently, very little was known about the genetics of the codling moth (Robinson 1971, Benz 1991), especially about their sex chromosomes. The only cytogenetic study (Ortiz and Templado 1976) reported that the number of metaphase I bivalents in males was n = 28. To fill this gap, a detailed analysis was made of codling moth chromosomes with a particular focus on the sex chromosomes (Fukova et al. 2005). This study confirmed that the codling moth karyotype consists of 2n = 56 chromosomes in mitotic metaphase, corresponding to n = 28 bivalents in metaphase I spermatocytes (Fig. 3a). Females are heterogametic with a WZ sex chromosome pair and males are homogametic with two Z chromosomes. While the Z chromosome is composed of euchromatin and resembles an autosome, the W chromosome consists largely of heterochromatin.

To develop transgenic sexing strains in the codling moth, a DCLM (transgene), has to be inserted into the W chromosome. Theoretically, the probability of the transgene inserting into the W chromosome is dependent on the size of this chromosome relative to the rest of the genome. In the codling moth, the W chromosome is one of the two largest chromosomes, comprising about 4% of the female genome. This should make it a reasonable target for transgenesis with the probability of insertion of 1 in 25 (if only females are included), or with the overall probability of 1 in 50 (since both female and male embryos are injected). However, since the W chromosome is mainly composed of heterochromatin, silencing of the transgene expression is highly likely. Suggestions as to how to overcome this problem are discussed in Marec et al. (2005).
To further characterize the codling moth W chromosome advanced methods of molecular cytogenetics i.e. genomic *in situ* hybridization (GISH) and comparative genomic hybridization (CGH) were employed. GISH detected the W chromosome as evidenced by strong binding of the Cy3-labelled, female-derived DNA probe (Fig. 3b). With CGH, both the Cy3-labelled female-derived probe and Fluor-X labelled male-derived probe evenly bound to the W chromosome. This suggests that the W chromosome is composed mostly of repetitive DNA sequences that are also distributed on other chromosomes but have accumulated in the W chromosome (Fukova et al. 2005).

Finally, W-specific probes were prepared by laser microdissection of the W chromatin followed by amplification using degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) and DOP-PCR labelling. The probes stained the entire W chromosome with a high specificity. DNA fragments of the microdissected W chromatin were cloned and sequenced. The W chromosome sequence analysis revealed no homology to any DNA sequenced so far (Fukova et al. 2004). Several cloned sequences were found to originate exclusively from the W chromosome. These unique sequences can be very useful as molecular markers of the W chromosome in codling moth transgenesis.

6. Conclusions

The proposed transgenic approach may represent a straightforward method for generating genetic sexing strains in any lepidopteran pest to be controlled using SIT or inherited sterility. The codling moth, which is considered to be one of the best candidates for these technologies (Marec et al. 2005), was chosen to examine this approach. As reported here, all the basic research tools required for the development of transgenic sexing strains in this species are available. Future research will include generating transgenic lines, mapping the inserted transgene, and identifying and characterizing lines with the transgene located in the W chromosome.

7. Acknowledgements

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Sex Chromatin Body as a Cytogenetic Marker of W Chromosome Aberrations in *Cydia pomonella* Females

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ABSTRACT Genetic sexing techniques using transformation and a dominant conditional lethal mutation have been proposed for the codling moth *Cydia pomonella* (L.). This will probably require the isolation of females with T(W;Z) translocations. In *C. pomonella* there is no sex-linked morphological marker making the isolation of T(W;Z) translocations dependent on cytological identification of the aberration. The main objective of this study was to determine the possibility of using the sex chromatin body as a marker to identify translocated females. The appearance of the sex chromatin body and the analysis of sex chromosomes in F_1 females of irradiated *C. pomonella* females were investigated. Based on the appearance of this body, three mutant lines were isolated: lines with elongated, dispersed and fragmented chromatin bodies. The W chromosome was easily distinguished from the Z chromosome when the analysis of pachytene sex chromosome bivalents of *C. pomonella* females was carried out. The aberrations in the W chromosome directly influenced the appearance of the sex chromatin body in highly polyploid somatic cells of the isolated mutant lines. The results showed that the sex chromatin body could be used for sex determination and as a cytogenetic marker in *C. pomonella.*

KEY WORDS *Cydia pomonella*, translocations, cytology, genetic sexing, sex chromatin

1. Introduction

The codling moth *Cydia pomonella* (L.) is a very important economic pest of apples and other pome fruits. The sterile insect technique (SIT), and its variant the inherited sterility technique, are important components of insect pest management for this species (Bloem et al. 2001, Bloem et al. 2005) and it has been proposed that the development of genetic sexing strains for this species could increase the efficiency of these control tactics (Marec et al 2005). To date, the only genetic sexing strains available against lepidopteran insects are based on the construction of balanced lethal strains in the Mediterranean flour moth *Ephestia kuehniella* Zeller (Marec 1991) and for colour sorting in the silkworm *Bombyx mori* (L.) (Strunnikov 1975). These sexing strains, although quite effective, will not be suitable for inclusion in a mass-rearing system as two different strains will need to be reared and each with males from one strain being mated with females from the other to produce males for release. These systems exploit the sex determination system in Lepidoptera where females are the heterogametic sex (WZ) and males are homogametic (ZZ).

As an alternative to the use of a balanced lethal system, it has been proposed to use transgenesis to introduce a dominant conditional lethal mutation onto the female determining W chromosome so that the females could be killed when the restrictive conditions are applied (Marec et al. 2005). This approach has two weaknesses: (1) the W chromosome is heterochromatic which may interfere with the expression of any transgene, and (2) the prob-

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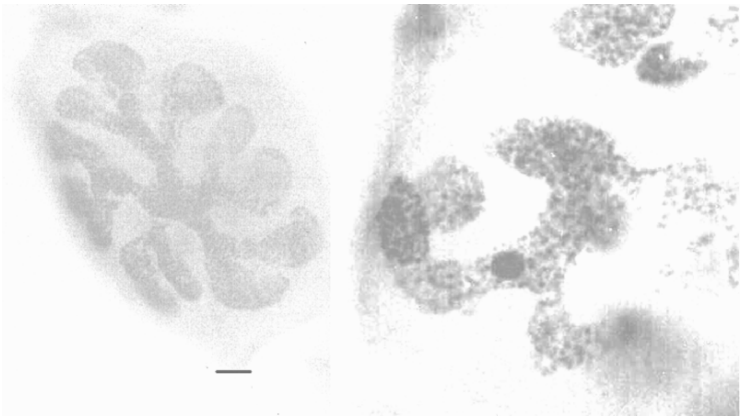

Figure 1. *Highly polyploid nuclei of the Malpighian tubule cells of* C. pomonella *from (left) a wild adult male without sex chromatin, and from (right) a wild adult female showing the sex chromatin body (Bar = 10 micrometer).*

ability of getting an insertion on the W chromosome is very small as there are 28 bivalents in the karyotype. It will therefore probably be necessary to have an insertion carrying the dominant conditional lethal mutation, preferably on the Z chromosome, and link it to the W chromosome by means of a translocation. This paper focuses on the development of cytological techniques for the identification of radiation induced translocations in *C. pomonella* as in this species there are currently no sex-linked visible marker mutations available to isolate and maintain T(W;Z) translocations.

Marec and Traut (1994) suggested that the female-specific sex chromatin body could be used as a cytogenetic marker of W chromosome aberrations. This body occurs in polyploid nuclei of female somatic tissues and is formed by multiple copies of the W chromosome (Traut and Marec 1996). In female *E. kuehniella* carrying a translocation, the appearance of the sex chromatin body is changed (Marec and Traut 1994). In this study, the effect of gamma rays on the appearance of the sex chromatin body was studied in *C. pomonella* female progeny of irradiated females with the aim of isolating T(W;Z) translocations, essential for the construction of a transgenic genetic sexing strain.

2. Materials and Methods

Newly emerged adult females were irradiated with 20 or 30 Gy. Twenty five females were exposed to each dose, with a further 25 newly emerged adult females being used as non-irradiated controls. Irradiated and non-irradiated females were singly paired with 1-day-old males and kept together until death. Eggs were removed daily, counted, and left to determine the percentage of egg hatch. All newly hatched larvae from each irradiated and non-irradiated female were fed on an artificial diet. The number of emerged adults and the sex ratio of F_1 progeny were determined.

2.1. F_1 Generation

Virgin F_1 females were crossed individually with normal males and F_2 families established. Immediately after death, F_1 females, which oviposited a sufficient number of eggs were dissected to detect the appearance of the sex chromatin body in the Malpighian tubule cells. Prior to pupation, four to five female last instar larvae from each family were dissected and their ovaries removed. Chromosomal analysis was carried out during the pachytene stage to identify the status of the W chromosome.

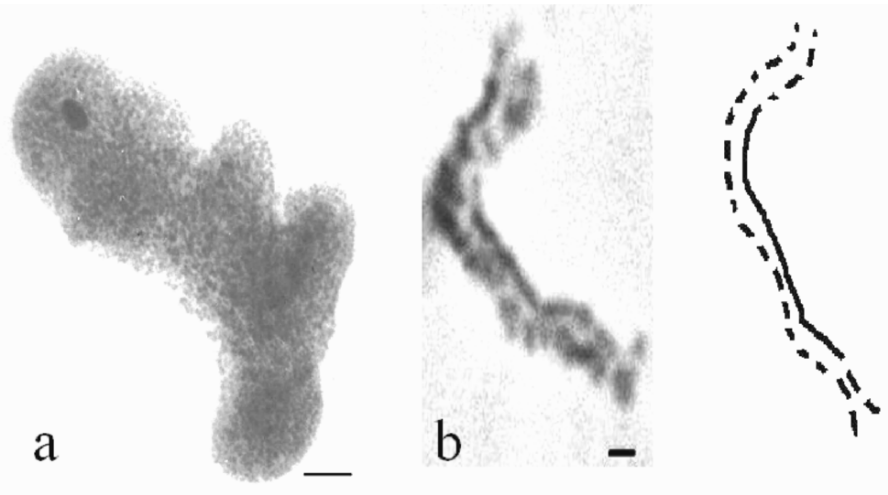

Figure 2. *(a) Highly polyploid nuclei of the Malpighian tubule cells of* Cydia pomonella *with an elongated sex chromatin body of an F_1 female larva that was irradiated as a parental (Bar = 10 micrometer), and (b) WZ chromosome bivalent with an elongated sex chromatin body in an oocyte of an F_1 female larva that was irradiated as a parental (Bar = 1 micrometer).*

2.2. W Chromatin Appearance

Malpighian tubules were dissected from female last instar larvae and adults and fixed in Carnoy's fixative (ethanol:chloroform: acetic acid in the ratio 6:3:1) for two minutes (Traut et al. 1986, Marec and Traut 1994). The tubules were mounted in lactic acetic orcein for five minutes, and then examined under a light microscope.

To detect the presence of sex-heterochromatin in *C. pomonella* males, highly polyploid nuclei of Malpighian tubule cells were taken from male last instar larvae and adults. Dissection, fixation and inspection were carried out as described for females.

2.3. Chromosomal Analysis

For analysis of the sex chromosome bivalent, preparations of spread pachytene oocytes were made from the progeny of normal and irradiated ovaries (Marec and Traut 1994). In brief, ovaries of female last instar larvae were dissected and fixed in freshly prepared Carnoy's fixitive for 30 minutes The ovaries were then transferred to a slide, and shortly before drying, a drop of 60% acetic acid was added and the ovaries completely macerated with fine tungsten needles. The slide was then placed on a heating plate at 45ºC, and the drop moved slightly by pushing it with a needle. At intervals of 30 seconds, this procedure was repeated for five minutes until all the acetic acid had evaporated. The preparation was then stained and mounted in lactic acetic orcein for five minutes, the cover glass sealed with nail polish and the morphology of the sex chromosomes examined under phase contrast and micrographs taken.

3. Results

3.1. Appearance of W Chromatin

Each highly polyploid nuclei of normal female larvae had a single spherical W chromatin body which was absent in males (Fig. 1). Also, there were no differences between last instar larvae and adults of both sexes in the presence and the appearance of W chromatin bodies.

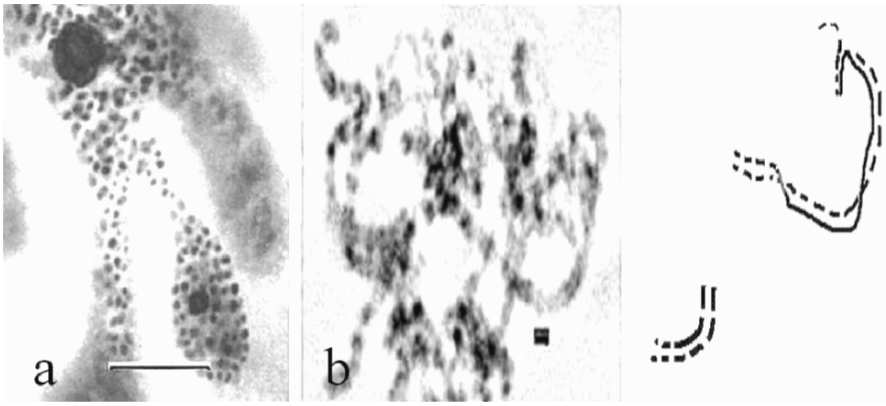

Figure 3. *(a) Highly polyploid nuclei of the Malpighian tubule cells of* Cydia pomonella *with a fragmented sex chromatin body of an F_1 female larva that was irradiated as a parental (Bar = 10 micrometer), and (b) WZ chromosome bivalent with a fragmented sex chromatin body in an oocyte of an F_1 female larva that was irradiated as a parental (Bar = 1 micrometer).*

Following radiation, polyploid nuclei of F_1 females manifested various shapes of the W chromatin body. A single normal or abnormal W chromatin body was seen in Malpighian tubule nuclei depending on the dose of gamma-radiation applied. Based on the appearance of W chromatin, F_1 females were classified into four different groups: with normal, elongated, fragmented and dispersed W chromatin bodies (Figs. 2a, 3a, 4a).

3.2. Chromosomal Analysis

From F_1 and F_2 progenies, four to five female larvae of each line were inspected to analyse the pachytene chromosome sets. In normal females there were 28 bivalents, comprised of 27 autosomal bivalents with each homologous bivalent having a homologous chromomere and an interchromomere pattern (Fig. 5). The sex chromosome bivalent ZW was easily distinguished in all pachytene chromosome sets, and it was very similar to that of *E. kuehniella* (Marec and Traut 1993). The W chromosome formed a deeply-stained heterochromatic thread while the Z chromosome displayed a chromomere/interchromomere pattern. The Z chromosome was longer than the W chromosome and in some cases it was twisted along the W axis (Fig. 5).

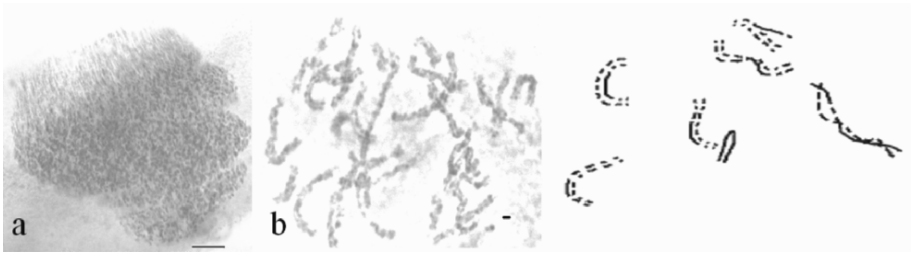

Figure 4. *(a) Highly polyploid of the Malpighian tubule cells of* Cydia pomonella *with a dispersed sex chromatin body of an F_1 female larva that was irradiated as a parental (Bar = 10 micrometer), and (b) WZ chromosome bivalent with a dispersed sex chromatin body in an oocyte of an F_1 female larva that was irradiated as a parental (Bar = 1 micrometer).*

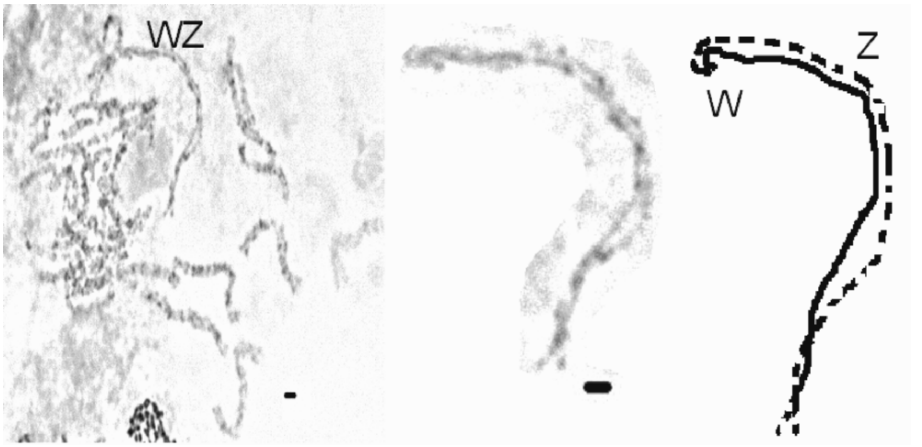

Figure 5. *Spread pachytene oocyte complement of a* Cydia pomonella *female larva, showing (left) the 28 bivalents, and (right) the WZ bivalents stained with orcein (Bar = 1 micrometer).*

In sex chromosome bivalents of females with an elongated chromatin body a clear translocated segment of the Z chromosome was observed in the terminal part of the W chromosome (Fig. 2b). The translocated Z segment was homologously paired with the corresponding region of the Z chromosome.

When sex chromosome bivalents of females with a fragmented chromatin body were inspected, two clear visible fragments, unequal in length, were observed in the W chromosomes (Fig. 3b). In females carrying a dispersed chromatin body, the deeply stained heterochromatic thread (W chromosome) was divided into several parts (Fig. 4).

The results showed that F1 females with elongated W chromatin bodies were detected at both radiation doses (Fig. 6) however at a significantly higher frequency in 30 Gy irradiated female parents. Females with dispersed W chromatin bodies were observed only in F_1 progeny of 20 Gy-irradiated female parents (Fig. 6). Females with fragmented W chromatin bodies were observed when female par-

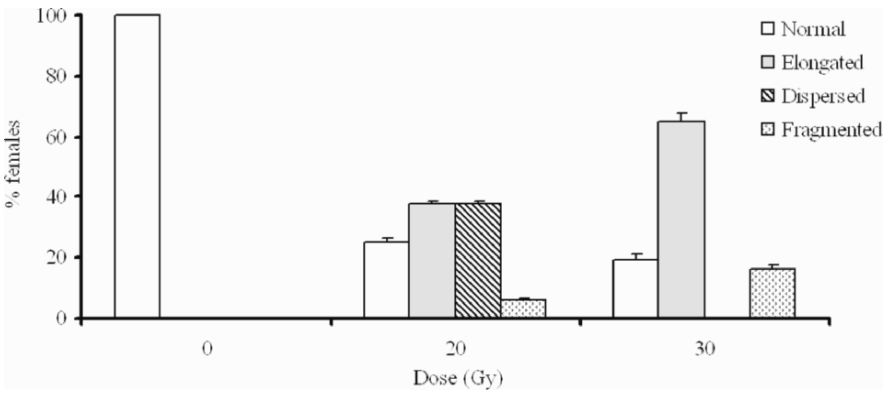

Figure 6. *Relationship between gamma irradiation and the percentage of* Cydia pomonella *females with normal, elongated, dispersed and fragmented W sex chromatin in F_1 lines.*

ents were irradiated at both doses, however the percentage was significantly higher at 30 Gy (Fig. 6).

4. Conclusions

This study showed that the sex heterochromatin body could be very easily observed in polyploid cells of *C. pomonella*. It could therefore be effectively used to: (1) determine sex during the early stage of larval development, (2) detect sex chromosome aberrations after irradiation and mutagen treatments, and (3) identify T(W;Z) translocation females. If suitable insertion lines carrying a dominant conditional lethal mutation on the Z chromosome can be produced then this technique can help to analyse these insertions.

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Potential Use of a Conditional Lethal Transgenic Pink Bollworm *Pectinophora gossypiella* in Area-Wide Eradication or Suppression Programmes

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ABSTRACT The sterile insect technique (SIT) has been used successfully for over 30 years to keep a large cotton growing area in the Central Valley of California, USA free of the pink bollworm *Pectinophora gossypiella* (Saunders). Releases of sterile pink bollworm as part of an integrated pest management programme were recently carried out on 36 400 hectares of cotton in Texas, New Mexico and northern Mexico for the eradication of this pest. The sterile releases will soon be expanded into Arizona and California. To achieve eradication and continue to effectively operate the Central Valley containment programme, a more effective and lower cost programme is needed. Irradiation of pink bollworm with a sterilizing dose greatly reduces mating competitiveness and the development of a conditionally lethal strain of pink bollworm as an alternative or supplement to sterilization by irradiation may allow a more effective and less costly programme. A pink bollworm strain carrying a conditionally lethal gene was recently developed using the "release of insects with a dominant lethal mutation" (RIDL) technology, and larval mortality levels ranging from 60 to 92% have been obtained in laboratory tests. With further development, a strain of pink bollworm carrying a conditionally lethal gene may serve as a complete replacement for radiation-based sterility, or as a supplement to the traditional SIT by providing a genetic sexing mechanism, a safeguard against accidental release, or as a means for lowering the radiation dose to produce a more competitive insect.

KEY WORDS autocidal biological control, RIDL, Lepidoptera, effects of radiation, pink bollworm, eradication

1. Introduction

Pink bollworm *Pectinophora gossypiella* (Saunders) infestations cost US cotton producers USD 47 million per year in direct losses and control measures (National Cotton Council, March 2005, unpublished brief). The United States Department of Agriculture-Animal Plant Health Inspection Service-Plant Protection and Quarantine (USDA-APHIS-PPQ) along with several states, and grower cooperative organizations are involved in two

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area-wide control programmes for pink bollworm that integrate the release of sterile moths. These are (1) an area-wide containment programme in the Central Valley of California, and (2) an eradication programme in Texas, New Mexico and northern Mexico combining the release of sterile insects with the use of *Bacillus thuringiensis* (Berliner) (*Bt*) cotton, mating disruption with pheromones, and pesticides. The use of the SIT was expanded to 36 400 hectares in 2005 when sterile releases were added to the eradication programme areas in Texas and New Mexico in the USA and the Juarez Valley in northern Mexico.

The SIT containment programme has been effective in keeping the Central Valley of California free of pink bollworm for 30 years (Bloem et al. 2005). However, increased cotton production costs, worldwide competition and the increasing demands of the expanded pink bollworm eradication programme require a more effective and lower cost programme, and one way to achieve this is by increasing the competitiveness of the insects in the field. The final competitiveness of the insect in the field is affected by a combination of stresses caused by many factors, e.g. long-term mass-rearing, sterilization, handling, transport and release. The relative effects of each of these factors on the final competitiveness in the field have not been determined for many insects. However, in Lepidoptera, where high doses of radiation are needed for full sterility, an alternative strategy is the use of inherited sterility (Carpenter et al. 2005). In addition, completely replacing radiation could be done provided the alternative strategy does not itself compromise other components of the biology of the insect. In this paper some of the negative effects of radiation on released pink bollworms in SIT programmes and how development of an autocidal biological control system (Fryxell and Miller 1995) to create a conditionally lethal pink bollworm could be useful to pink bollworm genetic control programmes, are summarized. Also, preliminary data are provided on several transgenic pink bollworms strains that carry a conditional lethal gene based on a system called ”release of insects with a dominant lethal mutation” (RIDL) (Thomas et al. 2000).

2. Radiation Effects on Pink Bollworm and Lepidoptera in General

Numerous studies have shown that the very high doses of radiation needed to sterilize male Lepidoptera are associated with decreases in quality, field performance, sperm transfer and dispersal ability in many species (North 1975, LaChance 1985, Carpenter et al. 1997, Bloem et al. 1999). Decreasing the radiation dose is associated with increased mating ability and superior sperm competitiveness (Carpenter et al. 1997) and this has led to the use of lower doses in field programmes. In pink bollworm, very early data showed that high radiation doses reduced longevity, decreased sperm transfer by males, decreased sperm receptivity by irradiated females, decreased female attractiveness and decreased control efficacy (Graham et al. 1972, Flint et al. 1973, Flint et al. 1974, Flint et al. 1977, Bartlett 1978, Miller et al. 1994). Because of the negative effects of radiation along with the negative effects of mass-production, handling, transport and release mentioned above, effective programme operation requires release ratios of 60 sterile males to one wild male (based on males caught in pheromone monitoring traps) and high frequencies of release (4-7 days per week). A more robust and competitive moth could reduce the release ratio and frequency required for effective control.

3. Development of a Strain of Pink Bollworm Carrying a Conditional Lethal Gene

The use of a conditional lethal or autocidal strain of pink bollworm created with transgenic technology as a possible supplement to, or replacement of, radiation-based sterilization may offer advantages that could improve the effectiveness of the SIT for pink bollworm.

A strain carrying a conditional lethal gene could also be used in combination with a conventional radiation strategy, as a safeguard or precaution to guard against an accidental release of fertile moths. This may become more important for operation of the current rearing facility as the eradication programme, when it expands westward toward Arizona and the area around the rearing facility therefore becomes free of pink bollworm. A moth carrying a conditional lethal gene used in this way would also allow the use of a lower radiation dose, helping increase the competitiveness of the released moths. Mortality associated with conditional lethality could not be used in an inherited sterility strategy (Carpenter et al. 2005) as F_1 progeny would die before passing on increased sterility to the next generation.

Lastly, while many researchers consider mixed-sex release desirable for effective SIT against lepidopteran pests, there is theoretical and empirical evidence that, when one sex of the sterile insects is more competitive than the other, single-sex release would be advantageous, either female only (Knipling 1979, Van Steenwyk et al. 1979, Henneberry and Keaveny 1985) or male only (Knipling 1979, Marec et al. 2005). The use of a sex-limited or sex-linked conditionally lethal system would make large-scale sex separation feasible in order to test these principles (Heinrich and Scott 2000, Thomas et al. 2000, Alphey and Andreasen 2002, Marec et al. 2005).

These last two examples of transgenic conditionally lethal technology for use in a pink bollworm genetic control programme would result in a hybrid programme where both the new technology and a standard SIT approach are mixed. While work continues to develop a conditionally lethal system that could potentially serve as a complete replacement for radiation-based genetic control of pink bollworm, these other approaches would be compatible with the existing programme. They may also have advantages where resistance due to public concerns about the use of genetically modified insects are a factor, and for refinements to the standard SIT strategy such as the addition of a sexing strain of pink bollworm

4. Development of Pink Bollworm Strains Carrying RIDL Constructs

Recently, twenty independent transformed lines of pink bollworm with RIDL constructs have been produced and tested. Of these, five lines with construct LA1124 express lethal phenotypes when reared on chlortetracycline-free diets. LA1124 is a lethal construct controlled by a tetracycline repressible transactivator protein (tTA). Lethality is produced by a positive feedback cycle, in which binding of tTA to its specific target sequence tetO drives production of more tTA. In the absence of tetracycline, this leads to lethality by high expression of tTA. When tetracycline is present, tTA does not bind tetO, and so the positive feedback cycle is not established and tTA remains at a low, non-lethal level (Gong et al. 2005 provide more details on the tetO-tTA system). Tetracycline (in the form of chlortetracycline) is a normal part of the pink bollworm artificial diet, so these strains of pink bollworm could readily be incorporated into the current mass-rearing system.

In the laboratory, pink bollworms, heterozygous for the LA1124 construct, were crossed to wild types to produce an F_1 generation with a 1:1 ratio of progeny of LA1124 heterozygotes and wild type. These were reared with and without chlortetracycline and mortality was scored at larval, pupal, and adult stages. This experiment was designed to simulate the mortality of progeny that would occur from the mating of a moth, homozygous for a RIDL construct with a wild-type pink bollworm after release of moths carrying RIDL constructs in a cotton field, while also including an internal wild-type control (the wild-type siblings of the heterozygous F_1 transgenics).

To date, over 37 000 individuals have been tested and significant levels of mortality were observed for progeny heterozygous for a RIDL construct (genotype = LA1124/+)

reared on a chlortetracycline-free diet. Most of the mortality occurred in the prepupal-pupal stage with mean levels of mortality of 60-92%. Rates of mortality in the control treatments (LA1124/+ progeny reared on the chlortetracycline TC diet, and +/+ progeny reared on chlortetracycline-free diet) were low at 2-15%. A more thorough description of the construction and testing of these transgenic pink bollworm lines with the LA1124 construct will be reported elsewhere.

5. Conclusions

Conditionally lethal strains of pink bollworm expressing partial mortality have recently been developed with RIDL technology. Although further improvements and testing of the strains are needed to determine if a fully functionally pink bollworm strain carrying RIDL constructs can be developed, the technology shows promise and may eventually serve as a replacement or supplement to the current technology using radiation sterilization. Work currently underway and planned for the future includes: (1) creating new pink bollworm strains doubly homozygous for the LA1124 constructs by crossing together individual lines with independent insertions of the LA1124 construct, (2) testing LA1124 doubly homozygous lines on a small scale on cotton plants in quarantine field cages to estimate mortality rates and control efficacy under the more realistic conditions of the actual plant host under field conditions, (3) testing adult longevity of LA1124 lines (important for both mass-rearing and field efficacy estimates), and (4) testing the mortality rates of constructs with autocidal effector genes other than tetO-tTA. The strategy is to combine the lethal effects of two separate effector genes into a single pink bollworm strain to assess if mortality rates can be increased or if lethality will occur at earlier larval stages when compared with the LA1124 strain construct alone. The results of these experiments will lead to a greater understanding of the function of RIDL constructs in pink bollworm and the potential for the incorporation of conditionally lethal pink bollworm strains into the existing control programme.

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Wolbachia-Induced Cytoplasmic Incompatibility to Control Insect Pests?

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ABSTRACT *Wolbachia* are a group of obligatory intracellular and maternally inherited bacteria of arthropods and nematodes, which have recently attracted attention for their potential as new biological control agents. *Wolbachia* are able to invade and maintain themselves in an enormous range of invertebrate species, including insects, mites, spiders, springtails, crustaceans and nematodes. Recent surveys using the polymerase chain reaction (PCR) suggest that perhaps over 20% of arthropod species may be *Wolbachia*-infected, making this bacterium the most ubiquitous intracellular symbiont yet described. *Wolbachia* can manipulate host reproduction by using several strategies, one of which is cytoplasmic incompatibility. *Wolbachia*-induced cytoplasmic incompatibility can be used in several ways: (1) to directly suppress natural arthropod populations of economic and public health importance, (2) as a tool to spread genetically modified strains into wild arthropod populations, and (3) as an expression vector, once a genetic transformation system for this bacterium is developed. A major research aim is to introduce *Wolbachia* into pest and vector species of economic and public health relevance and, through *Wolbachia*-induced cytoplasmic incompatibility, to suppress or modify natural populations.

KEY WORDS *Wolbachia*, cytoplasmic incompatibility, *Ceratitis capitata*, insect pests, biological control

1. Introduction

Wolbachia pipientis Hertig (denoted *Wolbachia* hereafter), is an obligate intracellular and maternally-transmitted bacterium (Werren 1997, Bourtzis and O'Neill 1998, Stouthamer et al. 1999, Stevens et al. 2001, Bourtzis and Miller 2003). *Wolbachia* are able to establish infections in the soma, but they mainly reside in the reproductive tissues of their invertebrate hosts (Fig. 1). *Wolbachia* cause a number of reproductive alterations such as parthenogenetic development, overriding of chromosomal sex determination to convert genetic males into functional females, killing male embryos at early developmental stages, and cytoplasmic incompatibility. Cytoplasmic incompatibility results in mortality of the embryos produced when uninfected females are mated to infected males or when females and males carry incompatible *Wolbachia* strains. Each of these reproductive alterations favours the transmission of the bacterium at the expense of the uninfected arthropod population. Elimination of *Wolbachia* through treatment of the infected hosts with the antibiotic tetracycline results in the restoration of normal reproductive phenotypes.

Wolbachia was first described by Hertig and Wolbach in the 1920s and 1930s as a microorganism infecting the ovaries of mosquitoes belonging to the *Culex pipiens* L. complex, hence the name *W. pipientis* (Hertig 1936). Polymerase chain reaction (PCR) surveys of arthropods, including insects, isopods and mites have indicated the abundance of *Wolbachia* in these organisms (Werren 1997, Werren and O'Neill 1997, Bourtzis and O'Neill 1998, Stouthamer et al. 1999, Stevens et al. 2001, Bourtzis and Miller 2003).

Sequence analysis of the 16S rRNA gene

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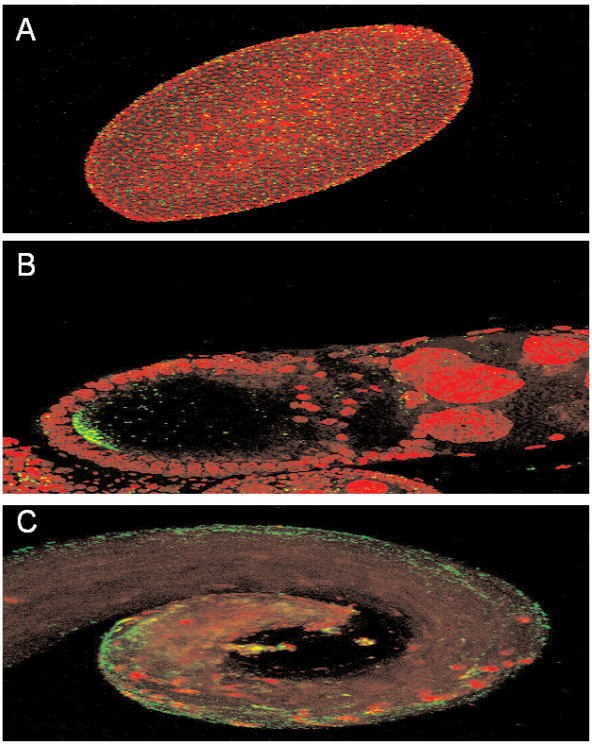

Figure 1. *Presence of* Wolbachia *in infected* Drosophila melanogaster, *(a) embryo, (b) ovary and (c) testes. Bacteria are visualized green-yellow and* Drosophila *nuclei red (Photos by Zoe Veneti and Kostas Bourtzis).*

has shown that *Wolbachia* belong to the alpha-2 subdivision of the Proteobacteria, forming a monophyletic group closely related to intracellular bacteria of the genera Anaplasma, Cowdria, Ehrlichia and Rickettsia (Breeuwer et al. 1992, O'Neill et al. 1992, Rousset et al. 1992). Many members of these genera are arthropod-borne pathogens of mammals. The phylogenies of *Wolbachia* so far generated have shown the existence of six major clades (A-F), which have been named "supergroups" (Lo et al. 2002, and references therein). Supergroups A and B include most of the parasitic *Wolbachia* found to date in arthropods. Supergroups C and D include the majority of the *Wolbachia* found in filarial nematodes. The E supergroup encompasses *Wolbachia* from primitive wing-less insects, the springtails (Collembola). Supergroup F is so far known to infect termites and the filarial parasite *Mansonella ozzardi* (Manson) (Casiraghi et al. 2001). More recently the existence of a new supergroup, named G, encompassing *Wolbachia* from some Australian spiders has been proposed (Rowley et al. 2004).

The mechanism(s) through which *Wolbachia* infects a new species in nature is not yet known. However, *Wolbachia* with a feminizing effect have been successfully transferred by simple haemolymph contact between closely related terrestrial isopod species. This suggests a natural route for inter-individual transfers (Rigaud and Jucqault 1995, Bouchon et al. 1998). Successful *Wolbachia* transfers between different insect species have also been performed. Micro-injection experiments were used to transfer cytoplasmic- and parthenogenesis-inducing

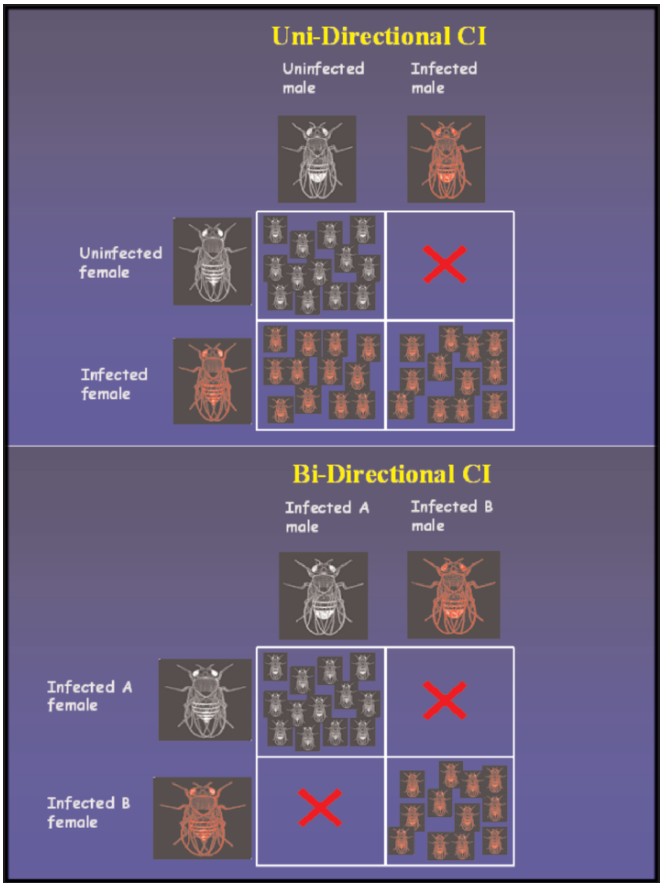

Figure 2. *Schematic representation of cytoplasmic incompatibility, (upper) unidirectional cytoplasmic incompatibility, and (lower) bidirectional cytoplasmic incompatibility (Drawing by Zoe Veneti and Kostas Bourtzis).*

Wolbachia strains between closely and distantly related species. Successful transinfection was followed by the expression of bacterial-induced reproductive phenotypes in the new hosts (Boyle et al. 1993, Braig et al. 1994, Chang and Wade 1994, Rousset and de Stordeur 1994, Giordano et al. 1995, Clancy and Hoffmann 1997, Grenier et al. 1998, Poinsot et al. 1998, Rousset et al. 1999, Riegler et al. 2004, Zabalou et al. 2004a,b). Very closely related *Wolbachia* strains have been found to infect some parasitoid wasps and the insects that they parasitize, which suggests another potential route for horizontal transfer (Werren et al. 1995). In addition, Huigens et al. (2000) reported evidence for horizontal transfer of parthenogenesis-inducing *Wolbachia* under natural conditions. When infected and originally uninfected *Trichogramma kaykai* (Perkins) larvae share a host egg, approximately 40% of the female offspring of the uninfected line acquire the infection and produce some daughters from unfertilized eggs. In subsequent generations, complete (100%) transmission of, and parthenogenesis induction by, *Wolbachia* was observed.

Despite the widespread distribution of *Wolbachia*, many important agricultural pests (e.g. *Ceratitis capitata* (Wiedemann) and

Bactrocera oleae (Gmelin)) and disease vectors (e.g. *Aedes aegypti* (L.) and *Anopheles gambiae* Giles) are not infected.

In this paper, the possible use of cytoplasmic incompatibility as a means for the biological control of insect pests is described, emphasizing the mechanism of *Wolbachia*-induced incompatibility.

2. *Wolbachia*-Induced Cytoplasmic Incompatibility

The phenomenon of cytoplasmic incompatibility was associated with the presence of *Wolbachia* in the 1970s (Yen and Barr 1971, 1973). Cytoplasmic incompatibility results in embryonic mortality in crosses between insects with different *Wolbachia* infection status. It can be either uni- or bidirectional (Fig. 2). Unidirectional cytoplasmic incompatibility is typically expressed when an infected male is crossed with an uninfected female. The reciprocal cross (infected female and uninfected male) is fully compatible, as are crosses between infected individuals. Bidirectional cytoplasmic incompatibility occurs in crosses between infected individuals harbouring different strains of *Wolbachia* strains, that is, strains with different modification and rescue properties. In most insects, the expression of cytoplasmic incompatibility is lethal to the developing embryo. In insects with haplodiploid sex determination (Hymenoptera), the result of cytoplasmic incompatibility is a sex ratio shift to the haploid sex, which is usually male.

Cytoplasmic incompatibility has been documented in diverse insect taxa including Coleoptera, Diptera, Hemiptera, Orthoptera, Hymenoptera, and Lepidoptera, as well as in the terrestrial isopod *Porcellio dilatatus* Brandt & Ratzeburg and in mites (Werren and O'Neill 1997). The host nuclear genome, the age of the male, repeated copulation of males and several environmental factors such as temperature, antibiotics, nutrition and larval density greatly influence the strength of the cytoplasmic incompatibility phenotype (Bourtzis et al. 2003). A correlation between *Wolbachia* density and the level of incompatibility has been demonstrated in several systems (Bourtzis et al. 2003).

The mechanism(s) by which *Wolbachia* causes cytoplasmic incompatibility have not yet been identified. However, a number of cytogenetic studies have described the events that take place during and shortly after fertilization in incompatible crosses. These studies described developmental defects and aberrant DNA structures in incompatible crosses as early as the first mitotic division, demonstrated that the paternal chromosome decondensation is delayed leading to improper paternal chromatin inheritance and to the production of embryos with aneuploid or haploid nuclei, and allowed observation of the direct interaction between *Wolbachia* and astral microtubules (Jost 1970, O'Neill and Karr 1990, Callaini et al. 1994, Kose and Karr 1995, Lassy and Karr 1996, Callaini et al. 1996, 1997). In a recent and very elegant study in Nasonia, Tram and Sullivan (2002) used real-time imaging and indirect immunofluorescence to visualize early developmental events leading to the expression of cytoplasmic incompatibility and consequent egg lethality and concluded that *Wolbachia* affects the timing of nuclear envelope breakdown prior to the crucial first gonomeric division. These and previous results showed convincingly that *Wolbachia* somehow modifies the paternal chromosomes during spermatogenesis (mature sperm do not contain the bacteria) thus influencing their fate during the first mitotic divisions and resulting in loss of mitotic synchrony.

Based on the genetic and cytogenetic data, Werren (1997) proposed the so-called modification/rescue model, which assumes the presence of two distinct bacterial functions. First, the modification function, a kind of "imprinting" effect, which acts in the male germ-line, probably during spermatogenesis, and second, the rescue function, which acts in the egg. Sperm imprinting may be due either to secreted *Wolbachia* protein(s) that modify the paternal chromosomes or to the removal of host protein(s) that are necessary for proper condensation/decondensation of the paternal chro-

mosomal set before and/or during zygote formation. Similarly, the presence of the same *Wolbachia* strain in the egg may result in the production and secretion of (a) rescue factor(s), or alternatively the recruitment of host molecules which are capable of rescuing the sperm "imprint" in a *Wolbachia* strain-specific manner.

Recently the genome sequence of two *Wolbachia* strains was reported: the first, *w*Mel, belongs to the *Wolbachia* strain infecting *Drosophila melanogaster* Meigen (Wu et al. 2004); the second, *w*Bm, belongs to the *Wolbachia* strain infecting the filarial nematode *Brugia malayi* (Brug) (Foster et al. 2005). The available genomic information provides the necessary tools to undertake comparative post-genomics approaches towards the identification of the genes involved in *Wolbachia*-host interactions thus deciphering the biology of this unculturable bacterium including the mechanism of cytoplasmic incompatibility, understanding *Wolbachia*-host symbiotic associations and uncovering the evolution of intracellular symbiosis.

3. Cytoplasmic Incompatibility-Inducing *Wolbachia* and Applications

Wolbachia has been suggested as a potential tool for the development of novel, environment-friendly strategies for the control of arthropod species that are major agricultural pests or disease vectors to humans, plants, and livestock or for improving beneficial species (Beard et al. 1993, Bourtzis and O'Neill 1998, Bourtzis and Braig 1999). Below is an outline of the potential applications for cytoplasmic incompatibility-inducing strains of *Wolbachia*.

3.1. Release of Infected Sterile Males

Wolbachia-induced cytoplasmic incompatibility might be used to suppress natural populations of arthropod pests in a way analogous to the sterile insect technique (SIT). The SIT involves mass-production and release of irradiated sterile insects and is one of the methods used within area-wide integrated pest management (AW-IPM) programmes for the control of insect pests (Dyck et al. 2005). Cytoplasmic incompatibility provides an alternative method to produce non-irradiated sterile males that may have improved competitiveness and so improve the efficiency of the SIT.

Recently the transfer of *Wolbachia* strains from the European cherry fruit fly *Rhagoletis cerasi* L. (Riegler and Stauffer 2002) led to stable infections in the Mediterranean fruit fly, following embryonic injection (Zabalou et al. 2004a). Austrian and Sicilian populations of *R. cerasi* were used as donors carrying different combinations of four *Wolbachia* variants (Riegler and Stauffer 2002, Zabalou et al. 2004a, M. Riegler and C. Stauffer, unpublished, cited in Zabalou et al. 2004a). Two out of initially eleven positive transinfected isofemale lines remained positive for the presence of *Wolbachia*, namely WolMed 88.6 (single infection with *w*Cer2) and WolMed S10.3 (single infection with *w*Cer4). At the time of writing, 47 generations (about 41 months) post-infection, both lines are stably infected with infection rates of 100%.

Test crosses were performed in different generations post-injection between transinfected lines and the parental uninfected Mediterranean fruit fly strains. All crossing experiments showed the same results: crosses between uninfected females and *Wolbachia*-infected males resulted in 100% egg mortality. Similar results were obtained in test crosses performed three years post-injection (unpublished). It has to be noted that complete cytoplasmic incompatibility has only been observed in very few *Wolbachia*-infected species such as *C. pipiens* (Laven 1967). This was the first report that a newly transinfected host species shows high stability of the infection and, at the same time, expresses 100% cytoplasmic incompatibility (unidirectional and bidirectional).

Laboratory cage populations of Mediterranean fruit flies containing different ratios of transinfected males:uninfected males:uninfected females were set up to deter-

mine whether cytoplasmic incompatibility expressed by the *Wolbachia*-infected lines could be used for population suppression. The caged Mediterranean fruit fly populations were suppressed by these single "releases" of incompatible males in a ratio-dependent manner. Population suppression was extremely efficient reaching levels greater than 99% at transinfected males:uninfected male release ratios of 50:1. Although these laboratory experiments are very encouraging they need to be extended to field cage systems where wild flies are used as the target population.

For effective *Wolbachia*-based population suppression, an efficient (100% effective) genetic sexing system producing only males is necessary and there are intensive efforts ongoing using both genetic and molecular approaches to develop such sexing systems in a variety of pest species. However as yet, none of the systems so far tested would meet the requirements needed in order to exclude the last female from the release males. Given this requirement and the numbers of insects that need to be released it is unlikely that an operational fail-safe sexing system can be developed. However using a sexing system such as that currently being used for the Mediterranean fruit fly (Franz 2005), several strategies could be considered. One solution may be to have two bidirectionally incompatible infected strains where males from the two strains are released alternately, so that even if an infected female of the one strain is released then in the next generation her offspring will most likely mate with males infected with the incompatible sperm. An alternative solution may be to combine radiation with incompatibility where the contaminating females can be sterilized with lower doses of radiation than males. In this way the released males could be more competitive as they will receive a lower dose of radiation. In tephritid species, females are sterilized by lower doses of radiation than males (Bakri et al. 2005).

Cytoplasmic incompatibility has been used in the past to introduce sterility into wild populations of mosquitoes. Indeed, several trials sponsored by the World Health Organization, were undertaken in the mid 1960s in Burma and India to eradicate the filariasis vector species *C. pipiens* and *Culex quinquefasciatus* Say. By mass-rearing and then releasing males that were incompatible with the target population, it was possible to effectively sterilize wild females. In one field trial, mosquitoes were completely eradicated from a Burmese village (Laven 1967). Also, in the 1970s, an international collaborative project took place in Central Europe, which evaluated cytoplasmic incompatibility as a method to control the European cherry fruit fly *R. cerasi*. Several successful field trials were performed, but for a number of reasons, this project was never completed (Blümel and Russ 1989, Boller 1989). In addition to these field experiments, a number of laboratory and warehouse experiments in the USA have successfully applied *Wolbachia*-induced cytoplasmic incompatibility as a means to control the stored product pest, the almond moth *Cadra* (*Ephestia*) *cautella* Walker (Brower 1978, 1979, 1980).

3.2. Release of Infected Fertile Male and Female Insects to Spread Wolbachia *in a Natural Pest Population*

Wolbachia-induced cytoplasmic incompatibility might be used as a mechanism to spread desirable genotypes into field populations (Turelli and Hoffmann 1991, Hoshizaki and Shimada 1995, Sinkins et al. 1995, Hoshizaki 1997, Rousset et al. 1999). The identification of the *Wolbachia* genes responsible for cytoplasmic incompatibility should allow the introduction of these genes into the host nuclear genome and the induction of cytoplasmic incompatibility without the presence of *Wolbachia*. Theoretical models suggest that nuclear-coded cytoplasmic incompatibility genes will lead to a spread of their host, replacing target naïve populations along with any other chromosomally-linked gene(s) (Sinkins et al. 1997, Curtis and Sinkins 1998, Sinkins and Godfray 2004).

Recently, Xi and colleagues reported the transfer of a *w*AlbB *Wolbachia* strain naturally occurring in *Aedes albopictus* Skuse, and

its establishment in *Ae. aegypti*, a naïve host (Xi et al. 2005). Crossing experiments indicated strong cytoplasmic incompatibility (100%): no egg hatch observed from more than 3800 eggs examined from crosses of uninfected females and *Wolbachia*-infected males. Laboratory cage tests demonstrated that *Wolbachia* can be spread into a targeted uninfected *Ae. aegypti* population, reaching infection fixation within seven generations. This is the second report that a newly transinfected host species shows high stability of the infection and, at the same time, expresses 100% cytoplasmic incompatibility. In addition, these data clearly indicated that *Wolbachia* can be used as a vehicle to drive transgenes into mosquito populations, and maybe other disease vector populations of medical importance.

3.3. Release of Male and Female Insects to Spread Paratransgenic Wolbachia

Wolbachia might be also used as an expression vector in paratransgenesis strategies. Paratransgenesis is a method that uses symbiotic bacteria as vehicles for the introduction and expression of genes of interest into a target arthropod species and has been suggested as an alternative approach for the genetic manipulation of arthropods (Beard et al. 1993, Ashburner et al. 1998). Symbiotic bacteria of arthopod species have already been used as expression vehicles (Durvasula et al. 1997, Cheng and Aksoy 1999) and a paratransformation approach is currently being evaluated for field releases of *Rhodnius prolixus* (Stål) aiming to reduce the prevalence of the causative agent of Chagas' disease *Trypanosoma cruzi* Chagas (Durvasula et al. 1997). The main obstacle to using *Wolbachia* in paratransgenesis approaches is that it cannot be cultured in a cell-free system, and a genetic transformation system is not yet available. The fact that these bacteria can now be maintained in different insect cell lines (O'Neill et al. 1997, Dobson et al. 2002) coupled with the recent isolation and characterization of endogenous phages and insertion sequences (Masui et al. 2000, 2001, Fujii et al. 2004), will certainly facilitate current efforts to genetically engineer *Wolbachia*.

4. Conclusions

Wolbachia-based applications may be broad since these bacteria are present in a wide range of arthropod species and can also be transferred into naïve hosts. It is possible that the ability of these bacteria to establish new infections and persist in their hosts for a long time may have to do with their ability to "escape" the host's innate immune system (Bourtzis et al. 2000). However, and despite the potential demonstrated in the above-mentioned earlier trials, there has been no consistent experimental follow-up with the exception of several review papers (Sinkins et al. 1997, Bourtzis and Braig 1999, Sinkins and O'Neill, 2000, Aksoy et al. 2001, Bourtzis and Robinson 2006). Therefore it remains to be demonstrated whether *Wolbachia*-based technologies will be used in the field and ever replace and/or complement existing operational AW-IPM programmes.

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Symbiosis-Based Technological Advances to Improve Tsetse *Glossina* spp. SIT Application

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ABSTRACT Tsetse flies, *Glossina* spp. are the sole vectors of the parasitic African trypanosomes, which cause devastating diseases in humans and animals. The sterile insect technique (SIT) is one pest control tool that, when integrated on an area-wide basis, is highly effective against tsetse populations. Several molecular techniques have the potential to enhance the application of this approach. In particular, the ability to engineer refractoriness into released strains would enhance the efficacy of this approach, especially in human disease endemic areas. In addition, natural mating incompatibilities between some populations could be exploited to enhance the fitness of released males, as the irradiation dose could be reduced to that required for female sterilization without compromising overall male sterility. The viviparous reproductive nature of tsetse flies has made direct germ-line transformation impossible. However, the symbiotic microorganism *Sodalis glossinidius* that lives in tsetse midgut tissue can be cultured and transformed. Because these symbionts live in close proximity to where parasites differentiate and replicate, gene products expressed and secreted by these microbes could have immediate impact. Fly midguts have been successfully repopulated with symbionts engineered to express foreign gene products. The complete genome sequence of *S. glossinidius* together with information on its population dynamics during fly development is now available. Gene expression experiments currently in progress now aid in identifying an expression system that does not reduce the fitness of engineered flies. This is done by making use of midgut-specific promoters. In addition to midgut symbionts, various field populations of tsetse harbour *Wolbachia* spp. In many insect species the presence of *Wolbachia* induces cytoplasmic incompatibility, a phenomenon that causes reproductive incompatibilities between infected and uninfected insects. To understand the impact of *Wolbachia* infections on tsetse, lines with and without *Wolbachia* should be developed for formal mating experiments. Field populations are heterogeneous for the presence/absence of the bacteria, and it may be possible to develop such lines directly from the field to evaluate the potential role of cytoplasmic incompatibility. In an attempt to identify such field populations, information was obtained on *Wolbachia* infections in *Glossina fuscipes fuscipes* Newstead.

KEY WORDS symbionts, refractoriness, tsetse flies, paratransgenesis, cytoplasmic incompatibility, *Wolbachia*, *Sodalis*, *Wigglesworthia*, population replacement

1. Introduction

Tsetse flies are the sole vectors of cyclical pathogenic trypanosomes in tropical Africa. Human African trypanosomosis, or sleeping sickness, is a zoonosis caused by the flagellated protozoa *Trypanosoma brucei rhodesiense* Stephen and Fantham in East and Southern Africa and *Trypanosoma brucei gambiense* Dutton in West and Central Africa. The World Health Organization (WHO) conservatively estimates that there are currently 300 000-500 000 cases of human African trypanosomosis, with 60 million people at risk in 37 countries covering approximately 40% of Africa.

During a devastating epidemic in the early 20th century, approximately one million people died of human African trypanosomosis. Following several decades of intensive surveillance, screening and treatment of patients combined with selective tsetse control efforts, the disease almost disappeared from Africa by the 1960s. However, there is presently another wave of human African trypanosomosis

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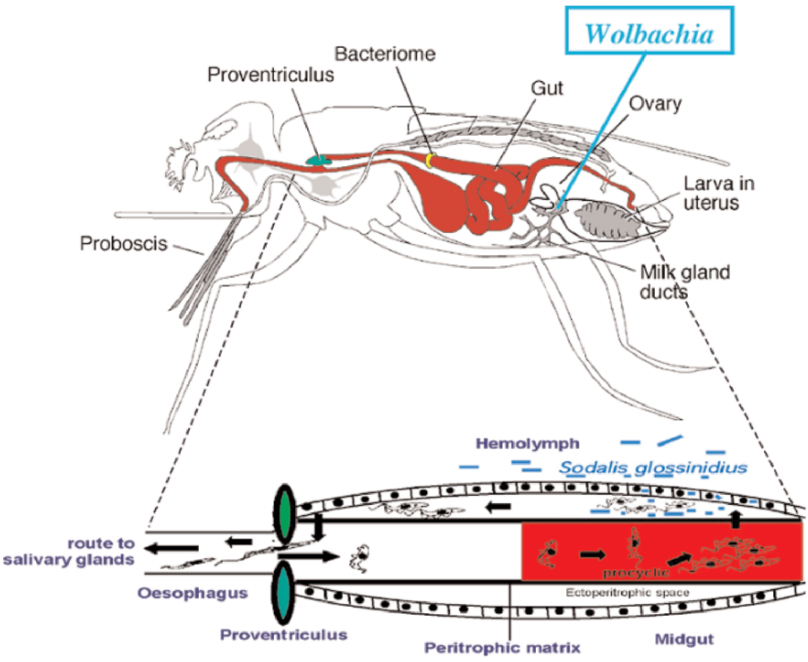

Figure 1. *Diagramme showing the digestive and internal reproductive anatomy of tsetse (modified from Rio et al. 2004).* Wolbachia *reside exclusively within reproductive tissues, while* Sodalis *and* Wigglesworthia *are associated with digestive tissues and hemolymph (the latter only being inhabited by* Sodalis*).*

epidemics, with a disease burden of 2.05 million disability-adjusted life years (Ekwanzala et al. 1996, van Hove 1996, Moore et al. 1999). Countries in Central Africa, especially Angola, the Democratic Republic of Congo, and Southern Sudan have been hardest hit, while Uganda is under threat with sporadic infections reported. The rate of new infections and mortality (55 000 deaths in 1993, 66 000 in 1999) shows no sign of decline. The collapse of health infrastructures and surveillance systems (Simarro et al. 2003), allied to the displacement of populations by war and natural disasters are important contributory factors to the present epidemic. Given that human African trypanosomosis affects often war-torn hard-to-reach rural populations that lack active surveillance, disease prevalence is generally considered to be grossly underestimated and the consensus view is that the situation may worsen (Smith et al. 1998, Barrett 1999, Stich et al. 2003).

In addition to their impact on human health, trypanosomes cause a wasting and fatal disease known as African animal trypanosomosis or nagana in cattle, domestic pigs and other farm animals. Nagana, caused by the related parasites, *Trypanosoma brucei brucei* Pimmer and Bradford, *Trypanosoma congolense* Broden and *Trypanosoma vivax* Ziemann, has restricted agricultural development and food production in sub-Saharan Africa, profoundly impacting the economy of much of the continent (Steelman 1976, Jordan 1986).

The prevalence of trypanosomosis relies on four interacting groups of organisms: (1) the human hosts, (2) the insect vectors, (3) the

pathogenic parasites, and (4) the domestic and wild animal reservoirs. While complex, this dependence on multiple players provides several opportunities for intervention since interruption of any of these interactions can potentially reduce disease transmission. Despite extensive research on the development of human and cattle anti-trypanosome vaccines, antigenic variation of the surface glycoproteins of the parasite within the mammalian host has hampered most efforts. Current management of human African trypanosomosis therefore still relies on active surveillance and treatment of infected patients along with extensive support from international organizations. These efforts have been constrained by the lack of inexpensive, easy to administer and effective drugs (Butler 2003, Docampo and Moreno 2003). In addition, the efficacy of available drugs is impaired due to increasing resistance detected in parasite populations (Anene et al. 2001, Geerts et al. 2001).

2. Tsetse Flies: Vectors of African Trypanosomes

Vector control at the local level does not play a primary role in the control of human African trypanosomosis (Gibson et al. 1978, Scott et al. 1983, Noireau et al. 1986, Fevre et al. 2001). This situation exists because the most widly used vector control strategies, such as the deployment of insecticide-impregnated targets and traps, rely on the participation of the village communities and tend not to be sustainable in the long-term. While the projects tend to start with enthusiasm, the maintenance of traps and targets dwindles over time as fly numbers decrease, inevitably resulting in the resurgence of vector populations.

For control of animal diseases, the availability of more effective trapping systems, coupled with strong economic interest, has provided the impetus for vector control programmes. The use of an area-wide integrated pest management (AW-IPM) strategy with a sterile insect technique (SIT) component is effective for tsetse, and following a successful programme on the Island of Unguja, Zanzibar (Vreysen et al. 2000), its application on the mainland is currently being pursued (Alemu et al., this volume, Kappmeier et al., this volume).

This article describes transgenic technologies that have the potential to improve the SIT implementation. It outlines current work in the field of tsetse transgenics, a powerful tool for use in engineering refractoriness traits into released flies. Also discussed are the potential naturally present mating incompatibilities conferred by *Wolbachia* infections. This phenomenon may allow the use of reduced radiation doses without sacrificing male sterility resulting in the release of more competitive flies.

3. Symbiosis in Tsetse

Tsetse flies harbour three distinct endosymbionts (Fig. 1), *Sodalis glossinidius* sp. nov. (Dale and Maudlin 1999), *Wigglesworthia glossinidia* sp. nov. (Aksoy 1995) and, in some populations, *Wolbachia* spp. (O'Neill et al. 1993, Cheng et al. 2000). *Sodalis* and *Wigglesworthia*, which both reside in the gut tissue of tsetse, are transmitted to tsetse's intrauterine larva through maternal milk gland secretions. Comparative phylogenetic analysis of *Wigglesworthia* with other insect obligate symbionts, such as *Buchnera* from aphids and *Blochmannia* from carpenter ants, indicates that they are descendents of free-living gamma-Proteobacteria (Chen et al. 1999, Aksoy 2000). *Sodalis* is a close relative of free-living microbes such as *Escherichia coli* (Migula) Castellani and Chalmers, *Salmonella* spp. and *Yersinia* spp. (Rio et al. 2004). *Wolbachia*, which is a member of the alpha-Proteobacteria (O'Neill et al. 1993, Cheng et al. 2000) is transovarially transmitted.

The relationship between *Wigglesworthia* and tsetse is ancient (some 50-80 million years old), as evidenced by the concordant evolution that members of the genus now display with their tsetse host species (Chen et al. 1999). As a consequence of its strict intracellular sequestration within specialized host

cells (bacteriocytes), the genome of *Wigglesworthia* has undergone a drastic size reduction to about 700 kilo bases and codes for only 621 predicted protein products (Akman and Aksoy 2001, Akman et al. 2002). However, despite its restricted functional capabilities, *Wigglesworthia*'s chromosome has retained 62 genes whose products are involved in the biosynthesis of cofactors, prosthetic groups and carriers. This symbiont has the potential to synthesize biotin, thiazole, lipoic acid, flavin adenine dinucleotide (FAD) (riboflavin, vitamin B_2), folate, pantothenate, thiamine (vitamin B_1), pyridoxine (vitamin B_6), protohaeme, and nicotinamide. These capabilities suggest that an important function of the *Wigglesworthia* symbiosis is to supplement essential vitamin metabolites to tsetse's single diet of vertebrate blood (Nogge 1981). It is now possible to rescue tsetse fertility in symbiont-free flies (fed antibiotics) by feeding them a cocktail containing vitamin products encoded on *Wigglesworthia'*s chromosome (unpublished).

Sodalis has a wider tissue tropism than *Wigglesworthia*, and is harboured both intra- and extra-cellularly, principally in the midgut tissue, but also in haemolymph and milk gland tissues (Cheng and Aksoy 1999). The genome of *Sodalis* has recently been sequenced and annotated (Toh et al. 2006). Although its 4 171 146 base pair chromosome is significantly larger than that of *Wigglesworthia*, it has only 2299 protein-coding sequences and a reduced coding capacity of 49%. Furthermore, over 20% of its genome encodes pseudogenes, a high proportion of which function in defence, and in carbohydrate and inorganic ion transport and metabolism. These functional erosions may reflect *Sodalis*' ongoing degenerative adaptations to its hosts immune environment and single nutrient source, vertebrate blood. This massive functional erosion will curtail the organism's viability outside of its tsetse host niche. *Sodalis*' genotype ensures this symbiont is a safe candidate for use in the paratransgenic strategy described below. With its genetic blueprint now available, functional analysis of its products will provide insights into its symbiotic biology, and at the same time, provide important information to enhance an applied paratransgenic approach.

4. Genetic Transformation Systems in Insects

Many insects have been genetically transformed by micro-injecting various transposable elements (plasmid or viral vectors) into syncytial embryos (germ-line transformation) (Jasinskiene et al. 1998). These transposable elements insert themselves randomly into insect DNA, resulting in germ-line transformation whereby the transgene is passed on to every individual cell of the genetically-modified organism. Marker genes carried by the transposable element help to identify transgenic individuals. By using tissue specific expression systems, transgene products can be ectopically expressed to affect parasite viability, i.e. in the gut (Moreira et al. 2000), salivary gland or haemolymph (Kokoza et al. 2001).

The viviparous reproductive biology of tsetse has hampered the application of a similar germ-line transformation technology. However, commensal *Sodalis* can be exploited to express foreign gene products in tsetse midgut, thus affecting parasite viability (Cheng and Aksoy 1999). Using this paratransgenic approach, insect cells are not transformed as in the germ-line transformation approach, but instead transgenes are expressed in the symbiotic bacteria (Rio et al. 2004). The specific associations that the symbionts have established with host populations, their physical proximity to the developing pathogenic agents in insect tissues and the vast quantity of information available on prokaryotic transformation and gene expression mechanisms make beneficial symbionts highly desirable expression vehicles that can be exploited for the control of a variety of vector-borne diseases. The symbiont *Rhodococcus* has been similarly exploited to express foreign gene products in triatomine bugs to block *Trypanosoma cruzi* Chagas transmission

(Beard et al. 1992). This approach has much promise for control of many agricultural diseases transmitted by plant pests that also house symbiotic microbes.

Some of the requirements for a successful symbiont-based insect transformation approach are: (1) presence of naturally harboured symbionts that can be isolated and cultured, (2) knowledge of the transmission mode as well as the growth and dynamics of the symbiont throughout the host life cycle, (3) an efficient symbiont transformation system that results in stable phenotypes upon reintroduction into the host, (4) genetic manipulation that does not alter either the role or the physiological impact of the symbiont on host biology. Likewise, the fitness of the transformed symbionts and of insects repopulated with them should not be compromised in comparison to their wild-type counterparts, (5) genetically altered symbionts should not pose a threat to non-target organisms, (6) characterization of effective anti-pathogenic products such as antimicrobial peptides or transmission-blocking monoclonal antibodies, (7) expression of such transmission-blocking effector molecules in insect tissues or compartments that impede pathogen life cycle, (8) an environmentally-sound implementation project for delivering transformed insects into the field

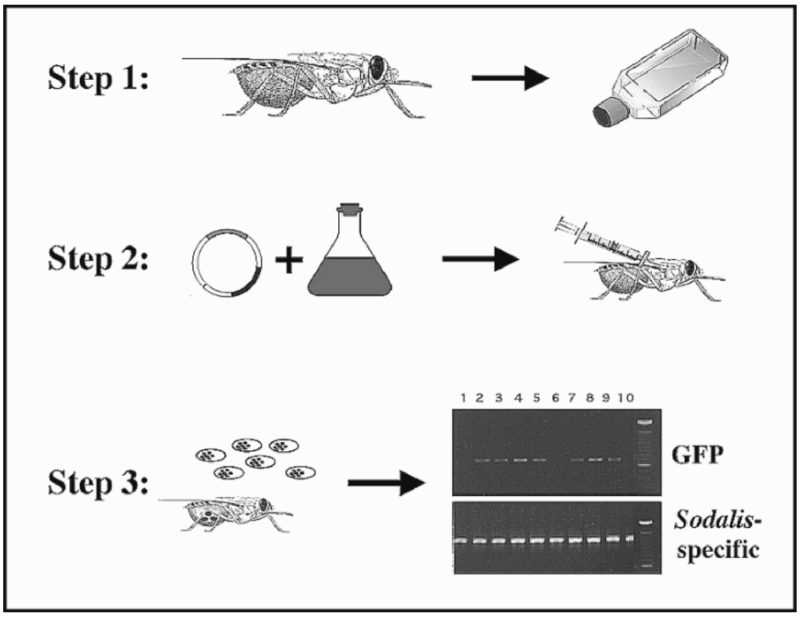

Figure 2. *Protocol used for the production of trypanosome-refractory paratransgenic tsetse flies. Step 1: a primary culture of* Sodalis *is established from the hemolymph of an adult fly. Cells are maintained* in vitro *under micro-aerophilic conditions. Step 2: cultured* Sodalis *are transformed with constructs that encode trypanocidal peptides. Recombinant* Sodalis *are then micro-injected into adult females, which are subsequently fed antibiotics and a mixture of B-vitamins. Step 3: pupae from injected mothers are collected and allowed to mature to adulthood. Foreign DNA can be detected in these offspring by polymerase chain reaction (PCR) (and in the case of green fluorescent protein (GFP), visualized under a fluorescent microscope), thus indicating successful vertical transmission of recombinant* Sodalis *from mothers to offspring.*

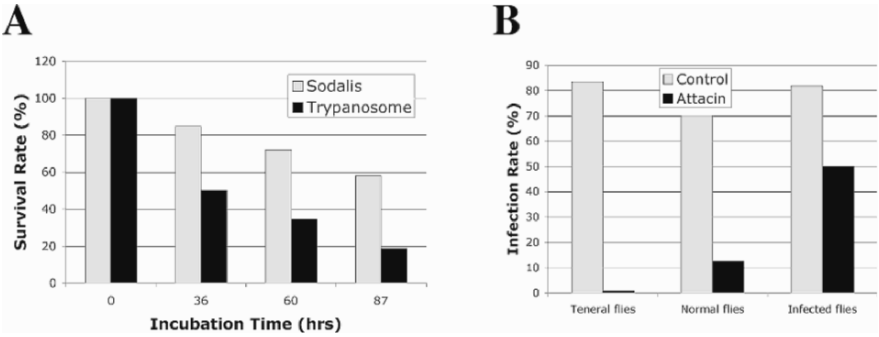

Figure 3. *Bio-activity of tsetse attacin (GmAttA1). (a)* In vitro *incubation of* Sodalis *and* T. brucei *indicates that the symbiont survives significantly longer than the parasite in the presence of GmAttA1. (b) Flies fed recombinant GmAttA1 at different times. The efficiency of inhibiting infection depends on when the flies were supplied GmAttA1.*

(effective gene-driving mechanisms), and (9) an ecological assessment of potential spread of transformed symbionts and barriers to dispersal.

4.1. Sodalis*-Based Transformation System*

Several aspects of *Sodalis'* biology make its use in a paratransgenic strategy highly desirable. Firstly, the availability of an *in vitro* culture system has enabled the development of a genetic transformation system that can introduce and express foreign products in *Sodalis* (Beard et al. 1993, Aksoy 2001, Aksoy et al. 2001, Aksoy 2003) (Fig. 2). Following transformation, recombinant *Sodalis* (rec*Sodalis*) are returned to their tsetse hosts. They are successfully acquired by subsequent generations of flies when micro-injected into haemolymph of the female parent, as determined by expression of the marker gene product, green fluorescent protein (Cheng and Aksoy 1999). Secondly, from a containment perspective, *Sodalis* is a fastidious microaerophilic endosymbiont that is difficult to maintain *in vitro*, presumably as a result of its adaptation to the symbiotic lifestyle. This organism has a low temperature optimum of 25°C and cannot grow in temperatures exceeding 30°C, eliminating the possibility of recombinant *Sodalis* strains colonizing warm-blooded hosts. *Sodalis* also has a slow growth rate both in the insect host (Aksoy, unpublished) and in laboratory culture. Thus, it would likely be a weak, perhaps unsuccessful, competitor outside of its host.

4.2. Expression of Trypanocidal Effectors in Sodalis

Because *Sodalis* lives naturally in close proximity to trypanosomes in tsetse's midgut environment, expression of trypanocidal products by this organism can potentially block parasite development. Presumably as a result of coevolution with its host immune system, *Sodalis* displays a high level of resistance to tsetse's immune arsenal. Among these immune products are the tsetse antimicrobial peptides attacin and diptericin, which are synthesized in response to microbial infections (Hao et al. 2001, Hu and Aksoy 2005). In fact, *Sodalis* demonstrates a remarkable resistance to a variety of insect-derived and non-insect antimicrobial peptides (Haines et al. 2003). Of practical value is the fact that unlike *Sodalis*, African trypanosomes are highly susceptible to the actions of these peptides, including tsetse attacin (GmAttA1) (Fig. 3).

During the course of a natural trypanosome

Table 1. *Trypanosome infection prevalence in* Glossina morsitans morsitans *after immune activation with* Escherichia coli*, lipopolysaccharide (LPS) or sterile phosphate buffered saline (PBS).*

Inoculation	Pooled[1]	Prevalence (SE)	Chi square-analysis[2]
Control	145	49.7% (5.0)	-
E. coli	99	11.1% (5.1)	$P < 0.0001$
LPS	152	27.0% (2.5)	$P < 0.0001$
PBS	106	50.0% (9.6)	$P = 0.94$

[1]Represents pooled samples from replicates

[2]The infection prevalence in control groups served as the expected data against which other groups were tested

infection, antimicrobial gene expression is induced in the proventriculus and fat body tissues. This expression continues in flies with established gut parasite infections. Many flies are able to cure their infections. However, in those few that are susceptible, either the concentration of these molecules does not reach levels high enough to eliminate the large numbers of parasites replicating in the gut, or these peptides remain mostly in the haemolymph without affecting the midgut environment where the parasites replicate. When flies are immune stimulated prior to infecting them with parasites, parasite prevalence was significantly lower because antimicrobial peptides were already being synthesized at the time of parasite acquisition (Table 1). The next step will be to see whether the constitutive expression of attacin in tsetse midgut symbionts will impede parasite development.

Table 2. Wolbachia *prevalence in several field populations of tsetse flies.*

Species	Location	Prevalence
G. f. fuscipes	Sudan	(4/8) 50%
G. f. fuscipes	Northern Uganda	(8/11) 75%
G. f. fuscipes	Southern Uganda	(4/10) 40%
G. brevipalpis	Mafia Island (Tanzania)	(4/7) 57%
G. brevipalpis	Mivumoni (Tanzania)	(0/2) 0%
G. m. centralis	Tanzania	(7/8) 88%
G. swynnertoni	Tanzania	(4/4) 100%

5. Gene-Driving Systems: *Wolbachia*-Mediated Cytoplasmic Incompatability

An important applied aspect of some transgenic approaches is the ability to spread laboratory-engineered phenotypes into natural populations. *Wolbachia*, which infects a wide range of invertebrate hosts (Werren et al. 1995), including several tsetse species, provides one potential drive mechanism. One unique consequence of *Wolbachia* infections is a condition called cytoplasmic incompatibility, which results in the death of zygotes during embryogenesis (Fig. 4). In an incompatible cross, the sperm enters the egg but does not successfully contribute its genetic material, and in most cases none or very few eggs hatch. Recently, it has been shown that *Wolbachia* can be transferred between different species and subsequently result in cytoplasmic incompatibility (Zabalou et al. 2004). *Wolbachia*-infected females have a reproductive advantage over their uninfected counterparts as they can reproduce successfully with both the infected and non-infected males. This will eventually allow the *Wolbachia*-infected insects to spread through natural populations (Sinkins et al. 1997, Sinkins and O'Neill 2000). As *Wolbachia* spreads itself into target populations, it can also drag other maternally linked traits and organelles such as mitochondria (Turelli et al. 1992). In addition, various models have been developed for other insects that describe the parameters contributing to the dynamics of cytoplasmic incompatibility-mediated spread. For example, population genetic models have been developed to explore the spread of reproductive incompatibility in *Drosophila* (Fine 1978, Turelli and Hoffmann 1991, 1995). The epidemiological impact of genetically modified symbionts that

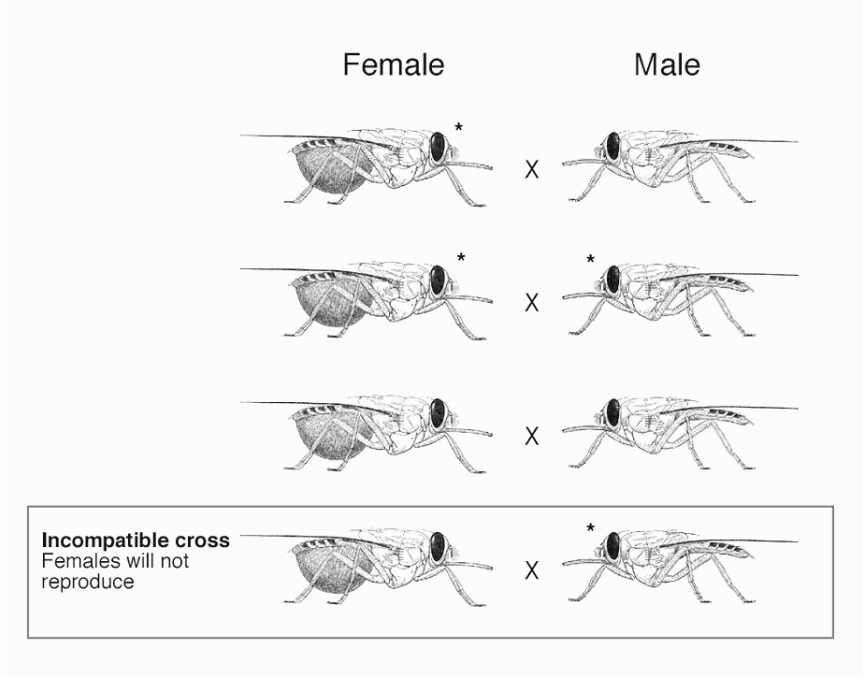

Figure 4. Wolbachia-*induced cytoplasmic incompatibility phenomenon in tsetse. Asterisks indicate* Wolbachia-*infected flies. The top three crosses result in viable offspring, while the fourth cross between an uninfected female and a* Wolbachia-*infected male does not. The reproductive advantage of* Wolbachia-*infected females drives the infected phenotype into populations.*

inhibit the transmission of trypanosomes still needs to be assessed in tsetse.

In a field survey, many tsetse populations were found to be infected by different strains of *Wolbachia* (Cheng et al. 2000). However, the extent of the cytoplasmic incompatibility phenomenon needs evaluation to assess the utility of this gene-driving system in tsetse. Analysis of laboratory colonies has shown that 100% of sampled individuals carry *Wolbachia*, making the analysis of *Wolbachia*-mediated effects impossible by traditional mating experiments (Cheng et al. 2000). The natural populations studied provide heterogeneities in *Wolbachia* infection status that should be further explored (Cheng et al. 2000). In 1999, *Wolbachia* prevalence in *Glossina brevipalpis* Newstead in South Africa was zero, while in Kenya it was 30%. In the colony maintained at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria, originally established from Kenya, infection prevalence was 100%. At the same study site in South Africa, the *Glossina austeni* Newstead population was 100% infected with *Wolbachia*, while in Kenya infection prevalence was at 50%. Analysis of the "now-extinct" *G. austeni* population from the Island of Unguja, Zanzibar (Vreysen et al. 2000) revealed none of the individuals to be infected. A recent analysis of *Wolbachia* infection prevalence in some field populations from East Africa is shown in Table 2.

Heterogeneous field populations may offer an opportunity to investigate the functional

biology of *Wolbachia* infections in tsetse by providing an ability to develop infected and uninfected lines from the field to subsequently evaluate cytoplasmic incompatibility. While no naturally-occurring horizontal transfer of *Wolbachia* has been observed, experimental transfer of this bacterium between different hosts, and even into insects with no prior infection history, is increasingly common (Sinkins et al. 1997). In addition, different *Wolbachia* strains confer mating incompatibilities among these infected insects (bidirectional incompatibility). The ability to introduce different strains of *Wolbachia* into insects makes possible the induction of multiple spreads, should the transgene ever be separated from its driver.

6. Conclusions and Future Directions

Knowledge accumulated on tsetse and its symbionts has the potential to provide unique disease management opportunities based on the control of parasite development in its natural vector. Of utmost importance is expanding the repertoire of trypanosome inhibitory products that can be expressed in tsetse's midgut using this symbiont expression system. Evaluation of the stability of introduced phenotypes is critical from an applied perspective. An aspect of transformation currently being followed involves using homologous recombination to incorporate foreign genes directly into the symbiont chromosome, thus ensuring additional linkage between the introduced transgene and *Sodalis*. Using the available genome sequence information, adult midgut-specific promoters are being identified. With these driving the constructs, genes would be preferentially expressed only at this host life stage, thus minimizing potential fitness costs of the transgene on the host. The ecological parameters that influence *Sodalis* density dynamics in tsetse biology need to be explored in field experiments. Similarly, cytoplasmic incompatibility needs to be further studied in field populations and laboratory experiments carried out to confirm the cytoplasmic incompatibility phenotype. To this end, the extensive heterogeneities in *Glossina fuscipes fuscipes* Newstead populations may provide such an opportunity.

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Colony Maintenance and Mass-Rearing: Using Cold Storage Technology for Extending the Shelf-Life of Insects

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ABSTRACT Implementation of area-wide pest control programmes using the sterile insect technique (SIT) is fundamentally dependant on the ability to rear large numbers of insects and to precisely release them, often at some distance from the production site. This process of producing purely biological agents for pest control frequently demands that periods of low temperature are utilized to store, stockpile or immobilize the insects to maintain quality and gain economy and effectiveness. Likewise, rearing and maintenance of often numerous laboratory colonies for the purpose of conducting research to develop and improve SIT programmes can also benefit from the use of this technology. Two approaches that can be used to maintain quality and to extend the utility of insects are cryopreservation and dormancy. Using either of these methods to extend the shelf-life of mass-reared or laboratory-cultured insects requires that they closely conform to the physiological and developmental capabilities and characteristics of a particular species. The technical aspects of this conformity are discussed, along with the advantages of using these two approaches for extending insect shelf-life. Both approaches have specific requirements for employment and both yield benefits relating to short- or long-term storage needs.

KEY WORDS cryopreservation, diapause, quiescence, shelf-life, dormancy, mass-rearing, *in vitro* fertilization

1. Introduction

Large financial and logistical commitments to insect rearing are being made on an international level such as the efforts involving extensive use of the sterile insect technique (SIT) within the framework of area-wide integrated pest management (AW-IPM) programmes supported by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA), the Animal and Plant Protection Service of the United Sates Department of Agriculture (USDA-APHIS) and the agricultural agencies of various countries around the world. For example, the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) mass-rearing and sterilization facility at El Piño, Guatemala has a production of over 2500 million sterile male flies per week, and the New World screwworm *Cochliomyia hominivorax* (Coquerel) facility in Tuxtla Gutiérrez, Mexico has the potential of producing 500 million sterile insects per week. While 60% of the type of rearing facilities like those operating in Guatemala and Mexico are found in the Americas, others have been established in Asia, Europe, Africa, and Australia (Hendrichs 2000). Mahon and Leopold (2002) have illustrated how AW-IPM programmes with an SIT component can benefit by having the technology to store insects for varying lengths of time. The establishment of a colony for mass-rearing purposes, maintenance of a variety of mutant lines for development of strains having specific qualities or uses, and banking of an array of geographic isolates for compatibility assessment of the

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mass-reared colony are examples of the endeavours that can be facilitated by having long-term storage capability. Further, situations involving potential hazards to maintenance of quality insect production such as genetic drift, disease, work stoppages and mechanical failure can be averted or alleviated with a variety of short- and long-term storage techniques that have been developed over the past 75 years (Leopold 1998).

As with AW-IPM programmes that integrate the SIT, the investments made for mass-rearing of insects as traditional biological control agents are also considerable. Hunter (1997) identified 142 North American commercial suppliers of 127 species of insects and mites for use in biological control of plant and insect pests in the greenhouse and in the field. The use of insects as bio-pesticides in the form of parasites, predators and phytophages is well established throughout the world, and the benefits of storage technology in production and release of biological control agents was recognized early last century by Flanders (1930) when rearing and releasing braconid wasps *Trichogramma* spp. for control of the codling moth *Cydia pomonella* (L.). The importance of having storage techniques to accumulate, store and ship beneficial insects for use in an IPM programme parallels that of SIT operations, especially when commercial entities are compelled to maintain the production of insects as a profitable enterprise.

The activity of rearing and maintaining colonies of quality insects under laboratory conditions for research purposes forms the foundation for development and testing of all potential pest control programmes. Accordingly, it is well known that maintenance of research colonies remains a necessary, but often costly, endeavour. With the advent of new technologies available to researchers to genetically transform non-drosophilid species (O'Brochta and Atkinson 2004), along with the advancing genomic sequencing of representative insects (Evans and Gunderson-Rindal 2003), the number of insect strains being created in the laboratory with unique characteristics will rapidly escalate. Strain maintenance will surely consume increasingly larger portions of research budgets unless storage methods are used for archiving the burgeoning array of mutant and transgenic strains as well as an increasing number of model insect species.

While the intent of this report is to raise awareness of the possibilities that cold storage presents for increasing the shelf-life of insects used as research models and in control programmes, the use of this technology is not limited to just facilitating the rearing of insects for these purposes. There are myriad of disciplines and enterprises including rearing insects for pet food, fish bait, forensic indicators, and the rescue of endangered species that can benefit by the incorporation of storage technology into the rearing and handling procedures. Therefore, it is hoped that the following information will provide a ready access to a basic description of the methodology and illustrate the potential that cryopreservation and dormancy induction offer for saving time, money and genetic resources.

2. Cryopreservation Techniques

Cryopreservation typically means storing or preserving cells, tissues and organisms in a cryogen such as liquid nitrogen. At very low subzero temperatures, such as that of liquid nitrogen (-196°C), all known cell processes are held in abeyance and storage time can be an indefinitely long period of time. To render biological systems cryopreservable, there must be a strict management of intra- and extracellular water. There are two basic techniques for accomplishing water management prior to liquid nitrogen storage and they are known as equilibrium freezing and vitrification. The following two subsections identify the major features of the techniques that are used for long-term storage of biological systems.

2.1. Equilibrium Freezing

Storage of cells, tissues and whole organisms at liquid nitrogen temperature generally

requires reduction of intracellular water to a negligible amount to avoid physical damage to the cells caused by the formation of ice crystals upon freezing. Water removal is accomplished by osmotic dehydration, usually in the presence of a controlled slowly falling temperature. Removal of intracellular water produces a potentially harmful situation by concentrating the remaining solutes in the cells. This situation is usually managed by the addition of a chemical cryoprotectant such as dimethyl sulphoxide, ethylene glycol or glycerol to alleviate the loss of water and protect the cells during the dehydration and freezing process. Conventional cryopreservation protocols balance the egress of water, the influx of cryoprotectant and avoid the occurrence of potentially lethal spontaneous ice crystal formation within cells by strict regulation of the cooling rate. Slowly lowering the temperature initiates extracellular freezing which, in turn, causes an osmotic disequilibrium between the external medium and the inside of the cells. Under these conditions, water flows out of the super-cooled cells into the external medium. Further, with most conventional equilibrium freezing protocols, the optimal cooling rate for a particular type of cell or organism proceeds at a rate that maintains a near vapour-phase equilibrium between the external residual liquid and the intracellular water (Mazur 1984).

There are various interconnected factors such as membrane permeability, surface to volume ratio, cooling rate and solute concentration in the external medium, which affect the efflux of water in the presence of a falling temperature (Mazur 1979, Farrant 1980). Cooling too rapidly when using the equilibrium freezing method before placement into liquid nitrogen can be lethal to cells by the induction of spontaneous intracellular freezing. Suboptimal cooling rates also produce irreversible damage in the form of shrinkage past a critical cell volume (Meryman 1974) and/or severe cell deformation caused by a low amount of residual water in the external unfrozen fraction (Mazur 1984). A number of cooling methods, besides simple rate variation, have been designed to accommodate the interconnected factors that affect cell dehydration. They include stepwise cooling rates directed to equilibrate water/cryoprotectant exchange, manual seeding to promote extracellular freezing and decrease intracellular ice formation, and quenching in liquid nitrogen at various subzero temperatures to terminate supercooling (Schiewe et al. 1991, Fahning and Garcia 1992, Trad et al. 1999).

Correspondingly, the rate of thawing cells and organisms during the recovery process from liquid nitrogen storage can also be a critical factor. Generally, rapid warming after using the equilibrium freezing method is required for survival. The advantage of a rapid over a slow thaw is thought to relate to the recrystallization of ice that occurs during warming (Farrant 1980, Mazur 1984). A rapid thaw may minimize the redistribution or growth of the ice crystals during recrystallization (Farrant et al. 1977, Shimada 1977). Liebo et al. (1974) have shown that the advantage(s) of rapid warming for frozen mouse embryos diminishes when excessive dehydration occurs after using suboptimal cooling rates. Thus, the interrelationship of freezing with thawing requires that close attention be given to optimizing the cooling and warming rates when using the equilibrium freezing method.

2.2. Vitrification

Vitrification is an alternative approach of cryopreservation that avoids the factors that generate intracellular ice formation by effecting dehydration using highly concentrated cryoprotectant solutions prior to cooling and employing ultra-rapid cooling and warming rates. The vitrification process solidifies liquids and the cytoplasm not by the formation of ice but by an extraordinary increase in viscosity, which results in an amorphous non-crystalline glassy state (Luyet and Gehenio 1940). Rapid cooling below the temperature at which solutions solidify without crystallization and rapid warming above the temperature where devitrification occurs (spontaneous crystal

formation) is required to gain survival of cells or organisms treated with vitrification protocols (Luyet and Gehenio 1940, Rall et al. 1980). Measured cooling rates in studies where organisms have been vitrified using liquid nitrogen range from 5100 to 4.2 x 10^{-4} °C/min (Steponkus et al. 1990, James 2004). Further, with *Drosophila* embryos, Mazur et al. (1993) have indicated that warming rates approaching 2.0 x 10^{-4}°C/min are needed to avoid devitrification and harmful ice crystal formation and are equally as important as cooling rapidly. In some cases, the critical warming rate for maximum survival may even be several orders of magnitude greater than the critical cooling rate (Baudot and Odagescu 2004).

Also crucial to the success of most vitrification protocols is the requirement that the system to be vitrified is resistant to desiccation by the multimolar concentrations of cryoprotectants necessary to produce glass transition upon rapid cooling (Engelmann 1997). Full permeation of the cells or organisms by the cryoprotectant(s) is usually not needed and may lead to solution effects such as osmotic damage and/or chemical toxicity (Rall 1987). Consequently, osmotic shrinkage of the cells to the extent that vitrification of the intracellular cytoplasm can be accomplished is normally sufficient. Two-step cooling/incubation methods have been developed to avoid the toxicity of concentrated cryoprotectants and/or to accommodate permeability barriers. James (2004) has extensively described the techniques and detailed the benefits gained by using the two-step method of cooling and incubating organisms in ethanediol before quenching in liquid nitrogen. He compiled studies of 11 parasitic helminths and insects that were cryopreserved when incubated in initial concentrations of ethanediol ranging from 10 to 40% at 25-37°C and then, as a second step, in concentrations ranging from 33-83% at 0°C before exposure to liquid nitrogen. An alternative method employing slow cooling to a sub-zero temperature of -31°C followed by an a 30 minute equilibration period in five molar dimethyl sulphoxide before quenching in liquid nitrogen was found to be an effective means for vitrifying for malaria trophozoites and shizonts (Wilson et al. 1977). Thus, in these ways, a variety of systems have been successfully recovered after vitrification and liquid nitrogen storage including: mammalian and invertebrate embryos (Rall and Fahy 1985, Wang et al. 2000), blood cells (Takahashi et al. 1986), corneal tissues (Bourne 1986), pancreatic islets (Jutte et al. 1987), parasites (James 2004) and plant tissues (Reed et al. 2004).

3. Insect Cryopreservation

There are a number of possible options for recovering insects or their germ-plasm after cold storage at cryogenic temperatures. These options include liquid nitrogen storage of: (1) sperm, (2) blastoderm and primodial germ cells, (3) gonads, (4) embryos, (5) postembyonic immature stages, and (6) adults. Except for the cryogenic storage of adults, there are successful examples for each of the other five options. The following describes significant research or observations recorded in the literature that relate to cryopreservation of the various forms of insect germ-plasm.

3.1. Sperm

It is patently obvious that cryopreservation of sperm or eggs is only a valuable asset when additional techniques are available to facilitate instrumental insemination of the female insect or *in vitro* fertilization of eggs. While it is commonly thought that the honey bee *Apis mellifera* L. is the only insect for which an instrumental insemination technique has been developed (Laidlaw 1977), similar methods have also been crafted for the yellow fever transmitting mosquito *Aedes aegypti* (L.) (Burcham 1957), the common bedbug *Cimex lectularius* L. (Davis 1965), the cotton boll weevil *Anthonomus grandis* Bohemann (Villavaso 1974), the fire ant *Solenopsis invicta* Buren (Ball et al. 1983), the silkworm *Bombyx mori* (L.) (Takemura et al. 1999) and three species of bumblebees *Bombus* spp.

(Baer and Schmid-Hempel 1999). This disparate assemblage of species having extremely dissimilar methods of sperm transfer during the normal male-female insemination process bodes well for development of additional methods for many other insects. For example, mosquito males transfer free sperm in seminal fluid; silkworm males transfer two types of sperm packaged into a spermatophore and the bedbug male practices "traumatic insemination" through the female's body wall into the haemocoel. Thus, it would seem that sperm cryopreservation, like the animals that are mass-reared in the livestock industry, would be an often-used technique for retaining insect germ-plasm. However, of these insects, sperm cryopreservation technology for use in instrumental insemination is currently available for only the honey bee (Harbo 1977, Kaftanoglu and Peng 1984) and the silkworm (Takemura et al. 1999).

The early work of Harbo (1977, 1979, 1981, 1983) on honey bee sperm cryopreservation indicated that considerable damage occurred when 10% dimethyl sulphoxide was used as the cryoprotective agent and the diluent was saline. The measured cooling rates ranged from 20-50°C/min and thawing was nearly instantaneous. Equilibration periods used were from 12-20 minutes and were followed by the rapid cooling. With this method, Kaftanoglu and Peng (1984) were more successful in gaining increased numbers of fertilized eggs and colony regeneration by changing the type of sperm diluent from saline to the Kiev medium, increasing the equilibration time and slowing the cooling rate to 3-4°C/min. Maintenance of colonies with queens receiving only cryopreserved semen was difficult but the F_1 queens reproduced normally. Considering the recent advances made on cryopreserving the semen of other animals (Arav et al. 2002), it would seem that more progress could be made on cryopreserving honey bee sperm. Recent work on identifying the innate factors which allow queen honey bees to maintain viable sperm within their spermatheacae for several years indicates movement in that direction (Collins et al. 2004).

In vitro fertilization has been accomplished with cryopreserved sperm in the sawfly *Athalia rosae* (L.) (Hatakeyama et al. 1994). This technique does not employ a cryoprotective agent and the sperm recovered from liquid nitrogen storage are non-motile. Nevertheless, diploid females were recovered by initially injecting sperm into mature oocytes. Essential to the successful employment of *in vitro* fertilization is having the capacity to activate the oocytes prior to the injection of the sperm in order that syngamy will occur between the two gametes. With this method, *A. rosae* oocytes were activated by placing in hot water (Sawa and Oishi 1989).

3.2. Blastoderm and Primordial Germ Cells

Yu et al. (1997) used the conventional equilibrium freezing process when cryopreserving totipotent nuclei from embryos of *A. mellifera*. Donor ooplasm containing preblastoderm nuclei was removed from 8-9 hour embryos with a transplantation pipette, which was then plunged into liquid nitrogen also without benefit of a cryoprotectant. After a rapid thaw the nuclei and ooplasm in the pipette were injected into recipient embryos. The incidence of chimerism using this method was 85% of the frequency obtained when non-cryopreserved nuclei were utilized. Further, two out of 157 surviving larvae expressed only the donor genotype and were presumed to be clones of the donors. An extension of this technology is to biopsy individual honey bee embryos by removing up to 80 nuclei and cryopreserving them (Yu et al. 1998). The biopsied embryos are then reared to adulthood as queens and submitted to a comprehensive phenotypic testing and screening for the traits desired for a highlevel of honey production. The totipotent nuclei that were held in liquid nitrogen storage are then used in the creation of germlines from those queens that had been determined to produce hives of superior quality during the screening process.

Unlike other animals, primordial germ

Table 1. *Current status of insect embryo cryopreservation.*

Species	Hatching	Pupating	Reproducing adults	References
Drosophila melanogaster Meigen	yes	yes	yes	Mazur et al. (1992b), Steponkus and Caldwell (1993)
Lucilia cuprina (Wiedemann)	yes	no	no	Leopold and Atkinson (1999)
Musca domestica L.	yes	yes	yes	Wang et al. (2000)
Cochliomyia hominivorax (Coquerel)	yes	yes	yes	Leopold et al. (2001)
Chrysomya bezziana (Villeneuve)	yes	no	no	Leopold and Mahon (unpublished)
Culicoides sonorensis Wirth & Jones	yes	yes	yes	Nunamaker and Lockwood (2001)
Anastrepha suspensa (Loew)	yes	yes	yes	Leopold et al. (unpublished)
Anastrepha ludens (Loew)	yes	yes	yes	Rajamohan et al. (2002)
Ceratitis capitata (Wiedemann)	yes	yes	yes	Rajamohan et al. (2003)
Spodoptera exigua (Hübner)	yes	no	no	Li et al. (2000, 2001)

cells of insects are not routinely cryopreserved. Techniques for transplanting and also isolating mass quantities of pole cells of *Drosophila* embryos have been in existence for over 25 years (Allis et al. 1977, Regenass and Bernhard 1980) and it would seem likely that creation of a cryopreservation protocol similar to that used for honey bee blastoderm nuclei could be easily accomplished.

3.3. Gonads

Methods for cryopreserving insect ovaries have been developed by Bruschweiler and Gehring (1973) for *Drosophila* and by Shinbo (1989) for the silkworm. Preservation of the silkworm germ-plasm was accomplished by adding increasing concentrations of dimethyl sulphoxide to larval ovaries in culture medium to reach a final concentration of 1.5 molar and freezing in liquid nitrogen vapour within a cryovial before placing into liquid nitrogen. Recovery from liquid nitrogen storage was by rapid thawing, and transplantation was into castrated 4th or 5th instar larvae followed by rearing to adulthood. Mochida et al. (2003) were able to obtain progeny from adult silkworm that had undergone transplantation of cryopreserved ovaries during their larval stage. Unlike the *Drosophila* technique, this method does not require that the donor ovaries connect with the recipient ovarian ducts because once the transplanted ovaries are mature, eggs can be surgically removed from the surrogate insects and either activated parthenogenetically or fertilized by *in vitro* fertilization.

3.4. Embryos

Dipteran embryos, and perhaps most insect embryos, are inherently intolerant to the use of the conventional equilibrium freezing technique for cryopreservation (Heacox et al. 1985, Meyers et al. 1988, Mazur et al. 1992a, Miles and Bale 1995). This intolerance relates to a lethal response to chilling when exposed to the slow cooling/dehydration regime required to implement equilibrium freezing. Leopold (1991) suggested that insect eggs having an abundance of yolk were especially sensitive to chilling and the reason later stage

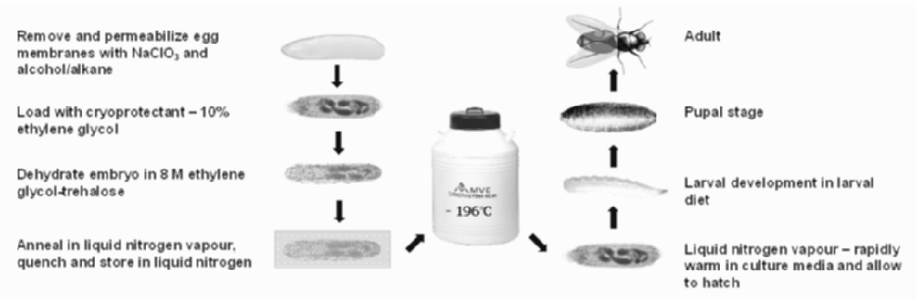

Figure 1. *Dipteran embryo cryopreservation method.*

embryos were able to be cryopreserved was that most of the yolk had been utilized during embryogenesis.

Steponkus et al. (1990) were the first to obtain survival of *Drosophila melanogaster* Meigen embryos after cryopreservation. They used an approach to avoid chilling injury by first loading 12-14 hour embryos with 2.1 molar 1,2-ethandiol at room temperature and then exposing them to 8.5 molar 1,2-ethanediol at 0°C to raise the intra-embryonic concentration of the cryoprotective agent to a high level by osmotic removal of additional water. With these preparatory steps, the embryos were then vitrified by rapid cooling in nitrogen slush. Recovery required equally rapid warming. The hatching of the embryos recovered from liquid nitrogen reached 19% with about 3% emerging as adults. Subsequent optimization of the vitrification technique by determining the optimum stage for cryopreservation, increasing the efficiency of the permeabilization process, improving the vitrification fluid, and changing the recovery methods increased hatching to 60-75% with about 40% of the larvae developing into adults (Mazur et al. 1992b, Steponkus and Caldwell 1993).

Wang et al (2000) attempted to use the *Drosophila* cryopreservation procedure to preserve house fly *Musca domestica* L. embryos and found that several significant modifications were required for survival. These modifications included eliminating the carry-over of alcohol into the alkane lipid-extraction procedure, substituting Schneider's cell culture media for the BD20 solution as a diluent, formulating a vitrification solution that contained 1,2-ethandiol, polyethylene glycol and trehalose, cooling the embryos by exposure to liquid nitrogen vapour for one minute prior to quenching, and adding foetal bovine serum to the recovery medium. With only slight alterations, Leopold et al. (2001) used this technique for cryopreserving the New World screwworm and also by Rajamohan et al. (2003) for the Mediterranean fruit fly. Fig. 1 is a visual summary of the general technique used for cryopreserving six of the ten species listed in Table 1.

Attempts to cryopreserve embryos of the moth *Spodoptera exigua* (Hübner), resulted in a low rate of less than 2.0% hatching (Li et al. 2000, 2001) However, it is encouraging that this technique may be applicable, with some modifications, to other insects besides dipteran flies. Obtaining a low yield of adults is not uncommon when attempting to adapt a method developed for another species. The relative diversity of the Class Insecta with respect to rates of embryonic development, complexity of egg membranes, tolerance to chilling, and the reaction to potentially toxic cryoprotective agents requires that each method for cryopreservation be tailored to fit a particular insect species.

3.5. Post-Embyronic Immature Stages

Hinton (1969) was successful in recovering live larvae of the midge *Polypedi1um vanderplanki* Hinton, after exposure to liquid helium. During the larval stage, these insects are able

to survive desiccation to about 3% total body moisture and hence they are not severely damaged by a -270°C temperature exposure. There are two other reports on the successful freezing and recovery of live whole insects at liquid gas temperatures in the absence of cryoprotectants. Tanno (1968) and Moon et al. (1996) used lengthy stepwise cooling regimes to freeze prepupae of the sawfly *Trichiocampus populi* Okamoto, and larvae of the drosophilid *Chymomyza costata* (Zetterstedt), before quenching in liquid nitrogen. These examples of freeze tolerance to liquid gas temperatures may be the exceptions to the rule for insects. Lee (1991) surveyed the literature on insect cold tolerance and, of the more than 35 reports that were listed, he found only two species were able to survive temperatures approaching -80°C and three in the -50 to -55°C range.

4. Storage Using Insect Dormancy

Dormancy is a strategy employed by insects to survive harsh environmental conditions such as cold temperature, and researchers and insectary managers can use it as a means to extend insect shelf-life. The ability of insects to survive a near- or freezing temperature in a dormant state usually involves an adaptive physiological response, which may be diapause, hibernal quiescence, and/or cold hardening. While diapause is a programmed obligatory or facultative interruption in the developmental scheme of an insect, quiescence is more of an immediate response to adverse conditions. Cold hardening, like diapause, is elicited by an external stimulus over time, but unlike insects that exhibit diapause, it is usually elicited proportional to the stimulus. Diapause is mediated by the endocrine system of the insect and, in the case of facultative diapause there is usually an all-or-none response to the external stimulus. The relationship of temperature with the induction and maintenance of insect diapause has been reviewed by Denlinger (1991, 2001). The techniques and research involved with storing insects under refrigerated conditions have also been previously reported (Leopold 1998, 2000, van Lenteren and Tommassini 2003). Therefore, since most of the more recent literature relates to storage of insect parasitoids and predators, only selected new developments in this area are presented here.

4.1. Storage Using Diapause and/or Cold Hardening

Using diapause and/or cold hardening as storage mechanisms requires that strict attention be given to incorporating the proper environmental cues into the insect rearing protocol. Denlinger (1991) outlined four possible situations whereby diapause may or may not be linked to cold hardiness. They are: (1) diapause in the absence of cold hardiness, (2) cold hardiness in the absence of diapause, (3) diapause and cold hardiness occurring coincidently, each requiring the same regulatory cues, and (4) diapause and cold occurring together, each requiring different regulatory cues. For an example of separate cues, the survival of the predatory mite *Euseius finlandicus* Oudemans to storage at -11.5°C was increased nearly five-fold by either rapidly cold hardening or slow preconditioning at a range of temperatures between 0 and 10°C after it had entered diapause (Broufas and Koveos 2001). Garcia et al. (2002) demonstrated the paradigm for shared regulatory cues with the egg parasitoid *Trichogramma cordubensis* Vargas & Cabello. A conditioning period for at least 30 days at 10°C induces diapause and survival at 3°C for six months, while exposure to seven or 12 °C for less than 30 days followed by 3°C induced quiescence for up to 40 days before survival began to decrease.

A notable observation relating to a host-parasitoid interaction and cold hardiness was reported by Rivers et al. (2000). The non-diapausing pupal parasitoid *Nasonia vitripennis* (Walker) survived exposure to sub-zero temperatures 4-9 times longer when fed on diapausing flesh fly pupae *Sarcophaga crassipalpis* (Macquart) having high concentrations

of glycerol as opposed to parasitoids feeding on non-diapausing *S. crassipalpis* pupae low in glycerol. Further, encasement of either diapausing or non-diapausing parasitoid larvae within the fly puparia afforded 2-3 times more tolerance to sub-zero temperature than those larvae removed from the puparia. Thus, those workers involved in the design of storage protocols for parasitoids or predators capable of entering diapause and acquiring cold hardiness must consider a myriad of interrelated factors that include: determining the various types and quality of environmental cues, identifying physiologically receptive and cold-tolerant stages of development, and clarifying the status of the host or prey cannot be accomplished when rearing on an artificial diet.

4.2. Storage Using Quiescence

The design of storage protocols using quiescence as a vehicle for extending insect shelf-life often involves the use of precise temperature control and determination of the lower threshold temperature for development of the insect. Some parasitoids have exceedingly low thresholds and the storage temperatures must be carefully adjusted to accommodate this factor. The two braconids *Binodoxys indicus* Subba Rao & Sharma and *Lipolexis scutellaris* Mackauer, the aphidiid *Lysiphelbus japonica* Ashmead, and the tachinid *Lydella thompsoni* Herting all have a lower threshold temperature that is less than 3°C (Cagan et al. 1999, Deng and Tsai 1999, Singh et al. 2000a,b). Leopold et al. (2004) reported that a change in storage temperature of only 0.5°C, from 4.0 to 4.5°C, increased the 10-day emergence of the egg parasitoid *Gonatocerus ashmeadi* (Girault), from 7 to 34%. Further, they demonstrated that cycling the in-storage temperature at 8-hour intervals (4.5, 6.0 and 7.5°C as opposed to the regime of 4.0, 6.0 and 8.0°C) increased 25-day in-storage parasitoid emergence to greater than 60%. Also, no parasitoids emerged after 20 days storage using the cycled regime starting at 4°C. The lower threshold temperature for *G. ashmeadi* was determined to be slightly less than 5°C since some emergence will occur from the host eggs of *Homalodisca coagulata* (Say) when held at this temperature (Leopold et al. 2003a). Renault et al. (2004) have suggested that fluctuating temperatures are an important factor in the repair of chilling injury in insects exposed to low storage temperatures. Nevertheless, the rigid chilling tolerances, such as those displayed by *G. ashmeadi*, would probably exceed the capabilities of refrigeration equipment and handling procedures if this type of protocol was to be scaled up for inclusion in a mass-rearing system.

5. Insect Quality and Cold Storage

To date, some in the entomological community have been slow to utilize the technology already available for increasing insect shelf-life and cryobanking. For example, techniques for cryopreserving *D. melanogaster* embryos have existed since 1992 (Mazur et al. 1992b, Steponkus and Caldwell 1993) and to this author's knowledge none of major stock centres located around the world are using the technology. Without doubt, the resources expended maintaining the various strains of *Drosophila* worldwide, estimated to be in the vicinity of 30 000 (Steponkus et al. 1990), are considerable. It is understandable that insectary managers would be reticent to incorporate new technology into established rearing and maintenance procedures without proper documentation of the quality of insects subjected to a particular storage protocol. However, it should be pointed out that insect quality can have different levels of acceptability. The type or extent of quality assessment required to determine whether a storage regime is harmful or beneficial can vary widely because it depends on the ultimate use of a particular insect strain or colony. For example, an insect geneticist conducting laboratory studies may only require that several genes on one particular chromosome remain stable each generation while a programme manager releasing sterile males in an AW-IPM programme has concerns about quality character-

istics related to production levels, mating competitiveness, flight ability, longevity under field conditions, etc.

Currently, studies on overall insect quality following storage in liquid nitrogen are meagre because it is a new and developing technology. In addition to the basic hatching, adult emergence and reproductive assessments, Houle et al. (1997) and Leopold et al. (2003b) have made preliminary studies on the genetic stability of dipterans following cryopreservation. Investigations examining tolerance to subambient chilling generally concern evaluation of post-storage lifespan, sex ratios, and reproduction, while the assessment of behavioural factors following cold storage has been limited (Leopold 1998). Yet, it should be reiterated that favourable laboratory tests on basic quality characters relating to lifespan, reproduction, behaviour, and genetic stability will still ultimately require retesting under field conditions if the stored insects are to be released as part of a control programme. Thus, the ultimate responsibility for confirming that the storage technology will be beneficial to a particular insect rearing system lies with the end-user.

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Improving the Efficacy of the Sterile Insect Technique for Fruit Flies by Incorporation of Hormone and Dietary Supplements into Adult Holding Protocols

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ABSTRACT The sterile insect technique (SIT) is a universally accepted method of control for tephritid flies. Improving efficacy of mating by sterile males would reduce costs significantly. This paper describes studies of the physiological mechanisms responsible for coordination of reproductive maturity and sex pheromone communication in males of the genus *Anastrepha* in order to develop methods for acceleration of reproductive maturity among sterilized males. These show that juvenile hormone III and its bisepoxide homologue are key hormones involved in coordination of reproductive development and pheromone-calling in both males and females. Additionally, incorporation of protein into the diet fed to sterile adults prior to release is critically important to improve pheromone calling, attraction of females and mating by sterile males. These results have led to development of a novel strategy to accelerate reproductive development of laboratory-reared sterile flies by incorporating hormone supplement therapy using mimics of juvenile hormone including methoprene and fenoxycarb and protein diets for use in mass-rearing protocols. This strategy resulted in accelerating reproductive development in males of the Mexican fruit fly *Anastrepha ludens* (Loew), the West Indian fruit fly *Anastrepha obliqua* (Macquart), the Caribbean fruit fly *Anastrepha suspensa* (Loew), and the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) fruit flies by 3-7 days. Incorporating the technology into mass-rearing will significantly improve the efficacy of area-wide integrated pest management progammes with an SIT component to control these pests.

KEY WORDS *Ceratitis capitata*, *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha suspensa*, juvenile hormone, protein diet supplements, sexual maturation, sexual signalling

1. Introduction

The interventionist approach of directly killing insect pests with toxic chemicals has been the prevailing control strategy for over 50 years. This has led to environmental contamination and pest resistance to the toxins while tolls due to pests grow higher, costs of treatment increase and profits decrease. Truly satisfactory and lasting solutions to pest problems will require a shift to understanding and promoting other means of control, such as the sterile insect technique (SIT). Indeed the SIT is a universally accepted and environment-friendly method for controlling a variety of insect pests. Better knowledge of the basic mechanisms that govern insect reproduction and a more thorough understanding of the

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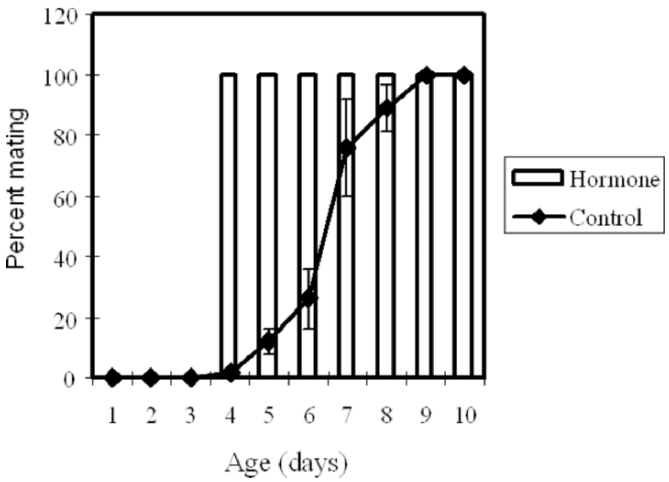

Figure 1. *Effects of hormone supplement therapy on mating by males of the Caribbean fruit fly* Anastrepha suspensa*. Males were either treated with just one microlitre of acetone or treated with five micrograms of methoprene on the day of eclosion. Six replicates of ten males per each treatment and age. Significantly more males treated with hormone mated on each of days 4-6. Data for fenoxycarb were identical to those of methoprene.*

physiology and chemical ecology of pests are necessary to develop improved methods to increase efficacy of the technique. To this end, research has been conducted in two areas: (1) determining the hormonal mechanisms regulating sexual maturity, and (2) determining the dietary requirements of adults to optimize pheromone calling and performance of males. The following describes the work conducted using tephritid fruit flies as model species.

2. Tephritid Fruit Flies of Economic Importance

Tephritid flies, including members of the *Ceratitis*, *Anastrepha*, and *Bactrocera* genera, pose a serious threat to agriculture in the USA. More than 260 different hosts, including stone fruits like plums and peaches, and cash crops like tomatoes and peppers, have been recorded for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) alone. All agriculturally important tephritids are under both national and international quarantine restrictions which, in the case of the Caribbean fruit fly *Anastrepha suspensa* (Loew), an established pest in Florida, have very significant impacts on the USD 1500 million citrus industry due to loss of world markets. The economic impacts of established populations of any quarantine tephritid in California or other citrus-producing states would be devastating. The cost of control of an established population of a single quarantined species of tephritid fruit fly to the USD 6800 million per year California citrus and vegetable industries has been estimated at USD 1000 million, and quarantine of California fruit and vegetables from foreign markets would result in the loss of 35 000 jobs and decrease output by USD 3600 million per year (CDFA 2006a,b).

Increased importation of fruit and vegetables from other countries and between states has magnified considerably the probability of populations becoming established. For example, in 2000 the California Department of Food and Agriculture declared an exterior quarantine for 100 fruits, vegetables and berries (including such common items as blackberries, figs, citrus, bell pepper, and tomato) from the areas in the State of Florida

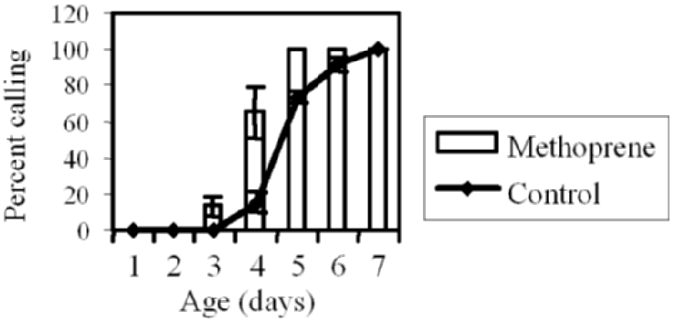

Figure 2. *Comparison of age (cumulative percentage) at which males of the West Indian fruit fly* Anastrepha obliqua *engage in pheromone calling when treated with methoprene or with just solvent (control) on the day of adult emergence (six replicates of ten males per each age).*

south of and including Hernando, Sumter, Lake and Volusia Counties, the major production areas for these commodities in Florida.

Strict monitoring protocols are in place to detect introductions of the pests. For example, the State of Florida deploys more than 13 000 attractant traps to detect potential invasions of the Mediterranean fruit fly alone. Once an outbreak is detected, the infested areas are subjected to immediate quarantine to eliminate movement of fruit to other areas, fruit from host plants are stripped from infested sites, and ground and aerial pesticide application control protocols are initiated. In the past, fumigant treatment of imported fruit using ethylene dibromide significantly limited invasions of these flies. However, registration for ethylene dibromide use has been withdrawn and no substitute has been found. Thus, three practical control methods remain: (1) stripping and destroying fruit from infested areas, (2) bait sprays using 10% malathion and more recently Spinosad®, and (3) release of sterilized males. The use of malathion bait sprays has come under constant criticism due to environmental and health related problems and several law suites have been filed to stop application.

Use of the SIT provides an environmentally safe and species-specific method to suppress or eradicate tephritid fruit flies of agricultural importance worldwide. In fact, California employs the technique on a routine basis for preventing and eradicating both Mediterranean and Mexican fruit fly outbreaks (CDFA 2006a) and also in Florida the technique is being used as a preventive method to ensure that the Mediterranean fruit fly does not become established. While the basic protocols associated with the SIT are well established, the technique is expensive in terms of time and money. An important goal is therefore to dramatically improve the efficacy of the SIT by developing methods to improve mating efficiency of sterile males and to reduce costs associated with implementation of control programmes.

3. Hormone Supplement Therapy

Until recently, few studies had been conducted on the endogenous regulation of sexual maturity and pheromone production in tephritid fruit flies. Studies on the Mediterranean fruit fly indicated that application of juvenile hormone accelerates ovarian maturity (Chang and Hsu 1982, Chang et al. 1988, Hsu et al. 1989). Also, the attractiveness of males to females was found to be reduced among males treated with precocene II (Chang and Hsu 1982, Chang et al. 1984). Application of juvenile hormone III reversed the effect of precocene II (Chang and Hsu 1982) suggesting that pheromone calling was affected by juvenile hormone in some, as yet unknown,

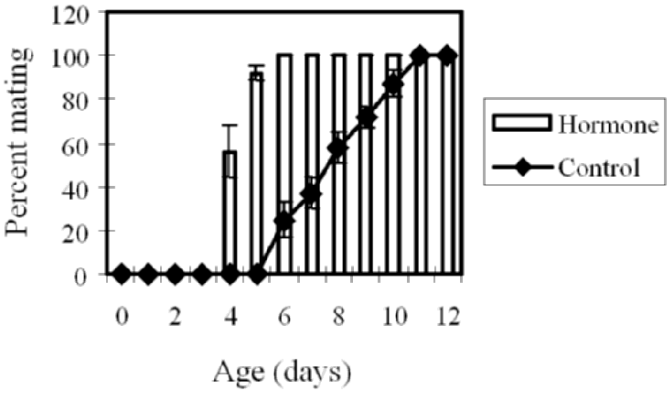

Figure 3. *Comparison of age at which male sterile Mexican fruit flies* Anastrepha ludens *mate when they are treated with hormone or with just solvent (control) on the day of adult emergence. All hormone-treated males (six replicate-treated groups of ten males per each age) mated by day five whereas only 86% of control males mated by day ten.*

fashion.

Research on the effects of juvenile hormone in regulation and acceleration of sexual maturity in tephritid flies has followed protocols developed for the Caribbean fruit fly (Teal et al. 2000). Results of studies on this fly have documented conclusively that juvenile hormone is a crucial hormone regulating development of reproductive competence and pheromone calling since topical application of juvenile hormone or the juvenile hormone mimics, methoprene and fenoxycarb, to newly eclosed adults or late stage pupae of the Caribbean fruit fly induced precocious reproductive development. Thus, Caribbean fruit fly males treated with hormone on the day of adult emergence mate 4-5 days earlier than untreated males (Fig. 1).

The same methods were followed to conduct studies on the effects of hormone supplement therapy on acceleration of reproductive development as indicated by age at which pheromone calling occurred using both the Mexican fruit fly *Anastrepha ludens* (Loew) and the West Indian fruit fly *Anastrepha obliqua* (Macquart) (Fig. 2). In both cases, application of methoprene or fenoxycarb induced a significant acceleration in reproductive development so that treated males mated earlier than their solvent-treated controls. These studies were conducted using flies that had not been irradiated. To determine if irradiated flies underwent the same acceleration in reproductive development, males of the Mexican fruit fly were treated with hormone on the day of adult eclosion and the same mating studies performed using fertile females. As shown in Fig. 3 essentially all hormone-treated flies mated by day 5, but even at day 10 only 86% of the solvent-treated sterile males had mated.

Field cage studies were conducted using the Mexican fruit fly to determine the competitiveness in mating with 12-day-old wild females of 5-day-old hormone-treated sterile males with 5-day-old untreated sterile males and 12-day-old wild males to mate with wild females. These studies were conducted using published standard protocols (FAO/IAEA/USDA 2003), except that each male and female released into flight cages was marked with a number so that the mating status of each individual could be assessed. The results of the study indicate that 6-day-old sterile males treated with hormone are fully competitive with both 12-day-old untreated sterile males (that matured naturally), and 14-day-old wild males in mating with wild females (Fig. 4).

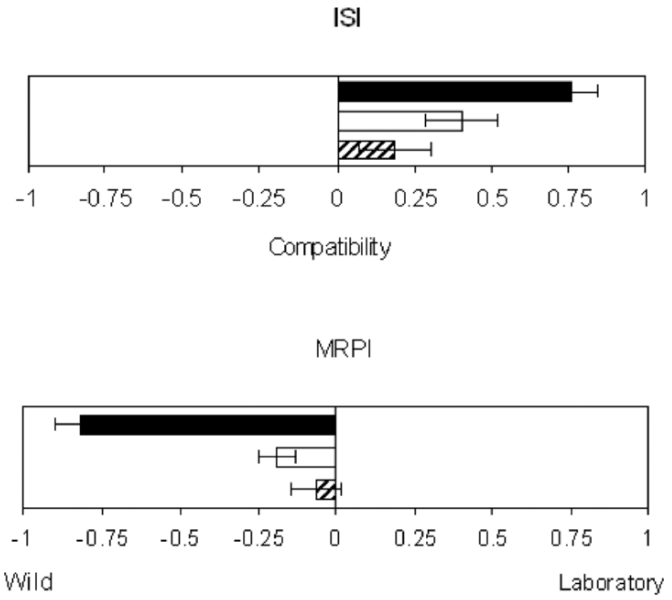

Figure 4. *Mating compatibility tests using Mexican fruit flies* Anastrepha ludens *comparing hormone-treated 6-day-old sterile males (open bars), control 6-day-old sterile males (hatched bars) and wild 14-day-old males (black bars). In all cases 6-day-old hormone-treated sterile males performed as well as 12-day-old untreated sterile males. Descriptions of tests conducted and of each of the performance indexes used are given in "Mating Compatibility Tests" in FAO/IAEA/USDA (2003).*

Preliminary studies were made on the effects of juvenile hormone supplement therapy on bisexual sex strain irradiated Mediterranean fruit flies obtained from shipments sent from Guatemala to Florida during 1998. In these experiments, the effects of application of juvenile hormone to newly eclosed males on pheromone production were monitored. It was found that 2-day-old males treated with hormone released twice as much pheromone as the solvent-treated controls.

Also monitored was the calling (pheromone release) period of treated and solvent-treated control sterile Mediterranean fruit flies. The pheromone release period is directly correlated with the mating period in this species. From this it was determined that flies treated with hormone began calling significantly earlier in the day than did untreated control sterile males. In fact the calling period of the hormone-treated flies occurred during the same period that has been determined as the reproductive period for wild Mediterranean fruit flies in Guatemala (data on natural calling period was obtained from data reported by Landolt et al. (1992)). If the trends for production and release of more pheromone and shifts to calling times that coincide with the wild flies are confirmed when sterile males are treated with hormone, then hormone therapy will significantly improve the mating performance of Mediterranean fruit flies.

Individual sterile males are often considered to have the potential to remove a single wild female from the reproductive population. Compounds in the accessory glands of the Mediterranean fruit fly have been shown to

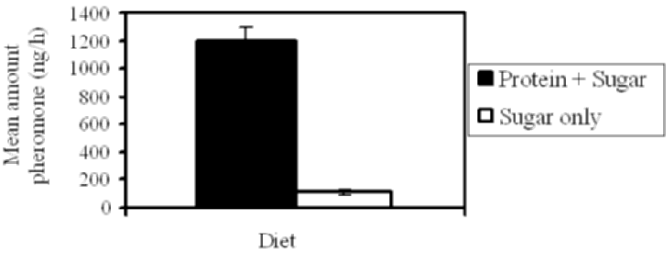

Figure 5. *Comparison of pheromone released by 14-day-old male Caribbean fruit flies* Anastrepha suspensa *fed either the dry diet composed of 1:3 protein plus sugar or sugar alone (N=6 replicates of each treatment).*

inhibit remating and to induce females to engage in searching for fruit and oviposition (Jang 1995, Jang et al. 1998, Miyatake et al. 1999). It is very possible that sterile males fail to replenish the secretions from the accessory glands in the reproductive system after mating. Wild males replenish these secretions within a few hours after mating. Juvenile hormone is known to induce replenishment of accessory gland secretions in other fruit flies. Thus, hormone supplement therapy with juvenile hormone should allow sterile males to rapidly replenish the accessory gland secretions after the initial mating. Therefore, sterile males could be capable of effectively mating more than once with wild females. Multiple mating by released sterile males will make the SIT even more cost effective.

4. Effects of Diet on Sexual Communication and Mating

Current protocols used for holding adult flies prior to release include feeding flies a sugar agar diet composed of 15% sucrose, 0.8% agar (a mixture of polysaccharides) and 84.2% water. No protein is included in the diet. The exclusion of protein from the adult diet was justified for three reasons: (1) it increased costs, (2) it was hypothesized that the protein benefited females greatly but had minimum effects on male survival (Galun et al. 1985), and (3) protein supposedly increased microbial growth in the diet which could lead to sickness. As a consequence the sugar agar diet is used to feed adult sterile flies prior to release. However, it has long been recognized that protein is of importance for achievement of sexual maturity by the adults of tephritid fruit flies (Bateman 1972, and references therein). Indeed, recent work on the Mediterranean fruit fly has shown that calling behaviour, sexual competitiveness and reproductive success are enhanced significantly when adult males from either wild stocks or laboratory-reared colonies are provided with protein in the adult diet (Warburg and Yuval 1996, Blay and Yuval 1997, Papadopoulos et al. 1998, Kaspi et al. 2000, Kaspi and Yuval 2000, Yuval et al. 2002). Similarly, males of the West Indian fruit fly and its relatives, the guava fruit fly *Anastrepha striata* Schiner and the sapote fruit fly *Anastrepha serpentina* (Wiedemann) have higher copulatory success when fed a diet containing protein hydrolysate (Aluja et al. 2001a,b).

Male Caribbean fruit flies will survive for at least 20 days when fed only sugar and water (Teal et al. 2004). However, the amount of pheromone produced and released by males fed only sugar is less than 10% of that produced and released by males fed both protein and sugar (Fig. 5). These authors also conducted studies in which flies were fed either on sugar alone or sugar plus protein for 11 days and their diets then changed so that sugar-fed flies were provided with protein plus sugar on days 12-14 and protein plus sugar-fed flies were fed only sugar for days

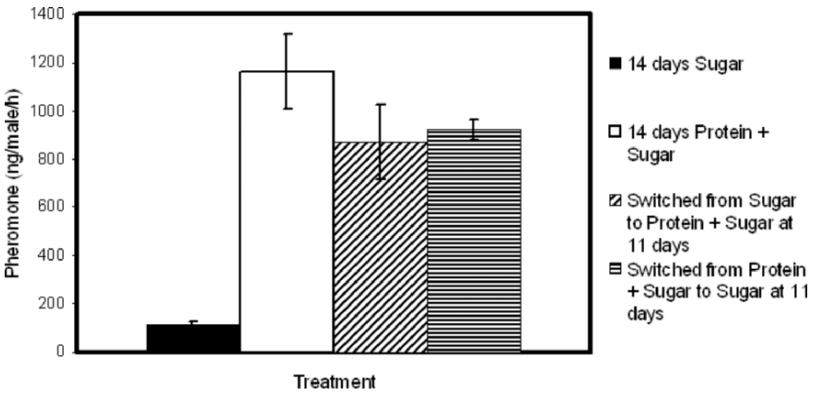

Figure 6. *Pheromone production by male Caribbean fruit flies* Anastrepha suspensa *fed sugar alone or sugar plus protein for 14 days, or by males switched from one diet to another on day 11 and allowed to feed until day 14 (N=8 replicates per treatment).*

12-14. On day 14, volatile pheromone released by these flies was collected and the amount released compared to that released by flies fed only sugar or sugar plus protein for 14 days. The results were of significance because adding protein to the sugar on day 11 caused flies fed only sugar for the first 11 days to produce as much pheromone as was produced by flies fed protein plus sugar for 14 days (Fig. 6). Additionally, flies switched from protein plus sugar to only sugar on day 11 produced as much pheromone as was produced by flies fed protein and sugar for 14 days. Clearly, providing protein is critical for optimizing pheromone release.

Flight tunnel studies have also been conducted in which 14-day-old females Caribbean fruit flies were released into the down-wind end of a 1.5-metre long flight tunnel and allowed to choose between the volatiles released by males fed on protein plus sugar or by males fed just sugar. All males were at least 11 days old. Volatiles were piped into the tunnel from holding cages held outside the tunnels. The outport of the volatile release tubes was attached to insect isolation traps lined with sticky paper to capture the female flies (Heath et al. 1993). Females were released early in the reproductive period (15:00 hours) and trap captures were recorded 23 hours later. The data showed that females were much more attracted to males fed protein plus sugar than to males fed only sugar.

5. Development of Hormone and Protein Delivery Techniques for the SIT

Although the results described above demonstrated that both hormone therapy and provision of protein supplements to male adults improved reproductive competence in experimental conditions, transfer of the technology to tephritid fruit fly factories requires that the methods are incorporated into practical systems. As indicated above, the diet currently used to feed adults prior to field release is a gel diet composed of 15% sucrose, 0.8% agar and 84.2% water. Therefore, it was critical that treatments developed could be incorporated into this agar-based diet.

The studies began by comparing the pheromone calling abilities in a flight tunnel and determining the amount of pheromone released by male Caribbean fruit flies fed the agar diet containing no protein or males fed the optimal diet for adult development, a dry diet composed of 3:1 sucrose and protein. For flight tunnel studies, females were released 1.5 metres downwind from traps releasing

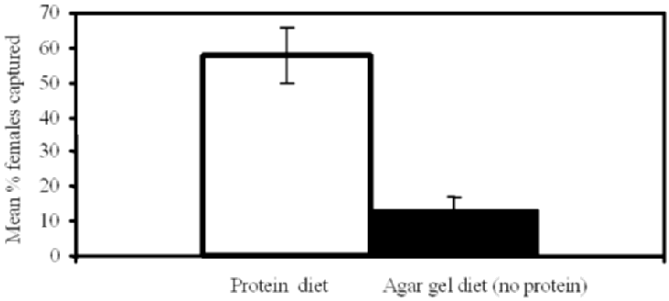

Figure 7. *Comparison of female Caribbean fruit flies* Anastrepha suspensa *responding to volatiles released by males fed the optimal dry diet or the agar gel diet as indicated by males captured in traps releasing pheromone from caged males held outside of the flight tunnel (N=8 replicates; 20 females released per trial).*

volatiles emitted from males fed either of the diets. As indicated in Fig. 7, females overwhelmingly chose traps releasing pheromone from males fed the dry diet. Additionally, males fed the dry diet released six times more pheromone than did males fed the agar diet. To determine if adding protein into the diet caused a change in pheromone calling, different amounts of protein were incorporated into the agar diets. Choice flight tunnel tests were conducted and the volatile pheromones collected from males. The results showed that addition of between 5-10% protein to the agar diet enabled males to compete equally with males fed the dry diet in attracting females and in release of pheromone (Fig. 8).

The effect of incorporation of hormone into the agar diet was then determined by incorporating a water-soluble formulation of methoprene at doses of 0.025, 0.05 and 0.1% active ingredient into the diet. Attraction of females to either the agar diet containing 10% protein or the agar diet containing 10% protein + either 0.025, 0.05 or 0.1% methoprene was compared in flight tunnel experiments. The data showed that females responded more

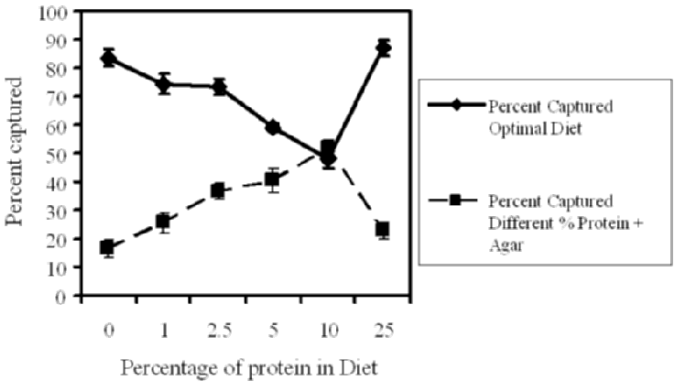

Figure 8. *Comparison of trap captures in flight tunnel studies where female Carribean fruit flies* Anastrepha suspensa *were offered the option of pheromones released by males fed the optimal dry diet or the agar gel diet to which various amounts of protein had been added (N = 6 replicates of each treatment).*

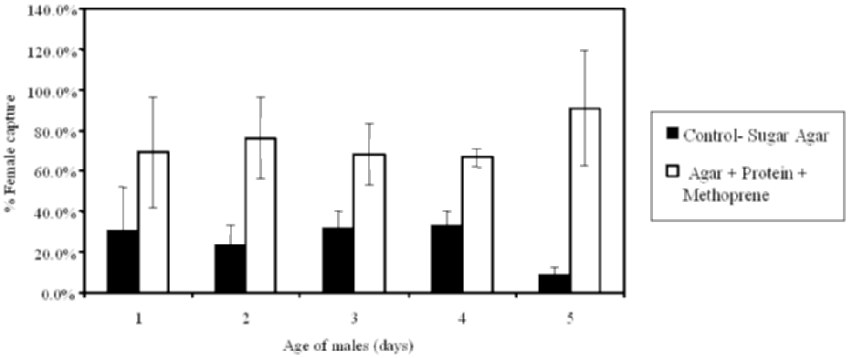

Figure 9. *Capture of sterile female Mediterranean fruit flies* Ceratitis capitata *in flight tunnel studies where females were given the choice of responding to males fed the control diet (sugar/agar gel) or the sugar/agar diet containing both 10% protein and methoprene. The numbers captured are significantly different on days 2-5 in a* t*-test (8 replicates).*

effectively when males had been fed any of the diets containing methoprene. Also, methoprene-fed males attracted females at much earlier ages. Analysis of pheromone released by males fed these diets showed that the amounts of pheromone were greater for all treatments that contained the methoprene.

Of course the most critical component of the SIT is effective mating. Therefore, mating studies were conducted using fertile females and sterile males fed the agar/protein plus methoprene diet or fed the agar/sugar diet that did not contain hormone or protein. Significantly more males that fed the agar diet containing protein plus hormone (48%, t = 3.16, d.f. = 8) mated. Only 30% of the males fed the agar only diet mated.

As with the Caribbean fruit fly, incorporating 10% protein into the agar diet fed to males belonging to the strain of Mediterranean fruit fly that carry a *temperature sensitive lethal* (*tsl*) mutation significantly improved the ability of males to attract sterile female flies on the second day after emergence, and these males produced approximately five times the amount of pheromone produced by males fed the agar diet alone. Adding methoprene to the agar/protein diet resulted in production of twice the amount of pheromone that flies fed the agar/protein diet produced (eight times more than flies fed the agar diet without protein), and this was reflected in flight tunnel studies indicating that females were far more likely to move to volatiles released by males fed the agar/protein plus methoprene diet than to males fed just the agar diet (Fig. 9). From this it was concluded that adding methoprene and protein to the agar diet fed to *tsl* male Mediterranean fruit flies improves their sexual competitiveness as was the case for the Caribbean fruit fly.

6. Conclusions

Although this research has demonstrated the need for protein in the diets fed to adult sterile males, and that incorporation of methoprene into the adult diet results in improved and accelerated development of pheromone calling, the technology has not yet been incorporated into mass-rearing systems. Currently, the cost effect of incorporating the technologies into sterile fly rearing programmes for both the Mediterranean and Mexican fruit flies is being assessed by the authors while numerous other research groups are examining the feasibility of the technology for different genera of tephritid flies. While it is believed that the technology will greatly improve the efficacy of the SIT for the Mediterranean, Caribbean, Mexican and West Indian fruit flies, a complete understanding of

the effects of hormone therapy and a benefit/cost analysis data are needed prior to employing the technology with other species of tephritids.

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Unfaithful Mediterranean Fruit Fly *Ceratitis capitata* Females: Impact on the SIT?

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ABSTRACT An understanding of the levels of remating and paternity skew in the field can be important for polyphagous pest species with a high colonization potential such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). The use of polymorphic simple sequence repeats on flies from two Mediterranean populations in combination with various statistical methods showed not only that Mediterranean fruit fly females remate in the wild, but most importantly, that the level of sperm precedence could influence the effect of remating itself since one male, presumably the last, tends to sire most of the progeny. Levels of remating and paternity skew may have important implications for the evolution of the species in terms of maintenance of genetic variability. Moreover, these features of mating behaviour may locally affect the efficiency of the sterile insect technique (SIT), which is a commonly applied control strategy against the Mediterranean fruit fly.

KEY WORDS multiple mating, sperm use, *Ceratitis capitata*, microsatellites, field populations

1. Introduction

Understanding the frequency of remating and the factors that may influence it, together with an understanding of the extent and mechanisms that regulate sperm use are particularly important for pest species such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) for which the sterile insect technique (SIT) is widely used as part of area-wide integrated pest management (AW-IPM) programmes (Krafsur 1998). The efficiency of the SIT depends, amongst others, on the sexual competitiveness of sterile males, in terms of mating competitiveness, sperm transfer, accessory gland secretion transfer, and sperm competition. Females mated with sterile males should ideally show the same remating behaviour as those mated with wild males (Cayol et al. 1999). If females mate more than once they can remate with either a fertile or a sterile male, however, the release of a huge excess of sterile males in Mediterranean fruit fly SIT programmes suggests that any female that does remate is likely to mate with a sterile male.

The Mediterranean fruit fly mating system is based on arboreal leks, where females encounter several courting males and freely select a mate (Yuval and Hendrichs 2000). Less commonly, an alternative "fruit-guarding" tactic is employed, whereby males occupy oviposition hosts and copulate previously mated females that alight on them to oviposit (Prokopy and Hendrichs 1979). It is hypothesized that female monogamy could be

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favoured over polyandry due to the highly male-skewed operational sex ratio at lek sites (Warburg and Yuval 1997), and the high copulation cost for females (Hendrichs and Hendrichs 1998). However, female remating has been reported in the laboratory (Saul and McCombs 1993, Whittier and Shelly 1993, Blay and Yuval 1997, Miyatake et al. 1999), in field cages (McInnis et al. 2002, Vera et al. 2003, Shelly et al. 2004), and in a wild population from the Greek island of Chios (Bonizzoni et al. 2002, Kraaijeveld et al. 2005). Depending upon the experimental conditions and the strain of flies studied, remating frequency ranges from 0.03 to 0.76. It has been proposed that renewal of female sexual receptivity is influenced by the efficiency of the previous mating, in particular by the quantity of the sperm transferred and the quality of male accessory gland secretions (Mossinson and Yuval 2003). Extensive studies have been done in *Drosophila*, where three seminal fluid peptides (the sex-peptide, ovulin and DUP99B) have been shown to play a major role in eliciting both short-term and long-term postmating responses (Liu and Kubli, 2003). Laboratory experiments using morphological mutants in the Mediterranean fruit fly have shown that, following remating, between 58 and 71% of the progeny was fathered by the male of the second mating, giving a second male sperm precedence (P2) of between 0.58 and 0.71 (Saul and McCombs 1993).

Little is known about whether Mediterranean fruit flies from different origins vary in remating frequency due to geography, climatic conditions, population density, and seasonal fluctuations. Studies conducted to date have focused on sexual compatibility among Mediterranean fruit flies from different localities, on sexual compatibility of wild flies versus sterile males (Cayol et al. 2002), and on the relative competitiveness of sterile versus wild males (McInnis et al. 2002, Vera et al. 2002, Kraaijeveld and Chapman 2004). Assessing the extent of Mediterranean fruit fly remating in the field is extremely challenging since, as in other field-based studies of multiple mating and sperm use, there is no experimental control over the number of times that a female mates, the interval between matings or the genetic identity of multiple fathers contributing to a brood.

However, the availability of high resolution molecular markers, such as microsatellites (simple sequence repeats) (Bonizzoni et al. 2000, Baliraine et al. 2003), coupled with efficient statistical methods such as GERUD (Jones 2001) or SCARE (Jones and Clark 2003), enables the assignment of paternity in an open field situation with considerable confidence to be made even from a relatively low number of insects sampled (Imhof et al. 1998, Bungaard et al. 2004, Bretman and Tregenza 2005, Schlötterer et al. 2005). The use of the most informative loci on the basis of their polymorphic information content – a parameter dependent on the number of the alleles at each locus and its heterozygosity (Hearne et al. 1992) – allows the inference of paternity by comparing the genotypes of wild-caught Mediterranean fruit fly mothers and their progeny produced in the laboratory.

2. Populations Studied

Two geographic populations from the Mediterranean basin, one on the Island of Chios in Greece and the other from Rehovot in the central coastal plain of Israel, were chosen to determine whether the frequency of remating varies between geographically different populations. These two sampling areas have been extensively used for Mediterranean fruit fly behavioural and demographic studies (Rivnay 1950, Katsoyannos et al. 1998, Israely et al. 2005). While both Chios and Rehovot populations follow a seasonal Mediterranean pattern, the slightly different climatic conditions influence the seasonal fluctuations of their population density (Katsoyannos et al. 1998, Israely et al. 2005). On the Island of Chios, Mediterranean fruit fly adults can be captured from June to mid January, with peak densities being reached between the beginning of August and the end of November. The population remains below a detectable level for the rest of the year, over-

wintering as larvae mainly in sweet oranges and mandarins (Katsoyannos et al. 1998).

In the Rehovot area, the seasonal fluctuation in population density is bimodal with the first adults appearing in April-May. Due to favourable weather conditions and host availability, the spring population rapidly increases until July when it goes through a strong bottleneck due to both high temperatures and a relative scarcity of hosts. A second, but minor population increase is observed in September, with a peak in October. In winter (January-March), the number of flies rapidly decreases to an almost undetectable level (Israely et al. 2005).

Mediterranean fruit fly females of unknown reproductive status were captured in BioLure® baited traps from the two areas. The sampling procedures in Chios were described by Bonizzoni et al. (2002). In Rehovot, traps were placed in September 2001 and in April-May 2002. The number of flies trapped was generally low (less than 50 flies). Twenty females in 2001 and 30 in 2002 were removed from the traps, transported to the laboratory and allowed to oviposit individually in appropriate fruits. In 2001 each female oviposited in one fruit whereas in 2002 the fruits were removed from the oviposition cages every two days and replaced by a fresh fruit. Females were allowed to oviposit until they died. Each female and all her adult offspring were killed and shipped to Italy in 100% ethanol.

To determine the level of microsatellite polymorphism of the two populations, two additional samples of 36 and 42 flies were collected in Chios in 2000 and in Rehovot in 2002.

3. Observed Remating Frequencies

Genotyping of the two random samples from Chios and Rehovot identified the most informative simple sequence repeat loci for paternity analysis, based on the number of alleles, their frequency and their polymorphic information content (Hearne et al. 1992). In Chios, these loci were *Ccmic 8*, *3*, *17* and *13*; and in Rehovot they were *Ccmic 2*, *6*, *15*, *17*, *25*, *3*, *32*, *13* and *23*.

Based on data from these most informative simple sequence repeats, combined exclusion powers (i.e. the probability of excluding a randomly chosen candidate parent from parentage of an arbitrary offspring, given the genotype of the mother and all her progeny), of 0.725 and 0.938 were derived for Chios and Rehovot, respectively. These same loci provided correct paternity assignments of 80.0% and 95.8% respectively in Chios and Rehovot (CERVUS simulation (Marshall et al. 1998)). The lower value of the predicted success rate for Chios could be due to its lower simple sequence repeats variability and its higher level of allele sharing (aS = 0.046 and 0.0002, in Chios and Rehovot; respectively (Palmer et al. 2002)), which could mask remating.

Seventy two percent of caught wild females (26 out of 36) in Chios produced progeny giving a total of 681 individuals. Paternity analysis performed on the progeny of the 18 mothers with at least 12 offspring (mean progeny size: 31.4) (GERUD (Jones 2001)) revealed a remating frequency of 5.5% (one out of 18 females).

In Rehovot, 15% of the females collected in September 2001 (three out of 20), and 30% from April-May 2002 (nine out of 30), produced progeny giving a total of 422 individuals. Paternity analysis performed on the progeny of the eight females with at least 12 offspring (mean progeny size: 36.5) revealed a remating frequency of 50% (four out of eight females; two from each of the 2001 and 2002 collections).

4. Observed and Expected Remating Frequencies

The probability of detecting two fathers was calculated from the population simple sequence repeats allele frequency data (GERUD (Jones 2001)) under different conditions of progeny size (10, 34 or 50) and paternity skew, assuming second male sperm precedence (50, 60 or 70%). The results indicated that the number of progeny influenced remating detection more than the

level of paternity skew (i.e. in Chios for a paternity skew P_1:P_2 of 3:7, the correct detection of the second father varied from 52.3 to 62.3%, with 10 and 34 offspring, respectively, while with a P_1:P_2 of 4:6, the range was from 54.1 to 62.3%. The same tendency was found in Rehovot (data not shown). The probability of detecting remating was higher in Rehovot than in Chios (Bonizzoni et al. 2006). Based on these simulations, 1.6 rematings should have been detected in Chios whereas only one was observed. In other words, among the 18 females analysed, 9% were expected to remate, but only 5.5% were observed. Consequently, the mean number of fertile matings per female (*M*) is estimated as 1.09 (18+1.6)/18, while the observed value was 1.06 (18+1)/18. In Rehovot, over the eight tested females, 4.3 cases of remating should have been detected compared with the four observed; *M* is estimated as 1.54 (4.35+8/8) versus the observed 1.50 (4+8/8).

G tests of independence were applied to test whether there were differences in the observed remating frequency between Chios and Rehovot. The test was significant at $P < 0.025$ ($G = 5.88$, d.f. = 1).

5. Breeding Behaviour

The SCARE program, which uses a Bayesian approach (Jones and Clark 2003) was used to estimate *M* and the proportion of offspring from the last-mating male (β). In Chios, the 95% confidence interval range for *M* and β were respectively 1.15-2.01 (mean 1.48) and 0.80-0.95 (mean of 0.89). In Rehovot, using the four unlinked loci with the highest polymorphic information content (*Ccmic3*, *6*, *15*, *17*), the 95% confidence interval ranges for *M* and β were: 1.61-4.09 (mean of 2.59) and 0.64-0.82 (mean of 0.73).

6. Is Remating Frequency Affected by the Genetic Background and/or Population Density?

Multiple matings ascertained both in Chios and Rehovot suggest that Mediterranean fruit fly females can copulate more than once in the field. Both the GERUD and the SCARE-derived estimates of *M* were lower in Chios than in Rehovot. The lower number of females analysed in Rehovot, associated with the higher rate of remating detected independently in the collections from two different years with respect to Chios, renders the observed difference in remating frequency even more reliable. It has been hypothesized that different strains of Mediterranean fruit fly differ in male stimuli and in female responsiveness (Jang et al. 1998), and that the tendency to remate may be adaptive and heritable (Saul and McCombs 1993). In laboratory experiments, Whittier and Shelly (1993) showed that remating was adaptive since multiple-mated females had a significantly higher reproductive output than once-mated females. Chios and Rehovot have well-established fly populations sharing a relatively recent common evolutionary history (Malacrida et al. 1998). Consequently, they show a very low level of genetic differentiation and a high gene flow estimate (Bonizzoni et al. 2004). However, the possibility cannot be excluded that the distinct genetic background of the two populations affected the extent of the remating estimates.

Although following a similar Mediterranean seasonal pattern, the fluctuations in Chios and Rehovot populations are influenced by slightly different climatic conditions (Katsoyannos et al. 1998, Israely et al. 2005). On the island of Chios, fly sampling was done in July 2000 when the population was still expanding but had already reached a moderate size (Katsoyannos et al. 1998). The flies from Israel were collected in September 2001 and in April-May 2002, i.e. at the beginning of the autumn and spring expansions respectively. Since the Mediterranean fruit fly has a high rate of population increase (Liedo and Carey 1996), it is reasonable to suppose that at the time of the collections the Rehovot population could have been biased towards younger flies. In Mediterranean fruit fly females, the receptivity to a second mating was shown to be negatively correlated with female age

(Chapman et al. 1998, Kraaijeveld and Chapman 2004). Moreover, mated males seem to be more efficient at inhibiting remating than virgin males (Vera et al. 2002). Therefore, a physiological parameter, such as the lower average age of the Rehovot flies may account for their higher frequency of remating.

According to sexual selection theory, females of a lekking polyphagous species such as *C. capitata*, should evolve highly discriminative mate choice based on male quality (Kaneshiro 1989). Kaneshiro (2000) suggested that under conditions of small population size, choosy females that cannot encounter males satisfying their courtship requirements, might undergo a physiological change thus increasing the chance of mating with less "successful" males and as a result will tend to remate. That such a scenario occurred in Rehovot during the time of sampling cannot be excluded.

7. Sperm Use

The SCARE simulations (Bungaard et al. 2004) on the two natural populations strongly indicate that Mediterranean fruit fly populations exhibit high sperm skew (mean $\beta = 0.89$ and 0.73 for Chios and Rehovot, respectively). Since results using morphological mutants have shown that the second male sired more progeny than the first (Saul and McCombs 1993), it is reasonable to assume in the present studies that it was also the second male that contributed in a higher proportion to the paternity of the offspring, as was assumed in the SCARE simulations (Jones and Clark 2003). In previous laboratory crosses, the proportion of offspring sired by the first and second male did not change over the females' life span or with different intervals between matings (Katiyar and Ramirez 1970), suggesting a complex system of sperm storage and use. Marchini et al. (2001) and Twig and Yuval (2005) analysed the functions of the two types of sperm storage organs (i.e. the two spermathecae and the fertilization chamber) in relation to insemination, fertilization and their control. Spermathecae function as long-term storage organs, while the fertilization chamber acts as a staging point for fertilizing sperm. Twig and Yuval (2005) concluded that the use of both sperm storage organs allows females to manipulate ejaculates by diverting and segregating them adaptively. Obviously, the storage of sperm from more than one male provides opportunities for sperm competition and/or sperm choice. Which mechanism is responsible for the high level of second male sperm precedence that was both observed and simulated is still an open question requiring further study. From the SCARE simulation, and at a very preliminary level also from the progeny analyses, the extent of paternity skew appears greater in Chios than in Rehovot where a higher frequency of remating was observed. This finding introduces the interesting question of understanding the evolutionary relationship (if any) among sperm allocation, remating frequency and sperm displacement in the Mediterranean fruit fly.

8. Conclusions

In the context of Mediterranean fruit fly control programmes based on the SIT, the low mating competitiveness of sterile males increases the probability of a female remating (Kraaijeveld and Chapman, 2004). The findings reported in this study indicate that in wild populations of the Mediterranean fruit fly (1) remating is occurring, and its level may vary, and that (2) there is preference for sperm from the second mating male. In relation to both the use of the SIT and in the analysis of the evolution of this species, these studies show the importance of acquiring knowledge on the mechanisms of sperm use and competition in Mediterranean fruit fly and couple them to studies on male mating competitiveness.

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Assessing Genetic Variation in New World Screwworm *Cochliomyia hominivorax* Populations from Uruguay

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ABSTRACT The New World screwworm *Cochliomyia hominivorax* (Coquerel) is an important parasitic insect pest in Neotropical regions. New World screwworm myiasis is caused by the larval stage of the fly infesting tissues of warm-blooded vertebrates. This species represents a serious threat to the livestock sector across its current distribution, which includes part of the Caribbean and all of South America (except for Chile). Knowledge of the extent and distribution of genetic variability of *C. hominivorax* is of great interest for the description of populations and for contributing to future strategies of control. This paper describes the analysis of genetic variability and structure of New World screwworm populations in Uruguay using two different molecular markers, mitochondrial DNA and microsatellites.

KEY WORDS New World screwworm, genetic differentiation, mitochondrial DNA, microsatellites

1. Introduction

The New World screwworm *Cochliomyia hominivorax* (Coquerel), one of the most important parasitic insect pests of warm-blooded vertebrates, causes invasive myiasis and is responsible for important economic losses to livestock rearing. The current distribution of the New World screwworm includes part of the Caribbean and all of South America (except for Chile). This species has been successfully eradicated from North and Central America using an area-wide approach involving the sterile insect technique (SIT) (Wyss 2000, Vargas-Terán et al. 2005). In 1988, the pest was introduced into Libya, but its spread to livestock and wildlife in the rest of Africa and the Mediterranean region was prevented by a successful SIT campaign using sterile flies shipped from the mass-rearing facility in Tuxtla-Gutiérrez, Mexico (Lindquist et al. 1992, Vargas-Terán et al. 1994).

In South America, however, this pest continues to affect the development of the livestock sector and wider economic development. An international effort is underway to evaluate the feasibility of eradicating the New World screwworm from endemic areas of the Caribbean and South America and to prevent invasions into screwworm-free areas. This involves *inter alia* collecting data on the damage and costs associated with control and on the distribution and density of the fly in these regions.

With respect to the latter, there have been speculations and conflicting reports about the existence of non-interbreeding populations and their possible effects on the control programme but to date there is no evidence that this situation exists (LaChance et al. 1982). To maximize the effectiveness of an eradication programme, it is essential to confirm that such populations do not exist in these new regions and to characterize the genetic variability of

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Figure 1. *The* Cochliomyia hominivorax *collection sites in Uruguay.*

target populations. Knowledge of the genetic structure of New World screwworm populations will also be useful for identifying their actual and potential routes of gene flow and thereby improve the implementation of area-wide approaches to control this insect pest.

In the past, Krafsur and Whitten (1993) examined isozyme loci in 11 Mexican New World screwworm populations and their estimate of Wright's *F*-statistics (F_{ST}) (Wright 1965) was not significantly different from zero. They concluded, therefore, that screwworm populations in Mexico belonged to a single panmitic population. Taylor et al. (1996) also used isozyme loci to study two Brazilian populations and compared the results with previous data from Costa Rica (Taylor and Peterson 1994) and partial data from Mexico (Krafsur and Whitten 1993). They also concluded that New World screwworm forms a single panmitic population.

However, subsequent analyses of four Brazilian populations using different types of molecular markers in the mitochondrial and nuclear genomes, suggested a different pattern of substructuring. Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) suggested that these

Table 1. *Field-collected samples of* Cochliomyia hominivorax *in Uruguay.*

Location	Number of individuals	Latitude	Longitude	Altitude (metres)
Bañados de Medina, Cerro Largo	24	32°23' 00 S	54° 21' 00 W	51
Cerro Colorado, Florida	29	33°52' 00 S	55° 33' 00 W	96
Colonia del Sacramento, Colonia	15	34°20' 00 S	57° 86' 67 W	213
Dayman, Paysandú	19	31°33' 00 S	57° 57' 00 W	27
Joaquín Suárez, Canelones	15	34°44' 01 S	56° 02' 12 W	203
Paso Muñoz, Salto	21	31°27' 00 S	56° 23' 00 W	55
San Antonio, Salto	15	31°24' 00 S	57° 58' 00 W	41

populations probably belonged to a single evolutionary lineage interconnected by reduced gene flow (Infante-Vargas and Azeredo-Espin 1995). The random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) technique was also used to detect genetic polymorphism and to select genetic markers to discriminate six Brazilian populations and one population from northern Argentina (Infante-Malaquias et al. 1999). In general, results from both mitochondrial and RAPD analyses were concordant in suggesting divergence among New World screwworm populations. Analysis of five Brazilian populations by means of isozyme loci revealed a high geographical differentiation across south-eastern Brazil with relatively low gene flow (Infante-Malaquias 1999).

One possible explanation for the discrepancies between the different data is that different levels of substructuring were present in different locations. Infante-Malaquias et al. (1999) suggested that South America could be the centre of origin of this species, explaining the high variability and the population structure found there whereas the homogenous populations of North America were possibly formed by a founder effect.

It is clear from the above that the available information is insufficient to infer patterns of genetic variation and structure among New World screwworm populations throughout their geographical range. Therefore, an effort was made to add to the body of information available on these aspects by using mtDNA and microsatellites to analyse the genetic variability and structure of Uruguayan populations of the New World screwworm.

2. Materials and Methods

2.1. Sampling of New World Screwworm in Uruguay

New World screwworm samples were obtained from seven distinct geographic locations in Uruguay with distances between them ranging from 50 to 418 kilometres (Fig. 1, Table 1). Larvae were collected directly from infested wounds in sheep, cattle and dogs in January 2003. Sampling of related individuals was avoided by choosing wounds in different animals and/or farms and by classifying larvae from the same wound by instar.

Larvae were transferred to the laboratory and reared until the pupal stage under standardized conditions (Infante-Vargas and Azeredo-Espin 1995) or fixed in 100% ethanol. Genomic DNA was extracted either from single adults, pupae or larvae using a phenol-chloroform procedure (Infante-Vargas and Azeredo-Espin 1995).

2.2. Mitochondrial DNA as a Molecular Marker

Diverse aspects related to the structure and evolution of mtDNA, such as simple and uniform organization, lack of recombination, maternal inheritance and high rate of nucleotide sequence evolution, have made it a valuable marker for estimating intraspecific genetic variability (Avise 1994).

RFLP analysis of mtDNA was previously used for New World screwworm populations and revealed a high level of genetic variation (Infante-Vargas and Azeredo-Espin 1995, Taylor et al. 1996). However, restriction analysis of mtDNA polymerase chain reaction products (PCR-RFLP) provides a faster and simpler method and has successfully been used for population analyses (Ross et al. 1997, Dueñas et al. 2002).

2.2.1. Mitochondrial DNA Variability in New World Screwworm Populations in Uruguay

Lyra et al. (2005) used mtDNA PCR-RFLP to examine the genetic variability among the seven Uruguayan populations sampled. Two regions of the mtDNA, the control region (A+T/12S) and subunits 1 and 2 of the *cytochrome oxidase* (*cox*1/*cox*2), were amplified and digested with the restriction endonucleases *Dra* 1 (A+T/12S), *Ase* 1 and *Msp* 1 (*cox*1/*cox*2).

Among the populations, nine haplotypes were observed. The mean nucleotide diversity (π) was 0.0229 and the haplotype diversity (*Hs*)

was 0.6355, indicating high mtDNA variability. The similarity index (F) was high (96.7%) and the estimate of nucleotide divergence between populations (δ) was very low (0.00055), suggesting a high similarity among samples from the different locations. The analysis of molecular variance (AMOVA) showed no evidence of population differentiation, indicating that New World screwworm forms a single panmitic population in Uruguay. Lyra et al. (2005) suggested that the distribution of New World screwworm in the extreme south of the species' occurrence and the fact that there are no geographical barriers or important climatic differences between studied regions were responsible for the lack of differentiation in Uruguay.

2.3. Microsatellite Markers

Microsatellites, or simple sequence repeats, are short sequences made up of a single motif with no more than six bases that is tandemly repeated (Goldstein and Schlötterer 1999). They are found in large numbers and are relatively evenly spaced throughout the genome of every eukaryotic organism analysed so far.

Among the several classes of molecular markers, microsatellite loci stand out as co-dominant markers with a high number of alleles per locus, high polymorphism and a high heterozygosity value. Due to these features, variation in these co-dominant markers has been increasingly used as the marker of choice to investigate questions regarding population structure, gene flow and mating systems even in populations which have low levels of allozyme and mitochondrial variation. The recent isolation and characterization of polymorphic microsatellite markers for New World screwworm (Torres et al. 2004, Torres and Azeredo-Espin 2005) enables genetic variability and population structure of this pest in Uruguay to be investigated.

2.3.1. Microsatellite Amplification and Genotyping of New World Screwworm Populations in Uruguay

Ten previously characterized microsatellite markers (Torres et al. 2004) were used in this study. The primer sequences and the procedures for microsatellite amplifications and analyses of PCR products were described by Torres et al. (2004).

2.4. Data Analysis

The number and frequency of alleles, the allele size range and the observed (H_O) and the unbiased expected (H_E) (Nei 1978) heterozygosities under Hardy-Weinberg equilibrium were determined per locus for each location. The software Micro-checker 2.2.0 (Van Oosterhout et al. 2004) was used to test for technical artefacts such as null alleles, stuttering and large allele dropout. Each locus and population was tested for deviations from Hardy-Weinberg equilibrium expectations using exact tests implemented in GENEPOP, a population genetics software for exact tests and ecumenicism (Raymond and Rousset 1995). Genotypic linkage disequilibrium among all pairs of loci within each site was investigated using Fisher's exact test as implemented in GENEPOP. An unbiased estimate of the exact probability was obtained using the Markov chain algorithm (Guo and Thompson 1992). Two indices of genetic differentiation were estimated between the localities, F_{ST} and R_{ST}, the former based on the absolute frequencies of alleles (Weir and Cockerham 1984) and the latter estimated from the sum of the squared number of repeat differences (Slatkin 1995). An unbiased estimate of F_{ST}, θ was calculated using the FSTAT computer programme (Goudet 1995). The significance of pairwise F_{ST} estimates was tested by permuting genotypes among populations (Goudet et al. 1996). The overall estimate of R_{ST}, ρ_{ST} was calculated using RSTCALC, a PC-based programme for performing analyses of population structure, genetic differentiation and gene flow using microsatellite data (http:// helios.bto.ed.ac.uk/evolgen/rst/rst.html). Significance levels for simultaneous statistical tests were corrected using the sequential Bonferroni method (Rice 1989). The isolation-by-distance model of population genetic structure was tested by

linear regression of pairwise F_{ST} /(1 - F_{ST}) against the natural logarithm of the geographical distance between population pairs (Rousset 1997).

3. Results and Discussion

3.1. Microsatellite Variation

The number of alleles and the expected and observed heterozygosity per locus and per population are given in Table 2. Analysis of 138 New World screwworm genotypes revealed a moderate degree of polymorphism across the seven sampling locations. Ten loci were used in the first analysis, but the locus *CH02* presented some ambiguous, non-reproducible patterns. For this reason, it was excluded from the statistical analysis.

For the nine microsatellite loci analysed, the number of alleles detected per locus and per population ranged from two to ten, with an average of six (Table 2). The observed heterozygosity (H_O), varied from 0.19 to 0.91 and the expected heterozygosities (H_E) varied from 0.37 to 0.87 (Table 2).

Significant deviation from the Hardy-Weinberg equilibrium (exact probability test, $P < 0.05$) was recorded for all sampling localities. In all cases, departures from expectations were due to an excess of homozygotes. Among the possible factors that might account for these deviations is the Wahlund effect, since the samples were collected from different farms at each location. However, such effects should be apparent in most of the loci across populations, which was not the case for this data set. Another factor that could also have caused the observed deviations is the presence of null alleles. These result from mutations such as substitutions, insertions, or deletions in one or both priming sites preventing the binding of the DNA strand and primers (Callen et al. 1993) and non-amplification of the allele. At the population level this can lead to a misinterpretation of the number of heterozygotes and consequently of Hardy-Weinberg deviations. Only the locus *CH10* presented a significant number of null alleles and the analysis excluding this locus was not significantly altered. Furthermore, these results are being confirmed by the preliminary analysis of new populations using these loci and additional loci (Torres and Azeredo-Espin 2005). The occurrence of demographic changes that affected New World screwworm populations may therefore be the main cause of the observed homozygote excess. These in turn could have arisen from decreases in temperature and humidity in the Uruguayan winter and/or persistent insecticide treatment which can cause mass-population mortality and local extinction of New World screwworm populations.

Linkage disequilibrium was found in only two of 252 comparisons among the loci and populations analysed, but no common pair of loci showed non-random associations in all the populations (data not shown).

3.2. Interpopulation Variability

Most variation was found within rather than between populations and the seven populations exhibited remarkably similar allele distributions. This is consistent with the results found by the PCR-RFLP of the mtDNA.

Two measures of interpopulation genetic differentiation were used in this study (F_{ST} and R_{ST}). The global multilocus estimate of R_{ST} was 0.015 and of F_{ST} was 0.031. Both estimates, although low, were numerically very similar and significantly different from zero ($P < 0.05$, for R_{ST} and $P < 0.001$, for F_{ST}), suggesting that little differentiation exists among these populations.

The relationship between local populations was tested by calculating pairwise F_{ST} estimates because it was demonstrated that F_{ST} yields the better estimate when the number of loci is small (< 10) or the sample size is small (Gaggiotti et al. 1999). F_{ST} estimates between populations ranged from -0.0005 to 0.0853 (Table 3) and for five of the ten population pairs were significantly different from zero at the 0.05 level.

These low levels of substructuring could be attributed to the high dispersal capacity of

Table 2. *Genetic diversity in* Cochliomyia hominivorax *from seven localities in Uruguay.*

Locus		Dayman 2N = 38	S. Antonio 2N = 42	Colonia 2N = 30	B. Medina 2N = 48	J. Suarez 2N = 30	C. Colorado 2N = 58	P. Muñoz 2N = 30
CH01	N_a	6	6	5	6	5	6	5
	H_O	0.3158	0.4762	0.5333	0.5833	0.6667	0.5517	0.5333
	H_E	0.6230*	0.7607*	0.7517	0.6809	0.6943	0.6031	0.7126*
CH05	N_a	5	5	6	7	4	7	5
	H_O	0.6316	0.5238	0.7333	0.4167	0.5333	0.5172	0.8000
	H_E	0.5874	0.6190	0.6667	0.6755*	0.4483	0.6636*	0.7747
CH09	N_a	4	4	4	7	2	6	5
	H_O	0.3333	0.4500	0.5333	0.7917	0.2000	0.5357	0.5333
	H_E	0.3762	0.4423	0.5724	0.7216	0.3701	0.6227	0.6299*
CH10	N_a	6	6	6	6	4	5	6
	H_O	0.3684	0.1905	0.2000	0.2917	0.6000	0.4000	0.4000
	H_E	0.6344*	0.5912*	0.7885*	0.7101*	0.6598	0.5167*	0.5011*
CH11	N_a	5	10	9	8	9	7	7
	H_O	0.6111	0.6190	0.7333	0.6364	0.6667	0.4286	0.4000
	H_E	0.6159	0.7317	0.7816	0.8245*	0.8276*	0.7247*	0.6943*
CH12	N_a	8	8	8	8	5	9	7
	H_O	0.8333	0.7143	0.6000	0.9167	0.6000	0.7241	0.8000
	H_E	0.8476*	0.8479*	0.8736	0.8475	0.6460	0.8100	0.7931
CH14	N_a	7	7	6	6	6	6	5
	H_O	0.5789	0.4762	0.6000	0.5217	0.6000	0.5714	0.5333
	H_E	0.8179*	0.6690*	0.7356	0.6135	0.6989	0.8013*	0.6713
CH15	N_a	7	7	6	6	3	6	5
	H_O	0.5556	0.4762	0.5333	0.3750	0.2667	0.2759	0.4000
	H_E	0.8302*	0.7607*	0.7862	0.7943*	0.5080*	0.7828*	0.7310*
CH20	N_a	7	7	5	7	5	6	4
	H_O	0.3684	0.4762	0.5333	0.5000	0.8000	0.6897	0.5333
	H_E	0.7084*	0.7120	0.6414	0.6835*	0.7540	0.7048*	0.6920
All loci	Mean N_a	55	59	55	61	43	58	49
	Mean H_O	0.5107	0.4892	0.5556	0.5592	0.5481	0.4816	0.5481
	Mean H_E	0.6723*	0.6816*	0.7331*	0.7279*	0.6230*	0.6922*	0.6889*

N_a, number of alleles

H_E, expected heterozygosity

H_O, observed heterozygosity.

* denotes a significant ($\alpha = 0.05$) deviation from Hardy-Weinberg equilibrium

New World screwworm, since migration is assumed to prevent genetic differentiation at neutral markers (Agis and Schlötterer 2001). However, the analysed populations showed no isolation by distance ($P = 0.6115$). Since restricted migration results in positive correlation between geographical and genetic distance (Slatkin 1993), simple migration models may not be sufficient to explain the low differentiation between New World screwworm populations. One factor that could be responsible for this pattern of genetic differentiation

Table 3. F_{ST} *estimates for all* Cochliomyia hominivorax *population pairwise comparisons.*

Study area	S. Antonio	Colonia	B. Medina	J. Suarez	C. Colorado	Paso Muñoz
Dayman	0.0080^{NS}	0.0171^{NS}	0.0194^{NS}	0.0497*	0.0209^{NS}	0.0281^{NS}
S. Antonio		0.0112^{NS}	0.0111*	0.0709*	0.0201*	0.0240*
Colonia			-0.0005^{NS}	0.0675*	0.0200^{NS}	0.0378*
B. Medina				0.0853*	0.0163^{NS}	0.0334*
J. Suarez					0.0664*	0.0533*
C. Colorado						0.0399*

NS, not significant
* significant at the 5% nominal level after standard Bonferroni corrections

is the passive migration of larvae by the movement of infested animals. However, an alternative explanation can be considered as responsible for the low differentiation and the lack of isolation by distance. It was noted (Slatkin 1993) that the absence of isolation by distance could be indicative of a recent recolonization event. Considering the hypothesis of mass-mortality by climatic conditions or insecticide treatment, a recolonization by a large founder population could cause a demographic turnover if this population spread rapidly over Uruguay during climatically favourable seasons. In this case, a very similar allele distribution would be expected over the country. To test this hypothesis it is necessary to compare Uruguayan New World screwworm samples collected during different hot/rainy seasons, as well as samples from intermediate and central populations which can be acting as stable sources of New World screwworm for recolonization events.

4. Conclusions

Information about patterns of genetic variation, structure and gene flow is needed before investing in large-scale efforts to control insect pests. This information can, to a large extent, be assessed using modern molecular techniques. Mitochondrial and microsatellite markers have helped to provide this information for New World screwworm populations in Uruguay.

The results presented here and elsewhere by Lyra et al. (2005) suggest that the seven populations from Uruguay are very similar, sharing homogenous haplotype (for mtDNA) and allele (for microsatellites) distributions. Although the mtDNA data indicate that this species forms a single panmitic population in Uruguay, results from microsatellite analysis yielded low, but significant, levels of subdivision between populations. These results can be explained by differences in the modes of inheritance of the two markers since the effective population size of mtDNA is only one quarter that of nuclear DNA (Sanetra and Crozier 2003). These differences, however, can also be explained by sex-biased gene flow among these populations. This would suggest that levels of female-mediated gene flow are slightly higher than male levels; consequently, mtDNA markers showed less structuring than the microsatellite polymorphisms. While Mayer and Atzeni (1993) described higher dispersal rates for New World screwworm females, this should be further investigated since microsatellite data also suggested that restricted migration might not play a significant role in population differentiation.

The results presented here provide some baseline data on genetic variation to which other New World screwworm populations can be compared. Analysis of other populations throughout its geographical distribution would determine if similar patterns of genetic variation and gene flow are observed and lay

the groundwork for future control strategies against this livestock pest.

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Emerging Mosquito-Borne Flaviviruses in Central Europe: Usutu Virus and Novel West Nile Viruses

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Short Communication

In Central Europe, four different mosquito-borne flaviviruses, i.e. Usutu virus (Weissenböck et al. 2002, Bakonyi et al. 2004), Rabensburg virus (Bakonyi et al. 2005), and two different unique West Nile viruses (Bakonyi et al. 2006) have recently emerged. During late summer 2001 in Austria, a series of deaths occurred in several species of birds, mainly Eurasian blackbirds *Turdus merula* L., reminiscent of the beginning of the West Nile virus epidemic in the USA. Dead birds were necropsied and examined by various methods. Pathologic and immunohistologic investigations suggested a West Nile virus infection. Subsequently, the virus was isolated, identified, sequenced, and subjected to phylogenetic analysis. The isolates exhibited 97% identity to Usutu virus, a rather unknown mosquito-borne flavivirus of the Japanese encephalitis virus group (Weissenböck et al. 2002). The Usutu virus has never previously been observed outside Africa nor associated with fatal disease in animals or humans. If it becomes established in Central Europe, this virus will have considerable effects on avian populations. Whether the

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Usutu virus has the potential to cause severe human disease is currently under investigation. The Usutu virus was isolated for the first time from mosquitoes in South Africa in 1959 and named after a river in Swaziland; it was sporadically isolated in Africa from several mosquito and bird species over the next decades and was once isolated from a man with fever and a rash.

A comparison of the complete genome sequences of the Austrian and a South African strain of the Usutu virus revealed 97% nucleotide and 99% amino acid identity. Phylogenetic trees were constructed displaying the genetic relationships of the Usutu virus with other members of the genus Flavivirus. When comparing the Usutu virus with other viruses of the Japanese encephalitis virus serogroup, the closest lineage was Murray Valley encephalitis virus (nucleotide: 73%, amino acid: 82%) followed by Japanese encephalitis virus (nucleotide: 71%, amino acid: 81%) and West Nile virus (nucleotide: 68%, amino acid: 75%) (Bakonyi et al. 2004).

West Nile virus is a member of the Japanese encephalitis virus group within the genus Flavivirus, family Flaviviridae. It is the most widespread flavivirus, occurring in Africa, Eurasia, Australia, and North America. Other members of the Japanese encephalitis virus group flaviviruses are Cacipacore virus, Koutango virus, Japanese encephalitis virus, Murray Valley encephalitis virus, Alfuy virus, St. Louis encephalitis virus, Usutu virus, and Yaounde virus. The West Nile virus was initially considered to have minor human health impact. However, human and equine outbreaks in Europe (Romania, Russia, France, Italy), Africa (Algeria, Tunisia, Morocco), and Asia (Israel) within the last ten years, and especially the virus's emergence and spread in North America since 1999, have lead to increased scientific interest. Phylogenetic analyses showed two distinct lineages of West Nile virus strains (which themselves subdivide into several subclades or clusters), isolated from different geographic regions.

The presence of the West Nile virus in central Europe has been known for some time. Serological surveys have detected specific antibodies to West Nile virus in several vertebrate hosts in Austria, the Czech Republic, Hungary and Slovakia during the past 40 years, and several virus strains were isolated from mosquitoes, rodents, and migrating birds. Human cases of West Nile fever were reported in the Czech Republic in 1997 and in Hungary in 2003. These countries are important transit areas or final destinations for migratory birds from the African continent, and hence may play an important role in the circulation and conservation of different West Nile virus strains. However, genetic information about the strains isolated in Central Europe has not hitherto been available.

A unique flavivirus strain closely related to West Nile virus, which was isolated from female *Culex pipiens* L. mosquitoes was collected ten kilometres from Lanzhot in the Czech Republic, after a flood in 1997. The complete genome sequence and phylogenetic analyses, as well as antigenic and mouse virulence characteristics of this strain were identified (Bakonyi et al. 2005). Since the collection site was very close to the Czech-Austrian border, about two kilometres from the small Austrian town of Rabensburg, this isolate was tentatively called Rabensburg virus. Another antigenically-identical or very closely related flavivirus strain was isolated from *C. pipiens* mosquitoes at the same location two years later.

The Rabensburg virus shares only 75-77% nucleotide identity and 89-90% amino acid identity with representative strains of West Nile virus lineages 1 and 2. The other flavivirus strain isolated in the same location two years later showed more than 99% nucleotide identity to the initially-isolated Rabensburg virus. Phylogenetic analyses of the Rabensburg virus, West Nile virus strains, and other members of the Japanese encephalitis virus complex clearly demonstrated that Rabensburg virus is either a new (third) lineage of West Nile virus or a novel flavivirus of the Japanese encephalitis virus group (Bakonyi et al. 2005).

An enzootic of encephalitis emerged in late

summer 2003 in a Hungarian goose flock resulting in a 14% mortality rate in six-week-old geese. Based on the histopathological alterations, serological investigations and nucleic acid detection by real time polymerase chain reaction (RT-PCR), West Nile virus was diagnosed as the causative agent of the disease. Although the presence of this virus in the country had already been known for 40 years, this was the first clinical outbreak of West Nile virus in Hungary. At the same time, an outbreak of West Nile virus was observed in humans in Hungary, which involved 14 reported cases diagnosed by serological methods (Bakonyi et al. 2006).

One year later, a Northern goshawk *Accipiter gentilis* (L.) showed central nervous system symptoms and died in a Hungarian national park. Using histopathological methods and RT-PCR, West Nile virus antigen and nucleic acid were detected in the organs of the bird.

To reveal the origin and relationship of the two West Nile virus strains, the complete genome sequences of the viruses were determined, and phylogenetic analysis performed. The goose (2003) isolate showed the highest (98%) identity with the West Nile virus strains isolated in 1998-99 in Israel and in the USA, causing endemics mainly in birds, humans and horses. These strains have been classified within lineage 1, clade 1a of West Nile virus. The other members of this clade have been isolated several times in Africa, Europe, and Asia during the last 50 years.

Interestingly, the goshawk (2004) strain showed the highest (96%) identity with the West Nile virus B 956 prototype strain, which had been isolated in 1937 in Uganda. This virus belongs to the lineage 2 of West Nile virus, and its close relatives have been detected exclusively in sub-Saharan Africa and in Madagascar so far (Bakonyi et al. 2006). Any possible laboratory contamination has been ruled out.

Based on the phylogenetic analysis of the two strains, it can be stated that the West Nile virus strains, which emerged in two consecutive years and caused avian mortality in Hungary are epidemiologically unrelated to each other. It is very likely, however, that the goose and human cases of 2003 are linked to each other, but in the absence of human isolates this was not proven. Because Hungary is a frequent transit station, and also final destination for migratory birds overwintering in Africa, the risk of the introduction of exotic West Nile virus strains to the country is quite high.

The results presented here demonstrate the threat of West Nile virus infection to animal and human populations in Central Europe. Therefore comprehensive investigations of the occurrence, ecology and epidemiology of the virus are of high priority. Results also show that these related viruses of transboundary nature, mainly resulting from long distance migration by birds, will continue to pose an increasing threat to animal and human health. In the event of a serious outbreak a comprehensive area-wide strategy will need to be developed to mitigate the effects of these viruses.

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Section 3

Modelling and Methods Development

The Role of Geographic Information Systems and Spatial Analysis in Area-Wide Vector Control Programmes

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ABSTRACT The success of area-wide interventions aimed at suppressing or eradicating insect populations rests largely on appropriate project planning and implementation - and this is as true in the context of vector-borne diseases as it is within the wider context of insect pest management. In either context, a successful control programme requires accurate knowledge of pre-existing distributions of insects (disease vectors) in time and space, on the appropriate design of insect control strategies, and on the development of suitable frameworks for monitoring and evaluation. Standard disease control operations, such as indoor residual spraying of insecticides or insecticide-treated bed nets for malaria, and the aerial application of insecticides or use of baited traps against the vectors of human and animal trypanosomosis, often include elements of area-wide planning because they target particular disease strata. Genetic control strategies (including the sterile insect technique (SIT)) are more intrinsically area-wide because they target specific vectors over delimited geographical areas delineated by biological criteria associated with colonization or dispersal potential. In either case it is argued that a strong geographical basis to planning and implementation is likely to improve the chances of programme success, as well as making more efficient use of resources and increasing cost effectiveness. Geographic information systems (GIS), global positioning systems (GPS) and remote sensing (RS) are allied technologies that together provide a means of gathering, integrating and analysing spatial data. To date, the application of these tools within traditional and area-wide programmes has been relatively limited, but this seems likely to change, particularly as GIS and GPS are already being used extensively in other areas of agroecological management and research. This paper examines potential areas for the application of GIS and associated spatial tools at various stages of planning and implementation of area-wide programmes integrating the SIT as a primary example, before going on to look beyond the SIT and to a number of examples of infectious diseases where GIS and spatial analysis have, to a greater or lesser extent, been employed within disease control efforts. With the help of these case studies the paper attempts to evaluate the extent to which the hype surrounding spatial tools has been (or can be) justified, and examines the barriers that remain in terms of further expansion of their use.

KEY WORDS spatial analysis, AW-IPM, SIT, geographic information systems, global positioning system, remote sensing, vector control

1. Introduction

Geographic information systems (GIS), remote sensing (RS) and the Global Positioning System (GPS) together represent a set of spatial tools that has commonly been touted as being vital for the proper planning and management of disease control programmes. Proponents of these tools suggest that a strong geographical basis to planning and implementation can improve the chances of a programme's success and increase its cost effectiveness by (1) providing more accurate information on pre-existing distributions of diseases and/or vectors in time and space, (2) contributing to the appropriate design of vector and disease control strategies, and (3) the development of suitable frameworks for monitoring and evaluation.

As a means of assessing the validity of

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these claims, this paper provides an overview of the specific contributions that GIS and associated spatial technologies can make within area-wide efforts to control vector-borne diseases, and assesses the degree to which this potential is being realized. The paper begins with a clarification of what is meant by the term "spatial tools" and describes briefly the core technologies it encompasses. The paper goes on to examine potential (and real) areas of application of spatial tools within a context of area-wide integrated pest management (AW-IPM) programmes integrating the sterile insect technique (SIT) and examines the extent to which spatial tools can aid the process of programme planning and implementation. The paper then goes on to look beyond the SIT, and to a number of examples of infectious diseases where GIS and spatial analysis have, to a greater or lesser extent, been employed within disease control efforts. With the help of these case studies the paper attempts to evaluate the extent to which the hype surrounding spatial tools has been (or can be) justified, and examines the barriers that remain in terms of further expansion of their use.

2. Spatial Tools for Control of Vector-Borne Diseases

Today's spatial "toolkit" includes three main components: GIS, GPS and RS. These are allied and overlapping technologies but are also separate tools in their own right. Within this toolkit, GIS can be defined as computer-based systems capable of capturing, cleaning, filtering, integrating, storing, retrieving, analysing and displaying spatial data. GIS incorporate spatial data and descriptive (attribute) data linked to the mapped features. What makes GIS distinct from other types of database is its ability to analyse data based on their location and spatial characteristics. Thus, while GIS may often be used solely for visualization, their functionality is likely to extend to much more sophisticated forms of spatial and statistical analysis. In this context, spatial analysis refers to the manipulation and transformation of GIS data as a means of extracting additional meaning from them. Common examples of spatial analysis include buffering map features (e.g. to define areas of exposure around vector breeding sites), interpolating between points (e.g. to produce climate "surfaces" from a network of weather stations) and overlaying a number of individual geographical coverages to produce derivative maps (e.g. suitability analysis and risk mapping).

There are a number of ways of getting spatial data into GIS, but arguably GPS and RS offer the most cost effective and flexible approaches. GPS receivers allow the collection of spatial and attribute information for points or more complex features on the ground with an accuracy of between 15 metres and a few centimetres depending on the hardware used. GPS receivers are often used simply to collect spatial data (coordinates) for geographical features, with associated attribute data being recorded separately and manually on survey forms. However, in many cases GPS receiver software now includes programmable data dictionaries, which can be used to capture attribute information directly. Alternatively, some GPS receivers can be linked up to personal digital assistant (PDA) devices or tablet computers. Both approaches greatly increase the speed and efficiency with which GPS data can subsequently be incorporated into existing GIS (Cox and Vreysen 2005).

RS is the process of gathering information about the earth's surface using electromagnetic sensors, typically on board satellites. Sensor data can be used in a relatively raw form, for example to derive land cover classification maps, or can be transformed into indices that constitute direct proxies for environmental variables such as rainfall, land surface temperature and vegetation status. Satellite images give objective, up-to-date assessments of surface conditions over large, sometimes inaccessible areas. Moreover, the repeatability of RS measurements makes RS particularly suitable for monitoring environmental conditions over time. There is now a

large variety of multispectral sensor data available – with each sensor offering different advantages in terms of spatial resolution (pixel size), temporal resolution (revisit time) and spectral resolution (the number, width and spacing of the spectral bands used by the sensor). With a number of new satellite sensors due for launch in the next five years it should become increasingly easy to match the specific data requirements of individual disease or pest control programmes with appropriate sources of satellite imagery.

3. The Utility of RS, GIS and GPS in Area-Wide Programmes Integrating the SIT

As set out at the beginning of this paper, the three areas where spatial tools are thought to offer most utility in terms of area-wide programmes are: (1) providing more accurate knowledge of pre-existing vector/disease distributions in time and space, (2) contributing to the appropriate design and implementation of vector/disease control strategies, and (3) facilitating the design of suitable frameworks for monitoring and evaluating control strategies. The following section seeks to translate these somewhat general areas of utility into specific activities relevant to area-wide programmes.

3.1. Knowledge of Pre-Existing Vector and Disease Distributions

Insect pest intervention (and pre-intervention) programmes require accurate, up-to-date information on the spatial and temporal distribution of target insects. GIS-based analysis can be used to bring together a wide range of information sources. These include climate, RS, land use and topographic data, historical data on vector distribution and abundance, disease prevalence, etc. It can also be used to develop modelled or empirical estimates of the temporal and spatial distributions of the pest or disease of concern (Cox and Vreysen 2005). The nature of this GIS exercise, and the data sources used for it, will reflect the stage to which pre-intervention planning has developed. At the very early stages of planning, for example, GIS modelling will almost certainly focus on identifying areas of relatively high risk at the national or regional level, using low spatial resolution environmental data in combination with available historical information on the insects and/or diseases of interest. In other cases, these broad assessments may be more suitable for directing more detailed risk modelling efforts using higher resolution geographic datasets and, possibly, prospective sampling of vectors, to specific areas of interest.

The use of low spatial resolution RS data to predict disease vector distributions at the regional scale began in the early 1990s to correlate distributions of tsetse and incidence of trypanosomosis to spatial variations in climate and vegetation indices – and later also to surrogates of land surface temperature and rainfall (Rogers 1991, Rogers et al. 1996, Robinson et al. 1997, Hendrickx et al. 2001). The outputs of these types of models constituted an important first step in terms of the spatial targeting of the SIT and other area-wide interventions. However, resource and technical constraints may mean that more specific information is required to identify priority areas for intervention or guide future sampling efforts to address levels of genetic variability among target insects, etc.

Regional or national-scale vector distribution models may fail to capture the often localized, patchy distribution of many insect pests in areas where the overall insect population density is low. Locating pockets of high-density populations, while vital for successful insect intervention campaigns, is a major challenge from a spatial analysis perspective. This is because the climate and RS datasets used to predict insect distributions over specific areas are rarely appropriate for work at larger scales. Although a limited number of studies have successfully used high spatial resolution RS data (e.g. from Landsat and Satellite pour l'Observation de la Terre (SPOT) satellites) to identify habitats associated with high insect densities (Rejmankova et al. 1995, Roberts et

al. 1996, Kitron et al. 1996), estimates of risk derived from this approach tend to be rather static as in the past it has either not been possible or practical to conduct multi-temporal analysis using these RS products. In future, however, the availability of multi-temporal RS data at respectable spatial resolutions (e.g. Moderate Resolution Imaging Spectroradiometer (MODIS)), should assist the development of dynamic population distribution models for predicting temporal and spatial population dynamics and to link spatial patterns with heterogeneity of habitat.

3.2. The Design and Implementation of Vector/Disease Control Strategies

The availability of temporal and spatial distribution models of the target species at a large spatial scale has implications beyond the design of efficient sampling frames. In particular, such models should facilitate a more efficient deployment of suppression tools as well as a better-targeted release of sterile insects. This increased efficiency should also translate into considerable economic savings in terms of logistics, personnel and sterile insects. A fuller discussion can be found in Cox and Vreysen (2005), but a brief summary of some of the main potential areas of utility for spatial tools is presented here.

In terms of selecting appropriate pre-release population suppression methods, it is worth noting that a variety of methods are often available for different species, but the appropriateness and effectiveness of each will depend on the characteristics of each target area. Spatial analysis can help identify the most appropriate suppression method (or combination of methods) for a given target zone – although the nature of this exercise will vary depending on the target species in question. In the case of tsetse, for example, spatial analysis could be used to (1) evaluate the likely impact of topography, wind velocity and direction, density of tree cover, etc. on the suitability of the sequential aerosol technique (SAT), (2) assess relative livestock and tsetse population distributions and densities in terms of likely impact on the efficiency of the live bait technology, or (3) use demographic data layers and tsetse population distribution models to evaluate likely efficiency or suitability of stationary bait technology (insecticide impregnated targets and traps). Alternatively, it may be possible to model the outcome of different combinations of suppression methods in target areas that are heterogeneous in terms of habitat composition, species composition, host distribution, demography and environment (Cox and Vreysen 2005).

Once an appropriate suppression method has been selected, spatial analysis can be employed directly to guide the suppression strategy, through, for example, determining appropriate sites for the deployment of traps and targets and by indicating required target/trap densities per surface unit in relation to environment and insect distribution. Spatial analysis may also obviate the need for uniform application of control measures such as the SAT over heterogeneous target areas and thereby reduce costs and any associated negative environmental impacts (Cox and Vreysen 2005).

3.3. Suitable Frameworks for Monitoring and Evaluating Control Strategies

Monitoring and evaluation are essential components of any area-wide programme but are time consuming and expensive in terms of materials, logistics and personnel. A careful balance has to be found between "cost efficiency" and the collection of "reliable data", which in most cases equates to restricted monitoring at carefully selected sites (Vreysen 2005). Spatial analysis can assist with the identification and selection of appropriate fixed monitoring sites. Mobile GIS and GPS technology can also make monitoring systems more efficient by allowing data entry in the field, for example using barcode scanners. Perhaps less obviously, spatial analysis can also be used to get "better value" out of available trap data – and a range of spatial analysis techniques employing both geo-statistics and GIS may be valuable at a landscape scale.

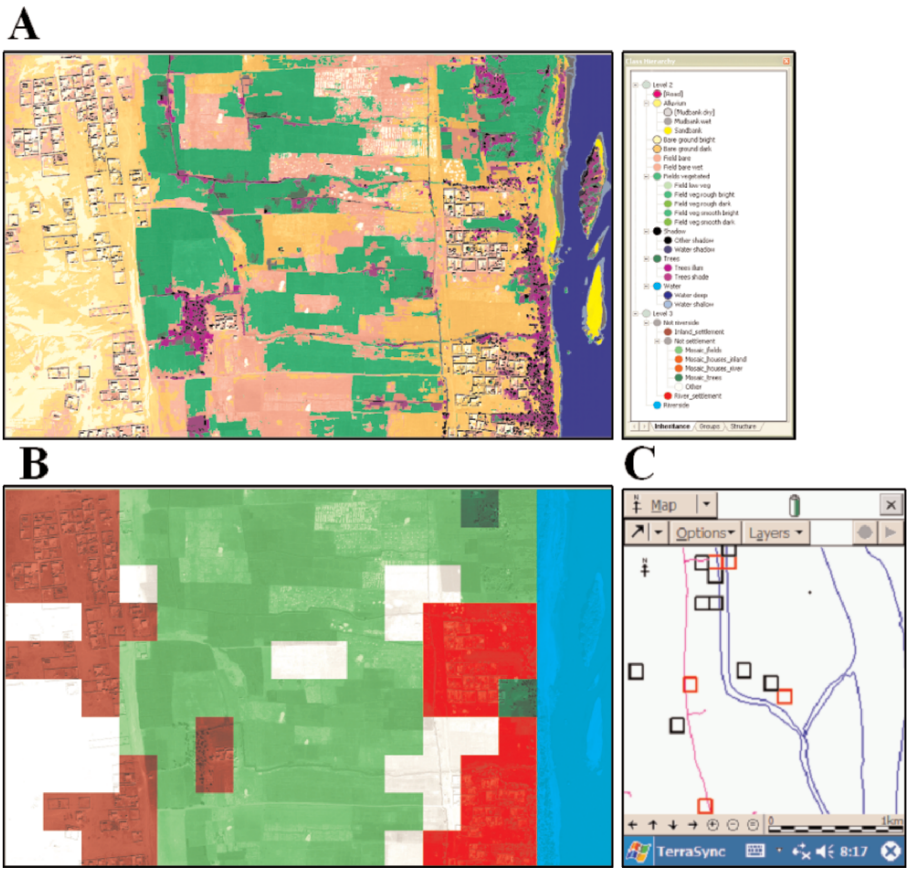

Figure 1. *An illustration of the use of GIS and RS for monitoring abundance of* Anopheles arabiensis *larvae in northern Sudan (Cox, unpublished). In this exercise the task was to generate a sampling approach to yield reliable baseline data on mosquito abundance within two defined reaches of the Nile River. (a) For each monitoring area, high resolution Quickbird RS data were processed using image segmentation and object-based classification techniques to derive land cover maps at sub-metre resolution, (b) which were then generalized to 100 × 100 metre cells conforming to a predefined sampling grid and a limited number of land cover mosaic types. Using these inputs it was possible to generate a random sample of grid squares, stratified by predominant land cover type, to be sampled over the course of a calendar year. (c) For each visit to the study area, field workers were able to upload maps of the specific grid cells to be sampled and use a pocket PC-based GPS/GIS system to navigate to and within the target cells.*

Interpolation procedures, for example, are ideally suited to the analysis of trap data, with output taking the form of contour maps or "surfaces" of insect density.

To date, examples of the application of spatial tools in programme monitoring are rare, but in principle the suitability of GIS for managing and interpreting data from a variety of sources makes it suitable for providing timely feedback on a large number of moni-

toring indicators (Cox and Vreysen 2005).

3.4. Uptake of Spatial Tools within Area-Wide SIT Programmes

From the information in previous sections it is evident that much of the potential utility attributed to spatial tools remains unproven in the sense that few published examples exist that demonstrate real uptake of the tools themselves. An exception – and an area where GIS and RS have been shown to have most impact – is the modelling of vector, disease and environmental datasets to produce spatial estimates of vector distributions or disease risk (Section 3.1). Of course, not all of this modelling effort has been carried out with SIT-based approaches in mind, and some of the early work was concerned more with methodological development than the utility of the outputs of models themselves. However, the academic nature of this work did ensure that the incremental development of modelling approaches could be tracked through the scientific literature. In contrast, it has been more difficult to assess the uptake of spatial tools with respect to project design, implementation, monitoring and evaluation within AW-IPM programmes (Sections 3.2 and 3.3) because the emphasis on the practical application of the tools, as distinct from their research application, usually precludes widespread publicity of experiences. In other words, in a properly designed programme, spatial tools should ideally be fully integrated tools that operate "under the hood".

That said, it would appear that even anecdotally there are relatively few cases where spatial tools have been explicitly incorporated into ongoing SIT-based programmes. The exceptions are most notably within programmes against the New World screwworm *Cochliomyia hominivorax* (Coquerel) (Philips et al. 2004), tephritid fruit flies (Orankanok et al., this volume), the painted apple moth *Teia anartoides* Walker (Suckling et al., this volume), and most recently, in the development of SIT against *Anopheles* mosquitoes. In such cases, spatial tools have been used primarily to provide navigational guidance and tracking for releases of the sterile insects and for navigation on the ground. In some cases GIS have also been used for mapping trapping sites and monitoring invasion routes (sometimes in real time), such as in the Mississippi boll weevil *Anthonomus grandis* Boheman eradication programme. Less commonly, spatial analysis has been used for selecting trapping sites, most commonly by overlaying grids on topographical maps or satellite images either manually (as historically in the case of the Zanzibar tsetse SIT-based AW eradication project), or more formally within GIS (as in the case of the ongoing SIT feasibility study for *Anopheles arabiensis* Patton in northern Sudan (Fig. 1). This approach is particularly well suited to situations where there is a strong justification for weighting sampling effort according to *a priori* assumptions about the effect of different land use and land cover types on insect distributions. In Panama, for example, Phillips et al. (2004) derived an optimal design of trap networks for monitoring adult New World screwworm flies based on RS-derived forest maps.

There generally seems little evidence for the widespread use of spatial tools for monitoring and evaluation, although within the Programa Moscamed (Guatemala-Mexico), work is ongoing to analyse sterile Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) performance under different environmental conditions, identifying hot spots of persistent high insect density and exploring insect behaviour. Otherwise, the use of GIS/RS as a decision support tool in AW-IPM programmes with an SIT component has so far largely been limited to the spatial display of data and has seldom been applied for planning, implementation of suppression and release programmes or analysis and modelling of the data.

4. Spatial Tools in Non-Genetic Approaches to Disease Control

Much scientific work has gone into geographical modelling of the distribution, abundance

and prevalence of diseases and their vectors at a variety of spatial scales (Hay et al. 2000, Lindsay and Thomas 2000, Malone et al. 2001, Elnaiem et al. 2003), while there has been rather less emphasis on incorporating spatial tools directly into control programmes. Arguably, this has particularly been the case for "high-profile" diseases such as malaria, where projects such as the Mapping Malaria Risk in Africa (MARA) collaboration have produced scientific outputs that have reached academic audiences through journal publications (e.g. Craig et al. 1999, Kleinschmidt et al. 2001) and have appeared to play important advocacy roles at international level. However, there is no clear evidence to suggest that these types of products have been used explicitly for planning control activities.

There are probably a number of reasons for this lack of uptake of risk model outputs. Most fundamentally, it is probable that in many cases decision makers simply do not trust the veracity of the models. In view of the methodological and data-related limitations of some published models, in some instances they are probably right not to. In other cases good models may have been produced, but decision makers simply do not find them useful, or even relevant. This is most often the case where modelling has been data-driven, rather than demand-led. Nevertheless, even in situations where researchers have attempted to "second-guess" the needs of policy makers, the results are not always fruitful. In other cases it may be that model results are too generalized from a geographical perspective to influence local-level planning or, conversely, it may be that models using data from specific geographical areas cannot be extrapolated in a reliable way to produce robust predictions of disease and/or vector characteristics over wide areas. It is of course also true that planners may be resistant to new sources of evidence in terms of their decision-making, in which case even highly reliable and pertinent models are likely to be ignored. In this respect it is important that the intended users of risk models are not viewed as "passive" consumers, but are instead brought in as active players in designing and carrying out risk mapping projects.

In reality, direct and productive use of spatial tools has tended to be limited to situations where planning is focused on coordinated disease eradication, or where a single, efficacious intervention is available for a disease and requires targeting. For human onchocerciasis (river blindness), for example, the Rapid Epidemiological Mapping of Onchocerciasis (REMO) developed by the Special Programme for Research and Training in Tropical Diseases/World Health Organization (TDR/WHO), has emerged as an important spatial tool for control planning (Ngoumou et al. 1994, Katabarwa et al. 1999). The Rapid Epidemiological Mapping of Onchocerciasis allows quick and cheap identification of communities at high risk of onchocerciasis using spatial information such as the locations of river basins. High-risk communities are then subsampled and rapidly assessed by screening individuals for onchocercal nodules. This enables communities to be classified into three categories: (1) priority areas which require community-directed treatment with ivermectin, (2) areas which do not require treatment, and (3) possible endemic areas needing further investigation. Results of the Rapid Epidemiological Mapping of Onchocerciasis have been incorporated into GIS to visualize priority areas for community-directed treatment with ivermectin and estimate the number of people needing treatment (Noma et al. 2002). This in turn has allowed the African Programme for Onchocerciasis Control to prioritize allocation of resources according to need.

Rapid mapping method approaches have also been developed for lymphatic filariasis, which is currently being targeted for eradication through the Global Alliance for the Elimination of Lymphatic Filariasis. As with onchocerciasis, identifying endemic localities is an essential first step to carrying out treatment programmes, and the Rapid Geographical Assessment of Bancroftian Filariasis (RAGFIL) was developed by TDR/WHO for this purpose. The Rapid

Geographical Assessment of Bancroftian Filariasis uses a spatial sampling grid with either 25 or 50 kilometres between sampled communities, together with rapid prevalence assessments using immunochromatographic card tests and geostatistical methods (WHO 1998, Gyapong et al. 2002). However, subsequent analyses have suggested that a smaller sampling grid may be required to successfully identify all high-risk communities (Srividya et al. 2002).

Perhaps the most impressive and most up to date example of the use of spatial tools in disease control planning comes from the Schistosomiasis Control Initiative, which is currently assisting the implementation of national programmes for schistosomiasis and geohelminth control in six African countries. In Uganda, where *Schistosoma mansoni* Sambon is widespread, GIS and RS have been employed to classify the country according to treatment strategy. Communities are classified according to three strategies: (1) annual treatment of schoolchildren and high-risk groups with praziquantel and albendazole where schistosomiasis prevalence is above 50%, (2) treatment every second year in communities with moderate prevalence (more than 20% and less than 50%), and (3) health facility based treatment of suspected cases in low prevalence (less than 20%) areas. Spatial analysis using RS data and climate surfaces showed that *S. mansoni* typically occurs only where average annual rainfall is more than 850 millimetres or where altitude is less than 1400 metres (Kabatereine et al. 2004), allowing remaining areas to be excluded from the control strategy. Modelling also showed that prevalence consistently exceeded 50% in areas within five kilometres of major lakes, justifying mass-treatment in villages within these areas without the need for further surveys. In intermediate areas individual communities are surveyed using lot quality assurance sampling (Brooker et al. 2005).

Elsewhere, the Schistosomiasis Control Initiative has used Bayesian geostatistical models to produce validated prevalence surface maps for both *Shistosoma haematobium* (Bilharz) and *S. mansoni* infections in northwestern Tanzania with the similar aim of guiding spatially mass-treatment with praziquantel (Clements et al. 2006). Bayesian spatial methods, although rarely applied in the context of vector-borne diseases, offer the distinct advantage of being able to incorporate spatial auto-correlation, as well as uncertainty through the modelling of both observed data and any unknowns as random variables. In this way, Clements et al. (2006) were able to use parasitological data from 143 schools to develop RS-based models for the two infections. Significantly, they were also able to investigate the confidence of the prevalence predictions and thereby inform decision makers on whether sufficient data were collected to exclude areas from mass-treatment.

5. Realizing the Potential of Spatial Tools within Disease Control Programmes

Although this paper in no way constitutes a thorough survey of ongoing area-wide programmes, the process of putting this review together has revealed that, to date, the application of spatial tools within area-wide programmes appears to have been relatively limited. Where spatial tools have been used, work has focused on using existing disease or vector datasets in combination with environmental data to model distributions of vectors and/or their associated diseases. It is likely that these products represent useful first steps in the planning of area-wide programmes (and particularly those aimed at tsetse). However, there is much less evidence for the direct use of GIS, GPS and RS in the planning, implementation, monitoring and evaluation of area-wide programmes. This is somewhat surprising given that these tools have been used extensively in other areas of agroecological management for many years.

Within non-genetic approaches to disease control there has also been a fairly research-oriented focus on spatial modelling of vector and disease risk. Nevertheless, as section 4 demonstrates, there are now a growing num-

ber of instances where an explicit spatial framework is being used for targeting public health interventions, and spatial tools have been critical in the development of these approaches. There are now validated instances where spatial tools are providing governments with a relatively low-cost approach to surveying and programme design. This can significantly reduce the cost of practical programmes through more precise geographical targeting and simplifying the processes of monitoring and evaluation. Spatial tools can reduce both the upstream (e.g. survey and design), and downstream (e.g. targeting, monitoring and evaluation) costs of programmes, while enhancing programme effectiveness. At the same time, it should be recognized that the uptake of spatial tools has been far from universal and that, in many cases, this uptake has occurred only very recently.

These findings beg the question of why it has taken so long for spatial tools to be incorporated within disease control programmes. Traditionally, the answer to this has lain in the large economic costs involved in obtaining GIS, RS and GPS hardware and software, in the need to generate spatial datasets from scratch and in a shortage of the necessary skills. However, spatial tools are now becoming increasingly accessible to non-specialists, while increases in computing power mean that even high-level GIS systems can be installed on a standard personal computer. Software costs, once a major disincentive, are now rarely prohibitive, and GIS and RS data are more widely available than ever before. Perhaps it is also significant that some of the "data vacuum" that has hindered the development of spatial disease models in the past is slowly being filled as basic mapping of public health infrastructure improves (e.g. through WHO's Public Health Mapping and GIS Programme (http://www.who.int/health_mapping/about/en/)) and surveillance systems for vector-borne diseases are increasingly incorporating an explicitly spatial dimension (Abeku et al. 2004, Gosselin et al. 2005).

It seems probable therefore, that the uptake of spatial tools will increase markedly over the coming years, and that much of the "hype" surrounding GIS, GPS and RS will be seen to be justified. Indeed, it could be argued that the potential benefits of spatial tools in terms of increasing programme effectiveness and, importantly, cost effectiveness, make it imperative that geographers and others continue to advocate the use of spatial tools for disease control.

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Optimizing Strategies for Eradication of Discrete-Generation Lepidopteran Pests Using Inherited Sterility

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ABSTRACT Population models were used to derive general principles for optimizing the success of the sterile insect technique (SIT) with inherited sterility against discrete-generation pest populations. Inherited sterility is predicted to be more effective than complete sterility whenever matings between irradiated-lineage partners are unsuccessful – this is rarely examined experimentally. Successful eradication also requires sufficient depression of fertility from matings between irradiated-lineage and wild partners, and that sufficient irradiated males are released to overcome the natural rate of increase of the wild population. A critical overflooding ratio Φ_c can be calculated to suggest the appropriate release rate, but because this is based on an assumption of equilibrium, the initial stages of an eradication programme with an SIT component must aim for a higher release rate than Φ_c suggests. Spatial modelling suggests that, given a finite number of irradiated males available for release, the best strategy is to release these as close to the wild populations as possible. However, if the locations of all wild populations are not reasonably well known, then many small releases, regularly spaced on an area-wide basis, are more certain to achieve eradication than few large release sites. In the latter case, the total number of irradiated males required is minimized when the maximum distance between adjacent release sites is approximately the same as the average dispersal distance of irradiated males.

KEY WORDS Lepidoptera, inherited sterility, eradication, irradiation, critical overflooding ratio, release rate, dispersal, model

1. Introduction

The sterile insect technique (SIT) was conceived by E. F. Knipling (1955) as a possible control method for suppressing or eradicating insect populations. In practice, the method usually consists of integrating various controls with the release of large numbers of sterilized male (and sometimes female) insects into the environment to compete for mates with the wild fertile males present. The approach has been used to successfully eradicate a range of insect populations from well-defined areas, most famously the New World screwworm *Cochliomyia hominivorax* (Coquerel) from the southern USA, Mexico and Central America (Wyss 2000, Dyck et al. 2005). The SIT has also been used to achieve area-wide suppression of pests such as Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and codling moth *Cydia pomonella* (L.) (Tan 2000, Dyck et al. 2005).

The process of sterilizing males typically involves irradiating laboratory-reared pupae or adults. Determining the optimal dose of radiation to use involves a trade-off between the level of sterility achieved in treated individuals, and physiological damage leading to suppression of competitiveness relative to wild individuals, both of which increase with

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radiation dosage. In some groups, notably the Lepidoptera, low doses of radiation may lead to partial sterility, in which irradiated individuals are highly competitive but produce fewer offspring than wild ones, and this reduced reproductive potential is passed on to the next generation. Knipling (1970) suggested that the use of such inherited sterility may be advantageous for achieving eradication of lepidopteran pests if reproductive ability is suppressed not only in the partially sterile males released, but also in their offspring.

A wide range of population models have been used to demonstrate the utility and limitations of the SIT (Barclay 2005), but few have considered inherited sterility (Walker and Pedersen 1969, Knipling 1970, Carpenter and Layton 1993). The present work aims to evaluate, by use of new population models, the conditions under which the release of partially sterile male Lepidoptera may be advantageous over releasing completely sterilized males, and to demonstrate how releases of sterile males may be optimized in time and space.

2. Methods

2.1. Analytical Model

The model assumes an insect pest control programme that includes the release of only substerile males. As a result, three different types of individuals may be present in the population: (1) the irradiated males which have been released, (2) those wild-type individuals with no irradiated male in their ancestry, and (3) those with at least one irradiated male in their ancestry, denoted "irradiated-lineage" individuals. Hence wild x wild matings were assumed to produce wild offspring, while all other mating combinations were assumed to produce irradiated-lineage offspring. All irradiated-lineage individuals were assumed to be equivalent no matter how many generations ago the original irradiated ancestor occurred. Irradiated-lineage x irradiated-lineage and irradiated-lineage x irradiated matings were assumed to produce no offspring, which is termed "irradiated-line incompatibility".

Let S denote irradiated adult males, F denote wild adult females, M denote wild adult males, X denote irradiated-lineage adult females, and Y denote irradiated-lineage adult males. Six possible mating combinations arise, with the relative viability of eggs resulting from each pairing denoted as f_{FM}, f_{FS}, f_{FY}, f_{XM}, f_{XS}, and f_{XY}, where the subscript refers to the pairing involved. By definition, $f_{FM} = 1$, and assuming irradiated-line incompatibility means that $f_{XS} = f_{XY} = 0$. Using similar symbology, the relative competitiveness of adult males of various lineages, in terms of locating and successfully mating with a female, may be denoted v_M, v_S, and v_Y, where $v_M = 1$. Now the probability of a mating involving a fertile male is $M/(M+v_S S+v_Y Y)$, while that of an irradiated-lineage male is $v_Y Y/(M+v_S S+v_Y Y)$. Assuming that mature females mate only once, or that their fertility is determined by their last mating, then a model for discrete generation populations is given by:

$$
\begin{aligned}
F_{t+1} &= \sigma\chi\lambda\left(\frac{M_t F_t}{M_t + v_S S_t + v_Y Y_t}\right)\\
M_{t+1} &= F_{t+1}\frac{(1-\chi)}{\chi}\\
X_{t+1} &= \sigma\chi\lambda\left(\frac{F_t(f_{FS}v_S S_t + f_{FY}v_Y Y_t) + X_t f_{XM} M_t}{M_t + v_S S_t + v_Y Y_t}\right)\\
Y_{t+1} &= X_{t+1}\frac{(1-\chi)}{\chi}
\end{aligned}
\qquad (1)
$$

where λ is female fecundity (eggs per female), χ is the sex ratio (proportion female), σ is the proportion of neonates surviving to maturity, and subscripts t and t+1 indicate the current and next generations, respectively. The net reproductive rate per generation R_0 is given by $R_0 = \lambda\chi\sigma$.

In some species the sex ratio becomes skewed in subsequent generations after a release of irradiated males, but this possibility was not included in order to keep this exploratory model simple and clear. Neither does the model include any direct density-

dependent feedbacks to fecundity or mortality since the SIT is typically used against small populations in which these are relatively unimportant. Note, however, that fertility is inversely density-dependent, since the number of offspring per female decreases as the ratio of irradiated to wild males increases. The analogous model for populations with overlapping generations has been developed, and will be published elsewhere.

2.2. Spatial Simulation Model

An integer-based version of the discrete-generations model was developed, using binomial and Poisson rounding of birth, death, mating and dispersal events to introduce demographic stochasticity. This was replicated across a square grid of 100 x 100 cells, each representing a semi-independent local population within a one hectare area, so that the model arena corresponded to 10^4 hectares or 100 square kilometres. By default the arena boundaries were absorbing, but this assumption was found not to affect the results.

Adult dispersal was assumed to occur prior to mating and oviposition. Therefore, each time step began with the mating post-dispersal adult stages to produce new generation neonates. These were divided up according to the sex ratio χ, and a proportion σ survived to maturity. At this point, S_t irradiated males were released in particular locations depending on the release strategy being simulated. Finally, each adult stage was assumed to disperse according to a Gaussian dispersal kernel (Clark et al. 1999):

$$A_i = \sum_j \left[\frac{A_j}{\pi d_A^2} exp\left(-\left(\frac{ij}{d_A} \right)^2 \right) \right] \qquad (2)$$

where A refers to an adult stage (*F*, *M*, *S*, *X*, or *Y*), subscripts *i* and *j* specify particular spatial locations, *ij* is the distance between locations *i* and *j*, and d_A is an average dispersal distance for adult stage *A*.

2.3. Spatial Release Strategies

The effects of two different spatial release strategies were investigated (Fig. 1). Under regularly spaced releases, irradiated males were released on a square grid with dimension d_{reg} between adjacent release points. Under a hot spot strategy, releases were made randomly within a radius d_{spot} of wild populations. A range of scenarios was investigated, each differing in the release pattern and in d_{reg} and d_{spot}. Simulations were initialized with ten

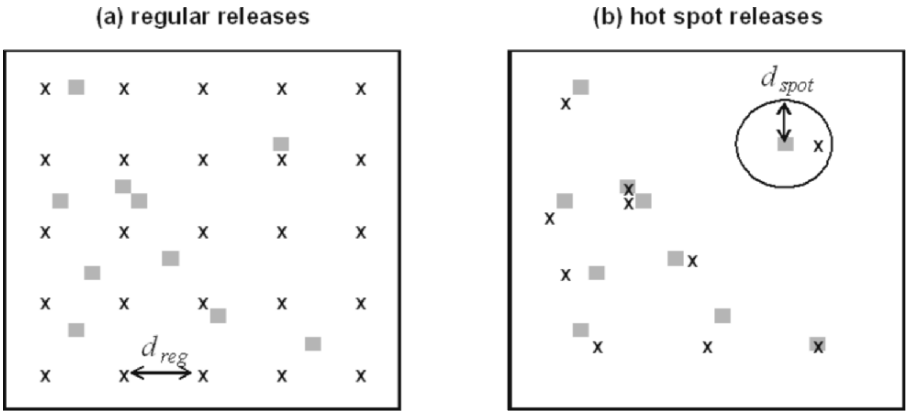

Figure 1. *Conceptual illustration of the regularly spaced and hot spot release strategies. Grey squares correspond to randomly-distributed wild populations, crosses indicate release sites.*

Table 1. *Model parameters for discrete-generation lepidopteran case studies. Italicized values are derived from other values in the table and those in brackets are based on other evidence.*

Parameter		Gypsy moth	Codling moth	
female fecundity (viable eggs/female)	λ	831[1]	144[4]	
sex ratio (females/total)	χ	0.5[1]	0.5[4]	
survival of larvae and pupae	σ	*0.0133*	(0.06)	
net reproductive rate (/generation)	R_0	5.51[2]	*4.31*	
irradiation dose (Gy)		100	100	200
relative competitiveness of:				
irradiated males	v_S	(0.9)	0.73[5]	0.63[5]
irradiated-lineage males	v_Y	0.9[1]	(0.73)	(0.63)
relative viability of eggs from:				
wild female x irradiated male	f_{FS}	0.563[1]	0.611[4]	0.363[4]
wild female x irradiated-lineage male	f_{FY}	0.062[3]	0.125[4]	0.009[4]
irradiated-lineage female x wild male	f_{XM}	0.005[3]	0.173[4]	0.083[4]
average dispersal distance (metres) of:				
females	d_F, d_X	1000[6]	300[8]	
adult males	d_M, d_Y	500[7]	1000[8]	
irradiated males	d_S	500	800[9]	
critical overflooding ratio	Φ_c	*1.18*	*1.04*	*2.04*

[1]Schwalbe et al. (1991), [2]Sharov et al. (1998), [3]Mastro and Schwalbe (1988), [4]Bloem et al. (1999b), [5]Bloem et al. (1999a), [6]Kamata et al. (2005), [7]Elkinton and Cardé (1980), [8]http://www.hortnet.co.nz, [9]implied from Bloem et al. (1999a)

randomly-distributed wild populations, each of 100 wild adults split according to the sex ratio χ. Release sites were fixed for the duration of each simulation.

Simulations were run until either global eradication (at generation t_e) was achieved, or it became clear that eradication was unsuccessful (at 20 generations). Each scenario was simulated 100 to 250 times to capture the variability in outcomes arising from the inbuilt demographic stochasticity. Each scenario was summarized in terms of the proportion of simulations leading to eradication p_e and the time to eradication t_e for each successful simulation.

2.4. Parameterization

The models were parameterized for two lepidopteran case studies: gypsy moth *Lymantria dispar* (L.) and codling moth *C. pomonella*. Both species have been targeted by the SIT (Bloem et al. 2005), and parameter estimates could be made from the literature (Table 1). It should be emphasized, however, that the aim was not to provide accurate models for these particular species, but rather to use them as a basis for assembling plausible parameter values with which to explore the models.

Female fecundity λ, was estimated as the average viable eggs laid per wild-mated female, being the total eggs laid per female multiplied by the proportion of eggs successfully hatching in control treatments. Similarly, the relative viability of eggs resulting from different mating combinations, f, was calculated from the number of viable eggs laid in irradiated treatments divided by the corresponding control treatment result. The assumption of irradiated-lineage incompatibility ($f_{XS} = f_{XY} = 0$) was supported by direct evidence for gypsy moth (Mastro and Schwalbe 1988), and implied by experimental results for codling moth (Bloem et al. 1999b).

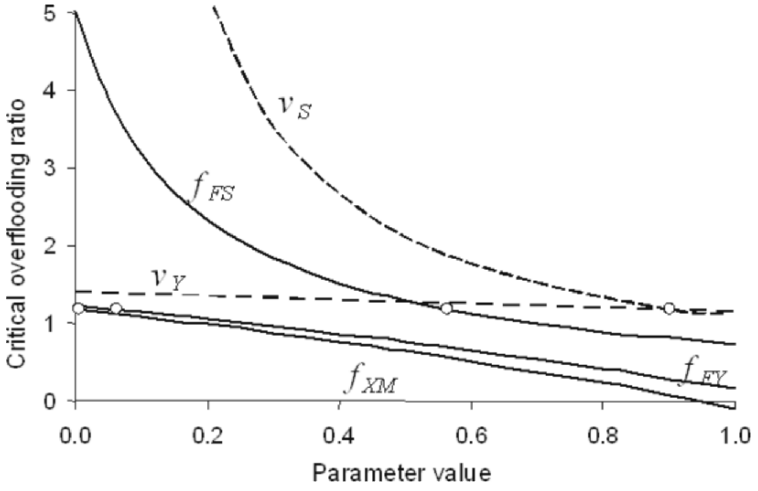

Figure 2. *Effects of inherited sterility parameter values on the critical overflooding ratio for the Asian gypsy moth. All parameters have their default values (indicated by open dots) except the one varied for each line. Solid lines show relative viability of eggs arising from different mating combinations: wild female x irradiated male (f_{FS}); wild female x irradiated-lineage male (f_{FY}); irradiated-lineage female x wild male (f_{XS}). Dashed lines show relative competitiveness of irradiated (v_S) or irradiated-lineage (v_Y) males.*

The relative competitiveness of irradiated and irradiated-lineage males was estimated in a number of different ways, depending on available information. Bloem et al. (1999a) monitored copulations with tethered codling moth females after a field release of irradiated males. Here, relative competitiveness, v_S, was estimated as the mean number of matings observed by irradiated males relative to that for releases of control males. In gypsy moth, there appears to be little effect of irradiation on the activity and searching patterns of males in field cages (Schwalbe et al. 1991), but the rate of irradiated-lineage larval development is only 90% that of wild moths (Schwalbe et al. 1991), and this was used as an index of relative overall male competitiveness. In all cases it was assumed that the competitiveness of irradiated-lineage males is the same as that of irradiated ones.

One of the most difficult parameters to estimate for the population models is σ, the average overall survival of larval and pupal stages in the field. For gypsy moth, σ could be estimated from the net reproductive rate per generation, $R_0 = \lambda\chi\sigma$, where this was available (Sharov et al. 1998). No data were available for codling moth survival, so $\sigma = 0.06$ was assumed as a representative value, based on a range of similar species.

The parameters for the gypsy moth were quantified from the European strain present in North America, in which the adult female is flightless. Of much greater concern for many countries is the Asian strain in which adult females can fly, as quantified by a recent unpublished study (Kamata et al. 2005). Therefore adult female flight was assumed (Table 1), as well as that this is the only substantial difference between the strains so that the estimated parameter values might be relevant for the Asian strain. The effects of short-distance ballooning by early-instar larvae were assumed to be negligible compared to the flight of adult females. The estimates used for codling moth dispersal were unreferenced

data from the website http://www.hortnet.co.nz and therefore should be treated with caution.

3. Results

3.1. Analytical Model Equilibrium and Critical Overflooding Ratio

Assuming that the same number of irradiated males is released each generation *S*, the equilibrium values for the analytical model are given by:

$$Y_{eq} = \frac{f_{FS} v_S S}{(1 - f_{XM} - v_Y f_{FY})} \qquad X_{eq} = Y_{eq} \frac{\chi}{(1-\chi)}$$
$$M_{eq} = \frac{v_S S + v_Y Y_{eq}}{(R_0 - 1)} \qquad F_{eq} = M_{eq} \frac{\chi}{(1-\chi)} \tag{3}$$

This equilibrium is unstable, with the population increasing from above the equilibrium or decreasing to eradication from below the equilibrium. Since the equilibrium is determined in part by the release rate *S*, successful eradication by SIT requires that sufficient releases are made that the unstable equilibrium exceeds the actual wild population, leading to population decline. The critical overflooding ratio, Φ_c, describes the number of irradiated males that needs to be released per wild male to prevent the population from increasing. For the analytical model, this is given by:

$$\Phi_c = \frac{S_{eq}}{(M_{eq} + Y_{eq})} = \frac{(R_0 - 1)}{v_S} \frac{(1 - f_{XM} - v_Y f_{FY})}{(1 - f_{XM} - v_Y f_{FY} + f_{FS}(v_Y + R_0 - 1))} \tag{4}$$

If Φ_c is exceeded then the population will decline, but if Φ_c cannot be achieved then the release of irradiated insects alone cannot achieve eradication. A model for complete sterility is recovered as a limiting case when $f_{FS} = 0$, in which case the critical overflooding ratio reduces to $\Phi_c = (R_0 - 1)/v_s$. Fig. 2 shows how the critical overflooding ratio for Asian gypsy moth is affected by each of the irradiation-related parameters.

3.2. Simulation Results of Release Strategy

The effects of release strategy and total number of irradiated males released on the probability of eradication and the time to eradication are shown in Fig. 3. For the strategies investigated, hot spot releases were more successful than regularly spaced releases. In each case, the probability of eradication p_e increased and the time to eradication t_e decreased as more irradiated males were released. However, both p_e and t_e asymptote, so that above a certain release rate there was no advantage in releasing more irradiated males. Eradication of ten populations of 100 gypsy moth or codling moth could not be achieved in fewer than approximately four generations, even with very high release rates.

4. Discussion

The ability to estimate the critical overflooding ratio (equation 4) is useful, not only for setting release rates, but also for optimizing the biological fitness of irradiated males. For example, parameter values were available for two different irradiation rates for codling moth, leading to two different Φ_c values (Table 1). These suggest that approximately twice as many irradiated males would need to be released at a 200 Gy dose to give the same suppressive effect of 100 Gy irradiated males.

Alternatively, a simple sensitivity analysis based on equation 4 can suggest which aspects of male irradiation biology might be targeted to give the best improvements in SIT performance. For example, Fig. 2 shows how the critical overflooding ratio for gypsy moth is affected by each of the radiation-related parameters. These results suggest that there would be relatively little advantage in increasing the competitiveness of irradiated males (v_S), but much to lose if this were to become depressed. Increasing the viability of eggs arising from wild x irradiated matings (f_{FS}) would perhaps offer the greatest relative bene-

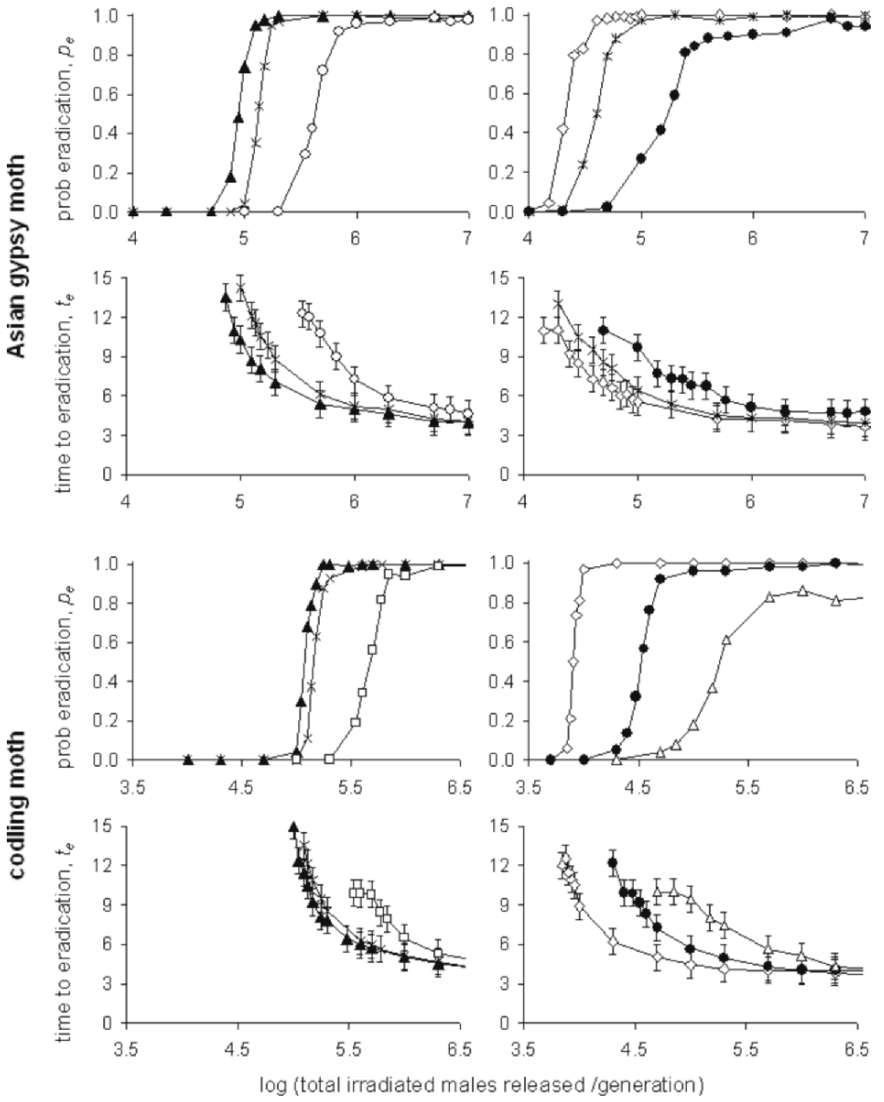

Figure 3. *Results from spatial simulations parameterized for Asian gypsy moth (upper graphs) and codling moth (lower graphs) treated at 100 Gy. Graphs show the effects of total irradiated males released per generation on the probability of eradication* p_e *and the mean number of generations to eradication* t_e*. Regularly spaced releases (left graphs) were made at* d_{reg} *= 500 metres (filled triangles), 1000 metres (crosses), 1500 metres (open circles)(for Asian gypsy moth) or 2000 metres (open squares) (for codling moth) intervals; hot spot releases (right graphs) were made within* d_{spot} *= 0 metres (open diamonds), 250 metres (crosses)(for Asian gypsy moth), 500 metres (filled circles), or 1000 metres (open triangles) (for codling moth) of wild populations. Each point represents 100 to 250 simulations and error bars show 95% confidence intervals.*

fits in terms of minimizing Φ_c, provided that the values of other parameters were maintained. On the other hand, v_Y has little effect on Φ_c, suggesting that the competitiveness of irradiated-lineage males might be sacrificed in order to optimize other aspects of irradiation biology.

The consistent negative slope of the lines in Fig. 2 suggest that the greatest benefits occur from maximizing the values of all parameters, but this relies on the assumption of irradiated-lineage-incompatibility providing an ultimate dead-end for the population. Without irradiated-lineage-incompatibility, the results may differ; other population models are being developed to explore this scenario. Meanwhile, this emphasizes the importance of assessing the success of irradiated-lineage x irradiated-lineage matings, which is rarely done (with the exception of gypsy moth (Mastro and Schwalbe 1988)).

The critical overflooding ratios calculated for the case study species (Table 1), are generally much lower than the targets of eradication programmes against these species. However, it is important to note that the overflooding ratio achieved at the beginning of the sterile insect releases is not a direct indicator of the success of the programme because the critical overflooding ratio is calculated for a theoretical equilibrium population in which irradiated-lineage individuals have been present for some time. In order to achieve eradication from a constant input of irradiated males, the target overflooding ratio in the early stage of the programme should be considerably higher than Φ_c to establish irradiated-lineage individuals in the population as it initially continues to increase. When measuring overflooding in the field, it may also be important to adjust the results to account for biased sampling of irradiated versus wild males, since, for example, v_S and $v_Y < 1$ suggests that irradiated and irradiated-lineage males may be less able to locate pheromone-baited traps than wild males.

A key factor in deciding whether to undertake a regularly spaced versus hot spot release strategy is likely to be whether the locations of wild populations can be accurately estimated, and the confidence that these finds represent the entirety of the breeding population. When the locations of breeding populations are poorly known, then a strategy that enables sterile moths to reach all potential breeding sites is essential, and an area-wide release with a regularly spaced grid may be the most efficient way of achieving this. In practice, a mixed strategy of hot spot plus smaller intermediate releases might be considered if there was a chance that unknown wild populations might be present. One method of quantifying the chance of undetected wild populations being present has been suggested by Kean and Suckling (2005), based on interpretation of zero trap catches in the light of temperature-dependent development rates and flight thresholds.

If a strategy of regularly spaced releases is used, it is important that the release sites are sufficiently dense to allow irradiated males to disperse across the entire area. As the spacing between release sites increases, the chance of a wild population remaining undetected increases, in which case the probability of eradication may never reach 100% regardless of how many irradiated males are released (Fig. 3).

For a given release strategy, there comes a release rate of irradiated males beyond which there is little benefit in increasing. Once the chance of eradication approaches 100%, there is little further reduction in the time to eradication possible from releasing greater numbers of irradiated males. It may be important economically, therefore, to be able to estimate this point of no further benefit, and the models presented here provide sufficient tools to do so. However, the number of irradiated males required depends critically on the size of wild populations, so accurate sampling technologies must be a prerequisite for conducting optimally-designed sterile insect releases as part of a control programme.

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A Diffusion Model for *Glossina palpalis gambiensis* in Burkina Faso

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ABSTRACT The dispersal of *Glossina* species is of interest to pest control personnel since these flies are the biological vectors of human and animal trypanosomes in Africa. The design of control and/or eradication programmes requires an accurate knowledge of the ecological characteristics of tsetse flies and the geographic structure of their populations. The present study attempts to model the dispersal process of a riverine tsetse species, i.e. *Glossina palpalis gambiensis* Vanderplank in Burkina Faso along an apparent homogeneous gallery forest. While for savannah species, dispersal is usually modelled as a two-dimensional random walk (in time and space) or diffusion (its continuous analogue), for riverine species, dispersal can be viewed more simply as a one-dimensional random walk. The data reported here show that the topology of the habitat, which is a system of tributaries rather than a straight line, has a great impact on the dispersal process. Moreover, since only a part of the river system can be observed in practice, the effect of partial observation when estimating dispersal parameters can be quantified. The results reported here were obtained using a data set from a mark-release-recapture experiment carried out with *G. p. gambiensis* on a tributary of the Mouhoun River in Burkina Faso. The model was fitted to field data and used to estimate the displacement of a fly during 10% of its lifespan (13 kilometres) and the probability of it dispersing more than 10 kilometres from its initial position ($P > 0.1$). The analysis was carried out by either taking into account, or ignoring, the fact that only part of the river system was observed during the mark-release-recapture protocol.

KEY WORDS dispersal, tsetse fly, *Glossina palpalis gambiensis*, diffusion model, mark-release-recapture

1. Introduction

In West Africa, climate and land use pressures result in fragmentation of tsetse fly habitats, especially at the northern end of their distribution areas (Hendrickx et al. 2004). Residual subpopulations, surrounded by semi-permanent barriers (particularly cotton crops), are increasingly isolated and can become a priority target for sustainable control. If the tsetse population is not isolated, barriers of traps or screens impregnated with insecticides can be deployed, to prevent reinvasion (Cuisance and Politzar 1983, Politzar and Cuisance 1983, 1984), at least until the adjacent tsetse populations are suppressed. To establish these barriers, traps or screens are placed every 100 metres along five to ten kilometre river sec-

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tions. Control campaigns preferably target areas where agricultural barriers already exist, and the methods used vary in their cost effectiveness depending on their reliability and the overall objective, i.e. eradication or suppression (IAEA/FAO 2001). Information about the degree of isolation between populations can be very useful for the design, implementation and monitoring of area-wide integrated pest management programmes (AW-IPM), especially when resources are limited.

The cotton belt of Mali and Burkina Faso has been identified as a priority area for tsetse control because of the potential benefits associated with the removal of trypanosomosis (Hendrickx et al. 2004). In Burkina-Faso, riverine species (*Glossina palpalis gambiensis* Vanderplank and *Glossina tachinoides* Westwood) are the main vectors of African animal trypanosomosis, since the only savannah species (*Glossina morsitans submorsitans* Newstead) is now restricted to protected areas in the south of the country. Current studies on the population dynamics of vector species therefore focus on the valleys and riverine forests, using remote sensing and ecological data to evaluate the feasibility of an eradication campaign based on area-wide principles and in support of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) recently initiated in Burkina Faso (IAEA/FAO 2001). In particular, researchers and programme managers are interested in the degree of isolation of vector populations, or conversely their ability to disperse as estimated by migration rates between favourable landscapes. For this purpose, both mark-release field experiments and population genetic analyses have proven to be very efficient tools (Solano et al. 2000, Krafsur and Endsley 2002, Krafsur 2003).

The methodology for estimating diffusion parameters by mark-recapture methods is now robust for species dispersing in two dimensions (Okubo and Levin 2001). For the savannah species of tsetse, such as *Glossina morsitans morsitans* Westwood, diffusion models have been successfully applied to model the dispersal process (Bursell 1970, Hargrove 1981, Hargrove and Lange 1989, Hargrove 2000). However, for tsetse flies dispersing along gallery forests, these are less well developed (Rogers 1977, Randolph and Rogers 1984, Cuisance et al. 1985).

Before addressing the problem of dispersal in fragmented landscapes, the present study analyses data obtained in a homogeneous riverine forest to understand the impact of river structure on the dispersal characteristics of riverine tsetse (Cuisance et al. 1985). In particular, account is taken of the fact that a river cannot be considered as a straight line but rather as a river system. As underlined by Buxton (1955):

> *The fly belt occupied by* G. palpalis *is nearly always along the waterside. […] It is known that the insect moves very freely up or down stream or up a tributary. […] Evidently then the width of the zones varies, but spontaneous movement of the insect is so closely confined to the vicinity of water that it is almost linear.*

2. Materials and Methods

2.1. Field Data

The mark-release-recapture data were collected in 1981 (Cuisance et al. 1985) on the Dienkoa River, a tributary of the Mouhoun River in Burkina Faso. Forty-three biconical traps were deployed at distances of approximately 500 metres over a distance of 20 kilometres along the main stream. The river section under study was bordered by a homogenous closed Guinean riparian forest. Trap locations (Fig. 1) were digitized from a Landsat Thematic Mapper image (pixel 30 metres x 30 metres) from December 2000 (cool dry season) using the original and very detailed field map (Cuisance et al. 1985).

For this study, the data obtained from the release of 8683 three-day-old male *G. p. gambiensis* were analysed. The flies were from the Centre de Recherche sur les Trypanosomoses Animales (CRTA) insectary and were fed once on a rabbit on the day before release. They were not irradiated before release. Ten weekly releases, which were not analysed by

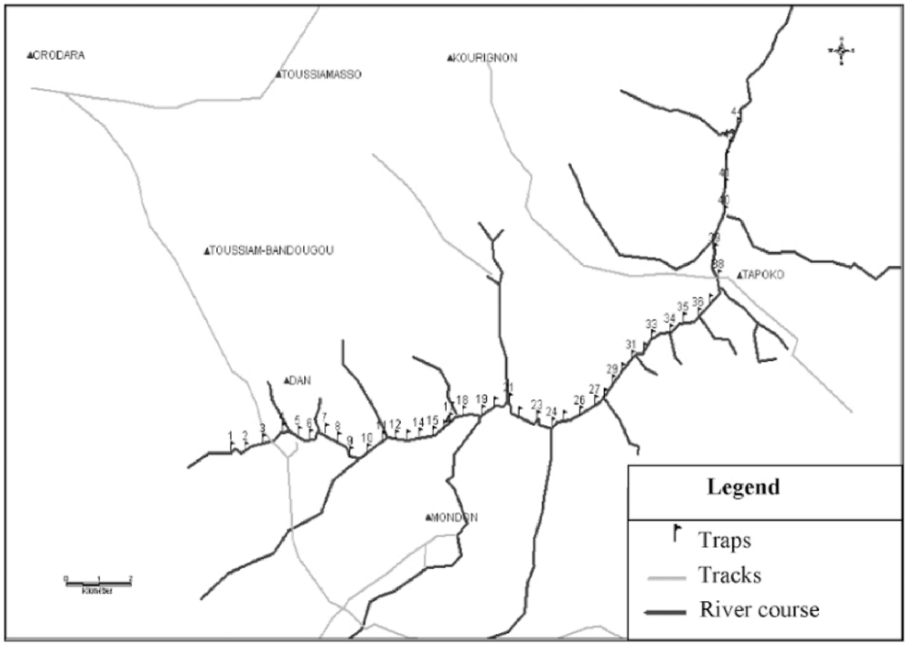

Figure 1. *Location of the biconical traps of the 1981 study along the Dienkoa river.*

Cuisance et al. (1983), were performed between May and August 1981. During these releases, the initial release point (Fig. 1) was located in the middle of the trapped section. Flies were marked on their pronotum with a dot of acrylic paint, the colour of which indicated the week of their initial release. Captures were performed three times per week with traps set from 08.00 hours to 16.30 hours each trapping day (On three days with heavy rain, traps could not be checked and only releases were carried out). Marked tsetse flies were released again at the location where they were captured.

Trapping was terminated at the same date for all cohorts so that data represent various times from the release of the cohorts (15 and four weeks for the first and last cohorts respectively). The data available are of two types: total trap catches by time elapsed from release cumulated over all ten cohorts (Cuisance et al. 1985), and a set of data obtained from Cuisance's diary corresponding to daily data cumulated for all traps.

2.2. Models

A discrete isotrope random walk (same probability of going up- or downstream one distance unit at each time unit), was used to model tsetse dispersal. It can be regarded as the simplest individual-based model of dispersal in one dimension. The model's assumptions are that, during one time unit (τ) a single fly will travel exactly one unit length (λ) either to the right or left with equal probability.

In this case, if the number of time steps n is large, and denoting:

$$x = m\lambda,\ t = n\tau$$

it can be shown that the probability density of the position at time t converges towards the density of a centred Gaussian random variable with variance $2Dt$, where D is the so-called diffusion coefficient and is equal to $\lambda^2/2\tau$ (Williams et al. 1992).

Since mortality appears as the main cause of the end of the diffusion process, zero diffusion was used to model the progress of fly dispersal. Such a model amounts to stopping an

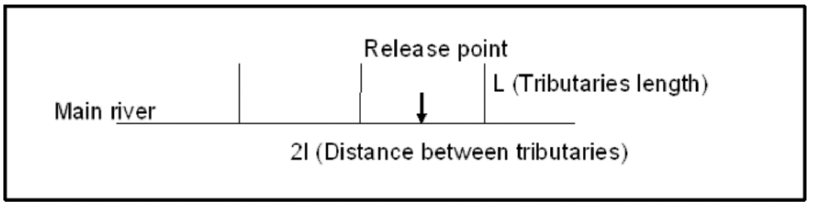

Figure 2. *Symmetric systems used for the simulations.*

individual's trajectory after a random time representing the individual's lifespan. Since the mortality rate between the first release and first recapture cannot be disentangled from the overall recapture rate, and since the mortality rate appeared almost constant during the later stages of the releases, a constant mortality rate, μ was used throughout (the model can however be easily extended to more accurate data on fly mortality). The parameter μ was roughly estimated as the log-regression coefficient of the number of recaptured flies against time. Based on the homogeneity of the gallery and trapping conditions, two strong assumptions had to be made, i.e. the time and space independence of capture and mortality rates.

The model was first applied on a theoretical system of tributaries, comprising an infinite main river where diffusion originates, and one or several tributaries connected to the main stream at definite locations (Fig. 2). Boundary conditions were isotropic at bifurcations (when a fly came to a junction between a tributary and the main river it had equal probability of going up the tributary, or up or down the main stream), and reflexive at the end of tributaries (when it reached the end of a tributary it always came back down at the next step). In order to assess the effects of geometry on the diffusion process, configurations with n = 2 and n = 10 tributaries were studied. To obtain a symmetrical process, the release was simulated in the centre of two tributaries distant of 2l (Fig. 2): the distance between the origin of the process and the next tributary was thus set to l = 1, and the diffusion coefficient was such that $2D = 1$. Several lengths of tributaries (L = 1, 3, 10) were used in the simulations.

The model's parameters (D, μ) were then estimated using the field data set and following two assumptions: (1) the trapping system allows a complete observation of the dispersal process which occurs only along the main river, and (2) the trapping system allows only a partial observation of the dispersal process which occurs throughout the whole river system (river basin with all its tributaries).

Finally, two "summary statistics" were calculated using the two-parameter estimations in a hypothetical case where two populations are ten kilometres apart on a straight river course without tributaries: (1) the distance to the origin exceeded during 10% of the fly's average lifespan, and (2) the probability of a fly travelling beyond a given distance to the origin. Details of the mathematical process will be described elsewhere (Sibert et al., in preparation).

3. Results

3.1. Theoretical Systems

The effects of considering only the main river for estimating D and μ while dispersal occurs throughout a river system was investigated using a variety of symmetric and asymmetric river systems. The results of the simulations depend on the complexity of the river system and the value of the diffusion coefficient. The results from the nine theoretical symmetric river systems (Fig. 3) are only illustrative but they use a realistic D, namely 0.5 square kilometres per day and a realistic river system,

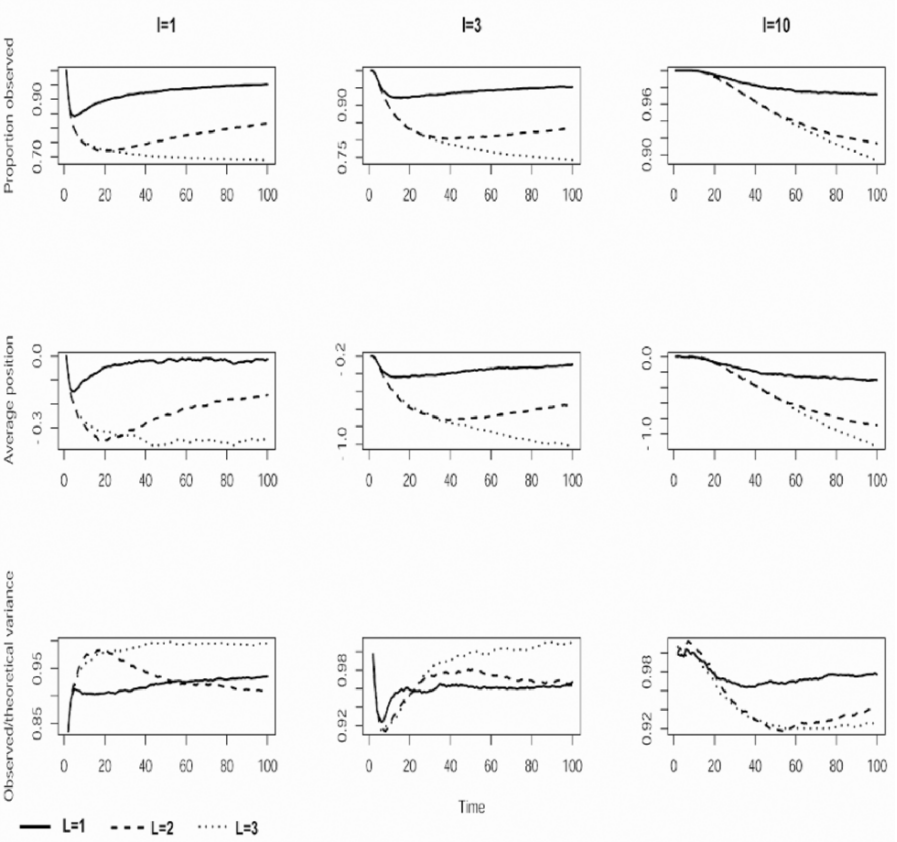

Figure 3. *(upper graphs) Proportion of flies observed on the main river, (middle graphs) average position in kilometres, and (lower graphs) ratio between observed and real variance in function of time (in days) in nine hypothetical river systems (n=10, L=1, 2 or 3 and l=1, 3 or 10).*

with ten tributaries. To appreciate the potential impact of partial observation on the estimation of D and μ, three variables are illustrated: (1) the proportion of flies observed on the main river (to illustrate the effect of partial observation on the estimation of mortality rates), (2) the average position of the population (to show that an isotropic random walk can lead to an apparent displacement of the population on the observed section), and (3) the ratio between observed and actual variance (to illustrate the impact of partial observation on the estimation of the diffusion coefficient (Fig. 3).

From the simulations, it is clear that spatial sampling, i.e. observing only the main river with traps while dispersal occurs on the whole river system, can lead to overestimation of mortality rates by up to 30% at day 40 (Fig. 3, first column, first line). The average position of the population on the main course can evolve up to one kilometre from the release position (Fig. 3, third column, second line), towards the centre of gravity of the system, leading to an apparent drift of the population, which would be impossible if the diffusion process occurred in an infinite symmetrical system. Finally, the variance in position (pro-

portional to the diffusion coefficient) can be underestimated by 15% in the case of complex systems (Fig. 3, first column, third line).

3.2. Real Data Set

With the real data set presented above, D and μ could be estimated depending on two different assumptions: (1) dispersal occurs only on the main river and the trapping system provides complete information, and (2) dispersal occurs on the whole river system and the data set is thus spatially sampled.

If dispersal occurs only on the main river, a complete observation of a random walk on a straight line can be implied. Daily mortality rate estimated over the first month is then 6.5%, corresponding to a mean lifespan of 15.5 days. The corresponding diffusion coefficient is 0.29 km^2/day.

In the alternative hypothesis (partial observation of a random walk on a tree), the estimated mortality rate is only 4.4%, corresponding to a mean lifespan of 22.7 days. The diffusion coefficient is 0.46 km^2/day.

The two estimation errors due to spatial sampling demonstrate the same tendency, i.e. an underestimation of the probability of long-distance movement in the case of partial observation. When the model coefficients are corrected for the partial observation bias, the average distance covered by a fly during 10% of its lifespan increases from four to 13 kilometres. At the same time, the probability of a fly reaching a population located ten kilometres away increases from less than 0.01 to more than 0.1. In other words, the (apparent) displacement – and the (apparent) probability of reaching another population – is much lower than it is if only the data from the main river are included.

4. Discussion

The estimates of mortality rate obtained from the raw data are 30% lower when a partial system of tributaries is assumed (0.040 versus 0.065). However, mortality remains very high for this highly favourable environment (closed and conserved Guinean gallery forest). Density-dependent factors may induce an increase of mortality rates next to the release points due to increased local density (Rogers and Randolph 1984). The assumption of a constant mortality rate in space is then not valid as the fly density will be higher at the release point, at least during the releases and maybe during the following days. In all cases, taking into account the spatial complexity seems very important when estimating mortality rates, in the case of sterile insect releases for example. Moreover, dispersal over the entire available system (including the tributaries) may be considered as a way to reduce mortality density-dependent factors as much as possible.

The structure of a river system is not static in time, but evolves with macro-climatic variations through seasons. In the hot dry season, the small tributaries are drained and become unsuitable for tsetse survival, leading to their concentration in the moister section, i.e. in the main river. While dispersing with the same diffusion coefficient on either the entire system or the main course only, the probability of long-distance movements on the main river will increase, thereby enhancing long-distance dispersal during the dry season. Thus, on the one hand, tributaries can act as brakes to longitudinal movements particularly during the rainy season while, on the other hand, the mortality rate will decrease as relative humidity increases during the rainy season. These two parameters have thus opposing effects on long-distance dispersal.

In the model used, the two-dimensional structure of the system was not taken into account, and the possibility cannot be excluded that some flies escaped from the gallery (main stream or tributaries) and re-entered the system at some other point. However, during the release period (beginning of the rainy season), 145 traps were set in two circles located at one and two kilometres from the release point in the neighbouring savannah (Cuisance et al. 1983), and only 1.1% (36 from 3228) of the captured flies were caught outside the gallery. Moreover, as movement in the savan-

nah is a two-dimensional diffusion, the latter environment can be considered less diffusive than the gallery itself and its contribution to long displacements along the main course can be neglected. During the rainy season however, riverine flies migrate into nearby savannah areas, especially while following their hosts, and their movement, perpendicular to the river systems, should be taken into consideration if attempting to estimate the probability of a river basin being "isolated" from another.

In the present work, the experimental site was chosen for its homogeneous, closed gallery. Provided that the spatial complexity is taken into account, a diffusion process was appropriate to describe tsetse fly dispersal. However, in fragmented landscapes, gallery forests are in fact very heterogeneous (Morel 1983). In such situations, a diffusion process may be unsuitable to describe fly dispersal, as the decision to disperse becomes a trade-off between a reduction in mortality-dependent factors and an increase in mortality-independent factors while moving from a favourable ecosystem (natural riverine forest) to a less favourable one (disturbed riverine forest) (Blondel 1995). Experimental releases integrating the lessons from the present work are presently being conducted in fragmented landscapes previously characterized by phytosociological analysis (Bouyer et al. 2005), to integrate spatial heterogeneity into dispersal models. In association with population genetics, these should be very useful in understanding the population structure within the Mouhoun river basin and may allow the identification of "natural" barriers in the planning of an AW-IPM programme in Burkina Faso.

5. Conclusions

A normal diffusion model can fit the data on riverine tsetse dispersal in homogeneous landscapes provided that the spatial complexity of the river system is taken into account. Daily mortality rate has an important effect on dispersal and especially on long-distance movements. Since they depend mainly on the season and the conservation status of the gallery forest, these elements may have a great impact on the tsetse dispersal process. Mark-release field experiments, supported by new techniques like dispersal models, remote sensing and geographic information systems (GIS) can be very useful in designing the sequential process of an AW-IPM project. They could also be used to optimize the releases of sterile flies during an eradication campaign.

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Current Advances in the Use of Cryogenics and Aerial Navigation Technologies for Sterile Insect Delivery Systems

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ABSTRACT Insect release methodologies have evolved into a more insect friendly process utilizing available industrial technologies to improve delivery mechanisms. The insect release activity has gone from a purely mechanical process to one that places greater emphasis on maintaining the quality of the sterile insect. Early systems depended greatly on mechanical displacement of flies using motors, augers, and moving belts. Complex heating, ventilation and air conditioning (HVAC) components were also used for air conditioning. Current systems are available that rely more on gravity and suction for fly transport, dry ice for cooling, and solid state circuitry for controls. Also, navigation using the Global Positioning System (GPS), geographic information systems (GIS) and computer technologies can be used to control release machine operation. Systems using new technologies are more reliable, less damaging to the sterile insects, and decrease the cost of the sterile insect technique (SIT) operations.

KEY WORDS sterile insect technique, release systems, gravity and suction, dry ice, GPS/GIS-based navigation

1. Introduction

A great deal of effort has been expended on the technology for mass-rearing sterile insects (Lindquist 2001, IDIDAS 2004). This is also the case for Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) at the mass-rearing facility in El Pino, Guatemala, where refining the rearing process is an ongoing activity (Cáceres et al. 2000). This effort is focused on producing a better sterile male fly as well as trying to achieve higher levels of efficiency and economy.

Producing sterile insects is only one activity in the overall process of area-wide fruit fly control applying the sterile insect technique (SIT) as part of an integrated pest management (IPM) approach. Quality processes and procedures need to be implemented from rearing to field release. Only under these conditions will fruit fly control with an SIT component be successful. In this paper, the current progress in aerial release technologies using cryogenics is presented and discussed with a focus on fly quality and handling, cost efficiency, and the logistics that make it feasible to operate medium- or large-scale SIT programmes worldwide.

2. Initial Technologies

2.1. Paper Bag Delivery System

The current energy being invested in Mediterranean fruit fly aerial release equipment has resulted in more emphasis on deliv-

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Figure 1. *Paper bags stacked in the aircraft cabin prior to release through a chute constitutes the paper bag delivery system.*

ering a viable fly to the target area. In the past, the major efforts of aerial release focused on the physical or mechanical act of getting the fly out of the aircraft. For many years, Mediterranean fruit fly has been aerially released using paper bags (Hentze and Mata 1987, Villaseñor et al. 2000). This flexible release container also functioned as the emergence container and fly emergence facilities were designed to accommodate the bulk of the paper bags. The bags were most likely the poorest delivery container since they often collapsed during ground transport and when packed into the aircraft (Fig. 1). The bags also maintained the sterile flies at or well above ambient temperatures depending on whether the bag was on top of or under the pile of bags. This simple handling and release procedure, while detrimental to fly quality, eliminated the need for complex release equipment. When the bag was discharged from the aircraft it was ripped open, either by an operator or hook on the exit chute or tube. The flies, paper bag, enclosed paper waste (to provide flies additional surface to expand their wings), and empty pupal cases were ejected from the plane into the environment. Despite the use of biodegradable paper, this resulted in an accumulation of a great deal of paper trash littering the countryside.

2.2. First Generation Chilled Adult Technology

First uses of cryogenics as a means of lowering temperatures in insect delivery systems were made through the release of chilled adults in Hawaii (Harris et al. 1975, Howell et al. 1975). In the early years of the Programa Moscamed (Hendrichs et al. 1983), such a mechanical delivery device was used. This initial design was capable of accommodating 5-8 million Mediterranean fruit flies. This equipment was installed in a Cessna 206 Beechcraft Baron, and in other light single- or twin-engine aircraft. The machine was composed of a cooling unit utilizing a compressor and other components similar to a car air conditioning system to provide a flow of cool air, storage box(es) for the flies, and a base with a set of augers to move the flies laterally as they were received by gravity from the storage box. Some of the earlier release machines used a moving band in lieu of the augers. This release system using the augers is still in operation in the USA for the Mediterranean fruit fly release programme in California and Florida and for the release of the Mexican

fruit fly *Anastrepha ludens* Loew in Tijuana, Mexico (USDA/FDACS 2004, CDFA 1999).

The machine was not ideal for use in humid areas or when the Mediterranean fruit flies needed to be cooled for extended periods of time, or when large quantities of sterile flies needed to be released every day. The inactive flies in the release boxes became increasingly more humid over time due to their metabolic activity and the influx of air from outside that contained more humidity than the air in the cooling system. This excess humidity and constant aircraft vibration caused the flies to compact. This condition resulted in the flies being aggregated when released instead of a steady stream of individual flies. The limited capacity of the boxes required numerous flights for large numbers of flies and its extensive use was costly since the cooling system required a great deal of routine maintenance and part replacement.

2.3. Larger Scale First Generation Chilled Adult Technology

A later version of the machine (Fig. 2) was built by a private vendor following a design provided by the New World screwworm *Cochliomyia hominivorax* (Coquerel) eradication programme. The machine was very similar but had a larger capacity holding up to 20 million Mediterranean fruit flies in two large boxes (FAO/IAEA 2007). This design, after additional modifications on site, provided the level of economy needed for massive releases of Mediterranean fruit flies but exhibited the same humidity and compaction problems of its smaller version.

Two of these machines could be placed in a Cessna Grand Caravan or Cessna 208 increasing the capacity of each flight to 40 million Mediterranean fruit flies. The augers (Fig. 3) mechanically delivered the flies to a common horizontal chute and suction extracted them through two exit tubes in the belly of the aircraft. However, utilizing two machines in one aircraft increased the electrical load close to the maximum working capacity (100 amps) of the aircraft's electrical system. Electrical problems in the 28 volt system were numerous until the system was modified to accommodate the fluctuating voltage/amperage.

Daily routine maintenance and cleaning

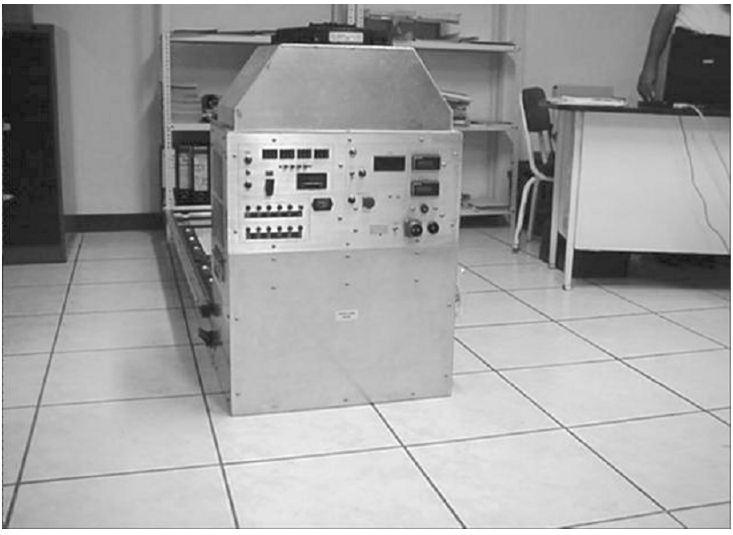

Figure 2. *Cooling unit for chilled Mediterranean fruit fly adult release designed after the New World screwworm release machine.*

Figure 3. *Screw auger system for chilled adult release of Mediterranean fruit fly.*

was crucial to continuous operation. When the augers jammed in flight due to an unmovable mass of humid flies the releases had to be terminated and the plane return to base.

3. New Developments: Second Generation Chilled Adult Technology

A new release machine was commissioned by the Programa Moscamed using a private vendor and completely different set of criteria. The criteria included a machine that was insect friendly, i.e. that would cause only minimal damage to the sterile flies, capable of carrying 20 million or more Mediterranean fruit flies, suitable to carry other sterile insects or parasitoids, able to control humidity and temperature, require a low electrical load, provide solid state circuitry, had a simple but more effective cooling system, metered gravity feed, and control assisted by a global positioning system (GPS) signal. International Fly Masters, a local contractor from Florida, undertook the design and construction of a prototype.

The resulting equipment is designed to fit in the cabin and/or cargo pod on the belly of a fixed wing aircraft (Fig. 4). The total capacity for the Mediterranean fruit fly is approximately 20-25 million, humidity can be controlled by a chemical drier or air circulation, electrical components are solid state and replaced as distinct units, and the electrical load was reduced to less than half. Cooling is accomplished by utilizing dry ice pellets manufactured from liquid CO_2 and a heat exchanger. Specific release instructions are provided by an AgNav® unit that also provides guidance to the pilot for navigation (Fig. 5). The unit is capable of releasing several species of insects together or as separate events.

The base of the unit is a fabricated aluminium frame. Internal parts of the release boxes are anodized and coated with Teflon®, moving plastic parts are made of special high wear polymers, and other structural plastic parts are made of composite materials. Controls are contained in a central console, remote motor control boxes, and the AgNav® navigation equipment. Overall monitoring of temperature, humidity, fly release rates, aircraft altitude, speed, and time is recorded by the software that is programmed into the AgNav® (Fig. 5).

Additional electronic supervision is

Figure 4. *Second generation chilled adult release system installed inside a Cessna 206 aircraft.*

obtained by the use of a portable self-contained computer (EDAC Electronics Ltd. Australia) that is connected to the system at the beginning of the flight and removed on its return. The information is then downloaded for later analysis or can be sent via email to a remote site for review. Several different units are available that can be accommodated in various aircraft and helicopters. A ground delivery system is also being designed using similar criteria.

The key to the successful delivery of sterile flies to the target area also includes the handling of the fly prior to and after "knock-

Figure 5. *Component of AgNav® aerial guidance system mounted in cockpit.*

down" in the cold chamber. Insuring that the heating, ventilation and air conditioning (HVAC) system in the emergence room is capable of reducing the excess humidity during peak fly emergence is critical. Emergence containers placed into the cold room with high humidity levels increases the load on dehumidification equipment. This high humidity can also limit the harvest of viable flies. The flies within the emergence containers that hold the pupae will collapse when overly humid, trapping healthy flies inside (Fig. 6).

The use of emergence towers instead of plastic aerial release containers (PARC boxes) (Salvato et al. 2004) would eliminate this problem since the tower does not use paper bags. Filling the release boxes with flies when humidity levels are above 30-40% and exposing release containers full of flies to excess humidity during later handling promotes "packing" or the tendency for the flies to become tightly packed in the release box. Prolonged ground transport of the release containers full of flies also adds to this problem. This packing will impact fly quality and can create difficulties during release. Tightly packed humid flies have less oxygen, retain more metabolic heat, impede cool air flow through the column of flies, and resist gravity flow (Fig. 6).

The flies need to exit the aircraft without being subjected to damaging physical trauma. The combination of gravity/ mechanical/ suction feed has to be balanced to facilitate a free flow of flies. Allowing the flies to flow through the augers via gravity instead of pushing them, moving them laterally by suction, and finally having them exit vertical tubes by gravity and suction minimizes fly damage. This seemingly simple process requires balancing the vacuum or suction created by the exit tubes protruding from the aircraft. Too much suction damages the flies and evacuates the cold air from the system. Too little causes the flies to accumulate in the horizontal exit tubes and ultimately blocks the tube.

The length and angle on the exit tube meeting the air-stream are also important in the generation of suction and insuring the flies leaving the tube do not impact the aircraft structure. Release times and flight procedures also play a role in successful delivery of the fly to the target area. Early morning flights in stable air minimize excessive wind drift and

Figure 6. *(upper) Mediterranean fruit fly emergence towers (Worley system), (middle) plastic adult release containers (PARC), and (lower) PARC boxes showing the pupae holding paper bags (middle and lower photo from F. Moscoso, reproduced with permission).*

Figure 7. *The versatile and dependable turbine Cessna Grand Caravan, that is being used as the primary release aircraft for Mediterranean fruit fly in the Programa Moscamed.*

the temperature shock of high ambient temperatures during midday.

Low altitude flying, rapidly changing mountainous terrain, variable airspeeds, and abrupt changes in flight attitudes (diving, climbing, steep turns) are procedures that can help locate the fly closer to the target but demands the best out of the equipment and pilot. Routine flights normally involve flying straight and level but can require that the pilot follow contours where high mountains or deep canyons interrupt the flight path.

Aerial delivery of the sterile insects or any other biological organism can be a costly event or a cost-effective one. Current Mediterranean fruit fly release costs vary from USD 30 per million flies up to USD 150 or more per million flies. The current Guatemala/Mexico Programa Moscamed enjoys the lowest release costs available. A timely investment in release technology has developed a unit that can hold 20-25 million fruit flies, and the choice of a spacious aerial platform that can hold two release machines permits loading 50 million Mediterranean fruit flies on each flight. The versatile and dependable turbine Cessna Grand Caravan is being used as the primary release aircraft (Fig. 7). This single engine turbine powered aircraft is approved for single pilot operation, flown extensively over populated areas and open water, has a seven hour range, capable of slow flight and routine landings on short unimproved mountain airstrips, fixed gear and low maintenance. The five Caravans currently flying for the Programa Moscamed in Guatemala and Mexico have completed thousands of hours conducting about 60 flights per week (one-three hours in length) for five years without accident or an engine/airframe failure.

4 Field Comparison of Technologies

4.1. Evaluation of Fly Distribution

One key factor in release effectiveness is the homogeneous delivery of sterile flies to the entire release block. A continuous stream of flies released within the entire block ensures that all areas with host are afforded the protection of sterile flies. Locations without flies are subject to infestation that can eventually defeat the purpose of the SIT. Controlling the

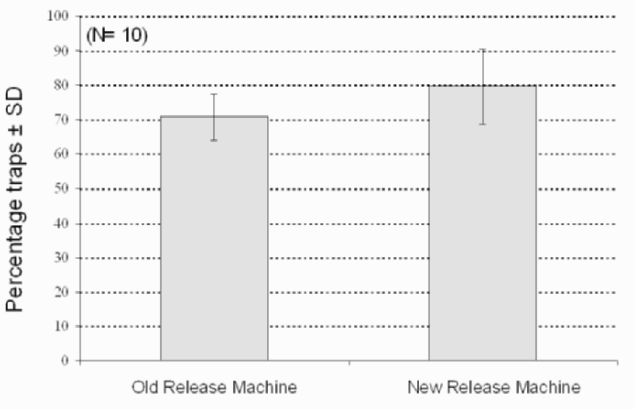

Figure 8. *Comparison of two release systems in terms of the resulting sterile insect distribution based on the percentage of traps that captured flies released with the old and the new release machine during ten consecutive weeks (n = 10).*

release of flies manually can lead to skips or areas without flies as well as wasting them due to releasing them outside the release block or host areas. Mountainous terrain or isolated infestations surrounded by non-host areas, present a high level of difficulty when not using electronic guidance for precise insect delivery.

Field comparisons of the two release systems were conducted during ten consecutive weeks (i.e. ten replicates) over the coffee area in south-western Guatemala. The total area subjected to releases was 104 square kilometres.

Pupae from the same production day of the male-only VIENNA7/TOLIMAN 99 strain that carries a *temperature sensitive lethal* (*tsl*) mutation, mass-produced at the El Pino rearing facility were dyed, prior to irradiation, with a fluorescent marker DayGlo^{TM} using two distinctive colours. Green (saturn yellow) was used to mark adults released from the new machine and yellow (arc yellow) for the old release machine. Twenty three million marked pupae of each colour were sent from the El Pino rearing facility to the emergence and release facility located at Retalhuleu. PARC boxes were loaded with pupae from each colour. For adult food the standard operational agar-water diet was provided. Pupae/adults were maintained inside emergence rooms for ca five days. Environmental conditions during the emergence and maturation period were the same for both batches of pupae/adults (temperature of 22 ± 1°C, relative humidity of 65 ± 5 %). After five days adult flies were taken to a cold room, (temperature ca 3°C for 45-60 minutes), a procedure which allowed removing dormant flies from the PARC boxes. Dormant flies were loaded on either old or new release cages and later loaded to each aircraft to conduct aerial releases. Aircraft flew over the same release area; the aircraft with the new release machine flying first and the aircraft with the old release machine second with an average delay of 90 minutes (n = 7) from each other. The order of plane releases was reversed in every consecutive week (i.e. the following week, the aircraft with the old release machine flew first). The release conditions for both releases were calm wind conditions with an average temperature of 20.3°C (n = 14). The release rate was ca 1500 sterile insects per hectare of each colour. A contour system release was used due to the characteristics of the terrain and elevation of aircrafts for the releases was aimed at ca 300 meters.

The following sterile fly distribution data (Fig. 8) demonstrate the increased effectiveness brought about by using GPS coordinates

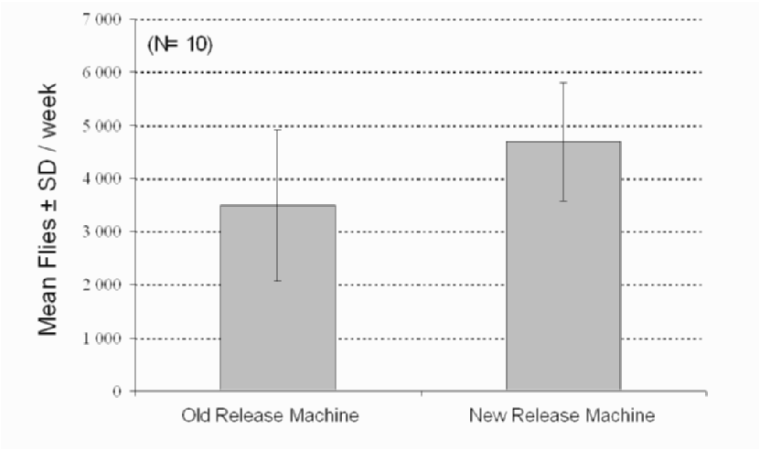

Figure 9. *Total sterile insects recaptured per week resulting from aerial releases of sterile males of Mediterranean fruit fly released with the old and the new release machine.*

and electronics to instruct the machine when to release the flies. As is obvious, the more traps with capture of sterile flies in the release block, the greater level of control and overall programme efficiency.

4.2. Evaluation of Fly Recapture

In addition to the even sterile fly distribution the overall quality of the fly after release is of primary importance. The new machine showed a much higher level of recapture in the traps indicating that longevity or survival, attributed to fly handling during the release activity, was much higher (Fig. 9). A healthy fly will perform better and live longer, and this is critical to overall programme performance. More flies in the release blocks, that are living longer and are overall in better physiological condition, facilitate effective and efficient control. These are management performance goals that allow the manager to receive the best return on the investment.

5. Conclusions

The success and growth of the SIT as a suppression, containment and eradication tool lies in its economy and return on investment. It is not just about releasing sterile flies from an aircraft, but rather one activity in a complex chain of events, which results in successfully controlling the pest. It is an environment-friendly option that is widely accepted but in the end, cost will play a major role in its implementation.

In the past little emphasis has been placed on the importance and role of engineering in SIT. Reviewing SIT publications over the years clearly demonstrates that the "mechanical connection" would profit from more attention. Mechanical engineering has successfully been used to improve adult emergence using emergence towers, egg production increased dramatically with special oviposition cages in use in the El Pino mass-rearing facility in Guatemala, and release systems are becoming more versatile and cost effective.

One key element to the future success of SIT is the search for biological solutions for insect-borne animal, plant and human maladies (malaria, dengue, sleeping sickness, etc.) and the role of engineering in providing effective and efficient delivery methods.

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Area-Wide IPM for Commercial Wheat Storage

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ABSTRACT The United States Department of Agriculture's Agricultural Research Service (USDA-ARS) funded a demonstration project between 1998 and 2003 for area-wide integrated pest management (AW-IPM) of commercial stored wheat in Kansas and Oklahoma. The AW-IPM concept is useful to stored grain because it reduces the mixing of infested and uninfested grain at the terminal elevator by controlling insect problems in small country elevators before the grain is shipped to the terminal elevator. This project was a collaboration of the USDA-ARS Grain Marketing and Production Research Center in Manhattan, Kansas, Kansas State University, and Oklahoma State University. The project utilized two elevator networks, one in each state. Over the five years of the study, researchers worked in approximately 55 country elevators and four terminal elevators, and collected and analysed more than 125 000 grain samples. Wheat at elevators was frequently infested by several insect species, which sometimes reached high numbers and damaged the grain. Fumigation using aluminum phosphide is the main method for controlling insect pests in grain elevators in the USA. Fumigation decisions tended to be based on past experience with controlling stored-grain insects, or were calendar-based. The best sampling method for estimating insect density in commercial elevators without having to transfer the grain from bin to bin was the vacuum probe sampler. Decision-support software "Stored Grain Advisor Pro (SGA Pro)" was developed that interprets insect sampling data and provides grain managers with a risk analysis report for their elevator that specifies the current and predicted risk for each bin. Recommended treatment strategies and economic analysis were presented to the elevator managers at six-week intervals. Elevators that followed the recommendations reduced the number of bins they normally fumigated by at least 50%. The AW-IPM programme was superior to calendar-based management because it ensured that the grain in each bin was only treated when insect densities exceeded economic thresholds. This approach reduced the frequency of fumigation while maintaining high grain quality. Minimizing the use of fumigant improves worker safety and reduces control costs and harm to the environment. A grain-scouting company was started that uses SGA Pro and the sampling tools that were developed in this project. The company is in its third year and has over 30 commercial elevators on contract.

KEY WORDS area-wide, integrated pest management, stored-grain, insect damage, decision-support software

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1. Introduction

Area-wide integrated pest management (AW-IPM) involves coordinating insect pest management programmes against a targeted pest population to reduce the overall densities of insect pests and to minimize the risk of initial infestation and reinfestation after insect pests have been controlled (Rabb 1978, Knipling and Stadelbacher 1983, Bellows 1987).

The USA is a world leader in wheat production. Stored-grain insects and moulds cause USD millions in losses annually in this multi-billion dollar industry. AW-IPM is particularly important for stored grain because insects are moved through the marketing system with the grain (Hagstrum and Flinn 1992, Hagstrum et al. 1999). Wheat is stored in large networks of country (smaller elevators) and large terminal elevators, and grain from several million hectares ultimately ends up in a few very large terminal elevators. Failure to control insects at country elevators early on can provide a source of infestation that can infest much larger quantities of grain as it is combined and blended at the terminal elevator. Often, the country and terminal elevators are owned by the same company, and by controlling insects in the country elevators, the company is assured of high quality grain by the time it arrives at the terminal elevator.

Blending infested grain with uninfested grain can increase the cost of insect pest management because many more bins will need to be fumigated. Currently, insect problems are managed at most elevators by calendar-based fumigations. These fumigations do not distinguish between high and low insect densities, and do not optimize the timing of the fumigation. Insect problems can be managed much more effectively, and with less fumigation, if insect are detected by sampling the grain at regular intervals, and fumigating bins in which insect problems are found.

From 1998-2003, the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) funded a demonstration project for AW-IPM for stored wheat in Kansas and Oklahoma. This project involved collaboration between researchers at the USDA-ARS Grain Marketing and Production Research Center in Manhattan, Kansas, the Kansas State University in Manhattan, and the Oklahoma State University in Stillwater. The project was conducted in working elevators and was designed to document the efficacy of current insect control practices, and test new approaches and technologies to further the adoption of integrated pest management (IPM) practices. An IPM approach involves data-driven decision-making based on benefit/cost ratios, especially when toxic pesticides are used. The USDA and the Environmental Protection Agency (EPA) promote IPM technologies because they bring increased profits while reducing pesticide use in many areas of US agriculture.

Over the five years of the study, project investigators worked in 55 country (smaller elevators) and four terminal elevators in Kansas and Oklahoma, collecting and analysing more than 125 000 samples. Special projects on grain aeration, grain fumigation, sanitation, pesticide use, and many other topics were carried out. The wheat at these elevators was harvested from over 32 400 hectares. Elevators were selected for the project so that the wheat could be followed as it moved from farm to country elevator to terminal elevator. Roughly, 70% of the wheat sampled at the country elevators moved to the four terminal elevators that were collaborating in the AW-IPM project.

The objectives of the study were to: (1) develop practical sampling methods for insects in elevator bins, (2) develop risk-analysis decision-support software, and (3) determine if a sampling-based risk analysis programme maintains grain quality and reduces fumigation compared to calendar-based fumigations.

2. Methods

Traditionally, elevator managers have depended on thermocouples to alert them of potential mould and insect problems. However, grain heating occurs only when large numbers of

insects or severe mould problems are present. Another insect monitoring and grain conditioning practice is to "turn" the grain from one bin to another, sampling the grain as it moves. However, grain turning is expensive. Fumigation is usually done as the grain is turned; thus, if the manager decides to turn, they often fumigate at the same time to save the cost of turning again to fumigate. For the AW-IPM study, a method was needed to monitor insect populations in grain bins without having to turn the grain. A newly developed gasoline-powered vacuum probe worked well. The vacuum probe was used to take a three kilogram sample from each 1.2 metre layer of grain as the probe was pushed down into the grain to a depth of 13 metres (about half the depth of grain in an average bin). Because of the large sample size (three kilograms), a specially designed inclined sieve was used to separate insects from the grain (Hagstrum 1989). In addition to the vacuum sampling, grain temperature and moisture were also monitored.

During the project, a decision-support software, Stored Grain Advisor Pro (SGA Pro) was developed and validated. This software interprets insect sampling data, and provides grain managers with a risk analysis report detailing which bins are at risk for insect-caused economic losses. This software uses rules to evaluate the risk based on current insect density, predicted insect density, grain temperature, and grain moisture. Insect density was predicted up to three months in the future using computer simulation models, based on grain temperature and moisture inputs (Flinn et al. 1997, Flinn et al. 2002).

3. Results and Discussion

3.1. Vacuum Probe Sampling

Data collected with the vacuum probe sampler were well correlated ($r^2 = 0.79$) with grain samples taken as the bin was unloaded.

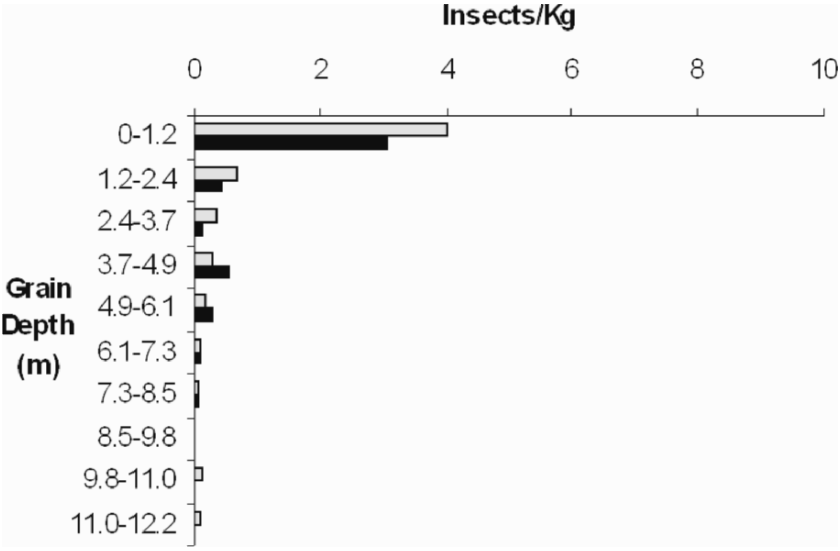

Figure 1. *Average insect density for* Cryptolestes ferrugineus *(grey) and* Rhyzopertha dominica *(black) as a function of grain depth in 30-metre-tall upright concrete grain bins in September. Ten, three kilogram grain samples were taken in each bin, and 172 elevator bins were sampled in September.*

The vacuum probe provided the most convenient and reliable method of routinely sampling bulk grain for insects without having to move the grain from bin to bin.

In general, insect density was found to decrease with grain depth, and in most cases, the majority of insects were found in the top half (13 metres) of the grain bin (Fig. 1). Therefore, it was not necessary to sample the entire depth of the grain bin, only the top 13 metres (most bins were over 26 metres in depth). The time required to probe a bin was greatly reduced by sampling only the top 13 metres of the grain mass instead of the entire 26 metres. Using the inclined sieve, samples were sieved at the elevator, and only the fine material containing the insects was transported to the laboratory for identification and counting.

The three most common insects infesting stored wheat in Kansas and Oklahoma were the rusty grain beetle *Cryptolestes ferrugineus* (Stephens), lesser grain borer *Rhyzopertha dominica* (F.), and red flour beetle *Tribolium castaneum* (Herbst) (Reed et al. 2003). Of these three, the lesser grain borer is by far the most damaging because the immature stages develop inside the kernel. These same three species have been reported as major stored grain pests in other counties, so the same IPM programme used in this study could be used in other parts of the world. The relative proportion of the species changed with time of year. In general, the rusty grain beetle was most common during the first months of storage. However, after about three to four months of storage, the lesser grain borer often became the dominant species (Reed et al. 2003).

The value of the insect monitoring programme to the elevator manager was evaluated by providing the managers with information about the insect density in each of their bins every six weeks. In some cases, this information was shared with the terminal elevator that was expected to receive the grain. How this information was used by the manager to make insect pest management decisions was also evaluated.

3.2. Fumigation and Aeration

In addition to fumigation, other methods, such as aeration to cool the grain, can be used instead of fumigation to suppress insect growth in bins with low insect densities (less than two insects per kilogram). Inexpensive automatic aeration controllers can be used to turn on aeration fans when outside air temperatures are below a set temperature threshold. Insect development and reproductive rates are directly related to temperature. The optimal temperature for stored grain insects is about 30-32°C. Most stored grain insects fail to develop and reproduce at temperatures below 20°C.

Currently, insect pests in stored grain are managed at most elevators by calendar-based fumigations. These do not distinguish between bins with high and low insect densities, and normally do not select the best time of year to fumigate infested grain. Fumigating grain with low insect densities in the summer increases the cost of elevator operations unnecessarily because the grain will probably need to be fumigated again in the autumn. Many elevators in this study fumigated grain within one month of storage. Based on sampling data, there were very low numbers of insects at that time. Elevator managers also believed fumigations to be very effective. However, the data obtained from this study showed that the efficacy of fumigation at two terminal elevators was quite variable. At one elevator, fumigation failed to reduce insect populations by 80% in all of the bins; while in two other elevators, insect populations were reduced by over 80% in nearly all bins. Insect problems can be managed much more effectively if the grain manager has information about which bins in their elevator have high insect densities, so that these bins can be fumigated before damage occurs. Often, the only indication of an insect problem is a hot spot indicated by the temperature cables. By the time the hot spot is noticed, significant insect damage has already occurred to the grain.

Determining when to fumigate grain for

insect control is complicated. A typical elevator has many bins, each with grain at a different temperature and moisture, with different insect densities, and stored for different time periods. These factors directly affect insect population growth. The problem with calendar-based fumigation is that bins either are fumigated unnecessarily, or are fumigated after the grain has already been damaged by high insect densities. IPM uses sampling to determine if insect numbers have exceeded an economic threshold. Turning grain from one bin to another can be used to sample grain. It is not cost-effective, however, to turn grain just for the purpose of sampling. There are costs for labour, electricity and shrink (loss of grain volume). Fumigating grain is normally accomplished by adding the phosphine pellets to the grain as it is turned from one bin to another empty bin. If grain is going to be turned, it is more cost-effective to simply add the fumigant pellets at the same time rather than having to turn the grain twice. However, the problem with this approach is that grain that does not need to be fumigated may be unnecessarily turned and fumigated (if the grain has not been sampled, the insect density is unknown).

What was needed for an elevator IPM programme was a cost-effective method to sample the grain for insects without having to turn the grain. A major objective of the study was therefore to determine whether insect-monitoring-based fumigation was more effective in reducing the risk of economic losses from insect problems than calendar-based fumigation. With calendar-based fumigation, some bins are fumigated too soon, and others are not fumigated soon enough. Newly harvested grain has a very low insect density and does not need to be fumigated. Grain that is fumigated early becomes infested soon after fumigation because there is no residual effect. Leaving newly-harvested grain undisturbed as long as possible minimizes the blending of infested and uninfested grain within a grain elevator. This occurred because the bottom half of the bin usually contained uninfested wheat, while the top of the grain was infested. Fumigating in the autumn is a good strategy because cooler air temperatures will lower the grain temperature following fumigation, which will reduce the growth rate of any surviving insect populations.

Sampling bins with a vacuum probe every six weeks ensures that bins are not fumigated unnecessarily, and that bins that have insect densities above economic thresholds (more than two insects per kilogram) are treated promptly. This way, the manager continually knows the status of the grain, and therefore does not have to fumigate their bins two to three times a year to reduce risk. In this project, sampling at elevators has shown that usually only a few bins have insect densities that justify fumigation. Thus, treating only the bins that require fumigation reduces the cost of pest management and results in better overall grain quality. The area-wide approach is also applicable within an elevator; by keeping overall insect density low for the entire elevator, the manager reduces the probability of insects spreading from one bin to another within the facility.

3.3. Validation of SGA Pro

The SGA Pro software was field-tested for two years. Every six weeks, the bins were resampled, the predicted insect densities were compared with actual insect densities, and the manager's bin treatment actions were recorded. To test the accuracy of SGA Pro's predictions, bins that were sampled at least twice were used, starting in autumn, in which the grain was not moved or fumigated. SGA Pro was used to analyse the data and to provide recommendations to the elevator managers. A report was generated for each elevator, and a bin diagram showed which bins were at low, moderate, or high risk (Fig. 2). Because grain managers were accustomed to working with bin diagrams, the output was intuitive to them. SGA Pro did an excellent job in predicting which bins were at risk for high insect densities. In Kansas elevators, the software correctly predicted that bins were either "safe" or at "high risk" in 285 out of 399 bins. It failed to

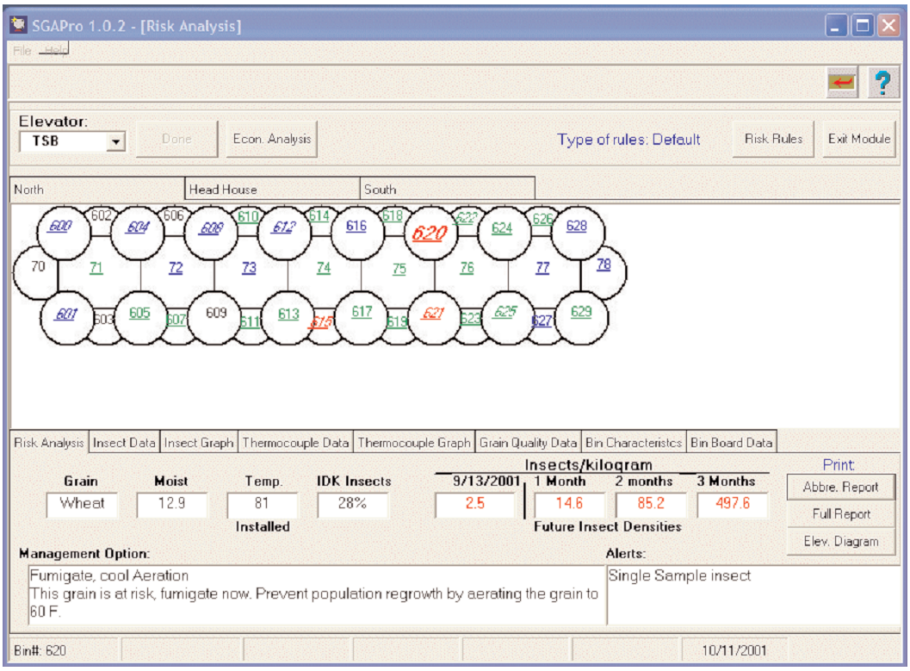

Figure 2. *SGA Pro computer screen shot: bin numbers in green, blue and red are predicted to be at low, moderate, and high risk for insect damage. In this figure, bin numbers in light grey are at low risk, bins 620, 621 and 615 are at high risk, and the rest are at moderate risk. In this example, the user has clicked on bin 620, and its data and recommendation are shown in the lower part of the screen.*

predict unsafe insect densities in only two bins (0.5%) and the insects in these isolated instances were mostly near the surface, suggesting recent immigration. That left 112 bins for which the software predicted moderate risk, and these bins did not have high insect densities when they were sampled six weeks later. Because the software recommended that the manager "consider fumigation and resample six weeks", it was not felt that this was a major concern, and that it was better to err on the side of caution then to not recommend treatment when it was necessary. All of the bins that the software indicated as being at high risk turned out to have insect densities greater than the threshold at the next sampling interval.

In Oklahoma, the SGA Pro correctly predicted bins that were safe or at high risk in 107 out of 133 total bins. All of the bins that the software indicated as being safe turned out to have insect densities below the thresholds six weeks later. Elevators that followed SGA Pro's recommendations reduced the number of bins fumigated by at least 50%.

3.4. Current Status of SGA Pro

Currently, a private company is using SGA Pro and the sampling methods developed in this project to provide services for over 30 elevators in several states. The company is in its third year of business and continues to expand. The consulting company has found that most grain handling companies in the USA are likely to have grain managers who

are conservative in their approach to pest control. However, a few early adopters of grain scouting, those who appeared to be open to major change in grain management because of unique market factors, embraced the entire information-based decision-making concept. In contrast, most clients perceive value only in parts or components of the software and services offered by the grain scouting company. Several of the scouting company's clientele purchased only grain sampling and grading services that had obvious benefits to the grain merchandiser. Some clients were initially attracted to the inexpensive grain quality information generated by SGA Pro, and only slowly began to perceive the potential benefits of the insect-sampling service. When presented with vacuum probe sampling data for their elevators, managers were often surprised at how few bins had damaging numbers of insects, and how ineffective fumigation could be. Grain managers were very interested in the spatial distribution of insects in their bins and the implication that insects are immigrating into their grain from the top of the grain bin.

To quantify the effect of the information-based approach to insect control, the incidence and density of insect-damaged wheat kernels in samples collected in the autumn of 2003, the first year of the scouting company's operation, were compared with samples collected during the same time period two years later. Data from four elevators were used in this analysis, providing 2132 data points. The frequency of samples with a high density (more than 10 per 100 g) of kernel damage was reduced by 24% (Chi square = 34.8, $P < 0.01$), and the difference in the mean density of kernel damage (2.5 per 100 grams versus 1.9 per 100 grams) was significant at $P < 0.05$.

AW-IPM will be adopted by grain elevator managers only if it is more effective and profitable than their traditional approach, and if it fits into their current marketing and grain management practices. Every effort has been made to determine how elevator managers might use insect-monitoring information to manage insect problems in their grain bins. The findings of the AW-IPM project have been communicated to managers through nine newsletters, at training programmes in Kansas, Oklahoma, Nebraska, and Minnesota, and at two recent International Grain Elevator and Processing Society annual meetings. Information gathered in this study was used to develop extension publications for stored grain integrated pest management. In addition, SGA Pro software is freely available to the public at the USDA-ARS, Grain Marketing and Production Research Center website: http://ars.usda.gov/npa/gmprc/bru/sga/. Learning to use the software is fairly easy; however, using the sampling equipment and identifying the insects does require some training.

4. Conclusions

Insect monitoring-based fumigation has several advantages over calendar-based fumigation. Treating bins only when insect densities exceed economic thresholds and treating only those bins that need to be treated can minimize the risk of economic losses from unexpected insect problems, while reducing the cost of pest management and the use of fumigant. Minimizing the use of fumigant improves worker safety by reducing exposure to phosphine, and reduces the probability that insect populations will develop resistance to phosphine. Aeration can also be used to cool grain to suppress insect population growth. Aeration should be started as soon as possible to reduce storage risk. SGA Pro software improved insect pest management by reducing the frequency of fumigation, while also maintaining grain quality. In addition, grain sampling provided unexpected benefits, such as the profiling of protein content in each bin, which allowed better grain blending. Improved cost-effectiveness of insect pest management, while maintaining grain quality, can improve the competitiveness of the grain company. Potential area-wide benefits include reducing the overall insect pressure within an elevator facility, and reducing the transfer of infested grain from small country elevators to large terminal elevators.

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Development, Validation and Use of a Simulation Model to Deliver National Predictions of Ovine Cutaneous Myiasis Risk in the British Isles

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ABSTRACT Forecasting systems represent an important approach to the sustainable control of a wide variety of insect pests and parasites. A computer model that simulates the seasonal pattern of ovine cutaneous myiasis (strike), by the blowfly *Lucilia sericata* (Meigen) was developed. The model is based on two sub-components. The first simulates the seasonal pattern of abundance of the insect pest, *L. sericata*, using quantified temperature-dependent development, mortality and oviposition rates. The second uses the range of key factors known to increase the susceptibility of ewes and lambs, to estimate the proportion of a flock at risk from strike. The two components are integrated to estimate a predicted strike rate per day. Key drivers in the model are the seasonal patterns of temperature and rainfall. However, parasitic nematode burdens and husbandry practices such as shearing and lambing dates, which are known to affect strike incidence, are also included. To validate the model, it was parameterized using average regional climate data and averaged regional patterns of lambing, shearing and insecticidal treatment. Its predictions were then compared with patterns of lamb and ewe strikes recorded over a year on 370 farms. The results showed that the model was able to account for the start of seasonal blowfly strike for both ewes and lambs and to explain a significant percentage of the variance in lamb strike incidence. To provide a national strike-risk warning system for the British Isles, a web site "strikewise.com" was established and run for three years. The web site carried basic information about strike and a regionalized map of England, Wales and Scotland. At the start of each month, the model was run using the accumulated weather data for each region and an expected strike incidence risk forecast generated and displayed graphically on the web site. Regular news releases in the sheep farming press were used to highlight the web site predictions.

KEY WORDS blowfly, *Lucilia sericata*, disease incidence, forecast, model, ovine cutaneous myiasis, simulation, weather, web site, strike

1. Introduction

Computer models are powerful practical and research tools, which can help to explain and predict the incidence of parasitic disease and improve the efficiency of control strategies. They allow the relative importance of the various factors, which determine the observed pattern of ectoparasite abundance and disease incidence, to be assessed. They also allow deficiencies in the available knowledge to be identified, enabling experimental work to be directed more appropriately.

Many existing models assume that there will be a close relationship between parasite abundance and the incidence of disease; they model the changes in parasite abundance alone and do not consider, explicitly, the role of host susceptibility in determining patterns of infection and disease. In reality, the degree of host susceptibility may be variable and may be influenced by a range of exogenous and endogenous factors causing it to fluctuate over time. Hence, the relationship between

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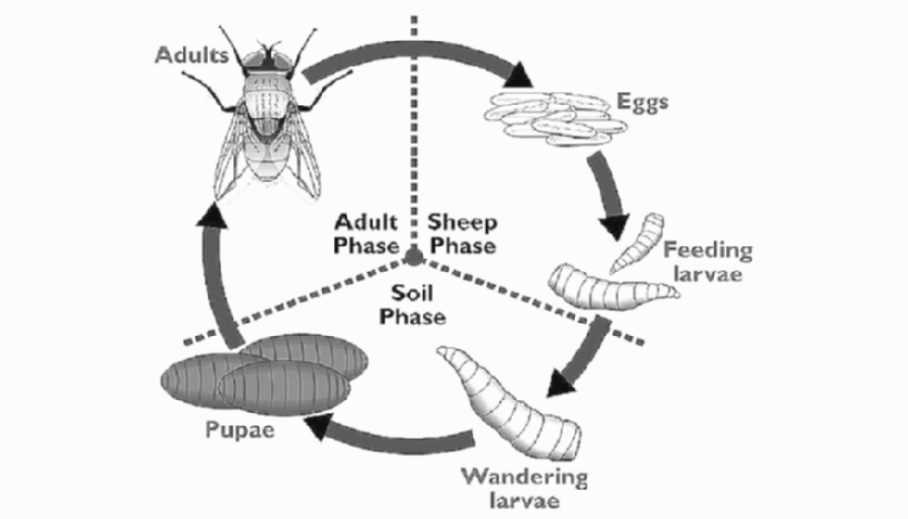

Figure 1. *Schematic representation of the sheep blowfly life cycle.*

parasite abundance and disease incidence may be complex. As a result, models which aim to predict or explain disease incidence for such parasite systems need to take into account both the dynamics of the parasite and changes in host susceptibility, if they are to work effectively. The development of such models, therefore, may be complex and require a detailed knowledge of the dynamics of the parasite and the forces that drive its patterns of abundance, in addition to an understanding of the husbandry of the host and the host-parasite interactions.

Ovine cutaneous myiasis is a familiar and widespread disease of sheep in many areas of the world (Hall and Wall 1995, Colebrook and Wall 2004). The primary agent of sheep strike throughout most of northern Europe is the blowfly *Lucilia sericata* (Meigen). A model which simulates the seasonal pattern of sheep myiasis on farms in Great Britain was developed (Wall et al. 2002). This model and its uses are described briefly here.

In the development of this blowfly strike model, a simulation approach was adopted; simulations are often of more practical use than analytical models since they allow predictions about the properties of particular systems to be made and allow computer-based experiments of control strategies. Simulation models of biological systems may, however, become complex relatively quickly and their construction does require detailed life history information.

2. Model Construction

The model is based on two sub-components. The first simulates the seasonal pattern of abundance of *L. sericata*. The second uses the range of key factors known to influence ewe and lamb susceptibility to estimate the proportion of a flock at risk from strike. The primary factors that determine susceptibility are considered to be presence of faecal material adhering to the wool, particularly around the anal and perineal regions (soiling), and the humidity within the fleece (French et al. 1994a).

2.1. Blowfly Component

Adult female *L. sericata* lay eggs in the wool of sheep close to the skin surface (Fig. 1). After hatching, the maggots pass through three stages, feeding on the epidermal tissues and skin secretions. When they have completed feeding, the third stage larvae drop to the

ground where they undergo a period of dispersal, before pupariating. Newly emerged adults mate, feed on protein and, after their ovaries have fully matured, females seek a suitable oviposition site on a host animal (Fig. 1).

In spring, as the temperatures increase, the model assumes that a new cohort of *L. sericata* emerges as adults. The model uses a sine wave fitted through the daily maximum and minimum temperatures to calculate the fraction of day-degree development completed each day for each life cycle stage and to calculate the daily mortality. Based on mortality rates observed in the field, adult blowflies are assumed to die at a rate of 2% per day degree (Hayes et al. 1999). Eventually, when sufficient day-degree development has been accumulated, the surviving females of this first cohort oviposit on susceptible hosts if available, laying 200 eggs each, half of which are female and half male. If insufficient oviposition sites are available, gravid females that are unable to oviposit on any one day remain gravid and oviposit on subsequent days as soon as oviposition sites become available. During the period when they are waiting for an ovipostition site, they continue to experience the same rate of mortality as other adult flies.

Egg hatch and the larval feeding stages, which occur on the sheep, are allowed three days for completion. This period is maintained as a constant since, in the protected microhabitat of the fleece, the rate of development of these stages is relatively independent of ambient temperature; survival for the larval stages on the host is set at 50% (Wall et al. 2001). Once the larvae are fully developed and have wandered off the host, once again, daily temperature is used to allow larvae and pupae to accumulate day degrees and estimate the time required to complete development prior to emergence of the next generation of adults. This continues on a daily basis throughout the year until, to simulate the effect of induced diapause, the larvae of females ovipositing towards the end of the season are assumed to enter diapause.

The model is stochastic and allows for variation between cohorts in day-degree requirements. To do this, the model uses a Monte Carlo simulation technique to assign random development rates to each life cycle stage, generated from the distribution of day-degree requirements described in empirical studies. In each simulation run, a function value for the number of day degrees required to complete each life cycle stage is generated from the specified distribution. The model is then run a specified number of times and the outputs are averaged across runs.

The model allows for some immigration and recruitment from carrion breeding sites since blowfly populations are unlikely to be completely isolated.

2.2. Sheep Susceptibility Component

Parasitic nematode burdens in sheep are an important cause of diarrhoea and scouring (Fig. 2). It is assumed that diarrhoea increases the probability that the wool will become contaminated with faeces, which in turn increases susceptibility to fly-strike (French et al. 1994a). The model assumes that faecal consistency is directly proportional to the level of parasitic nematode infestation in both the ewes and lambs. Patterns of faecal consistency used in the model are based on the seasonal pattern of nematode egg counts per gram of both ewe and lamb faeces from untreated animals maintained in contaminated pastures.

In the model, wool length is allowed to contribute to the levels of faecal contamination around the breech area, with longer fleece lengths increasing the probability that faeces will adhere to and contaminate the wool (Fig. 2). The seasonal pattern of lamb and ewe wool length is determined primarily by the date of lamb birth and the date of ewe shearing, respectively.

A further important contributor to susceptibility to strike is the humidity of the fleece (Fig. 2). In the model, two determinants of fleece humidity are considered: fleece length and rainfall. The model assumes that there is a direct relationship between wool length and baseline fleece humidity. However, humidity

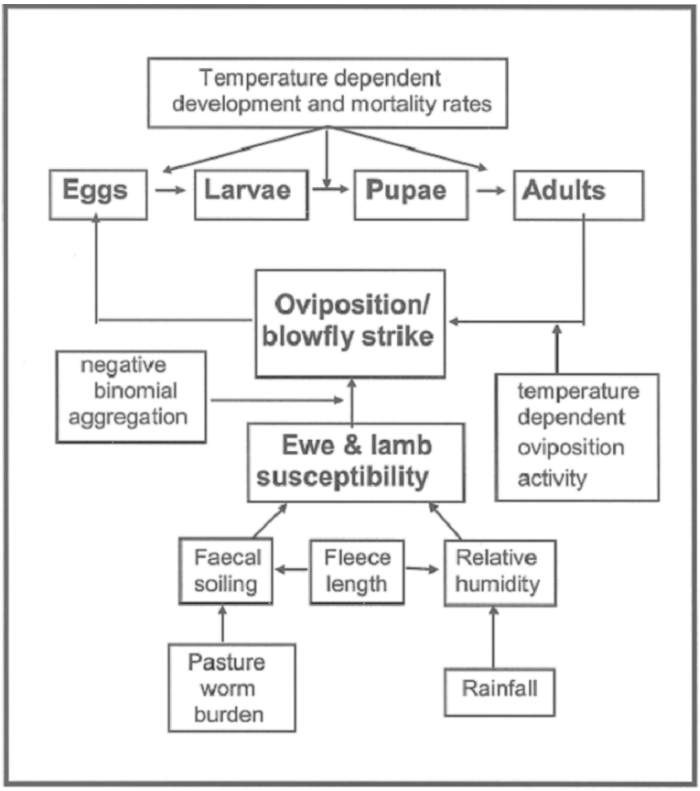

Figure 2. *Schematic representation of the sheep strike model.*

is also dependent on wetting due to rainfall, which is also affected by wool length. Hence on any one day, given the observed rainfall, an overall fleece humidity index is calculated as the baseline humidity, plus the humidity due to that day's rainfall, plus the residual humidity due to rain on the previous day, all of which depend on wool length.

A logistic regression equation is used to relate the effects of faecal soiling and wool humidity to the prevalence of susceptibility of ewes and lambs to strike (French et al. 1994a).

2.3. Calculation of Strike Incidence

In the model, the blowfly and sheep susceptibility components are brought together (Fig. 2). Each day, the fly component calculates the number of gravid females present while the susceptibility component estimates the number of susceptible ewes and lambs available. Individual ovipositions are assigned to ewes and lambs proportionately, according to the ratio of the number of susceptible sheep of that category to the total number of sheep in the flock. Hence, if there are no susceptible lambs, all ovipositions will occur on ewes; if there are no susceptible ewes, all ovipositions will occur on lambs. The model uses a negative binomial to determine the distribution of ovipositions among the susceptible animals (Fenton et al. 1999).

The model starts with a specified number of ewes and, from a given lambing date, an initial number of lambs. Lambs are weaned at a specified number of days after birth and then are removed at a specified rate, reaching zero on the last date of lamb sales. The date on

which ewes are sheared can also be specified in the model and normal variation in shearing date can be introduced to simulate the time on one farm, or a large number of farms in a region, over which shearing might occur.

In the model, the maximum number of ovipositions that any animal can receive on any given day can be specified. If the number of egg batches that an animal receives on any one day is equal to or greater than this value, the model assumes that the animal either will receive insecticidal treatment which kills off all eggs or larvae on that sheep, or that the animal will die. If the animal dies, it is removed from the host population. If the animal is treated, the model estimates the rate of decline in protection offered by the insecticide over time in days.

In addition to the treatment of individual struck animals, the model can also allow the effects of one or multiple insecticide treatments applied to all or specific classes of sheep, to be assessed as required (French et al. 1994b). To simulate the effects of regional patterns of treatment by farmers, the model can also incorporate measures of variance in the time of each treatment.

3. Model Validation

To validate the model, strike incidence data were obtained from a longitudinal questionnaire survey of sheep farmers in England and Wales carried out in 1991 (French et al. 1992). In this study, farmers had recorded cases of blowfly strike as they occurred throughout the year, as well as the size of their flocks, the type of animals struck, the dates of shearing, the dates of treatment and the mortality levels of struck animals. Only data from 101 farms in south-western and 87 farms in south-eastern England are presented here. From these data, the number of struck ewes and lambs per 10 000 animals per week and the mean dates of shearing, insecticide treatment and flock size were calculated.

The model was run from the 1st of January (day 1) with an initial spring ratio of one adult female *L. sericata* to ten ewes. An initial recruitment by immigration was set to one fly for the first spring generation and this was allowed to double in each subsequent blowfly generation (Smith and Wall 1998). The maximum number of oviposition sites on any one animal was set at five. If animals received five or more strikes, 2% were assumed to die as a result and 98% were assumed to be identified and treated (Wall et al. 2002). The parameter of aggregation, k, was entered as 0.01 (Fenton et al. 1999). Lambs were assumed to be born on day 60, the beginning of March, and weaned on day 122, the 1st of May. Lamb sales were not considered in the present study, since the data is presented as strikes per standard-sized host cohort. Averaged predictions were calculated from 50 runs of the stochastic model. For this validation, weather data was obtained from a weather station in each region (North Wyke, Devon, and Royston, Hertfordshire). For each region, data were analysed by linear regression of the log10 predictions of the model as the dependent variable against the log10 observed strike incidence as the independent variable.

In the south-western region, the first ewe strikes were observed in week 18 (Fig. 3, upper graph). Ewe strikes then rose to peak in week 22. They subsequently remained at a relatively constant level of about ten per 10 000 animals per week, before declining at the end of the season; the last strike was recorded in week 44. The first lamb strikes occurred in week 19 (Fig. 3, lower graph). The incidence of strike then increased gradually to peak in week 31 (early August) at about 30 cases per 10 000 animals per week. Lamb strikes then declined and the last cases were seen in week 42. For both ewes and lambs, the model predictions, indicate that the first strikes would have been expected about week 21, three weeks after they were first observed in ewes and two weeks after they first occurred in lambs. The predicted strike incidence then increases and peaks two or three weeks behind the observed data. Again for both ewes and lambs, there is a marked decline in the expected incidence of strikes between weeks 27 and 30, which is not apparent in the observed

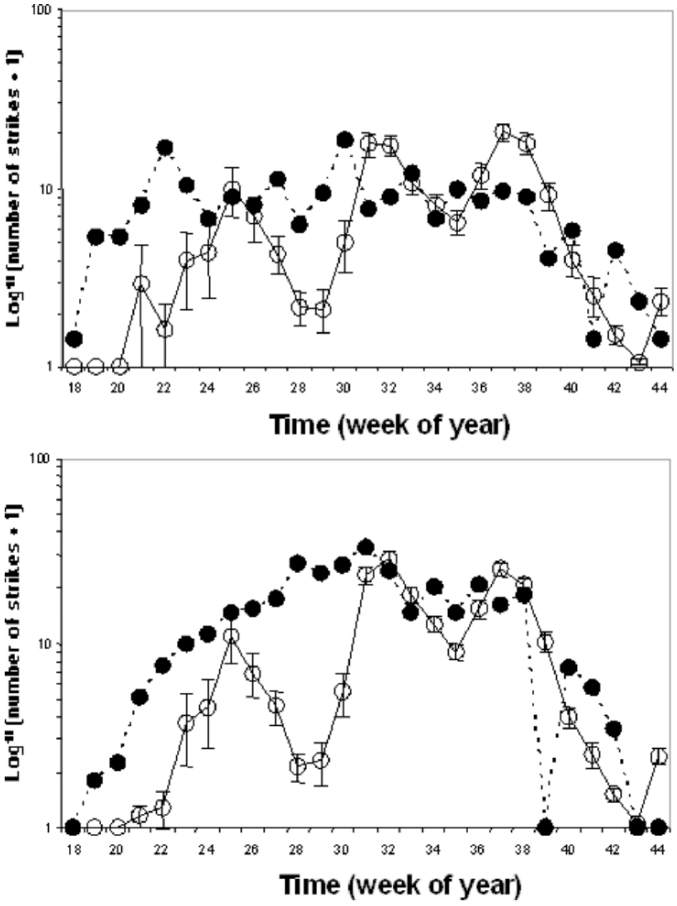

Figure 3. *The number of blowfly strikes per 10 000 hosts per week recorded in 1991 from a longitudinal questionnaire study of 101 farms in south-western England (dashed line, solid point) and the mean number (± SE) predicted by a stochastic simulation model (solid line, open point), in ewes (upper graph) and lambs (lower graph).*

strike incidence patterns. Nevertheless, after week 31 (late July-early August) there is subsequently a good fit between the observed and predicted strike patterns, until the end of the season. Overall, there are significant relationships between the predicted and observed strike incidences for ewes ($F = 6.63$, $n = 26$, $P < 0.02$, $r^2 = 20.9\%$) and lambs ($F = 16.02$, $n = 26$, $P < 0.01$, $r^2 = 39.1\%$).

In the south-eastern region, fly strikes in ewes were first observed in early May, week 18 (Fig. 4, upper graph). There were none in week 19, followed by an increase in the number of strikes in week 20. Ewe strike incidence then remained relatively stable at about ten strikes per 10 000 ewes per week throughout midsummer, although with increasing fluctuations in strike numbers between weeks 33 and 40 before strike declined at the end of the season in week 41-43 (mid October). For lambs in this region, the first strikes were recorded in week 20 (Fig. 4, lower graph). Lamb strikes then increased gradually over the summer reaching a peak between weeks 21-35

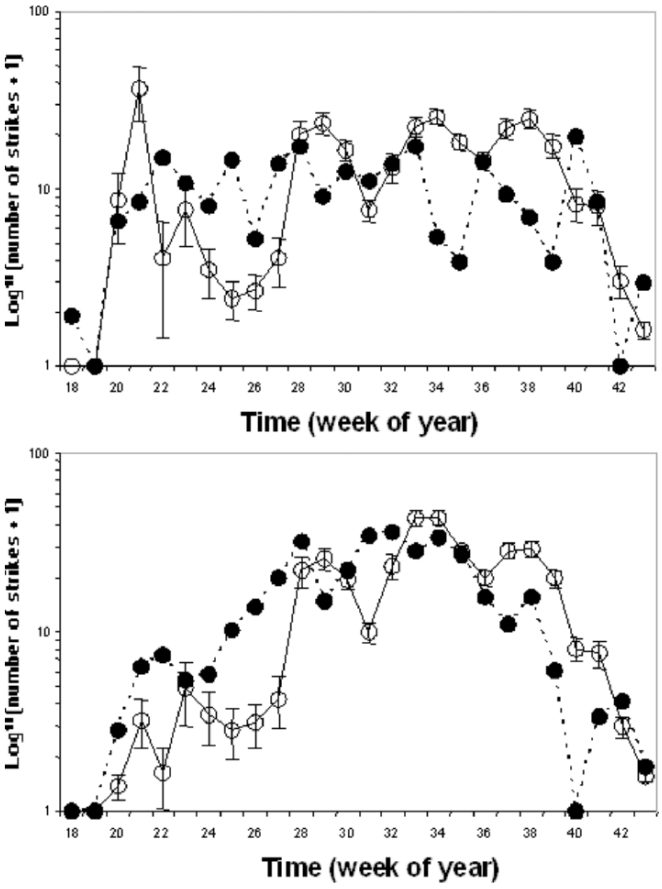

Figure 4. *The number of blowfly strikes per 10 000 hosts per week recorded in 1991 from a longitudinal questionnaire study of 87 farms in south-eastern England (dashed line, solid point) and the mean number (± SE) predicted by a stochastic simulation model (solid line, open point), in ewes (upper graph) and lambs (lower graph).*

(August) of about 30 to 35 strikes per 10 000 lambs per week, before declining with the last case recorded in week 43 (mid October). Linear regression shows that the model output is able to explain a significant percentage of the variance in the observed strike incidence for both ewes ($F = 7.83$, $n = 25$, $P < 0.01$, $r^2 = 24.6\%$) and lambs ($F = 26.74$, $n = 25$, $P < 0.01$, $r^2 = 52.7\%$). In both cases, the week of first strike (week 20) and the patterns of strike incidence and duration of the strike season are closely predicted by the model.

4. Strikewise Web Site

The model was then used to try to provide a national strike-risk warning system for the British Isles. To do this, a web site with the address "strikewise.com" was established. The web site carried basic information about strike and a regionalized map of England, Wales and Scotland (Fig. 5). At the start of each month, the model was run using accumulated weather data for each region and an expected strike incidence risk forecast was

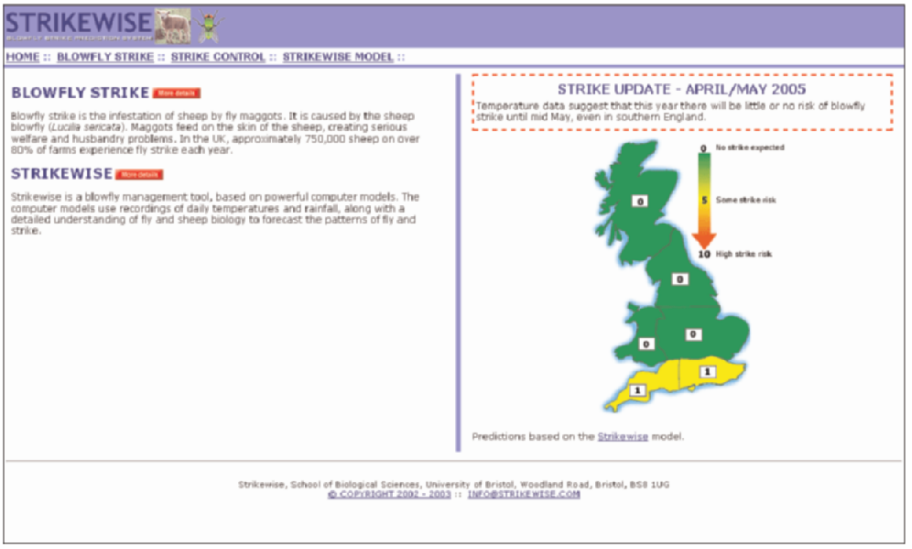

Figure 5. *The front page of the " Strikewise" web site.*

generated and displayed graphically on the web site. Weather data were transcribed from a range of open-access web sites. Regular news releases in the sheep farming press were used to highlight the web site predictions. The site remained operational for three years.

5. Discussion – Practical Implications

At a practical level, the model developed appears to be sufficiently accurate to provide useful information about the likely efficacy of new control techniques on strike incidence and to give advance warning to farmers of approaching strike problems. However, as with all climate-based models, the ability to forecast strike patterns into the future is dependent on the weather information that can be obtained and the accuracy with which it can be projected. In any one year, given weather data up to the end of June, for example, the model could predict what would happen in July, if the weather followed the same pattern, or followed a seasonal average. But clearly it would be unable to predict the effects of future unusual climatic events; the longer-term the forecast, the more approximate the prediction is likely to be.

A further limitation to its use is that, when applied on a regional basis, forecasts are guides to expected strike risk averages. However, the predictions are relatively crude, and for example, take no account of differences in physical factors such as altitude or husbandry factors. Farms geographically close, with a large altitude difference or very different husbandry regimes may have very different levels of strike risk. There is a need to refine the forecasts for more specific circumstances while also keeping the message it delivers simple. Individual farmers whose husbandry differs substantially from the average, for example in their lambing dates or stocking density, will find the generalized information that might be provided by the model relatively less informative for their particular farm. Only a detailed analysis, run at the individual farm level, would be accurate for that particular farmer's husbandry regime. Feedback from some farmers also suggested that they found the numerical output difficult to interpret in practical terms. In any future development, attention should be given to expressing the output as a clearer monthly incidence risk, for example as a percentage of

animals likely to be struck in the absence of treatment.

Running the model for each region and updating the web site took about two working days each month. Excluding model development costs, the costs associated with the initial set-up of the web site were approximately USD 2000 and subsequent maintenance of the registration of the domain name, host server and input from a web designer cost approximately USD 500 per year. While more frequent updates and more specific information would have made the site more useful to users and ensured more frequent revisits, it would elevate the recurrent costs associated with site maintenance.

Development of the web site was a valuable exercise in attempting to deliver national forecasts of disease risk. However, while some of the anecdotal expressions of interest from farmers, government and industry were good, improvements would be required to deliver the more specific information that is important to individual farmers more quickly, so that farmers find the web site sufficiently informative that they return to check it regularly. Nevertheless, this approach may become increasingly valuable and of interest to government, industry and funders in the future, as farmers are actively discouraged from reliance on chemical insecticides.

6. Acknowledgements

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Problems with the Management of the Golden Apple Snail *Pomacea canaliculata*: an Important Exotic Pest of Rice in Asia

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ABSTRACT The golden apple snail *Pomacea canaliculata* (Lamarck) is native to South America. It was introduced to farmers in the Philippines in the 1980s from Argentina via Taiwan, and to other countries in Asia, to increase farmers' income and enrich the protein in their diet, and also as an aquarium pet. Golden apple snail is expanding its distribution in Asia, threatening to invade Bangladesh, India, Pakistan, and also Australia. The Global Invasive Species Programme lists golden apple snail as one of the world's 100 worst invasive alien species. It has brought about economic losses to aquatic crops in the Philippines that are estimated to be up to USD 1200 million per annum without taking into account the non-crop damage to human health and natural ecosystems. It is also an environmental pest since to control this mollusc, resource-poor farmers resort to a "shot-gun approach", using toxic and non-specific agrochemicals and thereby aggravating ecosystem pollution, risking their health, and causing loss of aquatic biodiversity. The Philippine Rice Research Institute (PhilRice) focuses on (1) understanding the field ecology of the golden apple snail, and identifying "weak links" in its life cycle, and (2) using this information to manage the golden apple snail at the village level in ecologically sustainable, socially acceptable, and economically viable ways. This paper discusses how populations of this exotic pest species in transplanted irrigated lowland rice can be managed using locally available attractants during the vulnerable stage(s) of rice crop growth.

KEY WORDS invasive exotic pest species, *Pomacea canaliculata*, golden apple snail, rice, village level management

1. Introduction

The golden apple snail *Pomacea canaliculata* (Lamarck) is a freshwater mollusc native to Argentina. Its other common English names are golden miracle snail, jumbo snail, channelled apple snail, mystery snail, and South American apple snail. Some of these names have been used for more than one species of ampullariid. In South-East Asia, the common term for this species is the golden apple snail or GAS.

Golden apple snail species identification by shell morphology has limitations. This is because the ampullariid shells exhibit considerable intraspecific variation in shell colour and banding patterns. Genomic identification through DNA sequencing permits accurate and reliable discrimination and diagnosis of the true identity, geographic origins, and phylogenetic relationships of this invasive *Pomacea* species diversity through the analysis of a small segment of the genome. *P. canaliculata* and an unidentified *Pomacea* sp. were introduced to South-East Asia, aside from *Pomacea bridgesii* (Reeve) that was introduced into Sri Lanka. However, the *P. canaliculata* specimen did not cluster closely with the other species introduced into South-East Asia, and the following conclusions were arrived at: *Pomacea camena* Pain came from Venezuela, *Pomacea glauca* (L.) from Venezuela and Suriname, a species tentatively identified as *Pomacea haustrum* (Reeve) came from an introduced population in Florida, USA, and *Pomacea paludosa* (Say) is

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 257–264.

Table 1. *Global distribution of golden apple snails (*Pomacea *spp.).*

Country	Year introduced	Area affected in hectares	Cost of control in USD
Taiwan	1979	17 000 (1982) 171 425 (1986) >100 000 (annual)	8.3 million (1983) 30.9 million (1986) 1.0 million (1982-1990)
South Korea	1981	---	---
Japan	1981	63 559 (1998) 65 000 (2001) 68 000 (2002)	---
Philippines	1982	300 (1986) 426 000 (1988) > 800 000 (1996)	---
Thailand	1982	141 257 (2001)	---
China	1985	1700 (1988)	
Vietnam	1988	260 (1994) 64 623 (1996) 109 715 (1997) 50 000 (1999) 189 210 (2002) 214 519 (2003) 256 222 (2004)	---
Cambodia	1991	---	---
East Malaysia	1992	1817 (1999)	590 000
Lao PDR	1992	It appeared in 10 out of 17 provinces of the country	---
West Java, Indonesia	1995	1995 - only 12 districts were affected by the golden apple snail 1999 - 16 districts were affected	---
Hawaii, USA	1990's	---	---
South America		1993 - several hectares of wet seeded rice in Brazil were infested 1997 – the golden apple snail was found in all regions of Argentina	----

native to Florida (Cowie and Hayes 2004).

The golden apple snail is one of the 100 world's worst invasive alien species (GISD 2005). It has brought about economic losses to aquatic crops in the Philippines that are estimated to be up to USD 1200 million per annum without taking into account the non-crop damage to human health and natural ecosystems (Naylor 1996). Its invasiveness is related to its high reproductive rate and easy adaptation to harsh environmental conditions; its ability to colonize and invade diverse habitats by multiple pathways; its wide host range and voracious appetite; and the fact that there are no efficient biological control agents in its new habitat or competition with native snail

species and other native fauna (Halwart 1994).

A female golden apple snail can lay 50-500 eggs at a time, with an 80% hatchability rate, and 10-15 days of incubation. The golden apple snail has a gill and a lung-like organ enabling it to survive in or out of water. By closing its operculum and bedding in the soil, it can withstand drought for several months. It moves only when the depth of water is half or more of its shell height. Approximately twice as many females than males occur in the field, suggesting that females live longer than males (Mochida 1991, Estebenet and Cazzaniga 1992). It is a voracious nocturnal herbivore. When there is standing water in the field, it can destroy newly transplanted or direct-seeded rice. It cuts the base of young seedlings with its layered tooth (radula) and chews on the succulent, tender sheaths of rice. The damage that the golden apple snail can do to a rice crop depends on its size and density, and on the growth stage of the rice plant. Three snails in one square metre of rice can cause significant yield loss. Snails with a shell height of around 3.5 centimetres can eat up to 12 rice seedlings per day. This translates into crop losses over 50% if the snail density is high. Golden apple snails that are 20-40 millimetres long are the most destructive, regardless of the method of rice establishment (Joshi et al. 2002). The snail also feeds on a wide variety of other substrates including livestock feeds, decaying matter, animal flesh, and other crops.

This snail is a threat to human health since it is a host of the rat lungworm parasite *Angiostrongylus* (*Parastrongylus*) *cantonensis* (Chen) that causes eosinophilic meningoencephalitis (Mochida 1991). Hence, thorough cooking is needed if intended for food. Moreover, its sharp-edged empty shells can injure bare-footed farm workers (Joshi 2005a).

2. Global Distribution of the Genus *Pomacea*

Golden apple snails of the genus *Pomacea* have become one of the most important rice pests in countries where direct seeding has become more popular than transplanted rice, such as the Philippines, Thailand, and Vietnam (Wada 2004) (Table 1). In Asia, these

Figure 1. *Map of South-East Asia indicating the areas golden apple snails have invaded since 1979 and where the snail causes massive damage to rice, taro, and other aquatic plants.*

snails were first introduced to Taiwan in 1979 (Fig. 1) and it continues to expand in Asia with large rice-growing areas and water bodies of Bangladesh, India, Pakistan and Australia facing threats of golden apple snail invasion. These species and possibly several other related species, have invaded the Indo-Pacific from Hawaii to South-East Asia since 1979 and cause massive damage to rice, taro, and other aquatic plants.

3. Old Management Practices

3.1. Molluscicides

Molluscicides have been used to control golden apple snails, but they also kill non-target organisms. Consequently, in some countries molluscicides have been banned. Niclosamide, endosulfan, camellia seed cake (residue), and copper sulphate – often used to control golden apple snails in Asian countries – cannot be registered in Japan because of their negative effects on the environment (Wada 2004). Some farmers in the Philippines stopped using molluscicides because of their high costs and adverse effects on humans and animals. The chemicals that are used for the control of golden apple snails are mostly persistent, dose-cumulative organotins that create health effects such as falling out of nails, skin problems, blurring vision, and blindness (Mochida 1991).

Niclosamide 250EC is preferred over metaldehyde by rice farmers because of its "quick kill" action on the snail. Unfortunately, niclosamide 250EC is lethal to non-target beneficial water-inhabiting organisms such as frogs, fish, etc. Recently, Joshi et al. (2004) observed the detrimental effects of niclosamide 250EC applied at pre-seeding in direct-seeded rice culture. Treated seedlings had low and uneven emergence, and were stunted. Also, uneven crop establishment exposed seedlings to golden apple snail damage for a much longer time, and at all concentrations of niclosamide, underground effects on the seedlings included marked reductions in root growth and development.

Calcium cyanamide is used in Japan against golden apple snails in direct-seeded rice at 200-300 kg/ha. This is ten times higher than the rates of other chemicals used. However, it causes phytotoxicity if farmers did not apply it 7-10 days before sowing rice (Wada 2004), and it is less effective when the water temperature is lower.

Crude extracts of two botanicals, *Derris elliptica* (Wallich) Benth and *Azadiracta indica* A. Juss, have been evaluated for their effects on golden apple snails. Extracts from a number of African plants also are potential organic molluscicides. However, none of the botanical molluscicides identified has been produced commercially because syntheses of the compounds are currently not cost-effective and farmers' preference is for "instant-kill" chemicals.

3.2. Non-Chemical Methods

In 1989, the Food and Agriculture Organization of the United Nations (FAO), the International Rice Research Institute (IRRI), Visayas State College of Agriculture (now Leyte State University), and the Department of Agriculture-Philippine Rice Research Institute (DA-PhilRice) launched the strategic extension campaign. This introduced the use of non-chemical methods to control these snails, e.g. duck pasturing in rice fields after harvest, hand-picking, destroying egg clusters before final harrowing, transplanting older seedlings, and installing screens in water inlets. However, most of these practices remain untested in the rain-fed lowland, direct-seeded, and hybrid rice production environments. Moreover, farmers have observed that no single control tactic is better than the "best mix" of various management options, as each option has some constraints (IRRI 1991, Cagauan and Joshi 2003). For these and other reasons, it is important to stabilize and increase production in direct-seeded and transplanted rice systems by preventing golden apple snail damage through low-cost, technically effective, and environment-friendly options.

4. Golden Apple Snail in Direct-Seeded Rice: Possible Management Strategies

The shift from transplanted rice to direct-seeded-rice culture in Japan, the Philippines, Thailand and Vietnam has caused even greater golden apple snail problems, as control thresholds are much lower in direct-seeded rice than transplanted rice. Small snails, which are disregarded in transplanted rice, are harmful to sprouts and very young seedlings in direct seeding. These small snails are 150 times more serious in direct-sown rice fields that in transplanted fields. Good field levelling and shallow water management practices can reduce snail damage in transplanted irrigated lowland rice systems, but this practice is extremely difficult to carry out in direct-seeded and flood-prone areas. While farmers could experiment with a combination of preventive or corrective control measures, many of these are labour-intensive. For instance, missing hills (open areas created by dying rice plants) can be replanted up to four times, and could drain the physical and financial resources of rice farmers as it entails costs for additional seedlings and replanting time. The unprotected application of non-specific pesticides also does not help since snail populations recover quickly because they can avoid exposure by burying in the soil or simply crawling out of treated water onto clay clumps or standing vegetation.

There are few practices that can avoid or reduce damage in direct-seeded rice by golden apple snail. The rotary cultivator used during land preparation for tillage and soil puddling efficiently decreases snail density before rice planting. It results in 67-75% snail mortality compared with unploughed fields (Wada 2004). Draining fields for two to three weeks after seeding is the most effective way to avoid snail damage. However, there are two problems associated with this practice – the weeds and heavy rains followed by flash floods. Making ditches or ridges can enhance drainage but neither is highly successful. In such cases, pesticide application is indispensable, and a bait-type pesticide (metaldehyde) is currently used. Crop rotation, including growing an upland crop such as soybean, is a practical way to abate snail damage to the next rice crop as this reduces snail densities without pesticides.

5. New Strategies in Managing the Golden Apple Snail

5.1. Use of Attractants

For collection of golden apple snails, rice farmers use leaves of papaya, banana, and taro as attractants. However, such materials are not readily available and their excessive removal threatens plant biodiversity. Discovery of newspaper as a new snail attractant facilitates manual handpicking. Newspaper can be used in rice fields prior to crop establishment (direct-seeding/transplanting) and both types of attractant reduce further the misuse of synthetic commercial molluscicides (Joshi and Dela Cruz 2001), and encourage snail collection for food.

5.2. Vulgarone B

Recently, vulgarone B, a sesquiterpene from the plant *Artemisia douglasiana* Besser, has been shown to have molluscicidal activity against the golden apple snail (Joshi et al. 2005b). Laboratory bioassays showed that vulgarone B has molluscicidal activity at an LC_{50} value comparable to that of the commercial synthetic molluscicide metaldehyde. This corresponds to about 6.5 mg/litre of the vulgarone B and 4.4 mg/litre of the metaldehyde. In practical terms, a rice farmer using about 250 litres of water to spray one hectare will require 4.8 grams of pure vulgarone B for golden apple snail control. In addition, vulgarone B has fungicidal activity.

The potential of vulgarone B in controlling this agriculturally important mollusc species is high. Since it is not toxic to rice seedlings, it can be sprayed; it can also be put on attractant materials, or into ponds or rice paddy water. Furthermore, vulgarone B has the advantage of acting faster than metaldehyde against *P.*

canaliculata, since bioassays indicated mortality within 24 hours. Since vulgarone B is present as a major component in the essential oil of *A. douglasiana*, by steam-distillation, which is a simple and low-cost process, it can be concentrated to more than 80%. Also, since many *Artemisia* species are weeds in many parts of the world, the use of vulgarone B as an organic molluscicide could be both cost-effective and environment-friendly.

5.3. A Possible Agent for Paddy Weeding

Aside from being a pest, the golden apple snail has some profitable uses, e.g. in managing weeds in transplanted irrigated lowland rice fields, and as food for animals and humans. In transplanted irrigated lowland rice fields, the golden apple snail is a potential agent for paddy weeding. Two or three snails per square metre successfully control paddy weeds (Okuma et al. 1994a,b). Organic and some inorganic farmers in Japan, the Philippines, and South Korea grow rice without herbicides to produce organic rice by using the golden apple snail for paddy weeding. This approach is now spreading to other organic and inorganic farmers. The benefits from using the golden apple snail as a biological weeding agent far exceed those of using ducks or carp (Yusa et al. 2003). Joshi et al. (2005a) evaluated this innovation by Japanese and Korean farmers at PhilRice's Central Experimental Station using large-sized fields (each 0.5 hectare), and subsequently in several farmers' fields. When used in transplanted irrigated lowland rice systems, the golden apple snail changes its behaviour and is converted from a pest into a beneficial organism. However, fields must be well levelled to control movements of the snail, and seedlings should be sturdy and at the 3-leafs stage (21 days old).

Nevertheless, this approach is not appropriate and cannot be recommended for direct-seeded rice since rice and weeds sprout at the same time. Moreover, it cannot be carried out in an upland environment where the golden apple snail is in the soil, or in flood-prone areas where it is difficult to control water depth. A video, which documents step-by-step how Japanese farmers deploy *P. canaliculata* for paddy weeding in transplanted rice fields is available at: http://www.openacademy.ph/old_web/elearning/goldenkohol/. In addition, all available information on the golden apple snail has been compiled on a CD-ROM (Joshi et al. 2003), at http://www.applesnail.net under the "pest alert section", and at the National Biological Information Infrastructure golden apple snail page, which includes the option to download the database at the following URL: http://invasivespecies.nbii.gov/goldenapplesnail.html (Joshi 2005b).

5.4. A Palatable Food for Animals and Humans

The snails can replace meat meal or fish meal as a concentrate feed supplement for animals. Also, Mallard ducks can be pastured in rice fields after harvest so that they can feed on the golden apple snail. Duck herding and a little feed supplementation can yield up to 60-70% increase in egg production (Tacio 1987). A project to control golden apple snail infestations by turning the snails into a marketable processed product was implemented in Hawaii (Tamaru et al. 2004). In one field, the snails were given different types of food (lettuce and chicken, chicken feed, catfish feed, trout feed, or mahimahi feed), while in another field, they were fed taro tops, catfish feed, or trout and chicken feed. The taste and texture of the golden apple snail were tested using a "taste test" in Princeville Resort, Hanalei, Kauai, which is a four-star establishment. Using the snails from the feeding trials, the chef of the resort was asked to prepare dishes at his discretion; two dishes were created. It was found that snails fed with catfish feed were superior to all other snails in both taste and texture. In the Philippines, PhilRice developed a recipe called "chicharon" (cracker) which is unique compared with other golden apple snail recipes in that it is devoid of water, has no offensive odour, has a longer shelf-life, and can be used as an ingredient in

other Filipino recipes.

6. Conclusions

Once established, the golden apple snail is not easy to control. The economic, health, and environmental problems it causes are irreversible, and the cost of dealing with it is enormous. Old management practices to manage the snail are labour-intensive, uneconomic and non-sustainable, and many are harmful to the environment. However, new options are now being introduced, and these measures are environment-friendly and cost effective. They include using the golden apple snail for paddy weeding and as food for humans and animals. An area-wide approach that integrates the above described systems with existing farmer-based pest management practices, especially in direct-seeded rice systems where managing the golden apple snail is still difficult, could be a promising new direction to control this invasive exotic pest in the future.

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Mass-Rearing and Field Performance of Irradiated Carob Moth *Ectomyelois ceratoniae* in Tunisia

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ABSTRACT An artificial diet for rearing the carob moth *Ectomyelois ceratoniae* Zeller was developed composed of wheat bran, yeast, sucrose, salt mixture, vitamin C, aureomycine, methyl paraben, lysine, glycerine and distilled water. Carob moths, mass-reared on this artificial diet showed a similar performance to moths reared on this diet as single pairs with respect to larval developmental time, percent adult emergence, adult weight, longevity, percentage egg hatch and sex ratio. However, fecundity and fertility of adults reared on the artificial diet was significantly lower than single-pair reared moths. In addition, an assessment was made of the propensity of male carob moths to respond to the female pheromone during field cage studies using non-irradiated and irradiated males in the presence of virgin non-irradiated females. The data indicated that irradiated males responded equally well to the virgin females as untreated males, irrespective of the male ratio. The dispersal of partially sterile carob moth males was assessed in a pomegranate orchard using mark-release-recapture tests. A similar dispersal between irradiated and normal males was observed for distances between 40 to 80 metres from the release point. Significant differences between dispersal of irradiated versus untreated males were obtained for distances exceeding 100 metres from the release point.

KEY WORDS sterile insect technique, artificial diet, rearing, inherited sterility, dispersal, mark-release-recapture, pomegranate, *Ectomyelois ceratoniae,* carob moth, Tunisia

1. Introduction

The carob moth *Ectomyelois ceratoniae* Zeller is a major insect pest of dates, pomegranate and several other host plants in Tunisia (Dhouibi 1989). Larvae are polyphagous and attack both stored products and field crops in the Mediterranean basin and countries in the Near East region (Gothilf 1969). This pest causes great economic losses and yearly infestation rates range from 20% in dates to 80% in pomegranate in Tunisia (Dhouibi 1982, 1992) (Fig. 1a).

Many control methods have been used to keep populations below economic threshold levels. Controlling the carob moth with insecticides is not efficient because larvae feed and develop inside the fruit, where they are protected (Dhouibi 1989). Moreover, the harmful effect of broad-spectrum insecticides on the environment and the risk of developing insecticide resistance further restrict the use of this control method. Furthermore, oases are fragile ecosystems and since the 1970's, the use of broad-spectrum insecticides has been banned by the Government of Tunisia for dates intended for export. There is therefore a great need to develop alternative control methods, which are both effective and friendly to the environment. These include sanitation, bagging, spraying with *Bacillus thuringiensis* (Berliner) (*Bt*) and the release of natural ene-

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mies. Dhouibi (1992) showed that in an oasis ecosystem, *Bt* spraying could decrease the level of carob moth infestation by 60% when applied against first instar larvae, i.e. before the moth larvae migrate into the dates. Bagging of date clusters decreased carob moth infestations in both oases and pomegranate orchards by 6.0 and 4.5%, respectively (Dhouibi 1982). The success of this control method depends on the timing of bagging and the material used, i.e. to be efficient, bagging should be done in July before the third annual generation of the insect (Dhouibi 1982), and Kraft paper, mosquito netting or plastic films should be used. However, bagging is expensive and therefore unsuitable for large areas. It also requires that infested and fallen fruit are discarded to avoid new infestations the following year (Gothilf 1970, 1984). Finally, biological control using natural enemies provides good control of the pest. The experimental use of two parasites *Habrobracon hebetor* (Say) in pomegranate orchards and *Phanerotoma flavitestacea* (Kohl) in date oases lead to a high level of parasitism (Jemmazi 1994, Charni 1995) and a 40% reduction in infestation level.

In 1999, research was initiated to develop the sterile insect technique (SIT) as a component of area-wide integrated pest management (AW-IPM) for the control of carob moth in Tunisia (Dhouibi and Abderahmane 2001, Abderahmane 2002, Mediouni 2005). The research programme initially focused on improving the mass-rearing of the carob moth, with special emphasis on the development of an artificial diet. In this respect, rearing techniques that had been developed in Canada for the codling moth *Cydia pomonella* (L.) (Proverbs and Logan 1970) were adapted for carob moth rearing. To improve the quality and production of mass-reared insects, various modifications were made to the mass-rearing system and to the artificial diet. An experimental population was established in the laboratory of entomology at the Institut National Agronomique de la Tunisie in Tunis in 1999 from infested field-collected dates.

Studies were initiated to assess the field performance of irradiated male moths. Using field cages, the response of irradiated and non-irradiated carob moth males to the female pheromone was assessed (Robinson and Proverbs 1975, Abderahmane 2002). In addition, the dispersal of released irradiated males was studied using mark-release-recapture tests (Weissling and Knight 1994, Bloem et al. 1998). These field assessments were done over a period of three years (Mediouni 2005). This paper reports the progress made in Tunisia during the last two years with mass-rearing of the carob moth and in assessing the performance of irradiated substerile males in the field.

Table 1. *Composition of carob moth diet: quantities per 1000 grams of diet.*

Ingredient	Weight (grams)
Wheat bran	600.0
Sucrose	120.0
Yeast	23.0
Salt mixture	20.0
Vitamin C	6.7
Aureomycin	6.7
Methyl paraben	1.3
Lysine	3.0
Glycerine	150 millilitres
Distilled water	250 millilitres
Calco Red dye	41 millilitres

2. Mass-Rearing the Carob Moth

2.1. Diet Composition

Research on the development of an artificial diet for rearing the carob moth was initiated many years ago (Gothilf 1969, Dhouibi 1989, Abderahmane 1997). Several formulae based on leguminous seeds such as soybeans, beans, pods of carob and acacia, or on cereals like wheat germ or bran, have been tested. These diets were abandoned because they were costly and prone to bacterial and fungal attacks.

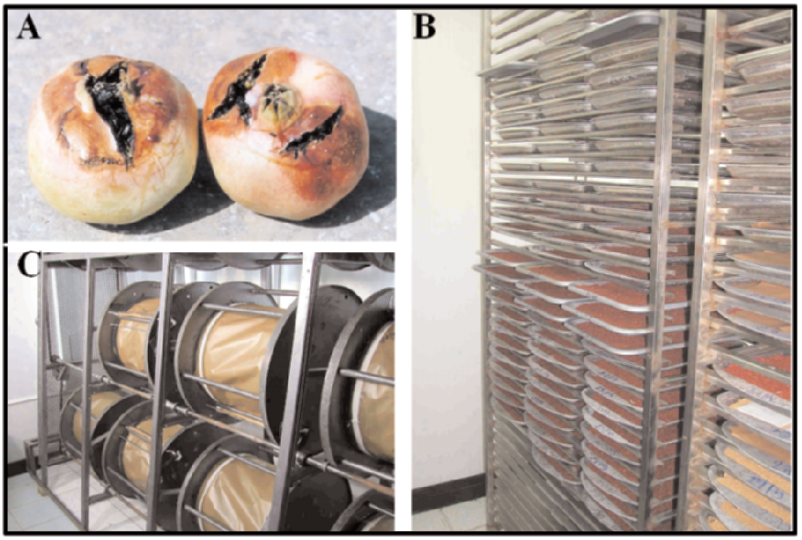

Figure 1. *(a) Damage in pomegranate caused by larvae of the carob moth, (b) larval diet trays stacked in trolleys, and (c) round, slowly rotating oviposition cages (Photos b and c from M.J.B. Vreysen, reproduced with permission).*

Therefore, using the work of Fenny and Brinkman (1967) and Abderahmane (2002), a new artificial diet was developed for mass-rearing the carob moth with wheat bran, sucrose, glycerine and water as major components (Table 1).

2.2. Diet Preparation

The wheat bran was first sterilized at 120°C for two hours. After that, all solid ingredients (Table 1) were weighed and mixed until a homogenous consistency was reached. Glycerine and water were then added followed by Calco Red dye to stain the digestive tract of the larvae and thereby distinguish between wild and released insects. The Calco Red dye was prepared by dissolving 5 grams of Calco Red powder in 80 millilitres of oil and heated to boiling point under continuous agitation. The resulting red suspension was then mixed with the diet. To avoid bacterial and fungal contamination, methyl paraben and aureomycin were added to the diet. Five kilograms of the artificial diet were prepared each day to seed eggs.

2.3. Rearing Procedures

The experimental rearing unit consisted of four rooms each of 9 square metres, i.e. two larval holding rooms, one oviposition room and one adult collection room. In the first step of the production process, virgin female moths were placed together with fertile male moths in locally constructed oviposition cages (dimensions 25 (diameter) x 65 (height) centimetres) for four days (Fig. 1c). The wall of the cylindrical oviposition cages consisted of removable paper sheets (Kraft paper), which were highly suitable for the deposition of eggs. The moths were provided with a sugar solution on moistened rolls of cottonwool. A series of these cages was kept in an acclimatized room (temperature of 28 ± 1°C, a photoperiod of 14:9 (L:D) and 45 ± 5% relative humidity (RH)), where they were slowly rotated on rails to ensure an equal light distribution. The system was highly efficient as shown by the good fecundity of the female moths and the random distribution of the eggs on the paper sheets. The approach is a modification of the system that was developed for

codling moth in Canada (Proverbs and Logan 1970).

After several days, when the female moths had deposited sufficient eggs, the paper sheets of the oviposition cages were removed. The sheets were then cut into equal-sized pieces, which were deposited on the trays with the larval diet at a density of 1500-2000 fertile eggs per one kilogram of diet. Up to 85 of these larval trays could be stacked in locally constructed trolleys (2 x 1.5 metres) (Fig. 1b). With an average emergence rate of 80%, it is estimated that between 160 000 and 170 000 adult moths emerged from the pupae held in each trolley. The larvae were reared under the following conditions: a temperature of 28 ± 1°C, photoperiod of 15:9 (L:D) and 75 ± 5% RH.

Dishes with pupae were moved into the collection room, which was kept at 30°C, continuous low light and without any humidity control. The room had a capacity of 819 dishes placed into seven cabinets. Emerging moths were attracted by a light source in the ceiling of the collection room and sucked into a duct system leading to a conical collector in an adjacent room, kept at 0°C. The low temperature immobilized the moths making the collection each morning easy. The collected insects were transferred to the oviposition cages at a density of 30 grams of adult moths per cage, corresponding to approximately 1500 adults per cage.

3. Performance of Carob Moths Reared on Artificial Diet

An assessment was made of various biological performance indicators of carob moths maintained under mass-rearing conditions and single-pair cultures (Tables 2 and 3). The larvae of both experimental groups were reared on the new artificial diet. For all experiments, the data were subjected to Duncan's test at $P < 0.05$ to assess significant differences between the parameters using the statistical package SPSS 10.0 for Windows.

The total development time of moths maintained under mass-rearing conditions (33.6 days) was slightly longer than that of moths maintained under single-pair cultures (34.2 days) (Table 2). These differences could mainly be attributed to the longer development time of the egg (3.1 days versus 3.0 days) and pupal stages (7.3 days versus 7.0 days) (Table 2). The first instar stage (L_1) showed the longest development time and the highest mortality rate (12.4 and 5.9% for the mass-reared and single-pair reared moths, respectively). The development time of the L_2, L_3,

Table 2. *Duration of the different development stages of* Ectomyelois ceratoniae *reared on the artificial diet under single-pair and mass-rearing conditions.*

Developmental stage	Mean duration (days): mass-rearing	Mean duration (days): single-pair rearing
Eggs	3.14 ± 0.2 b (325)	3.01 ± 0.1 a (300)
L1	5.01 ± 0.1 a (275)	4.99 ± 0.3 a (255)
L2	4.58 ± 0.8 a (241)	4.55 ± 0.9 a (240)
L3	4.56 ± 0.5 a (213)	4.54 ± 0.7 a (235)
L4	4.67 ± 0.6 a (208)	4.63 ± 0.8 a (235)
L5	4.89 ± 0.1 a (208)	4.85 ± 0.4 a (235)
Pupae	7.33 ± 0.9 b (203)	7.01 ± 0.2 a (235)
Duration	34.18 days	33.58 days

Values in parenthesis indicate the number of insects used. For the same stage, means followed by the same letter are not significantly different (Duncan's Test at $P < 0.05$).

Table 3. *Biological performance indicators of carob moth maintained under mass-rearing and single-pair rearing conditions on artificial diet.*

Biological parameter	Number of insects used	Mass-rearing	Single-pair rearing
Male pupae weight (milligrams)	1000	35.7 a	36.1 a
Female pupae weight (milligrams)	1000	41.3 a	42.2 a
Adult male weight (milligrams)	1000	16.1 a	17.2 a
Adult female weight (milligrams)	1000	24.3 a	25.2 a
Percentage of egg hatching (%)	1000	93.0 a	95.0 a
Percentage of adult emergence (%)	1000	85.0 a	85.21 a
Male longevity (days)	500	5.8 a	6.2 a
Female longevity (days)	500	8.8 a	8.6 a
Fecundity (number of eggs per female)	1000	115.6 b	182.5 a
Fertility (number of fertile eggs per female)	1000	95.9 b	140.0 a
Sex ratio	1000	1:1 a	1:1 a

Comparison is made for each biological parameter in mass-rearing and single-pair cultures. Means followed by the same letter are not statistically different by Duncan's test.

L_4 and L_5 stages were similar for the mass-reared and single-pair reared moths. The observed duration to complete the life cycle under mass-rearing and single-pair cultures resulted in a total of ten generations per year.

Results presented in Table 3 also indicate that all biological parameters of performance, with the exception of fecundity and fertility, of moths maintained under mass-rearing conditions were similar to those from single-pair cultures. Females kept under mass-rearing conditions produced significantly fewer eggs and these had a lower hatch rate as compared to single-pair rearing. The lower fertility level was most likely correlated to the lack of space necessary for the copulation process. Indeed, Dhouibi (1989) observed that carob moth copulation required adequate space for males to be able to fertilize females. In addition, differences were observed between the biological performance indicators of the two sexes, i.e. the weights of female pupae and adults were higher than those of males and females survived longer than males. However, the sex ratios of moths reared under the different rearing systems was similar.

These initial data on the performance of carob moths reared under mass-rearing conditions and maintained on the wheat bran-based artificial diet are encouraging, with most of the performances similar to those of insects reared as single-pairs. Nevertheless, more research is needed to further increase the fertility and fecundity of the moths to make the mass-rearing of carob moth more efficient.

4. Field Performance of Substerile Carob Moth Males

Male field dispersal was studied in a five hectare pomegranate field using 48 Delta traps (Biological Control System Ltd. Treforest, Mid Glamogan. CF37 5SU U.K.), baited with the synthetic female carob moth pheromone (Dhouibi 1989) and deployed in trees at a height of about 1.5 metres. Dispersal trial releases were undertaken from July to September in 2001, 2003 and 2004 using approximately 1000 irradiated and non-irradiated males per hectare per week. Male moths were treated with a gamma radiation dose of 400 Gy, which is the minimum required to induce complete sterility in female and substerility in male carob moths. The radiation

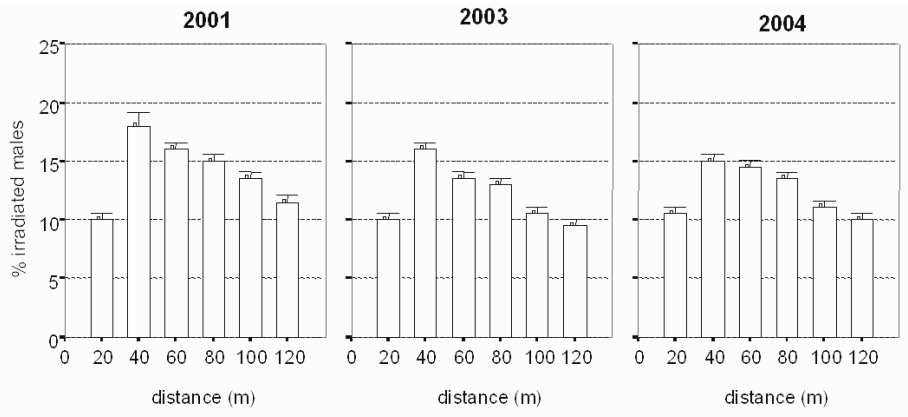

Figure 2. *Field dispersal of irradiated carob moth males in an pomegranate orchard in 2001, 2003 and 2004.*

treatment was given in a ^{60}Co irradiator at a dose rate of 46 Gy per minute.

From a release point chosen in the pomegranate field, six distances (20, 40, 60, 80, 100 and 120 metres from the release point), were selected for male capture using eight traps at each distance in a cross layout. All traps were checked once a week and total numbers of irradiated and non-irradiated moths were counted. Released insects were distinguished from wild ones by their red-marked digestive tract. The dispersal experiments using irradiated and non-irradiated males were conduced in the same area but at different times. The dispersal of irradiated and non-irradiated males was compared at each distance using the SPSS program (version 10.0) (Duncan's Test at $P < 0.05$).

In addition, two large field cages (3 metres (diameter) x 2.5 metres (height)) covered with a mosquito net were placed over two medium-sized trees to study male field competitiveness. In this experiment, the propensity of males to respond to female pheromone (expressed by the number of males caught) was evaluated. In each cage, a Delta trap baited with two virgin non-irradiated females was deployed. Four ratios of irradiated to untreated males were investigated: 1:1, 2.5:1, 5:1 and 10:1. Experiments were carried out from April to July each year and each ratio was replicated five times. For each replicate, the number of captured males was counted three days after the release of irradiated and non-irradiated males into the cages. Comparisons were made between the percentages of captures using the SPSS programme (version 10.0) (Duncan's Test at $P < 0.05$).

4.1. Male Dispersal

The data in Fig. 2 showed that the substerilizing dose of 400 Gy did not affect the ability of males to disperse under field conditions. Indeed, irradiated males were able to reach traps placed at 120 metres from the release point. The percentage of captured males (irradiated and non-irradiated) depended on the distance from the release point, maximum captures being obtained between 40 and 80 metres for the three years (Fig. 2 and Fig. 3). Statistical analyses showed no significant differences in captures of irradiated and non-irradiated males at distances 40, 60 and 80 metres. However, for distances of 20, 100 and 120 metres from the release point, significant differences were recorded in captures of non-irradiated and irradiated males (Duncan's Test at $P < 0.05$). These results were similar for the three years of observations (2001, 2003 and 2004).

Numerous researchers have studied the impact of gamma radiation on the dispersal of

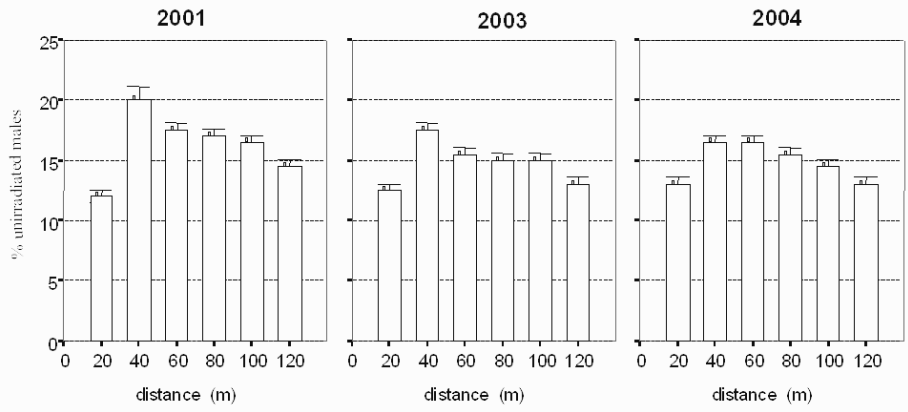

Figure 3. *Field dispersal of unirradiated carob moth males in an pomegranate orchard in 2001, 2003 and 2004.*

irradiated Lepidoptera. Qureshi et al. (1993) indicated a similar dispersal between irradiated and non-irradiated males of the pink bollworm *Pectinophora gossypiella* (Saunders). In addition, Carpenter and Gross (1993) indicated that the number of irradiated released and recaptured males of the corn earworm *Helicoverpa zea* (Boddie) was negatively correlated with the distance between the release point and the trap.

4.2. Propensity of Substerile Males to Respond to Female Pheromone in the Field

For AW-IPM programmes that integrate the SIT, assessing the field competitiveness of irradiated males and the optimal release ratio is essential to ensure that the sterile insects can compete with wild males under natural conditions and are released in appropriate numbers. The results obtained here indicate that when the ratio of irradiated to non-irradiated males increased in the cage, the irradiated to non-irradiated male ratio of the catch in the traps likewise increased (Table 4). During 2001, 24 and 26 irradiated and untreated males were recaptured, respectively at a 1:1 irradiated to non-irradiated male ratio. At this ratio, the difference between the number of captured males was not statistically significant. At other ratios, the captures of irradiated males were higher than those of non-irradiated males, due to increases in the number of irradiated males in the cage. During 2003 and 2004, similar results were obtained. Consequently, after irradiation at the substerile dose of 400 Gy, males of *E. ceratoniae* remain attracted by virgin females and were able to respond to the female pheromone.

5. Conclusions

Dates are an important and valuable export commodity for Tunisia but high infestation rates with the carob moth are causing significant economic losses. Current available control methods are either banned (broad-spectrum insecticides), being phased out (methyl bromide for postharvest fumigation) or have serious limitations (e.g. bagging of the clusters, *Bt* spraying, sanitation, etc.). The SIT could offer a viable, efficient and economic additional component of an integrated approach.

As reported in this paper, important progress has been made with the rearing of carob moth. The development of a wheat bran-based artificial larval diet, of an efficient adult oviposition cages and of an automatic adult moth collection system were critical steps in progressing towards an efficient

Table 4. *Number of irradiated and non-irradiated males of* Ectomyelois ceratoniae *responding to female pheromone under field cage conditions during 2001, 2003 and 2004.*

Ratio IM:NM	Insects per cage	2001	2003	2004
1:1 (5x)[1]	10:10	24/26 a	26/24 a	23/27 a
2.5:1 (5x)	25:10	88/37 b	90/35 b	92/33 b
5:1 (5x)	50:10	221/29 b	218/32 b	225/25 b
10:1 (5x)	100:10	490/10 b	486/14 b	491/9 b

IM: Irradiated males, NM: Non-irradiated males

[1]Values in parenthesis are the number of replicates

Catch ratios of irradiated to non-irradiated males are shown for each year. Comparisons were made between these ratios. Ratios in the same row accompanied by the same letter are not statistically different by Duncan's Test.

mass-rearing system. The fecundity and fertility of the moths maintained under mass-rearing conditions is still inferior to moths reared as single-pair cultures and therefore more work is needed to further improve the performance of moths maintained under a mass-rearing system.

A dose of 400 Gy was needed to obtain full female sterility and substerile male moths. This dose is indicative of the high level of resistance of the carob moth to radiation. However, experiments have shown that despite the high dose, irradiated carob moths responded well to the female sex pheromone, dispersing well up to 120 metres from the release point and showing a similar dispersal pattern to that of non-irradiated moths. Further research will focus on assessing the competitiveness of the irradiated moths under field conditions.

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Autodissemination of Semiochemicals and Pesticides: a New Concept Compatible with the Sterile Insect Technique

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ABSTRACT ExoSex technology utilizes inert particles of materials that have the ability to adhere to the arthropod cuticle. The ExoSex Autoconfusion™ system has been developed as an insect control method that differs from all other mating disruption systems in contaminating the target pest with electrostatically chargeable powder formulated with pheromone. The technique has now been evaluated in the field against a range of lepidopteran species, including the codling moth *Cydia pomonella* (L.), the grape berry moth *Lobesia botrana* Denis and Schiffermueller, and the Asiatic rice borer *Chilo suppressalis* Walker. Research into the mechanisms of autoconfusion shows that habituation, inhibition of courtship and delay in mating are important mechanisms in confusion and population reduction. The ExoLure™ system utilizes adhesive particles as carriers for synthetic or biological pesticides and can be used as a lure-and-kill technique for insect pest control. Ways are suggested in which Exosect technology can be integrated with the sterile insect technique (SIT) to provide new powerful hybrid techniques for area-wide insect pest control.

KEY WORDS autodissemination, mating disruption, autoconfusion, electrostatic powder

1. Introduction

The use of charged particle technology (UK patent application No. EP0650322) was tested in preliminary trials against the codling moth *Cydia pomonella* (L.) and tephritid fruit flies using a pheromone lure to attract insects into dispensers and electrostatically charged powder formulated with slow-acting insecticide as a killing agent (Howse and Underwood 2000). The latter was spread by contact to non-contaminated insects. Having proven the potential of the technique (Chandler 2004a,b, Chandler and Howse 2005, Armsworth et al. 2006), regulatory approval has been granted for use against certain species in the USA and UK, and further applications are pending. This technology also suggests other applications, e.g. the dissemination of biopathogens from bait stations and, coupled with this, the use of sterile insects as carriers for semiochemicals or biopathogens.

2. Mechanism of Autoconfusion

In the proprietary powder formulation Entostat™ there are approximately 1.5 x 1010 particles per gram and the diameter particle size is in the range of 5-20 microns. Taking into account the known threshold response of *C. pomonella* to pheromone there is theoretically sufficient pheromone in one particle resting on the surface of the antenna to initiate habituation. An insect carrying approximately 1800 particles would then be liberating sufficient pheromone in the short term to constitute an attractive source for another male moth. Therefore one ExoSex

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dispenser (containing around three grams) is capable of contaminating about 21 million male moths with enough pheromone to make them attractive sources to other males. Autoconfusion is believed to be due to habituation of male responsiveness as a result of constant sensory stimulation, and males acting as false lures or mobile dispensers (Chandler 2004a). Possible components of autoconfusion in codling moth are listed below.

2.1. False Trail Following

This also occurs with conventional mating disruption, where discrete pheromone sources effectively act as female mimics thereby distracting males in their search for calling females. However, in the case of ExoSex Autoconfusion™, it is the contaminated males themselves that act as dispensers rather than discrete sustained release devices. The males are mobile, and so progressively increasing their density will greatly decrease the chances that newly arriving males will locate calling females.

2.2. Habituation

Again, this occurs in conventional mating disruption by raising the threshold for detection of aerial plumes of pheromone originating from calling females. Habituation may involve the sensory pathways or the central nervous system, or both. Thus the sensory receptors may "saturate", or the sustained input to the insect's brain may induce a long-lasting blockage of response. In ExoSex Autoconfusion™, the pheromone sources are in intimate contact with the sensory receptors on the antennae, which provide a sustained high level of sensory input. Reversal of the response (dishabituation) cannot therefore occur when the insects are in clean air outside the crop or in a windy situation.

2.3. Trail Masking

Together with habituation effects, the presence of a dense concentration of pheromone in the air will mask the individual pheromone trails from calling females. An analogy can be made with the so-called "cocktail party effect" in which a high level of background noise makes it impossible to pick out a voice a few feet away. This is, in other words, like habituation, a form of "chemical deafening". In ExoSex Autoconfusion™ this effect will be of minor significance compared with habituation, and there is therefore no requirement to release the extremely high quantities of pheromone into the environment to achieve trail masking.

2.4. Sensory Imbalance

With ExoSex Autoconfusion™, there may be a strong imbalance of sensory input due to unequal contamination of the two antennae, particularly where the amount of powder picked up is very small. This may interfere with the guidance mechanism of the male, causing it to deviate to the most heavily stimulated side.

2.5. Inhibition of Courtship

In the laboratory the percentage of females mating when confined in a small space with males is substantially reduced when the males are contaminated with Entostat™ powder (Howse and MacDonald 2005). Courtship in Lepidoptera normally involves an exchange of stimuli at close range, including release of male-produced pheromone (Howse et al 1998). This is less likely to occur. It has also been hypothesized that males releasing a high concentration of female pheromone are less sexually competent (Knight 2003).

2.6. The Role of Foliage

Suckling and Karg (2000) found that apple leaves could absorb and release sufficient pheromone from nearby dispensers to enhance mating disruption of the light brown apple moth *Epiphyas postvittana* Walker. This effect can also be seen after removal of dispensers. The same phenomenon is known to

occur in the pea moth *Cydia nigricana* (F.), where the former sites of monitoring traps remain attractive for long periods (Wall et al. 1981).

2.7. Enhancement of Predation

Mating disruption when used alone does not interfere with the action of predators and parasites. Knight (1997) reported levels of egg predation around 20% higher in orchards treated for mating disruption of codling moth, compared with orchards under conventional insecticide treatment.

2.8. Delay in Mating

Recent evidence indicates that very low levels of pheromone in the environment, may delay mating. Brunner (2003) and Knight (1997) believe that this delay in mating results from interference in mate location.

3. Relevance to the Sterile Insect Technique (SIT)

In any mating disruption technique, the main constraints are the cost of the materials and the labour-intensive process of placing dispensers in the crop. In these respects, the Exosex system is considerably more cost-effective than other techniques. In the case of codling moth, for example, the total amount of pheromone dispensed per hectare is between 80 and 200 milligrams in 25 dispensers. By comparison, most conventional techniques dispense between 45 and 192 grams/hectare, i.e. around 1000 times as much, in 400-1000 dispensers (Chandler 2004a,b).

Area-wide integrated pest control programmes using mating disruption may be made more efficient by using sterile insects as mobile dispensers of adhesive particles, carrying behaviour-modifying chemicals. When they are coated with sex pheromone, they can achieve control by mating disruption, in which they act as female mimics, outcompeting the females (or males) in the natural population that are releasing their own sex pheromones. By using sterile insects as living dispensers of pheromone, the level of mating in the natural population is reduced, and when matings do occur they are likely to be between sterile males and females of the natural population.

Sterile insects can also be released at a density sufficient to raise the concentration of the sex pheromone throughout the crop to a level high enough to achieve mating disruption by trail masking. This would then compare with the spraying of pheromone in, for example, Hercon flakes or Consep Checkmate macrocapsules for pink bollworm *Pectinophora gossypiella* (Saunders), where saturation of the environment is achieved at between 12 000-50 000 sources per hectare (Hall and Marrs 1989).

Sterile insects of one species could be used to control populations of a different pest species. For example, in a zone where both Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and codling moth are established, sterile Mediterranean fruit flies can be released coated with particles containing the codling moth sex pheromone. The Mediterranean fruit fly does not pose a threat to the crop concerned but they act, as before, as mobile dispensers of codling moth pheromone, decoying males away from females. The main advantage here is that Mediterranean fruit flies are much cheaper to mass-rear than most Lepidoptera, including codling moth.

Sterile insects could be coated with powder containing a slow-acting chemical pesticide, or spores of a fungal entomopathogen. Males and females of the natural population attempting to mate with the released flies become contaminated with the pathogen, which they pass to partners in subsequent mating attempts.

Possible advantages of the above applications: (1) environmental contamination is reduced because the active ingredients are applied to insects released into the environment and are not sprayed onto the crop; (2) the amounts of active ingredients used are much

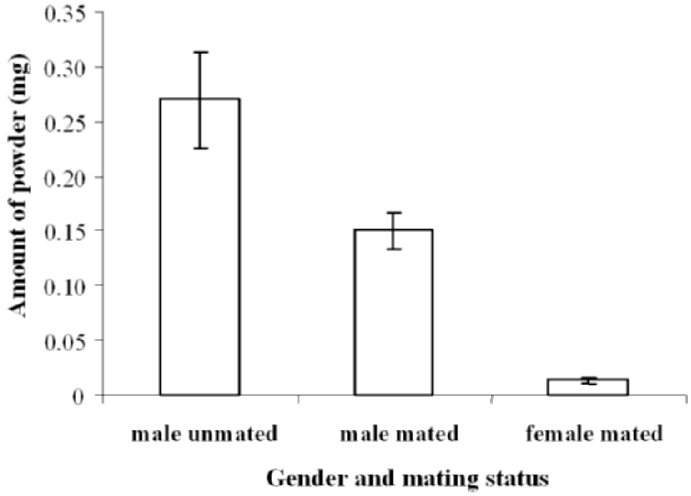

Figure 1. *Transfer of dyed adhesive particles to female Mediterranean fruit fly* Ceratitis capitata *during mating, measured using a fluorimetric assay. Ten virgin males artificially contaminated with adhesive particles were introduced to a fly cage with ten virgin females. Mating pairs were removed and the experiment terminated when five pairs had mated. Closed bars represent standard errors (n = 5).*

lower than in conventional mating disruption systems or lure-and-kill techniques; (3) as the method depends on the use of odours and visual stimuli to which the particular pest is strongly attracted, it is also highly selective, protecting, in particular, beneficial insects; (4) because sterile insects are used as carriers for insecticides or behaviour-modifying compounds they pose no risk to the build-up of pest populations: all mating result in sterile eggs; (5) the insects are biodegradable and do not need to be removed from the crop, unlike plastic traps and dispensers commonly used in insect control; (6) both sterile males and sterile females can be released (unless the females cause damage themselves); (7) quality control of sterile male competitiveness becomes less important if the main route of control is through transfer of materials between individuals. It should be borne in mind that dispersal and survival will strongly influence the efficacy of the technique; and (8) the numbers of sterile insects released per unit area can be substantially reduced while still achieving efficacy of control. This may give rise to substantial cost savings in production, although the more insects are produced in a rearing facility, the cheaper the insect becomes.

4. Lure-and-Kill Applications with Mediterranean Fruit Fly

The efficacy of a control method for the Mediterranean fruit fly (or any insect) depends on several factors: the number of particles picked up, the number transferred to another insect, the concentration of active ingredient in the particles, the rate of loss of particles from the cuticle of the secondarily contaminated insect, and (in a lure-and-kill system) the LT_{50} of the pesticide.

Entostat^{TM}, electrostatically chargeable powders are readily picked up by Mediterranean fruit flies. Entostat^{TM} was dyed with a fluorescent dye and flies were artificially contaminated with the dyed powder in glass vials. The amount of dyed powder picked up by flies was determined by retriev-

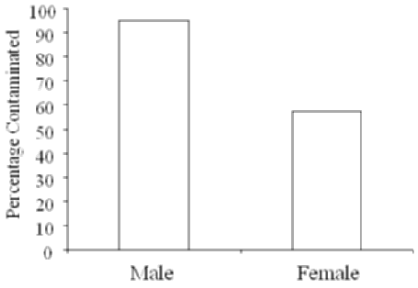

Figure 2. *Percentage of contaminated Mediterranean fruit fly* Ceratitis capitata *after five days exposure to an ExoLure™ dispenser in a field cage (Supplied courtesy of Karen Underwood).*

ing the powder in solvent and running samples in a fluorimeter and comparing the samples to a calibration curve. It was determined that Mediterranean fruit flies picked up a mean average of 130 micrograms of Entostat™ powder. This amounts to approximately 10 000 particles (P. Howse, unpublished). There is therefore little doubt that such insects can support quantities of attractants that are well above the threshold of attraction for others of the same species.

Transfer of electrostatically chargeable powders between individual Mediterranean fruit flies has been demonstrated both in the laboratory and in the field (P. Howse, K. Underwood, and C. Jackson, unpublished). In the laboratory, ten virgin males coated with dyed adhesive particles were introduced into a cage with ten virgin females. After 1 hour 40 minutes, half the flies had mated. It appears that males lost particles during mating and the mated females gained between 9.2 and 21.3 micrograms, i.e. around 10% of that retained by unmated males (Fig. 1). Similar results have been obtained with tsetse flies (*Glossina austeni* Newstead), (P. Howse and K. Underwood, unpublished). In field cage experiments carried out in Mallorca, male Mediterranean fruit flies made repeated visits to a bait station containing trimedlure and electrostatically chargeable powder, and the powder was also transferred to females (Fig. 2).

Fig. 3 shows the rate of loss of dyed adhesive particles from Mediterranean fruit flies in a controlled environment (laboratory conditions of 20-25°C and 60-80% relative humidity) assessed using a fluorimetric assay. This conforms to a pattern the authors have also observed in other Diptera of an initial loss of 50-60% of the material, followed by exponential loss over a period of from about one hour to at least one week. Most of the initial loss is due to larger particles being lost from the surface of cuticular hairs. These figures then suggest that approximately 40% of the initial amount transferred from one insect to another will remain on the secondarily contaminated insect for at least a week.

5. Conclusions

The Exosect technique can be used to coat different insect species with certain types of powder that readily adhere to the cuticle and studies are ongoing to assess the dynamics of powder pick-up and transfer between individuals. The final goal is to show that particles formulated with semiochemicals or insecticidal materials can be distributed throughout a pest population by the insects themselves. It is proposed that this technique can be extended to use sterile insects as mobile dispensers, or vectors, of semiochemicals or pesticides. Work has now begun to establish dosage and transfer rates so that the efficacy of combining Exosect technologies with the SIT can be investigated.

6. Acknowledgements

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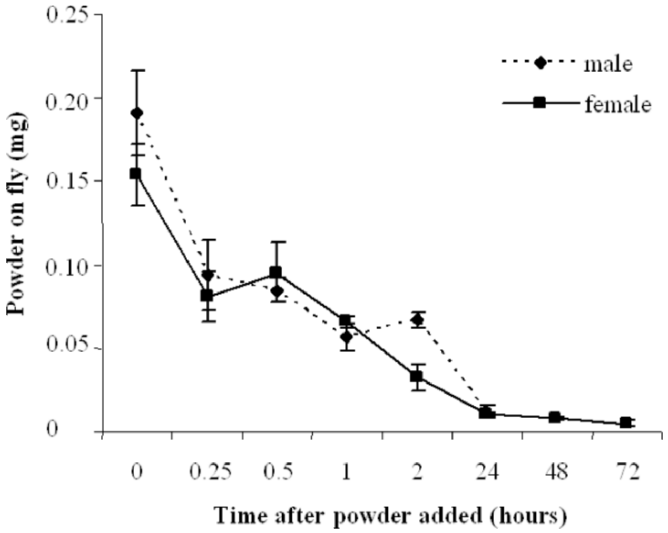

Figure 3. *Loss of dyed Entostat™ powder from artificially contaminated male and female Mediterranean fruit fly* Ceratitis capitata *over time in a laboratory fly cage, measured using a fluorimetric assay. Closed bars represent standard errors (n = 5).*

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Section 4

Feasibility Studies

Strategies to Control the Desert Locust *Schistocerca gregaria*

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ABSTRACT The desert locust *Schistocerca gregaria* (Forskål) can infest an area from Mauritania to India and roughly from the Mediterranean to the Equator. Plagues with many swarms of adults and very many bands of hoppers are separated in time by recessions, when most of the locusts are scattered and confined to the 16 million square kilometre arid central belt. Widespread and heavy rain in the recession belt may lead to outbreak breeding. This may be followed by an upsurge during which successful breeding occurs in areas to which the adults of successive generations migrate. Further population increases lead to a plague. In spite of recent studies, the cost of the damage caused by the desert locust is not known and it is not clear whether the costs of control balance the costs of the damage that is prevented. Nevertheless, the impact on individual farmers may be devastating. Local crop protection is not feasible and financial compensation is difficult. However, the main importance of the desert locust is "political" since swarms are both dramatic and migrate between countries. The current strategy is to prevent plagues by controlling the outbreak or the early upsurge, despite the lack of field evidence that this is effective, and theoretical studies that suggest it is not. Adherence to such a preventive strategy, the irregular occurrence of plagues, and the fact that even during a plague many countries will escape, has led to plague crisis management. Donors have supplied aid, usually in the form of insecticides and aircraft, during upsurges and plagues with no adequate assessment of the need or of the capacity of the recipient country to use what is supplied. Much insecticide has remained in stock after plagues, creating a difficult disposal problem. Recent research and development has concentrated on: (1) processing of survey data with geographical information systems (GIS), (2) survey and application techniques using global positioning systems (GPS) and precision spraying, (3) physiological and ecological studies focussing on phase change, (4) barrier applications with new persistent insecticides, (5) use of biopesticides, especially mycopesticides and insect growth regulators, and (6) environmental monitoring. Hope for the discovery of some novel solution to the desert locust problem should not detract attention from improving campaign organization, as this will be required with any feasible new research finding. However, priority needs are to gather more information about recession populations, evaluate control campaigns, elaborate a new strategy for outbreak prevention, develop contingency plans for a plague campaign, and provide training in the execution of such plans when the need arises.

KEY WORDS desert locust, *Schistocerca gregaria*, strategy, outbreak, upsurge, plague, prevention, insurance, contingency plan

1. Introduction

The desert locust *Schistocerca gregaria* (Forskål) can infest a huge area (29 million square kilometres) putting more than 65 countries intermittently at risk (Fig. 1). During recession periods when densities are low, the solitarious form of the desert locust generally are scattered over a broad, 16 million square kilometre, belt of arid and semi-arid land extending from the Atlantic Ocean to northwestern India and covering more than 25 countries.

The desert locust lives in an arid environment and needs a certain amount of rainfall for the environment to become favourable for breeding. Since it has no resting stage to wait out prolonged periods of drought, it has to

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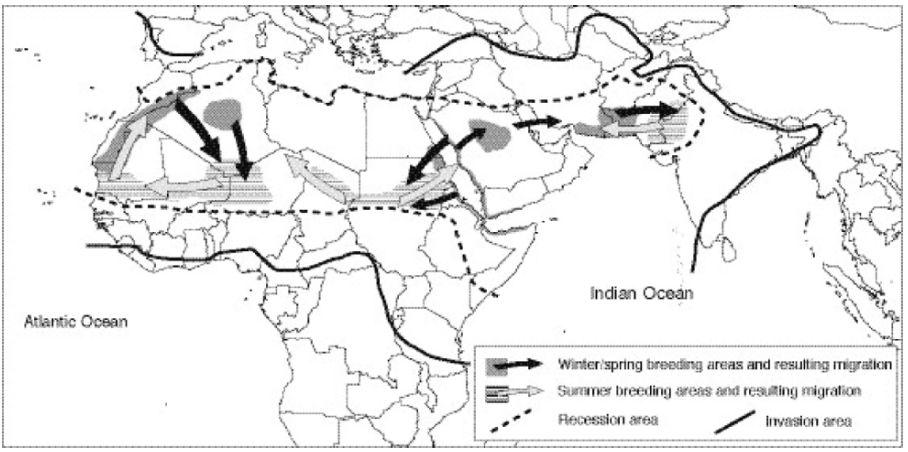

Figure 1. *Desert locust recession and invasion areas with recession migration circuits. Map modified after Symmons and Cressman (2001).*

migrate after one or sometimes two generations from areas that, were temporarily favourable for breeding but have dried out, to newly favourable areas where recent rains have fallen. During recession periods, in which overall populations are low and the locusts are in their solitarious stage, these migrations are inconspicuous.

How do plagues develop? The change from the solitarious to the fully gregarious form takes a number of generations, and progresses from local outbreaks via upsurges to plagues. Outbreaks occur locally after unusually heavy rains, with the resulting vegetation allowing successful breeding and the development of gregarious behaviour in the form of very many small "patches" of nymphs (aggregations covering an area of about ten square metres). Upsurges occur when widespread and heavy rains fall in successive generations or adjacent breeding areas. This may finally result in a plague, characterized by many adult swarms and very many nymphal bands (also called hopper bands). A plague may last between three and 22 years. The last major plague occurred in large parts of Africa from 1986 to 1989. Insecticides sufficient to treat 260 000 square kilometres in the Sahel region and North Africa were supplied during this period, at a cost of about USD 315 million (Gruys 1991). The last upsurge developed following heavy rains in most of the Sahel region in 2003 and continued in 2004.

2. Economics of Locust Control

2.1. Costs and Benefits of Locust Control

A bioeconomic simulation model developed from historical data and an expert assessment commissioned by donors concluded that the costs and benefits of locust control were of the same order of magnitude (FAO 1998). Another study concluded that control investments exceeded potentially preventable losses (Herok and Krall 1995). However, both studies were not refereed and included a large number of uncertain assumptions. The desert locust is a "political pest" in the sense that its occurrence as a plague and its resulting damage are spectacular, creating popular demands for action. Furthermore, swarms can migrate great distances making the desert locust a transnational problem. Consequently, desert locust plagues receive much attention from the press, and therefore from politicians.

Issues that need to be taken into account when deciding to manage desert locust populations include: (1) interrelations, since failure to control in one area has negative effects on

others. In fact, effective preventive control operations conducted simultaneously over the whole distribution area of the desert locust (i.e. area-wide) has never been implemented. This is because some infested areas are too remote, others are insecure and some are located in countries having desert locust control units that exist in name only. A more coordinated approach may change the above-described relationship between costs and benefits; (2) real costs, since it has been suggested that to detect and control early upsurges throughout the recession area may be too costly to be feasible (van Huis 1994); and (3) environmental and health issues arising from control campaigns, since the effect of spraying on the environment, the risk to operators and others, and the hazard associated with surplus insecticide storage, also have to be taken into account.

2.2. Insurance Schemes to Mitigate Risks?

Would the damage caused by desert locust qualify for insurance schemes in the context of disaster management? The inherently unpredictable nature of locust damage and the low overall probability of an individual farmer or village being affected, mean that crop insurance is an obvious means in principle to mitigate the risks (Hazell et al. 1986). In practice, high operating costs and premiums, especially when the infrastructure is weak (Hardeweg 2001), would render formal public or private insurance schemes impractical in the context of locusts threatening semi-arid farming systems (Anderson and Dillon 1992). Assistance towards community level informal risk mitigation (e.g. tenancy contracts or extended family networks) has better potential. The appropriateness of insurance schemes would probably also depend on the countries (Maghreb or Sahel) and the type of agriculture (cash or subsistence crops).

Hardeweg (2001) developed a conceptual framework for economic evaluation of desert locust interventions, suggesting that farmers would be willing to participate in insurance schemes. Another issue raised was whether the humanitarian aspect of people affected by locust invasions would be taken sufficiently into account (FAO 1998). Would the international donor community favour engagement in insurance schemes or food aid in preference to supporting control efforts? LeCoq (2001) and Krall (1995) found these options unrealistic and little adapted to the economic and social realities of the countries involved. Commercial insurance depends on statistical data on risk and cost; what is your house worth and how often does a house burn down? There is no basis for estimating the risk of a field being invaded by locusts, and even estimating the loss that might result would be difficult.

3. Technical Strategy to Control Locusts

3.1. Outbreak Prevention

The current official strategy is to prevent plagues by control at the local outbreak or early upsurge stage. This is superficially attractive and it seems sensible to control when numbers are relatively low. However, control is not a matter of numbers but of the area needing treatment, of the difficulty of finding the targets, of the control methods that are appropriate, and of the time available to muster and deploy resources. For example, the swarms of a plague might number perhaps twenty with a total area of about 1000 square kilometres (with 5×10^9 locusts). The "patches" of an outbreak might be distributed over very many times that area although the total number of insects would be only a small fraction of the number in a plague population.

Symmons and van Huis (1997) calculated the efficiency of controlling an outbreak in an area of 20 000 square kilometres (of which 4000 square kilometres was green) treating either blocks using vehicle-mounted ultra-low volume sprayers or individual patches using hand-held ultra low volume sprayers. Spraying patches using a vehicle is not effective since they are too small; therefore blocks

have to be identified that contain sufficient patches to make spraying efficient. Results indicated that one team with two vehicles could spray no more than 0.28 square kilometres during a day, and during a whole campaign not more than seven square kilometres. To effectively treat either all infested blocks or all patches would require more than 75 vehicles to control only 50% of the population.

During two contingency planning workshops in Egypt and Mauritania (FAO 2002a, 2004) when field monitoring was carried out with experienced survey operators, it was proven that the above-mentioned values were probably far too optimistic. It is very difficult to demarcate infested blocks and when deciding on treating each patch individually by hand-held sprayers, the problem is that these patches are very difficult to find. Magor (1999) also indicated the difficulty of finding locust infestations during early outbreaks. Besides, outbreaks in the immense recession area (Hemming et al. 1979), where the rain needed for breeding is often sporadic, appear suddenly (Roffey and Popov 1968), and are therefore often not detected. Therefore, outbreak prevention – namely the control of gregarizing locusts that might cause an outbreak – is almost certainly not possible.

3.2. Upsurge Prevention

So, what would be the appropriate control strategy when outbreak control to prevent upsurges or plagues is not possible? During outbreaks and the early stages of an upsurge only a certain proportion of the population behaves gregariously. During this stage numerous small gregarious hopper bands are scattered over a large area. During the development of an upsurge the bands and swarms become progressively larger and more cohesive, and the infested area diminishes. This can be illustrated using data from the development of the 1968 plague. Locust numbers during three generations increased from 2000 million to 30 000 million while the total area infested decreased from over 100 000 square kilometres to about 5000 square kilometres (Bennett 1976).

The crucial question is at which stage during the development of a plague a sufficient proportion of the population would be in the gregarious phase and become suitable targets for control. At which population densities and degrees of gregarization does upsurge prevention end and upsurge elimination begin? Upsurge prevention by outbreak elimination is almost certainly not feasible at reasonable costs. In practice, probably upsurge elimination is what is normally attempted, and if that fails, plague suppression. Upsurge elimination is difficult to define and probably difficult to carry out. The reason is that the populations are a mixture and the mix itself changes as the upsurge develops. At the hopper stage there may be medium-sized bands, small bands, groups, patches and scattered hoppers. Adults may be scattered, in groups, in “light flights”, in low-density swarms that may well disperse and reform, as well as in small cohesive swarms. The definition of ”prevention” is not clear and can be taken to mean different things (van Huis 1994, LeCoq 2001). It may include all control efforts up to upsurge elimination (Posamentier and Magor 1997).

3.3. Swarm or Hopper Control

During late upsurges and plagues, adult swarm spraying is much more efficient than hopper control and it has been claimed that it may be the only feasible method of achieving general population reduction (Courshee 1990, Symmons 1992). Symmons (1992) pointed out that ten square kilometres with a 5% band infestation would need to be treated to prevent one square kilometre of swarm on the probably generous assumption that one square kilometre of band would give rise to two square kilometre of swarm. Despite this, most campaigns concentrate on hopper control. This is unavoidable in the early stages of an upsurge when swarms are small and perhaps transitory. In late upsurges, swarm control is theoretically attractive. However, although swarm control is very effective, there is a risk that

swarms will escape control because of their mobility and the limited time frame available for control actions. Control of swarms requires timely action and excellent organizational and logistic capabilities (van Huis 1997).

3.4. Strategy Question Unresolved

The Food and Agriculture Organization of the United Nations (FAO) established in 1994 an Emergency Prevention System (EMPRES) for Transboundary Animal and Plant Pests and Diseases in order to minimize the risk of such emergencies developing. During a meeting of EMPRES aimed at desert locust plague prevention in the central region (those countries bordering the Red Sea), it was concluded that an optimal control strategy could not be identified, due to insufficient data, and called for the collection and analysis of data during future outbreaks and upsurges (FAO 1997). However, during the past seven years only recession populations could be studied.

An outbreak occurred in 2003 in West Africa, an upsurge was underway in 2004 and ended due to adverse climatic conditions during the spring of 2005 in the Maghreb countries. However, the impact of the upsurge control actions in 2004 has not been quantified. At times when control is needed there is an understandable demand to devote all available resources to control. Therefore, the question when to intervene remains unresolved.

4. Contribution of Research to Improve Control

In 2000 a group of desert locust experts identified the following research areas (FAO 2000): (1) population dynamics (migration monitoring, intervention threshold parameters, genetic studies to characterize populations and to understand migration), (2) mapping of gregarization biotopes, (3) changes of ecological factors in recession areas, (4) mortality factors, (5) alternatives to synthetic insecticides (mycopesticides, semiochemicals and botanicals), (6) application techniques in particular barrier treatments, (7) environmental impact assessment, and (8) economic impact assessment.

The main constraints in conducting desert locust research are the lack of a coordination body, lack of partnership between southern and northern institutes, operational locust data not being utilized, the difficulty and costs of conducting field research, and the lack of continuity of research not only in terms of the unpredictable occurrence of locust infestations but also of funds. The longer the recession period, the less local authorities and international donors are willing to support research.

4.1. Forecasting, Monitoring and Early Warning

Ground and aerial survey teams transmit their data to the national plant protection organizations (NPPOs) for collation and analysis as well as to FAO headquarters, where analysis and forecasting is carried out at the international level. In 1996, FAO introduced the Schistocerca Warning Management System (SWARMS), a geographic information system (GIS) for desert locusts. This allows storage of data in several databases, display of current data on maps consisting of various thematic overlays (e.g. rainfall, habitat conditions, locust incidence), and comparison to historical data (Cressman 1997).

In terms of monitoring and early warning, progress has been made with the development of a GIS called Reconnaissance and Management Systems for the Environment of Schistocerca (RAMSES) (Rosenberg 2000). The system is a computerized application that allows the nationally designated locust information officer to store, view and retrieve locust-related data for his/her country. The use of eLocust (a handheld computer that the locust officer uses in the field to enter data and transmit it to RAMSES) has further improved data management capability within the country. RAMSES can also display and analyse remote sensing imagery. The combination of satellite images, complementary geographical

information such as soil maps, and locust data gives information about the regions at risk and assists in planning field surveys and may facilitate an area-wide approach that eventually may reduce or prevent plague development.

4.2. Physiological and Ecological Studies

A number of physiological and ecological studies have been carried out during the last ten years to gain a better understanding of the behaviour of desert locust populations. Of particular importance were studies conducted on the causes of shifts from solitarious to gregarious behaviour. It appears that patchiness of vegetation and insufficient nutrition increases activity and crowding of locusts (Despland and Simpson 2000), and the resulting mechanical stimulation of the hind femur seems to play an important role in the phase transition (Simpson et al. 2001).

Woldewahid et al. (2004, 2006) found a strong association between the occurrence of solitary locusts and a certain plant community that covered only 5% of the area. Application of these findings to surveys will considerably improve their efficiency. Various authorities have suggested that the apparent association of outbreaks and "drought-breaking rains" may be caused by an unusually high quality of food plants at such times.

4.3. New Insecticides

New products have been investigated such as semiochemicals, botanicals, and mycopesticides. Mycopesticides are currently operationally used in Australia against the Australian plague locust *Chortoicetes terminifera* (Walker). Against the desert locust, a consortium of donors supported for 13 years a programme called Lutte Biologie Contre les Locustes et les Sautériaux (LUBILOSA) to develop a mycopesticide based on an African strain of the fungus *Metarhizium anisopliae* var. *acridum* (Gams and Rozyspal). It is applied as an oil suspension and can be sprayed using standard ultra low volume spinning disk spray equipment. It has a shelf-life of more than five years under refrigeration and approximately one year at 30°C. The biopesticide kills 70-90% of treated locusts within 14 to 20 days with no measurable impact on non-target organisms (Lomer et al. 2001).

Phenylacetonitrile inhibits pheromonal communication of gregarious nymphs resulting in loss of their gregarious behaviour (Hassanali et al. 2005). Phenylacetonitrile also seems to increase the vulnerability of locusts to conventional insecticides. Combining phenylacetonitrile with the biopesticide would enable the concentration of the biopesticide to be reduced by a factor of four (Pettit and Jenkins 2005). However, it is unlikely that the biopesticide can be effectively used in large-scale desert locust plague situations due to the time necessary to produce the product and its delayed control action. Its use will probably be restricted to environmental sensitive areas (Lomer et al. 2001).

Skaf et al. (1990) reasoned that since barrier spraying with the very effective dieldrin, which is both extremely persistent and biocumulative, was no longer possible there would be major technical, logistic and financial problems in containing plagues. Fipronil and insect growth regulators (IGRs) have been proposed as replacements to dieldrin (which is not being produced and used anymore). However, there are concerns that these products have negative environmental impacts. For example, the environmental impact of fipronil was tested in large-scale, long-term field trials mimicking locust control operations and found to have devastating effects on termite and ant populations (Tingle et al. 2000, Peveling et al. 2003).

It is often tacitly assumed that barrier treatment solves the block demarcation problem. In fact, it makes that task more difficult. Blocks must be larger and percentage infestation less, and the lower the percentage infestation, the more difficult demarcation becomes. The alternative is to define the area for treatment on the basis of reports of mature swarms. However, that might lead to treating hundreds of thousands of square kilometres to

control the resulting hoppers from those swarms. Barrier treatment will not work during an outbreak and would probably not be successful during an upsurge, since it relies on hopper mobility and at those stages hoppers move little.

4.4. Contribution of New Techniques and Products to Control

Control application techniques have been improved by the use of global positioning systems (GPS) and differential GPS. Details of these and other new techniques are given in the Desert Locust Control Committee (DLCC) reports and by LeCoq (2001). Whatever control method is going to be used, most of the above-mentioned products are contact insecticides meaning that targets have to be sprayed very likely in blocks. However, finding the targets and demarcating them in blocks is a prerequisite to being able to use them. Therefore, LeCoq (2001) stated that locust control depends more on political and institutional choices than on scientific and technological innovations. It is probably better to say that control depends more on organization and technical choice than on innovation, but the organization and technical choices are influenced by politics.

5. Sustaining Locust Capacity During Recession Years

When there are no upsurges or plagues it may be difficult to convince national governments of the necessity to maintain a locust unit, in particular when other crop protection problems arise. However, due to the transboundary character of the plague, a minimum capacity is required to ensure that locusts are monitored to find outbreaks and upsurges and that the country is prepared when populations are building up (continuous actualization of emergency action plans). This minimum level of activity should be determined by each country (in terms of number and capacity of human resources, and of survey, control and communication equipment). National governments should commit themselves to sustain such small flexible locust units. This has the attention of EMPRES. Separate anti-locust units in countries within the invasion area but outside the recession area are not considered necessary.

6. Upsurge and Plague Control

During plagues and upsurges the main actors are: national governments, FAO, regional locust organizations, and donors. What are the roles and responsibilities of each of these organizations?

6.1. National Governments

Many locust-affected countries are among the poorest in the world. In several of these countries locust control has no priority. In any given country a plague will be a rare event. Few countries in the recession area have organizations, in a permanent state of readiness, capable of combating such locust plagues. It follows that when a plague does threaten there is a need to either expand or create a powerful anti-locust capability quickly. The issues to be dealt with at the national level are: (1) justification, (2) scale of operations, (3) definitions of threats, (4) selection of survey and control methods, (5) resources needed and available during recessions (within locust unit and elsewhere), upsurges and plagues, (6) sources of funds, (7) time to acquire resources, (8) organizational arrangements, (9) responsibilities at different levels of threat, and (10) timetables (Annex 3 in Symmons and van Huis 1997).

Previous plagues have been a matter of crisis management with the approach being reactive instead of proactive. During the 1986-89 plague, mismanagement resulted in a waste of resources and non-judicious use of insecticides (OTA 1990, Joffe 1995). Was there any improvement 15 years after the 1986-1989 plague events?

6.2. FAO

The desert locust was considered the first pri-

ority problem to be addressed by EMPRES (see section 3.4) and activities were initiated in the central region of the desert locust distribution area since this was where most desert locust plagues had originated in the past. The nine frontline countries along both sides of the Red Sea and in the Arabian Peninsula are included in the programme. In 2006 its activities were handed over to the FAO Commission for Controlling the Desert Locust in the Central Region (CRC). There are 16 member countries in the CRC with a secretariat based in Cairo, Egypt. The desert locust component of EMPRES in the western region commenced in 2005 and involved nine frontline countries in the Maghreb and the Sahel. Activities and management are closely linked to the FAO Commission for Controlling the Desert Locust in the Western Region (CLCPRO). There is an EMPRES office in Dakar, Senegal while the commission's secretariat is located in Algiers, Algeria.

The main objectives of the desert locust component of EMPRES are to harmonize regional cooperation, to enhance national and regional communication, to improve early warning and information systems, to improve survey systems, to set-up and implement contingency plans, to introduce efficient and environmentally safer control methods, and to develop systematic methods of campaign evaluation. Strengthening national locust units in training, survey and research is the main objective. The overall programme goal was redefined in February 2000 (FAO 2002b) as:

> *To strengthen the capabilities and capacities of the national, regional, and international components of the desert locust management system to implement effective and efficient preventive control strategies based on early warning and timely, environmentally sound, early control interventions.*

Although the term "preventive" is subject to many interpretations, there is no doubt that this statement refers to outbreak and early upsurge control, tacitly assuming that this is feasible. In practice, those now running the programme have come to realize that prevention can fail and therefore plague campaign contingency plans are needed. Therefore regional contingency workshops were conducted in Egypt and Mauritania and guidelines provided to countries.

A contingency plan or an emergency action plan is the crux of any locust plague campaign. Without such a plan, the establishment of locust units would be a waste of time. Often locust campaigns against major upsurges and plagues have been compared to a war. What is needed during a war are: (1) weapons (insecticides), (2) people who know how to use them (trained people), (3) means of finding the enemy (monitoring capacity), (4) intelligence in the information sense and analysis (information service), (5) a command structure to allow deployment and redeployment (an efficient and effective control organization), and (6) adequate resources in personnel, equipment and materials (resources to survey and control).

Having a clear understanding of these issues is most important for containing locust plagues. It means that for different levels of threat it should be clear what is available and what should be done. For example: (1) existence, procurement and distribution of insecticides, control equipment, vehicles, planes, camping equipment etc. (from national plant protection organizations, other ministries, and donors), (2) the kind of actions needed in terms of information, training, monitoring and control, and (3) who is responsible for what (FAO headquarters, national plant protection organizations, ministries of agriculture, finance and defence, donors), etc.

Another complicating issue is that when bands and swarms exist, all efforts are concentrated in eliminating them. Estimates of the total number of locusts present or the total area infested are only rarely made (Bennett and Symmons 1972). Such estimates are not easy, but without them, assessments of the impact of campaigns are not possible. The effectiveness of individual treatments has not been monitored, and moreover there is often inadequate knowledge of the technique of "incremental" spraying of concentrated insec-

ticides (ultra low volume spraying) against locusts. This means there is not yet a clear strategy for locust control.

Locust plagues are an international event. Uvarov (1953) stated

> *Locusts recognize no frontiers...*

and

> *...in many cases, the ability of locust swarms to cross frontiers is more readily admitted when they are entering a country than when they are leaving it for the neighbouring one.*

The organization responsible for, and the only organization capable of international coordination is FAO. Currently, apart from its EMPRES-related desert locust and other long-term coordination activities such as those embodied in the Desert Locust Control Committee, FAO's main involvement is forecasting. This is done by the Desert Locust Information Service (DLIS) at FAO headquarters based on rainfall and habitat information provided by satellite images and locust data provided by countries. However, the difficulties for the countries to provide adequate information should not be underestimated because of the logistical problems involved in surveying immense areas.

In emergencies, the Locust and Other Migratory Pests Group of FAO becomes the Emergency Centre for Locust Operations (ECLO) with additional competencies to manage the locust situation. The most important issues to be dealt with by FAO during an emergency are: (1) the collection, analysis and dissemination of information, (2) the assessment of the situation through provision of consultant services, (3) the assessment of survey and control efficiency, (4) the procurement and management of contingency funds from donors, (5) donor coordination, (6) the arrangement of international meetings for all stakeholders, (7) insecticide management (procurement, contingency stocks, distribution, and avoiding the problem of obsolete products), and (8) contact with commercial organizations (provision of aircraft, equipment and insecticides). The donors could collaborate by making available a permanent "desert locust emergency fund", large enough to start emergency operations.

6.3. Regional Locust Organizations

There is only one operational regional locust organization: the Desert Locust Control Organization for East Africa (DLCO-EA). It specializes in aerial surveillance and control of the desert locust, grain-eating birds and the tsetse fly. Member countries can apply for assistance in control activities. Another regional organization Organisation Commune de Lutte Antiacridienne et de Lutte Antiaviare (OCLALAV) in West Africa is not operational.

6.4. Donors

Donors are particularly interested in evaluating the effectiveness and efficiency of desert locust campaign operations. The following aspects are considered important: (1) organizational issues – role of local, regional, international and donor organizations, cooperation, coordination, communication, logistics, training, contingency planning, (2) technical issues – collection, analysis and dissemination of information, improved forecasting, integrated pest management approaches, efficacy of insecticide applications, locust population dynamics, crop damage, and sustainability, (3) financial issues – national and emergency funds, management of funds, and contingency planning, and (4) health and environmental issues – insecticide banks, obsolete insecticides, biodiversity (pollinators and biodiversity), alternatives to synthetic insecticides, safety procedures (food residues, water contamination, toxicological monitoring of staff, existence of protocols), procurement of insecticides, insecticide stocks, disposal of insecticides, and quality of insecticides.

To record what has been done and to evaluate the impact of any action undertaken is the crux of locust control. Independence of surveys and control, the extent of the area infest-

ed and the populations of locusts therein should be determined. This is the only way to determine which part of the population was destroyed during an outbreak or upsurge, and whether control is making any sense. Probably the best criterion is the extent (area) of the infestation, more than the density of the population.

Campaign evaluation during outbreaks/upsurges, and outbreak prevention during recessions are other crucial issues that need to be addressed (effectiveness of control operations). The way in which this can be done most effectively has to be considered. Probably the best approach for regional coordination would be to establish an independent group responsible for these activities, but hosted at FAO.

7. Conclusions

Popov (1972) stated that the objectives of locust surveys are simple:

> *To find economically important locust populations and destroy them as efficiently as possible.*

It often seems that miracles are expected from technical innovations. Large funds have been spent on satellite imagery, remote sensing, new control agents and application techniques, etc. to improve future prevention capacities that avoid plague development. However, the core business of locust control still depends on the organization, logistics, finances, and politics of controlling upsurges/swarms. Locust affected countries, FAO, and donors need to be organized for locust prevention and control (contingency plans).

During upsurges and plagues it is very common for national governments and FAO to request funds for buying insecticides, and survey and application equipment. However, almost never is it indicated how these will be used. Donors should only provide funds to governments of locust-affected countries when there is a plan showing how these resources will be used to find and efficiently destroy economically important locust populations. Equally important, they should follow up to see that some attempt has been made to execute the plan. Such plans of course concern the items already listed. The execution of a campaign requires flexibility. No battle ever goes according to plan. During recession periods there is enough time to develop contingency plans to execute an effective campaign in time of need.

Another issue of crucial importance is the strategy of locust control. Campaigns need to be evaluated independently during outbreaks/upsurges to determine the extent of infestations and the effect of control efforts. The other issue is how to prevent outbreaks during recessions. Can surveys be better targeted? When done effectively on an area-wide basis could this lead to a new strategy of outbreak prevention?

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The Mountain Pine Beetle *Dendroctonus ponderosae* in Western North America: Potential for Area-Wide Integrated Management

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ABSTRACT Epidemics of the mountain pine beetle *Dendroctonus ponderosae* Hopkins have occurred within the pine forests of western North America four times during the last century. Considerable resources have been directed toward suppression of populations due to the extensive tree mortality associated with outbreaks. However, management efforts have been largely unsuccessful. A framework for successful control based on simple population processes is proposed and used to evaluate past management efforts. The compounding effects of increasingly susceptible forests due to climate change and the legacy of forest fire suppression are discussed. Due to the increase in the amount of susceptible pine, the mountain pine beetle epidemic that began in the 1990s has spread over nine million hectares. Because of its size, control efforts against the present major outbreak are largely irrelevant. However, a swift and aggressive area-wide control strategy is required to limit the spread of isolated populations east of the Rocky Mountains. This strategy must integrate advances in remote sensing technology that permit the earliest possible detection of small incipient epidemic infestations with novel forms of direct control and aggressive sanitation harvesting. In the long term, mitigation of impacts will only be possible through the area-wide management of the amount of susceptible pine.

KEY WORDS mountain pine beetle, direct control, forest fire suppression, climate change, range expansion, area-wide management

1. Introduction

The mountain pine beetle *Dendroctonus ponderosae* Hopkins. is the most destructive biotic agent of mature pine forests in western North America. Normally it is innocuous, infesting only a few suppressed or damaged trees scattered throughout a forest. However, populations periodically erupt into large-scale epidemics capable of causing the mortality of trees over many thousands of hectares. In Canada, the most extensive outbreaks have occurred in the southern interior regions of British Columbia, while in the USA the largest outbreaks have manifested in the Rocky Mountain states. In addition to extensive timber losses, mountain pine beetle epidemics may increase fuels for wild fires, alter successional patterns of forest growth, affect watershed quality, wildlife composition, and recreational values (Safranyik et al. 1974).

The mountain pine beetle is broadly distributed in western North America from northern Mexico to north-western British Columbia, Canada. Throughout its range, it breeds in virtually all species of native or introduced pine (Furniss and Schenk 1969). However, lodgepole pine *Pinus contorta* var. *latifolia* Engelmann is considered to be the beetles' primary host due to the size, intensity and commercial impact of epidemics in that forest type.

During the past century, four significant mountain pine beetle epidemics have occurred in North America (Taylor and Carroll 2004). Given the extensive tree mortality associated

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with outbreaks, considerable resources have been directed toward their control. Unfortunately, very few management efforts have achieved population suppression (Klein 1978). The objectives of this paper are to (1) establish a theoretical framework for successful control of the mountain pine beetle derived from simple population processes, (2) utilize this theoretical framework to assess previous efforts at control (insofar as the literature permits), (3) examine the challenges for successful control associated with changing climate and the legacy of past forest management practices, and (4) discuss the relevance of future, area-wide control efforts in the short- and long-term given the current status of mountain pine beetle in North America.

2. A Population-Based Framework for Successful Control

2.1. Mountain Pine Beetle Population Processes

Normally, the mountain pine beetle must kill its host to reproduce successfully. It is a subcortical herbivore that feeds within the phloem (i.e. the vascular tissues between the bark and the sapwood). Mature, large-diameter trees are preferred due to their thicker, more nutritious phloem (Amman 1972). However, these trees are normally the most vigorous and therefore the most resistant to attack within a stand (Safranyik et al. 1974). As attacking beetles bore through the bark of a potential host, the tree responds by producing copious quantities of toxic resin (Berryman 1972). If the density and/or rate of arrival of attacking beetles are low, they may be flushed from the tree or encapsulated in resin-soaked tissues beneath the bark (Safranyik et al. 1975).

The mountain pine beetle has evolved two complex adaptations that facilitate the colonization of often highly resistant trees. First, beetles employ aggregation pheromones to ensure that they arrive and initiate attacks simultaneously (i.e. mass-attack), thereby overwhelming host defences (Raffa and Berryman 1983). Second, the beetles have evolved a mutualistic relationship with phytopathogenic ophiostomoid fungi. Attacking beetles introduce fungal spores that rapidly invade the phloem and sapwood, thereby compromising the expression of tree defences (Safranyik et al. 1975). The combination of larval tunnelling within the phloem, and rapid fungal colonization following mass-attacks invariably cause tree mortality before the onset of winter.

Following successful colonization, mated females oviposit in niches chewed along vertical galleries. Eggs hatch within several days and larvae mine circumferentially around the bole, developing through four instars. The beetles typically overwinter as late-instar larvae, before completing their development during the following spring and early summer (Reid 1962).

There are four distinct phases in the population cycle of the mountain pine beetle: endemic, incipient epidemic, epidemic (i.e. outbreak) and post-epidemic populations (Safranyik and Carroll 2006). The endemic and incipient epidemic phases represent distinct population states regarding interactions of beetles with individual host trees and stands, whereas the latter two population phases mainly represent differences in population size and spatial extent.

Following the collapse of outbreaks during the post-epidemic phase, and before populations increase to incipient epidemics, the mountain pine beetle is considered to be in the endemic phase. An endemic population can be defined as one with insufficient beetles to overcome the resistance of any large-diameter tree within a stand. Beetles are therefore restricted to low-quality host trees with little or no defensive capacity. Natural enemies, weather, inter- and intraspecific competition combine to balance mortality and reproduction rates so annual changes in population and damage levels are minimal. Since female mountain pine beetles produce an average of 60 eggs, and two-thirds of offspring (i.e. 40) are female (Reid 1962), then given that only

one female offspring needs to survive to achieve replacement, approximately 97.5% generation mortality (i.e. 39/40) is required to keep endemic populations static (Safranyik and Carroll 2006).

The incipient epidemic phase begins when mountain pine beetle populations have grown to a minimum size sufficient to successfully mass-attack a single large-diameter tree within a stand. The main factors that permit populations to increase to the incipient epidemic phase are those that cause either a decline in tree resistance or an increase in beetle population size. For example, a number of consecutive years of warm and dry weather, favouring beetle survival and compromising tree resistance (through drought), have been associated with sustained increases in beetle populations (Thomson and Shrimpton 1984). Interestingly, only a very small rise in survival is required for populations to increase dramatically. For example, if generation mortality declines from 97.5 to 95.0%, then populations have the potential to double in size (Safranyik and Carroll 2006). Once populations have gained access to large-diameter trees, their potential rate of increase is often extremely high. During the transition from the incipient epidemic to the epidemic phase, local populations often increase more than eight-fold, yearly.

An outbreak forms as incipient epidemic infestations coalesce over the landscape. This involves emigration of mountain pine beetles from localized points of increase into neighbouring stands, thereby facilitating the endemic-incipient epidemic transition of resident populations. If large areas of susceptible host trees coincide with sustained favourable weather conditions for beetle survival, epidemics will spread over vast areas. Due to the sheer number of beetles, epidemic populations can rebound from a large-scale mortality event.

The post-epidemic phase comprises the collapse of outbreaks, generally as a consequence of depletion of the large-diameter trees within stands and/or unseasonably cold weather conditions during the period from late autumn to early spring. In the final stages of the post-epidemic phase, increased mortality from natural enemies and competitors can hasten population collapse (Safranyik and Carroll 2006).

2.2. The Framework

Knowledge of the basic population processes associated with the mountain pine beetle is essential for effective control efforts. In populations where conditions have changed such that reproduction outweighs mortality, unless a sufficient amount of additional mortality is introduced, the infestation will expand. Given that beetles spend the vast majority of their life cycle beneath the bark of trees, the only viable means (to date) of adding mortality involves destroying beetles in infested trees before they can complete their life cycle and disperse to new hosts. More specifically, this entails the treatment of single trees or small groups of trees through felling and burning (or bark removal), removal and processing (where transport to a mill is economical), or the application of a systemic insecticide. Larger groups of trees are felled, transported to a mill and processed; a practice known as sanitation harvesting. The relative success of these tactics is dependent upon the state of the beetle population.

Initially mountain pine beetle populations appear to grow relatively slowly. For example, a stand with one infested tree and a population where the generation mortality has declined slightly to allow it to double each year (i.e. a rate of increase $R = 2$) will have 512 dead trees after ten years. This represents less than 2% of the trees within a 20-hectare stand, and therefore the population may escape detection or concern for a number of years. If the infestation was detected and, in an effort to control it, 37.5% of the infested trees were removed during the 4th year, 194 fewer trees would be killed by year ten, but the population would continue to expand (Fig. 1). From this example, the question arises: what level of mortality must be added and how often, to slow or stop an increasing population?

The general concept is straightforward. To maintain a static population, a proportion of infested trees (*P)* must be removed in each year equivalent to:

$$P = 1\text{-}1/R \qquad (1)$$

where *R* is the yearly rate of increase in the population. In other words, if the population is expected to triple yearly (*R* = 3), then two-thirds of all infested trees would have to be removed and destroyed before the flight period each year. Obviously, if a reduction in the size of the population is the goal, then removal rates must exceed two-thirds. The concept is presented graphically in Fig. 2. For any measured rate of increase, unless sufficient mortality is introduced that equals or exceeds the yearly growth in a population, it will continue to increase.

With the above framework in mind, control efforts must be considered in light of the population phases described previously. In the endemic situation, population increase is usually constrained to unity. This is the state where management efforts can have their greatest impact. Beetles are restricted to a few weakened or damaged trees within a stand, so relative to the potential rate of increase and the number of trees involved, removal of any of the infested stems would suppress the population (Fig. 2). Thus, provided they can be detected, endemic populations are amenable to virtually any management strategy.

By virtue of their larger size and more obvious impacts, incipient epidemic populations are relatively easy to detect. Because they have gained access through mass-attack to healthy, large-diameter trees, their rates of increase are often between two and four per year. Typically, when these populations are first detected, the number of trees involved is still small (less than 500), and the area they occupy is well defined (Safranyik and Carroll 2006). Yearly rates of increase can be easily assessed through ground and/or aerial surveys of the number of trees or area infested. To limit the potential for increase if *R* = 4, then more than 75% of the infested trees must be treated every year (Fig. 2). If 500 infested trees were found, then at least 375 stems must

Figure 1. *Number of trees killed by mountain pine beetle versus age of an incipient epidemic population doubling in size yearly (solid line). The broken line represents the same population with the removal of 37.5% of infested trees in year four.*

be treated that season, and a similar proportion in subsequent seasons provided *R* remains constant. If there is ready access to the infestation, it is highly amenable to direct control.

An incipient epidemic population may take only two to three years to develop into an outbreak if left untreated and rates of increase remain high. During an outbreak, the number

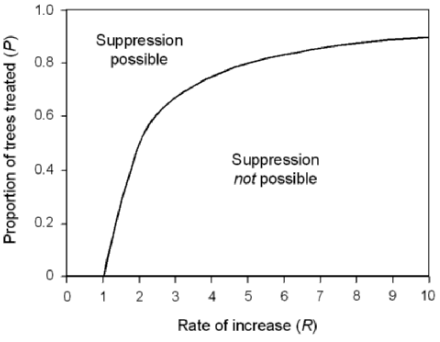

Figure 2. *Graphical representation of the proportion of a mountain pine beetle population (*P*) that must be removed in relation to the yearly rate of increase (*R*) to suppress population growth (*P = *1-1/*R*). The area below the curve represents treatment levels where suppression is not possible; treatment levels above the curve (applied yearly) will suppress populations.*

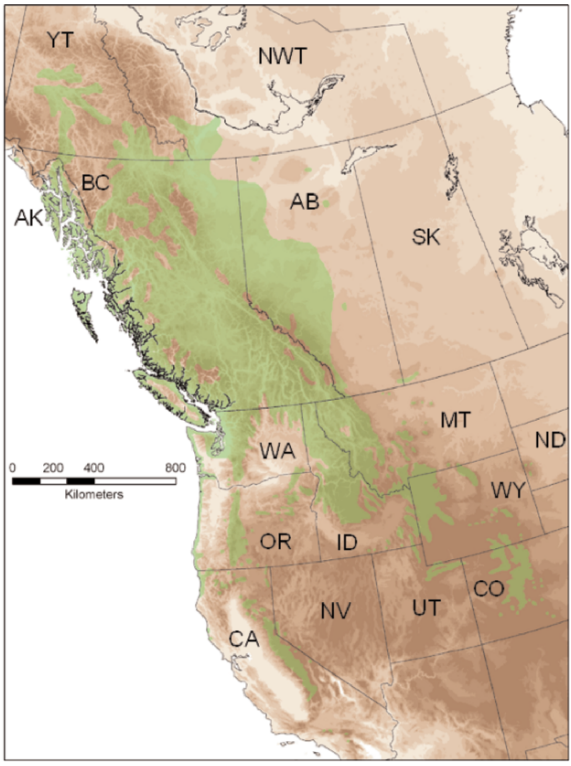

Figure 3. *Distribution of lodgepole pine in North America (adapted from Little 1971).*

of trees killed annually is often in the millions and may encompass hundreds of thousands of hectares. The rate of increase may not be more than that of an incipient epidemic population, but its sheer size renders most management tactics ineffective. As an example, if an outbreak is spread across 300 000 hectares and $R = 2$ (a conservative rate during peak outbreak years), then 150 000 hectares of infested trees must be harvested in each year just to keep the infestation static. Logistically, detection and removal of such a vast number of infested trees is impossible.

3. Trials and Errors: Lessons from the Past

Lodgepole pine forests occur over approximately 160 million hectares of western North America (Fig. 3). The mountain pine beetle is an ubiquitous component of mature stands over much of this area. Despite the vastness of the region in which mountain pine beetle populations exist, epidemics normally initiate and spread from well defined epicentres (Aukema et al. 2006). Therefore, direct control tactics aimed at controlling developing epicentres in the incipient epidemic phase are theoretically amenable to a suppression strategy.

Despite many significant efforts at direct control of mountain pine beetle populations during the previous century, most authors concluded that suppression was seldom if ever achieved and, at best, the rate of tree mortality was reduced only marginally (Craighead et al. 1931, Amman and Baker 1972, Klein 1978, Amman and Logan 1998). A brief examination of historical control activities in light of

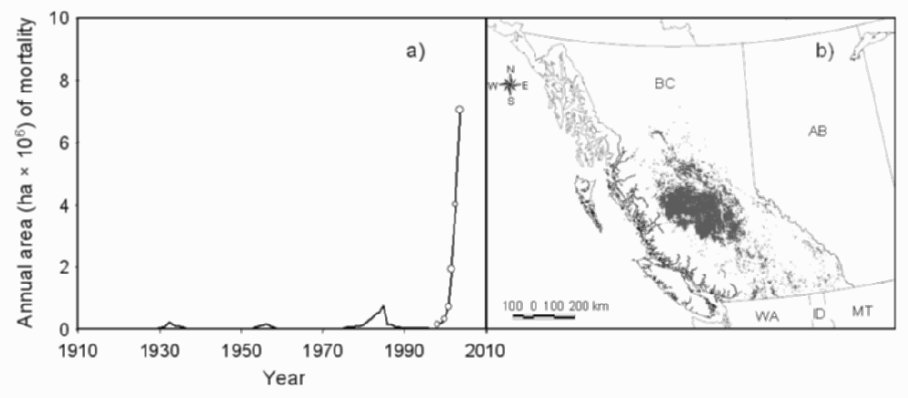

Figure 4. *(a) Historic occurrence of mountain pine beetle epidemics, and (b) cumulative area of mortality from 1992 to 2003 in western Canada.*

the framework proposed above reveals three major shortcomings.

First, most efforts targeted removal of infested trees as either a fixed percentage of the total or of the area involved (Klein 1978). Without assessments of the yearly rate of increase of a population, the treatment levels were most often insufficient. Second, even when a sufficient proportion of a population may have been removed in one year, the efforts frequently did not persist in subsequent years (Craighead et al. 1931). Since incipient epidemic populations often have very high rates of increase, and conditions amenable for increase typically persist for more than one year, then a single aggressive intervention may slow the development of an epidemic, but not prevent it (Fig. 1). Finally, early control programmes suffered from the inability to accurately detect and delimit increasing populations. As a consequence they were often abandoned when populations "erupted" in adjacent unsurveyed jurisdictions (Evenden 1944). In recent years, thorough systematic aerial survey techniques have been developed that provide accurate, real-time quantification of the condition of mountain pine beetle populations over the landscape. In addition, incorporation of these data into geographic information systems (GIS) along with detailed forest "inventory" data (e.g. species composition, stand age, stem density, etc.) have facilitated effective integrated management efforts over large areas (Wulder et al. 2005).

Interestingly, there is one documented example of successful suppression of a mountain pine beetle population. During the early 1940s, an incipient epidemic was detected near Banff, Alberta, Canada. Every tree in the vicinity of the infestation was assessed over two years, and any with evidence of mountain pine beetle attack was felled and burned. During the third year, no beetles could be found (Hopping and Mathers 1945). Although rates of increase were not considered, it is not surprising that such an aggressive and consistent intervention was successful.

4. Changing Rules? Altered Disturbance Regimes and Global Warming

Although mountain pine beetle populations have erupted several times in the past, the latest outbreak that began in the early 1990s has reached levels that are nearly an order of magnitude greater than any previously recorded (Fig. 4a). Its consistent rate of increase quickly outstripped the resources available for its management. Indeed, populations have been doubling each year for the past eight to ten years such that the cumulative area impacted

comprises well over nine million hectares of lodgepole pine forests in western Canada alone (Fig. 4b). As outlined above, for an epidemic to occur there must be an abundance of susceptible host trees in combination with a sustained period of favourable weather. Both of these conditions have coincided in recent years in western North America. Moreover, evidence suggests that these conditions have been exacerbated by anthropogenic activities.

Virtually all lodgepole pine forests originate from stand-replacing wild fires (Smith 1981). However, due to aggressive fire suppression, the average yearly area burned in lodgepole pine in western Canada has declined to less than 1% of historic levels during the last five decades (Taylor and Carroll 2004). This dramatic reduction in the rate of disturbance has allowed pine forests to age to the extent that nearly 70% of current stands are more than 80 years old – a significantly greater proportion than that expected from a natural wild-fire regime (Taylor and Carroll 2004). Since mountain pine beetles preferentially attack trees more than 80 years old (Safranyik et al. 1974), fire suppression has dramatically increased the amount of susceptible trees. In fact, it has been estimated that there was 3.3 times more susceptible pine at the start of the present mountain pine beetle epidemic than in 1910 (Taylor and Carroll 2004).

In addition to an abundance of suitable hosts, climatic conditions have steadily improved for mountain pine beetle populations in recent years. Historically, the extent and severity of epidemics have been limited by insufficient summer temperature accumulation and/or minimum winter temperatures below a critical mortality threshold (Carroll et al. 2004). It has recently been shown that during the past three decades relevant climatic conditions have improved for the beetle over large portions of western Canada (Carroll et al. 2004). More importantly, as a consequence of changing climate, populations have expanded into formerly climatically unsuitable habitats, especially toward higher elevations and more northerly latitudes. Indeed, large parts of the current epidemic occur in areas that were climatically unavailable prior to 1970, despite the presence of susceptible host trees (Carroll et al. 2004).

Previous large-scale mountain pine beetle epidemics have collapsed as a consequence of localized depletion of suitable host trees in combination with the adverse effects of climate (Carroll et al. 2004). Given that the occurrence of an adverse weather event sufficiently severe and widespread to affect the epidemic is improbable, the current outbreak in western Canada is projected to continue unabated until 2015 when approximately 80% of susceptible pine could be killed (Eng et al. 2004). Due to the sheer size of the epidemic, efforts to control it have been largely abandoned and redirected toward salvage of dead stands. However, aggressive tactics aimed at slowing the spread of the outbreak at its periphery continue and remain important to minimize the potential spread of the mountain pine beetle into new habitats.

5. Area-Wide Control: Relevance in the Short- and Long-Term

Although the current mountain pine beetle epidemic in western North America renders irrelevant any available control tactic, recent expansion of the epidemic beyond the geo-climatic barrier presented by the Rocky Mountains (Carroll et al. 2004) demands an aggressive area-wide control effort. During 2004, mountain pine beetle infestations were discovered in the Peace River region of north-eastern British Columbia, Canada (Fig. 5); an area that was historically considered climatically unsuitable for mountain pine beetle (Safranyik et al. 1974, Carroll et al. 2004). Assessments of these infestations revealed that they originated in 2002 (i.e. did not increase from local populations), most probably as a consequence of long-distance dispersal from epidemic populations located several hundred kilometres to the south-west, across the Rocky Mountains (Fig. 5). The expansion by the mountain pine beetle into this previous-

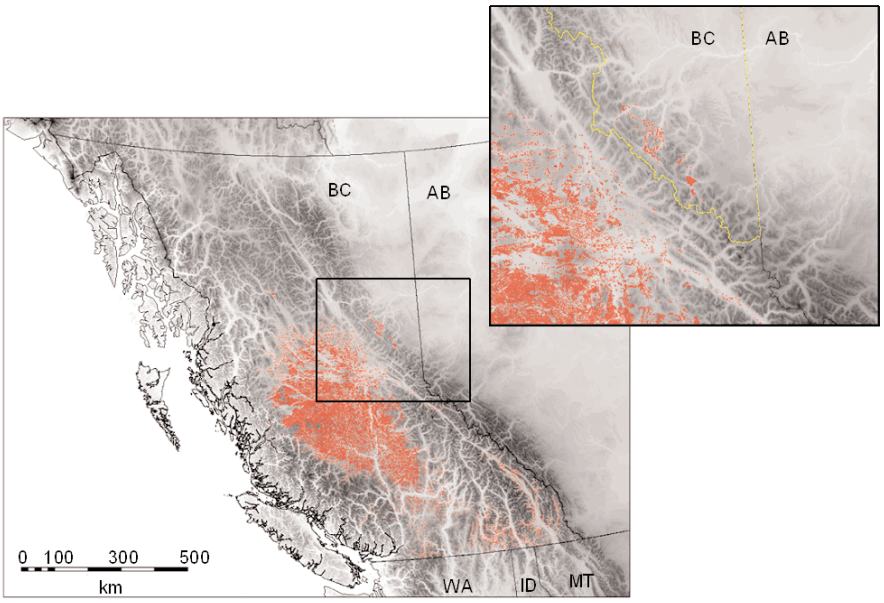

Figure 5. *Distribution of the mountain pine beetle epidemic in western Canada in 2004, and (inset) the isolated population established east of the Rocky Mountains as a consequence of a long-distance dispersal event in 2002.*

ly unoccupied region is a source of considerable concern. Immediately adjacent the Peace River region, lodgepole pine forests intermingle and hybridize with jack pine *Pinus banksiana* Lamb., a species susceptible to mountain pine beetle (Furniss and Schenk 1969), and a major component of the boreal forest that encompasses most of northern North America.

Several aspects of the Peace River population make it amenable to a variety of area-wide control techniques: (1) it is isolated from the epidemic to the west. Although additional inputs of beetles into the region via long-range dispersal are possible, it has not occurred subsequent to the original introduction, and the probability of the occurrence of such an event diminishes yearly as the source population to the west declines due to host depletion, (2) in relative terms the population is small, consisting of scattered incipient epidemic infestations, and confined to an area of approximately 2000 square kilometres along the north-eastern slopes of the Rocky Mountains (Fig. 5), (3) the region is almost entirely comprised of publicly owned land, facilitating an area-wide approach, and (4) the state of the beetle population, its distribution and its rate of increase are extremely well quantified based upon aerial and ground surveys (A.L. Carroll, unpublished). Since this final aspect is critical to successful area-wide management, it is worthy of further examination.

Once mountain pine beetle populations reach the incipient epidemic state, the number of infested trees can be used as a reliable index of beetle population size (Safranyik 1988). Infested trees display several distinct symptoms (Safranyik and Carroll 2006). The most obvious and easiest to detect over large landscapes by standard analogue (i.e. aerial surveys) or digital (i.e. aircraft- or satellite-borne sensor) remote sensing techniques, comprises gradual “fading” of foliage from green through yellow to reddish brown as

leaves become chlorotic and die (reviewed by Wulder et al. 2006).

Although quantification of the number of infested trees based on crown fading can provide a simple and accurate indication of the location, size and yearly rate of increase of mountain pine beetle infestations, by itself it has limited value in beetle management programmes due to a significant delay in the onset of crown fading following attacks. Adult beetles disperse, colonize and establish broods in new trees during mid to late summer of each year. However, reliable visible signs of foliage fading do not manifest until early summer of the year after attack, leaving only an 8- to 12-week period following detection within which to plan and apply control tactics before emergence and dispersal of the next beetle generation. This narrow temporal window generally precludes application of effective control tactics, particularly in remote and inaccessible areas such as the Peace River region.

In recent years, significant research efforts have focused on developing techniques to detect infested trees before the onset of visible changes to foliage (reviewed by Wulder et al. 2006), thereby increasing the temporal window for direct control efforts. Unfortunately, reliable differentiation of non-visual stress due to mountain pine beetle attack from other sources of stress has so far been impractical with existing technologies. To maximize the time available to access and treat infested trees, traditional mountain pine beetle management programmes employ a hierarchical approach involving: (1) aerial surveys to ascertain the location of infestations based on visual crowns symptoms, (2) systematic ground surveys around identified infestations to locate newly colonized trees based upon evidence of attack on the bole (Safranyik and Carroll 2006), and (3) prioritization and application of treatments based on an integrated management plan intended to encompass ecological and economical objectives (Hall 2004). Given the significant potential for the Peace River population to expand eastward toward the boreal forest (Carroll et al. 2004), traditional detection and monitoring systems have been applied with unprecedented rigour, thereby providing reliable information on the state of the beetle population and making possible an area-wide control programme.

Although research into novel tactics for direct control continues (Borden 1995), the eruptive nature of mountain pine beetle requires that any intervention against the Peace River population be swift and aggressive. Consequently, in the short term the suite of available area-wide control tactics will be limited to conventional techniques such as aerial and ground surveys to detect infestations, followed by felling and burning, or harvesting and processing. However, to minimize the probability of continued spread toward the boreal forest, management in the near future must integrate existing operational control tactics with emerging techniques for detection/monitoring and novel forms of direct control. For example, advances in remote sensing technology that permit early detection of endemic or small incipient epidemic infestations throughout the region combined with prompt application of fell and burn treatments. Alternatively, if larger incipient epidemic infestations are detected, aggregation and anti-aggregation pheromones should be deployed to concentrate beetles in accessible stands followed by aggressive sanitation harvesting (Borden 1995). In the longer term, management efforts should focus on an area-wide approach to reduce the susceptibility of host trees through silvicultural modification of existing pine stands to increase their vigour and, therefore, resistance to the beetle, and/or harvest planning or prescribed wild fire to create landscapes with a mosaic of age classes or tree species.

In spite of the most aggressive efforts, eradication of the Peace River population using conventional approaches to direct control will be virtually impossible given the challenges associated with detection and treatment of low-density populations in remote forested landscapes. Based on the preponderance of susceptible hosts (Taylor and Carroll 2004) and the potential for continued

improvements to climatic conditions due to global warming (Carroll et al. 2004), the mountain pine beetle population will likely persist in this new habitat, and the threat of spread to the boreal forest will remain. If the Peace River mountain pine beetle population can be controlled, and its spread limited in the short term, other tactics may emerge that ultimately facilitate its eradication. For example, sterile insect releases have been successfully employed in the area-wide control of coleopteran species (Setokuchi et al. 2001), although the feasibility of this technique has not been explored against bark beetles.

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A Strategy for an Area-Wide Control Campaign with an SIT Component to Establish a Tsetse- (*Glossina austeni* and *Glossina brevipalpis*) Free South Africa

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ABSTRACT A strategy is proposed to create an area free of *Glossina brevipalpis* Newstead and *Glossina austeni* Newstead in the southern-most tsetse fly belt in the province of KwaZulu-Natal, South Africa. The concept is based upon an area-wide integrated pest management (AW-IPM) approach that integrates several tsetse suppression techniques, such as insecticide impregnated odour-baited targets, mobile targets, the sequential aerosol technique (SAT), and the release of sterile insects (sterile insect technique (SIT)). The prerequisites for the proposed programme are described and include the development of sampling and control tools, ecological studies, entomological field surveys, feasibility studies and the development of adequate tsetse rearing capacity. The proposed AW-IPM strategy suggests the division of the 12 000 square kilometre tsetse-infested area into four zones of manageable size and the successive implementation of four phases (pre-suppression, suppression (population reduction), release of sterile males and post-eradication activities) in each of these zones following the "rolling carpet principle". Assuming a minimum release density of 100 sterile males per square kilometre, tsetse colonies of around 4.5 million producing *G. brevipalpis* females, and 5.5 million *G. austeni* would be required to sustain the releases. The entire programme would require an annual budget of USD 3.35 million for the duration of eight years. The creation of a tsetse fly-free area in South Africa and southern Mozambique would result in significant improvements to the livelihood of communal farmers owning around 350 000 cattle.

KEY WORDS KwaZulu-Natal, South Africa, *Glossina austeni*, *Glossina brevipalpis*, tsetse-free area, area-wide eradication, feasibility, strategy

1. Introduction

Tsetse presence in KwaZulu-Natal, South Africa, represents the southern-most limit of *Glossina* distribution in Africa. The area consists of several game reserves and conservation areas, including surrounding rural cattle farming areas. The two tsetse species, *Glossina austeni* Newstead and *Glossina brevipalpis* Newstead, inhabit riverine and coastal forests in the north-eastern parts of KwaZulu-Natal province, and are solely responsible for the cyclical transmission of nagana or African animal trypanosomosis (AAT). It is estimated that 350 000 cattle, mostly belonging to communal farmers, are at risk of contracting AAT (Kappmeier et al. 1998).

A severe outbreak of AAT in 1990 resulted in high cattle mortalities in the communal farming areas. The outbreak was brought under control by treating cattle with try-

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panocidal drugs, combined with tsetse control efforts using odour-baited insecticide impregnated targets (Green 1994), as well as pour-on treatment of cattle with pyrethroid insecticides (Holmes and Torr 1988). As the area has a well-established diptank network, with a compulsory fortnightly dipping regime for tick control, the dipping component amitraz (a tick-detaching agent) was replaced by the pyrethroid cyhalothrin. This was applied for two years only to prevent ticks developing resistance against pyrethroids (Kappmeier et al. 1998). The temporary nature of this control approach is, however, evident from the current prevalence of trypanosomosis, which has reverted to the high levels of 1990 (Van den Bossche et al. 2006) recorded before these temporary control measures were instigated (Kappmeier et al. 1998). In 2003, veterinary staff administered more than 10 000 prophylactic doses of trypanocidal drugs to livestock (R. Bagnall, personal communication).

In 1992, the National Directorate of Veterinary Services contracted the Agricultural Research Council-Onderstepoort Veterinary Institute to develop a long-term strategy to control *G. brevipalpis* and *G. austeni* in KwaZulu-Natal. In view of the continuous cost and limitations of the applied approach (disease surveillance, animal treatment and pyrethroid dipping/pour-on), it has become obvious that the only sustainable, long-term solution to the trypanosomosis problem is to completely eliminate the two vector species from KwaZulu-Natal.

2. The Concept

Area-wide integrated pest management (AW-IPM) involves the integration of several control tactics against an entire insect pest population within a delimited geographical area (Klassen 2005). The theoretical basis of AW-IPM was defined by Knipling (1972) as: the uniform suppressive pressure applied against the total population of the pest for a period of generations will achieve greater suppression than a higher level of suppression on most, but not all, of the population each generation.

AW-IPM is therefore a more efficient way of pest control than localized IPM that is applied on a field-by-field approach. This is exemplified by the long list of successful AW-IPM programmes, some of which have integrated the release of sterile insects against major insect pests of veterinary and agricultural importance with other methods (Klassen and Curtis 2005). The approach has not only been used with great success for pest eradication (e.g. the New World screwworm *Cochliomyia hominivorax* (Coquerel) from the southern USA, Mexico and Central America (Wyss 2000), or the tsetse fly *G. austeni* from Unguja Island, Zanzibar (Vreysen et al. 2000)), it has also been applied successfully for pest containment (e.g. the pink bollworm *Pectinophora gossypiella* (Saunders) containment programme in California (Hennebery 1994)), for pest suppression (e.g. suppression of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in the Hex River Valley in South Africa (Barnes et al. 2004) and the Arava/Araba valley in Israel/Jordan (Cayol et al. 2004)), and for the prevention of the establishment of an invasive pest in a given area (e.g. the Mediterranean fruit fly preventive release programmes in California (Dowell et al. 2000) and Florida (IPRP 2003)).

History confirms that the elimination of tsetse from delimited geographical areas is not only feasible but also sustainable, provided the control tactics are directed against an entire tsetse population, i.e. according to the area-wide concept. Classical examples include the elimination of *Glossina pallidipes* Austen from South Africa in the 1950s by means of aerial spraying of residual insecticides (DDT and HCH) (Du Toit 1954), the elimination of *Glossina morsitans submorsitans* Newstead, *Glossina palpalis palpalis* Robineau-Desvoidy, and *Glossina tachinoides* Westwood from 200 000 square kilometres in northern Nigeria, mainly by the selective spraying of resting sites from the ground and air (1955-78) (Spielberger et al. 1977), and the elimination of *G. austeni* from Unguja Island, Zanzibar by integrating the

sterile insect technique (SIT) with other control methods (1994-1997) (Vreysen et al. 2000). With the exception of possibly some parts in Nigeria, all treated areas have remained free of the target tsetse species until today.

In stark contrast to these successes stand the many, small-scale, localized control efforts, which were mainly based on community participation at the farm or village level, but which have rarely been sustained (Barret and Okali 1998). This, despite the availability of several tsetse suppression methods that can be applied by the communities and that can achieve significant reductions in tsetse populations, i.e. insecticide-impregnated odour-baited targets (Vale 1982, Vale et al. 1988), and the use of mobile targets (application of residual insecticides as a pour-on on livestock) (Bauer et al. 1995). A number of technical and sociological reasons have been identified for this lack of sustainability (Brightwell et al. 2001). However, most importantly, none of these programmes was implemented according to the area-wide concept, which resulted in constant invasion pressure from neighbouring tsetse-infested areas.

It is indeed tempting to assume that in the short term, the most suitable control option in the communal farming areas of KwaZulu-Natal would be intensive disease surveillance combined with a regime of animal treatment with trypanocidal drugs and tsetse suppression when surveillance shows a rise in trypanosomosis incidence. However, this reactive approach would require continuous vigilance, a large permanent work force and the ever-increasing risk of the development of tick resistance to pyrethroids.

It seems, therefore, that the only long-term solution to the AAT problem in KwaZulu-Natal would be the elimination of the entire populations of the two vectors using an area-wide integrated approach, i.e. the removal of tsetse not only from the communal farming areas but also from the neighbouring game reserves and conservation areas. Area-wide suppression techniques such as the temporary aerial application of ultra-low volume non-residual insecticides (sequential aerosol technique (SAT)) could be combined with community-based control tactics in the more densely populated areas (see section 4.1.). The sterile insect technique (SIT) could be integrated as the final component of the campaign to eliminate the remaining fly pockets (Vreysen et al. 2000).

Figure 1. *The H-trap for sampling* Glossina austeni *and* Glossina brevipalpis.

3. The Requirements

AW-IPM programmes with an SIT component are complex, management intensive and have many interdependent components. An appropriate strategy can only be developed and implemented provided several prerequisites have been fulfilled (Vreysen et al., this volume). As part of the planning phase, a feasibility study was carried out to collect all necessary data to develop a suitable control strategy.

3.1. Sampling Tools and Ecological Studies

A basic requirement for tsetse surveys and ecological studies is the availability of an efficient sampling device. Studies were therefore carried out to develop a suitable trap to sample both tsetse species in adequate numbers (Fig. 1). This was especially challenging for *G. austeni* in view of its reluctance to enter traps that are efficient for other tsetse species. Sticky panels were the only alternative available for sampling and monitoring purposes, but these are very cumbersome to use and not

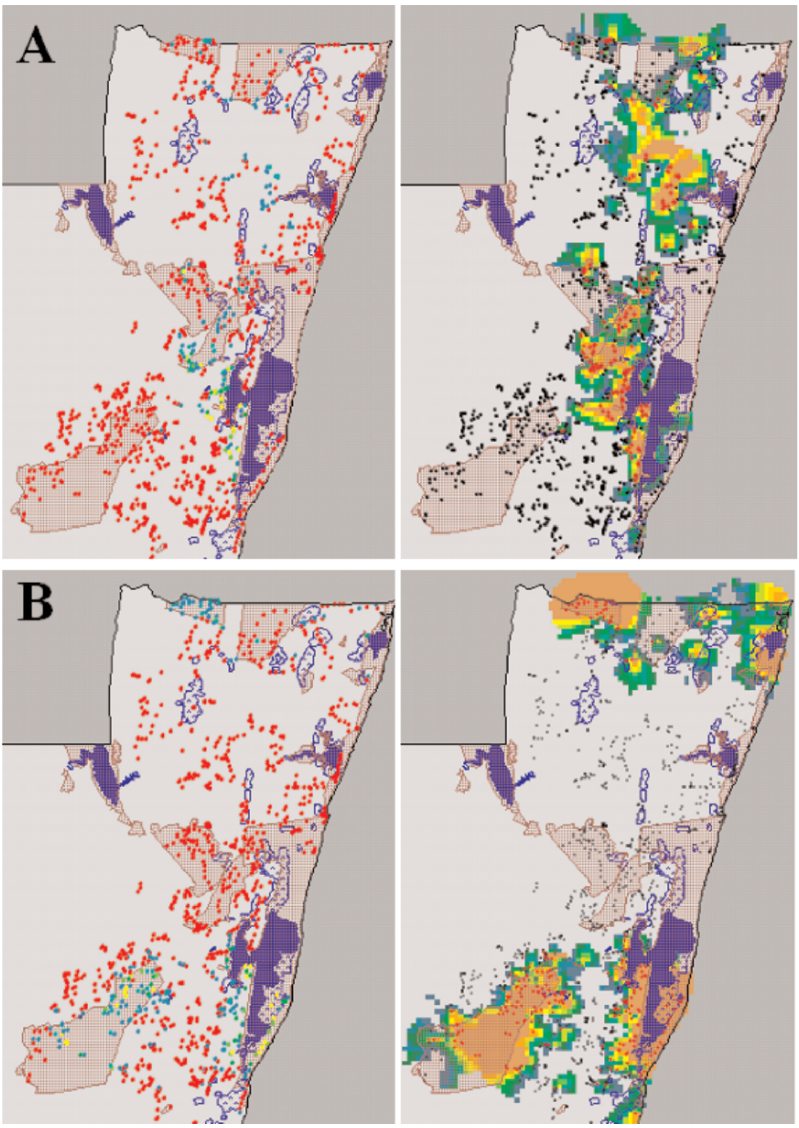

Figure 2. *Survey data (left) of (a)* Glossina austeni *and (b)* Glossina brevipalpis *in KwaZulu-Natal (Red dots - tsetse absent, Blue - Green - Yellow dots low to high density) and the presence-absence prediction model (right) indicating the probability of presence of (a)* G. austeni *and (b)* G. brevipalpis *(Shaded polygon: conservation area; Blue areas: lakes and dams; Blue contours: major marshes; Blue - Green - Yellow - Orange - Brown: low to high probability of occurrence; Black spots: negative trap catches; Grey in the East: Indian Ocean; Grey in the North: Mozambique) (Map adapted from AVIA-GIS 2002).*

suitable for ecological studies using release-recapture methods (Schönefeld 1988, Madubunyi 1990, Vreysen et al. 1996, 1998). A new sampling device (H-trap) (Fig. 1) was

therefore developed that was suitable for research, survey and monitoring activities (Kappmeier 2000) and that allowed the collection of live flies, a prerequisite for mark-release-recapture studies (Kappmeier Green 2002). As an example, the trap was used to collect data on the tsetse flies' potential to negotiate areas of unsuitable habitat located between pockets of forests and other suitable habitat and which could act as natural barriers between controlled and infested areas. It was shown that *G. austeni* was more confined to densely shaded areas but that it still traversed short distances of unsuitable habitat between pockets of vegetation. *G. brevipalpis* was less confined to the densely shaded areas and was found to be a much more mobile fly (Kappmeier Green 2002).

3.2. Entomological Field Surveys

The distribution of *G. austeni* and *G. brevipalpis* was determined through entomological field surveys that were conducted from 1993 until 1999 in north-eastern KwaZulu-Natal (Kappmeier Green 2002). Fly sampling was done in natural conservation areas (game reserves) and communal farming areas, using blue and black cross-shaped XT sticky panels baited with the synthetic ox odour developed for tsetse in South Africa (Kappmeier and Nevill 1999a). The highest densities of flies were found within the game reserves and natural areas. The resulting field data were used to develop a satellite-derived probability-of-presence model (AVIA-GIS 2002, Hendrickx et al. 2003), which clearly showed that the distribution of *G. austeni* is fairly continuous throughout the central part of north-eastern KwaZulu-Natal, while the *G. brevipalpis* population is restricted to two distinct bands (Fig. 2). The model confirmed that *G. brevipalpis* is mainly confined to the game reserves and natural areas whereas this is not necessarily the case for *G. austeni*.

The results of the surveys and the subsequent model clearly indicated that both *G. brevipalpis* and *G. austeni* are confined to restricted, isolated areas of north-eastern KwaZulu-Natal, making the creation of an area free of the two vectors an attractive long-term solution to the AAT problem.

3.3. Suppression Methods

The survey data on relative abundance indicated that suppression of the native tsetse population, prior to the release of sterile males, would be necessary. As such, the appropriateness of various tsetse suppression methods was assessed, taking into account local geographical characteristics.

Stationary attractive devices (e.g. traps and insecticide-impregnated targets) (Vale 1982, Vale et al. 1988) (Fig. 3) aim to exert a modest daily mortality of 2-3%, in addition to the natural mortality (Hargrove 1988) on the female tsetse fly population by attracting them to a device and inducing a landing response (Green 1993). The flies are killed by contact with a lethal dose of insecticides applied to the surface of the target or by heat or starvation after being guided to a non-return cage of a trap (Laveissière and Couret 1981, Dransfield et al. 1990). The AW-IPM programme against *G. austeni* on Zanzibar already highlighted the limitations of insecticide-impregnated blue cloth screens to suppress *G. austeni* in primary forest habitat (Vreysen et al. 1999). Therefore, an odour-baited insecticide-impregnated target system was developed for the suppression

Figure 3. *An odour-baited insecticide-impregnated target developed to control* Glossina austeni *and* Glossina brevipalpis *in localized areas of KwaZulu-Natal.*

of both *G. austeni* and *G. brevipalpis* (Kappmeier and Nevill 1999b) (Fig. 3). The black-blue-black target was tested against the two species in a pilot trial, covering an area of 80 square kilometres. Whereas *G. austeni* could be effectively controlled with 4-8 targets per square kilometre, these target densities were insufficient to effectively control *G. brevipalpis* (Esterhuizen et al. 2001, Esterhuizen et al. 2006).

The deployment of these targets could therefore be considered for localized use in some areas or to create barriers between tsetse-free and infested areas to prevent reinvasion, but the required high target densities (especially for *G. brevipalpis*) precludes their deployment on a large scale, i.e. it would be impractical, uneconomical and requiring a huge infrastructure.

The SAT relies on the aerial application of non-residual insecticides in ultra-low volumes. The goal of SAT is to kill adult tsetse in the first spraying cycle by direct contact and then kill emerging flies in subsequent spraying cycles before the emerged flies can deposit a larvae (Holmes and Torr 1988). The SAT is a delicate operation and has to be conducted at night during periods of temperature inversion. Recent advances in satellite-guided navigation systems (e.g. SATLOCK's Air Star System), have made the application of these insecticides much more accurate and effective (Cox and Vreysen 2005). The SAT appears to be the best method for the suppression (possible eradication in certain areas) of the two species in the natural and commercial game areas as the vegetation in these areas is not very diversified and the maximum elevation does not extend beyond 500 metres (Parker 2003). In addition, all available evidence to date suggests that the environmental impact of SAT is small and temporary (Grant 2001). However, the presence of the many protected game areas in KwaZulu-Natal and the opposition of a strong environmental lobby will make this option very challenging in terms of perceived or real environmental impact.

In the communal and commercial livestock farming areas, insecticide-treated cattle may be a practical alternative to the use of insecticide-impregnated targets and SAT, as KwaZulu-Natal has well developed functional dip facilities at which cattle are regularly presented for examination by an effective provincial veterinary service.

3.4. Other Feasibility Studies

In addition to the entomological activities, a socio-economic study and an environmental impact assessment (EIA) were carried out. The data of the socio-economic model clearly showed significant economic benefits occurring from the area-wide tsetse eradication effort with a breakeven point reached during the 8th year. A cumulative total net benefit (net present value, taking into account a discount rate of 8%) of USD 51 million and an overall benefit to cost ratio of 3.4 would be obtained over a 15-year time frame, not taking into account additional benefits, such as improved agricultural productivity due to improved health of draft animals. As from year nine, the project reaches the maintenance phase and benefits will be fully established; the annual benefit to cost ratio fluctuates from 90-493 per USD invested (year nine to year 15). Moreover, the project has an internal rate of return of 23%, meaning that the discount rate could be almost three times higher than the estimated value of 8% and the project would still break even in a 15-year time frame (Knight 2006).

The EIA was carried out, as South Africa is a signatory to the 1992 International Convention to conserve biodiversity. These important issues cannot be ignored and require an objective and balanced approach if practical strategies are to be formulated.

3.5. Development of Tsetse Rearing Capacities

Area-wide IPM programmes with an SIT component require large colonies of the target insect to produce sufficient numbers of sterile males for release. Due to the slow reproductive capacity of tsetse flies (each female pro-

duces only one offspring every 10 days), seed colonies of the target species have to be initiated in the early phases of the programme. Therefore, membrane adapted colonies of *G. brevipalpis* and *G. austeni* were initiated at the Agricultural Research Council-Onderstepoort Veterinary Institute, derived from colonies maintained at the Entomology Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria, and the Tsetse and Trypanosomiasis Research Institute in Tanga, Tanzania. In late 2006, the *G. austeni* colony had 33 000 reproductive females that produced approximately 15 000 pupae per week, while the size of the *G. brevipalpis* colony was 17 000 reproductive females with a pupal production of about 7000 per week.

Discussions were held with the Nuclear Energy Corporation of South Africa (NECSA), located in Pretoria, to explore possibilities for the establishment of a large tsetse mass-rearing factory. Several abandoned buildings at NECSA seem to be suitable for conversion into such a facility, which would reduce significantly the initial investment. The selection of NECSA as a location to establish a mass-rearing facility for tsetse was based on several criteria, i.e. the land area allows for easy expansion; the environment is not conducive to the survival of tsetse (no biosecurity needed); temperature and humidity are modest, thereby reducing the rearing costs; radiation facilities are present on site; large cities are in the vicinity facilitating the purchase of supplies and equipment; and an airstrip is available which would facilitate the transport of sterile tsetse flies from Pretoria to KwaZulu-Natal (FAO/IAEA 2004).

4. Development of an AW-IPM Strategy

The data emanating from these studies strongly suggest that a sustainable solution to the AAT problem in the province of KwaZulu-Natal in South Africa could be found through the creation of an area free of *G. brevipalpis* and *G. austeni* using an AW-IPM approach with an SIT component. KwaZulu-Natal not only has high agricultural potential as evidenced by the many communal farming areas, but the tsetse-infested area is situated at the southern-most limit of the tsetse distribution. Except for a limited extension of the fly belt into Mozambique (Fig. 2), the tsetse-infested area in KwaZulu-Natal is completely isolated from the rest of the tsetse belt. Any area-wide control programme in KwaZulu-Natal would therefore require a collaborative agreement with Mozambique, and efforts have been initiated to develop a common strategy. In addition, the total size of the infested area (ca 12 000 square kilometres) is small enough to be manageable, it has only two species of tsetse which are already adapted to artificial rearing conditions, and the programme can be conducted in four consecutive phases, each treating fairly isolated blocks.

4.1. The Strategy

Using the data and the results of the various studies, a potential strategy to create an area free of *G. austeni* and *G. brevipalpis* was proposed (Kappmeier Green 2002, 2003). The proposal suggests the division of the total tsetse-infested area of KwaZulu-Natal, which comprises around 12 000 square kilometres, into four Zones, each having a manageable size of 2600-3500 square kilometres, which could be addressed sequentially (Fig. 4). Zone I (2600 square kilometres) contains only *G. brevipalpis*, whereas Zone III (3500 square kilometres) is mainly infested with *G. austeni* and Zone II (2200 square kilometres) and Zone IV (3000 square kilometres) contain both tsetse species. Each project phase would include pre-suppression (preparatory activities), suppression, release of sterile males and post-eradication operations (verification). Each of these phases would be implemented successively in the four blocks according to the rolling carpet principle described by Hendrichs et al. (2005) (Fig. 5). Control efforts would begin in Zone I and proceed progressively eastwards to Zone II and then northwards to Zones III and IV (Fig. 4). The

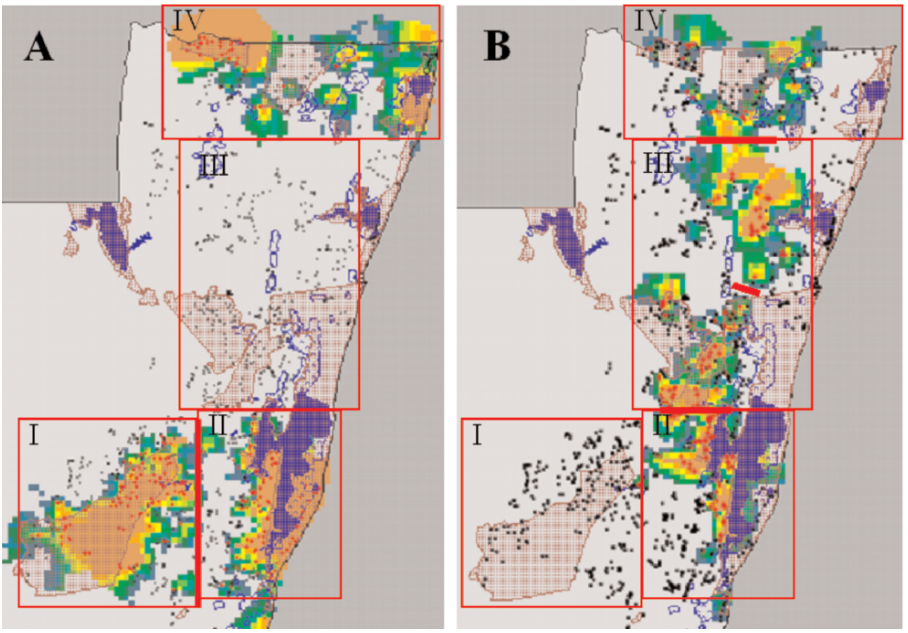

Figure 4. *The areas of KwaZulu-Natal infested with (a)* Glossina brevipalpis *and (b)* Glossina austeni *and the four control zones of the AW-IPM strategy.*

four operational activities (pre-suppression, suppression, release and verification) would be carried out sequentially in a phased manner (Fig. 5), with activities occurring simultaneously in the four zones, until all have been successfully freed of tsetse flies. Available data indicate that the northern *G. brevipalpis* and *G. austeni* populations extend to only about 25 kilometres northwards into Mozambique (up to around 26° 15' S), which is then interrupted by a natural fly-free zone before tsetse are once more found (Sigauque et al. 2000). This extension of the tsetse belt into Mozambique will have to be incorporated in Zone IV. It is estimated that six years of operational field implementation will be needed to eliminate both tsetse species from KwaZulu-Natal and southern Mozambique (Fig. 5).

4.2. Population Reduction

Reduction of the tsetse population is recommended to be done by two or three cycles of spraying with non-residual insecticides (SAT), using fixed-wing aircraft and helicopters in the more challenging areas (e.g. valleys of the Lebombo range). The spraying cycles in each block will be separated by less than 14 days. The cost to implement these spraying cycles is estimated at USD 170/km^2. It is anticipated that this operation will be implemented through a contractual agreement with a private company, which has the necessary expertise and equipment. Spraying operations in each block will be preceded by several months of preparatory activities. The budget for the spraying activity is estimated to be on average USD 480 000 for each zone.

4.3. Sterile Male Requirements

Using the data from the AW-IPM programme that eliminated *G. austeni* from Unguja Island, Zanzibar, it is estimated that each square kilometre will require 100 sterile males for a total of 78 weeks. The total weekly sterile male *G.*

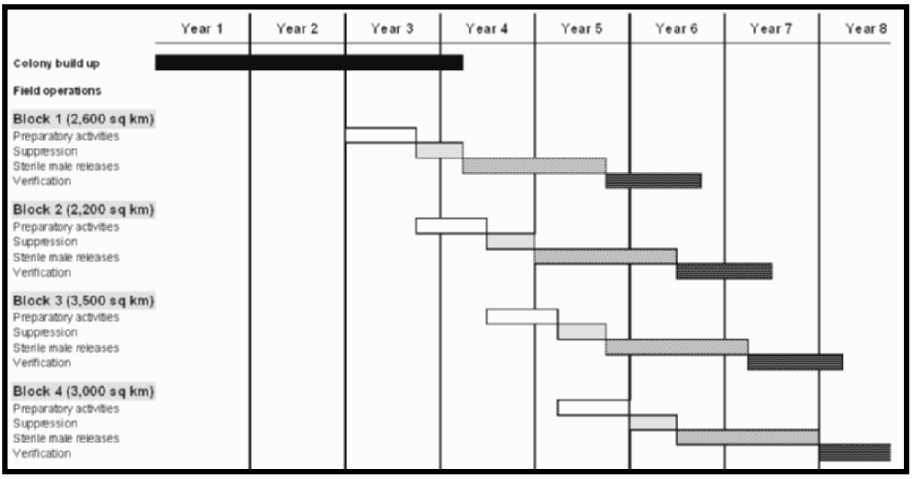

Figure 5. *Temporal diagramme of the control strategy in the four control blocks according to the rolling carpet principle (Hendrichs et al. 2005).*

brevipalpis requirements will therefore be 260 000, 200 000 and 300 000 tsetse for Zones I, II and IV, respectively. For Zones II, III and IV a total of 200 000, 350 000 and 200 000 sterile male *G. austeni* will be required per week, respectively. Taking into account the overlap in the various phases and in the different zones, a colony of 4.5 million *G. brevipalpis* and of 5.5 million *G. austeni* producing females will be needed to provide sufficient sterile males for the intervention. The time required to reach the projected colony target sizes will depend on the initial size of the colony and the production parameters (i.e. colony growth) (Fig. 6). It will take 41 months to reach a colony size of 5.6 million tsetse with an initial colony of 15 000 producing females and a 16% increase in colony size every month (i.e. corresponding to a doubling time every 5-6 months), whereas with an initial colony of 100 000 females and a 25% increase in size every month (doubling time every 3-4 months), this target will be reached after only 19 months.

The price per sterile male fly was estimated at USD 0.15 and includes the refurbishment costs of the buildings at NECSA, the equipment, operating costs and local staffing. A total of 59 million sterile male *G. brevipalpis* and 58 million *G. austeni* will be needed to cover the total area with 100 sterile males per square kilometre and the estimated budget for this component is USD 2.2 million per annum. However, if the sterile male requirements could be reduced to only 75 sterile males per square kilometre (e.g. as a result of very effective suppression), the required annual budget would be reduced to USD 1.6 million.

4.4. Sterile Male Transport and Dispersal

The sterile male flies will be transported with light aircraft from Pretoria to KwaZulu-Natal. It is assumed that (1) each zone can be covered by two releases every week, (2) the total ferry time is eight hours per week, (3) the dispersal flights can cover 500 square kilometres in one hour and, (4) the cost of each flying hour is USD 400. The annual budget for this activity (for four years of releases) is estimated at USD 425 000.

4.5. Management and Budget Requirements

Area-wide intervention campaigns with an SIT component are inherently complex with many important and essential programme

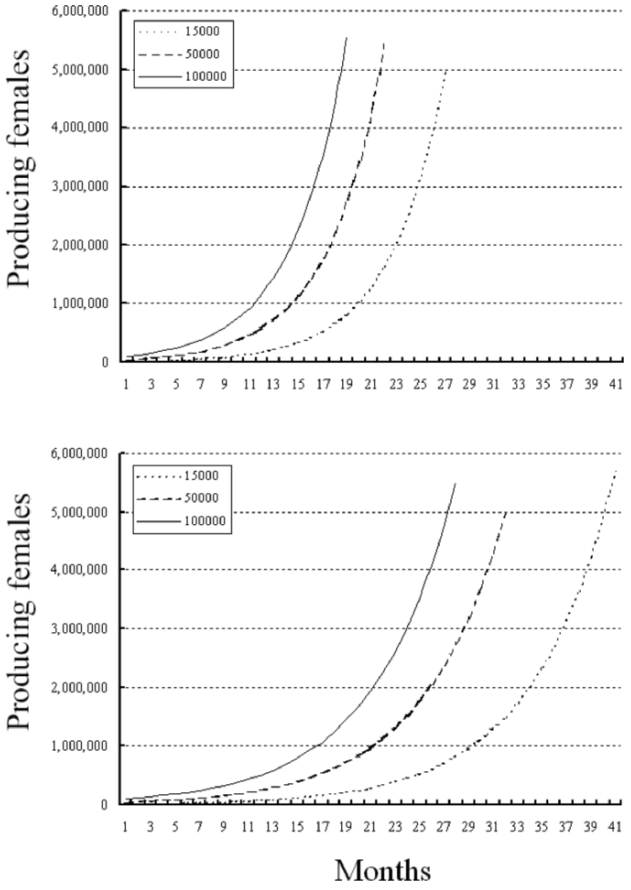

Figure 6. *Colony development with an initial colony size of 15 000, 50 000 and 100 000 producing females and assuming a 25% (upper graph) and 16% (lower graph) increase in colony size every month.*

components. Failure to implement one component will jeopardize the entire operation. Such programmes usually require a management structure that is politically and financially autonomous and independent of government bureaucracies and politics (Dyck et al. 2005). Establishment of management structures like the screwworm commissions (established between the USA and Mexico and the Central American countries), or the Screwworm Emergency Centre for Northern Africa (SECNA) office established by the Food and Agriculture Organization of the United Nations (FAO) for the management of the screwworm programme in Libya, that have the flexibility but also the responsibility and accountability for implementing all programme components, would be a prerequisite for success (Vreysen et al., this volume). The annual cost to operate a commission with a foreign and local director, six rearing specialists, two release experts, two monitoring experts, and adequate fleet of vehicles, office equipment and operating costs, is estimated at USD 572 000 (this excludes the national field and rearing staff). The estimated budgets of

the various project components have already been indicated above. It is estimated that an annual budget of USD 3.35 million would be required for the total duration of the programme (eight years).

5. Future Challenges and Prospects

Whereas the benefits of a sustainable tsetse-free area in KwaZulu-Natal are obvious, not all are convinced of its usefulness, and it is anticipated that the proposal will face serious opposition from some sectors of society. South Africa is a signatory to the UN Convention on Biological Biodiversity, the World Heritage Convention and the Ramsar Convention. There are eighteen protected areas gazetted within the boundaries of KwaZulu-Natal, some of them in the tsetse-infested areas, and some with the status of a World Heritage Site. Thus, there is a strong environmental lobby in South Africa, which has raised many concerns with respect to the removal of tsetse and the preservation of biodiversity. The use of insecticides is strongly opposed and there is great concern about the direct and indirect effects of the applied control techniques on non-target species. However, Grant (2003) pointed out that the aerial spraying (SAT) of the wetlands in this region is unlikely to present a real threat to the biodiversity of the ecosystems and that the biggest risk for terrestrial biota is the change in land use. Therefore, the apparent conflict between those that advocate the preservation of the biodiversity and those who favour socio-economic development is an important one that cannot be ignored. It will require unbiased scientific analysis and an objective and balanced approach if practical strategies are to be formulated and implemented.

The implementation of the control campaign according to the proposed strategy will entail the maintenance of a colony of ten million producing tsetse females. It is well known that the mass-rearing of tsetse flies is challenging but feasible, as was demonstrated by the maintenance of a colony of up to one million female *G. austeni* at the Tsetse and Trypanosomiasis Research Institute in Tanga to provide the required number of sterile males for the eradication campaign on Unguja Island, Zanzibar (Msangi et al. 2000). In South Africa, first but important steps have been taken to develop a rearing capacity for tsetse, i.e. a thriving seed colony of both species of tsetse has been established at the Agricultural Research Council-Onderstepoort Veterinary Institute, initial training has been provided to key staff, and serious interest has been expressed by NECSA to provide facilities for the development of a mass-rearing complex. However, the lack of an adequate pool of trained and experienced rearing staff (both senior and at the technician level), and the current lack of funding (both for the refurbishment of the building at NECSA and for the operating costs), remain important bottlenecks that will have to be removed. In addition, consideration needs to be given to the time needed to expand the colony to its required size (Fig. 6).

Similarly, the successful implementation of the field component of the programme will require a sufficient number of skilled and trained personnel in the field. The training component should already be implemented prior to the start of an eradication programme. Another prerequisite for success is the political willingness to set up independent, flexible management structures, and to contract private companies for the SAT operations and the release of sterile flies.

The above-mentioned challenges become meaningless without the political and financial commitment of the central Government of South Africa. Vision and leadership are needed to advocate and promote the approach of creating a tsetse-free area as the most sensible and cost-effective way to solve the AAT problem in South Africa (as opposed to an attitude of resignation and a philosophy of "learning how to live with the problem"). The disease is restricted to only one province in South Africa and is consequently not perceived as a national priority. The Provincial Department of Veterinary Services of KwaZulu-Natal will

therefore have to play a leading role to promote this programme and create awareness of its political significance and socio-economic dimensions, up to the highest political level. Only then can adequate funding be expected through the national Department of Agriculture and outside donors and partners. Further political support from the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), and the technical support of international organizations such as the FAO and the International Atomic Energy Agency (IAEA) will be valuable but essential contributions to the cause. With sufficient commitment from the government, the donor community and international organizations, it will be feasible to eradicate the two species from this region. This would enhance agricultural advancement in the 12 000 square kilometre area currently infested with tsetse flies and would improve the health and productivity of 350 000 cattle belonging to farmers in communal farming areas and 9000 cattle on commercial farms. Should the proposed eradication strategy be initiated and implemented, the end result would be a permanent removal of tsetse flies from South Africa.

The implementation of such an area-wide programme would be challenging. However, if successful, it could serve as a motivation and model for the development of similar area-wide programmes in neighbouring countries with similar isolated fly belts (although each situation would be unique) and would certainly generate the required financial support. The proposed strategy has been designed according to data available at present, which may be adapted as new or updated data becomes available due to newly developed techniques.

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Area-Wide Control of Tsetse and Trypanosomosis: Ethiopian Experience in the Southern Rift Valley

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ABSTRACT In 1997, the Ethiopian Government – assisted by the International Atomic Energy Agency (IAEA) – initiated a project in the Southern Rift Valley called the Southern Tsetse Eradication Project (STEP). Its long-term objectives are: (1) to create a tsetse-free zone in a 25 000 square kilometre area under agricultural development, and (2) to develop adequate national capacity for applying the concept of area-wide integrated pest management (AW-IPM) with a sterile insect technique (SIT) component to other parts of the country affected by the tsetse and trypanosomosis (T and T) problem. This project will require consistent commitment and inputs by major stakeholders over a period of at least 15 years. The project was initiated with the collection and evaluation of entomological, veterinary, environmental and socio-economic baseline data which reconfirmed the presence of only one species, i.e. *Glossina pallidipes* Austen, in the main valley, and the positive socio-economic and agro-ecological impact anticipated. This situation generated international acceptance of the Southern Rift Valley as a high priority area for the control of T and T and for related sustainable agriculture and rural development. A colony of *Glossina pallidipes* Austen originating from the Southern Rift Valley was also initiated. In 2002, community-based tsetse suppression was initiated in localized areas using insecticides on cattle and on blue-black-blue fabric targets that attract tsetse flies. These localized tsetse suppression activities have been expanded to all operational grids of the 10 500 square kilometre STEP block-1 area. Limited entomological and veterinary monitoring in 15 sites suggests that the apparent density of *G. pallidipes* in these localized control sites may have been reduced by 92%, while the prevalence of trypanosomes in livestock in those areas decreased by 58%. An analysis using geographic information systems (GIS) has indicated that the community-based tsetse suppression does not cover all of the tsetse-infested areas in the STEP block-1 and it is therefore assumed that some cattle herds remain with high disease prevalence in areas that were not adequately covered by the community fly control measures. The operational programme will include the introduction of a set of implementation rules and regulations conducive to the special needs of an operational AW-IPM campaign, i.e. an efficient management structure and the provision of adequate financial flexibility.

KEY WORDS *Glossina pallidipes*, *Glossina fuscipes fuscipes*, baseline data collection, sterile insect technique, tsetse-free zone, area-wide IPM

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1. Introduction

Ethiopia's economy is largely based on agriculture, which contributes 40-50% to the country's gross domestic product and provides 85-90% of the employment opportunities in the country. Agriculture accounts for 73% of rural incomes and 90% of foreign exchange earnings. Most agricultural products are provided by integrated crop-livestock systems and the livestock subsector alone contributes 40% of the agricultural production of the country, excluding draught power and manure and 16% of the country's total gross domestic product.

Livestock are vital as an energy source for most agricultural processes as they contribute to traction, cultivation, transport and threshing. Nevertheless, the economic potential of the livestock subsector is not fully realized mainly due to the widespread occurrence of livestock diseases. Among these, tsetse-transmitted African animal trypanosomosis (AAT) causes severe losses and prevents productive agriculture in some 200 000 square kilometres of western and south-western Ethiopia. Trypanosomosis limits the expansion of the national herds and farming practices by denying access to vast woodland and savannah areas with good grazing and agricultural potential. Consequently, there are high concentrations of livestock and people in the Ethiopian highlands, which results in overgrazing and environmentally unfavourable farming practices leading to both soil erosion and a progressive decline in average farm size. Over the last three decades, various attempts have been made to control tsetse and trypanosomosis (T and T) in Ethiopia but these were rather fragmented and uncoordinated and lacked the proper policies and strategies needed to produce significant and sustainable impact. Indeed, during the past 25 years, tsetse flies have expanded their distribution limits and have advanced towards the tsetse-free highlands by some 200 metres (Kassaye et al. 1995, Vreysen et al. 1999).

As part of the Government's programme for sustainable development and poverty reduction, high priority is given to the appropriate utilization of fertile land areas that are infested with tsetse. To this end, the decision was made to adopt an area-wide integrated pest management (AW-IPM) approach involving the release of sterile males in the final phase (Feldmann 2004, Klassen 2005). The project was initiated in 1997 and is called the Southern Tsetse Eradication Project (STEP).

2. The Southern Tsetse Eradication Project (STEP)

2.1. Project Objectives

The project has the following objectives: (1) to establish capacity at national and regional levels for sustainable removal of the T and T constraint by integrating different methods, including the sterile insect technique (SIT), (2) to introduce and apply these techniques on an area-wide basis to remove the T and T constraint from an area of 25 000 square kilometres in the Southern Rift Valley of Ethiopia, and (3) to create conditions for reducing pressure on highland resources and promoting sustainable agriculture and rural development in the Southern Rift Valley.

2.2. Project Area

The project area comprises about 25 000 square kilometres of the Southern Rift Valley, located between 4° 45' and 7° 15' northern latitudes and 36° 40' and 35° 20' eastern longitudes (Fig. 1).

This fertile lowland area, which is as low as 460 metres above sea level, is surrounded by highlands that rise to 4200 metres above sea level. The climate varies between humid and arid and annual precipitation is between 300-700 millimetres in the lowlands and 500-1200 millimetres in the highlands. Average temperatures range between 11 and 38°C. Flora and fauna are abundant both within and outside the conservation areas that are situated within the project area such as the Nechisar National Park.

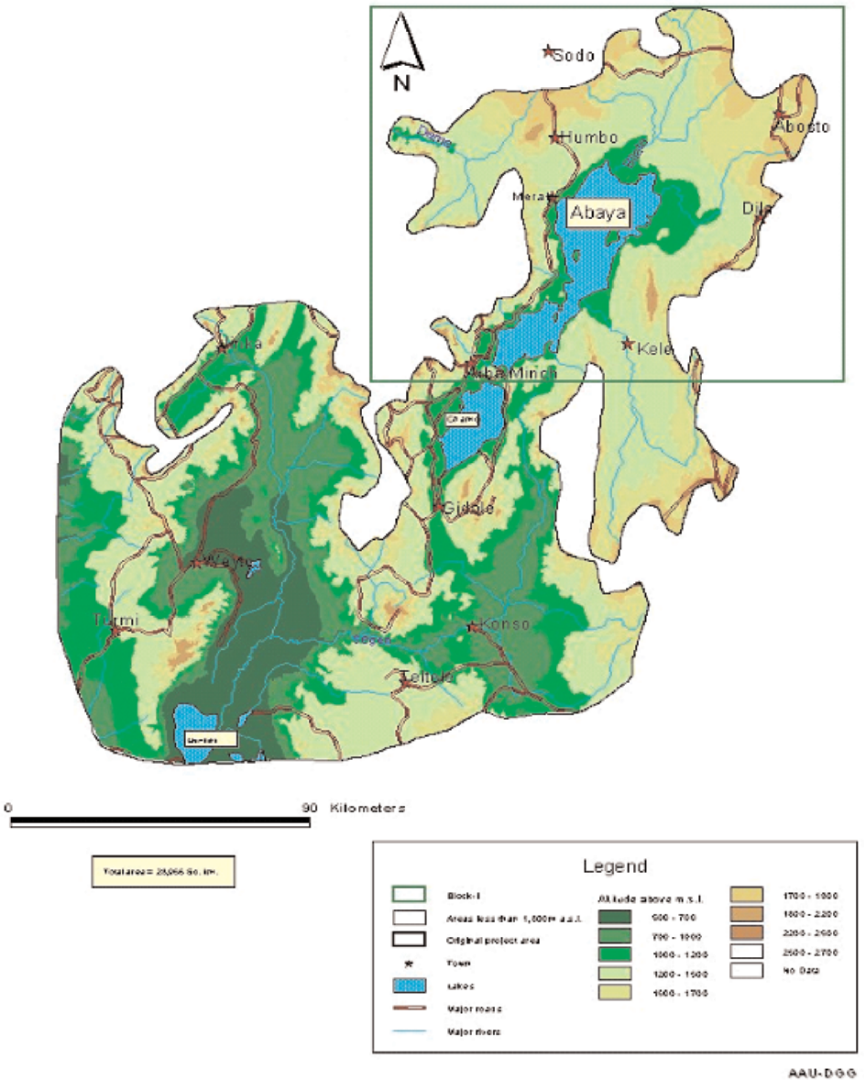

Figure 1. *The Southern Tsetse Eradication Project (STEP) area. The area within the green box around Lake Abaya represents the 10 500 square kilometre STEP block-1 area.*

In the Southern Rift Valley, the creation of a T and T-free zone (FAO 2002) is feasible as the target area is confined by high escarpments in the east, north and west and by vast arid land in the south, substantially reducing the risk of reinvasion from other tsetse-infested areas. Furthermore, in the main valley only *Glossina pallidipes* Austen has been found, although *Glossina fuscipes fuscipes* Newstead occurs in the Deme basin; a smaller valley in the north-western part of the Southern Rift Valley, which is connected with the Omo

River basin in the west.

2.3. Approach

Planning and implementation of project activities are based on the AW-IPM concept and require: (1) flexible management with clear lines of authority at national and regional level (Dyck et al. 2005), (2) adequate training and capacity building in management, sterile male mass-production, sterile male releases, and field monitoring, (3) methods development and refinement on tsetse mass-rearing, suppression, barrier systems, monitoring and overall quality assurance, and (4) technical activities such as baseline data collection, area-wide tsetse suppression, mass-production of tsetse flies, aerial releases of sterile male tsetse flies (Vreysen et al. 2000), and (5) entomological and parasitological monitoring. In addition, internationally agreed standards and procedures (Barclay et al. 2007) will be followed to verify eradication.

2.4. Organizational Structure and Role of Stakeholders

The project is managed by the Ethiopian Science and Technology Commission (ESTC) in close interaction with the authorities of the Southern Nations Nationalities and Peoples Regional State (SNNPR) and the Oromiya Regional State. Additional technical and research support is provided by national and international research institutions and mandated specialized United Nations (UN) agencies. A steering committee, made up of representatives of relevant regional agricultural bureaus, the Federal Ministry of Agriculture, the Ethiopian Institute of Agricultural Research (EIAR), the ESTC, the International Atomic Energy Agency (IAEA), the Food and Agriculture Organization of the United Nations (FAO) together with the project management is responsible for providing strategic administrative and technical policy decisions. It is assisted by a technical advisory committee, consisting of representatives of academic, national and international research and UN institutions/organizations, and by national regulatory institutions. A national project coordination office, headed by a national coordinator manages day-to-day activities in addition to relevant national and regional capacity building efforts. The mass-rearing and irradiation facility at Addis Ababa-Kaliti is responsible for colony establishment, mass-rearing (including blood collection and diet processing), irradiation of male tsetse flies and preparations for release operations. The regional project coordination office in Awassa is responsible for the planning, implementation, monitoring, evaluation and overall coordination of field operations. There are also field operation teams located in Arba Minch, Dilla and Sodo.

Community awareness is important for generating acceptance among, and support from, the beneficiaries (Dransfield and Brightwell 2004). This concerns particularly human resources, without which project staff would not be in a position to implement the various activities at the necessary scale. The existing "grass root" regional government structures, including the agricultural extension services at district administrations and in the peasant associations, are instrumental in generating the required awareness and support in the communities.

3. Project Activities and Results

3.1. Capacity Building and Training

Besides extensive technical training of project staff, for which IAEA provided substantive support, major emphasis was on awareness generation and training of communities. In total, 3444 community members and district leaders participated in local awareness courses and in training exercises on community-based tsetse suppression. This aimed to reduce theft and vandalism of traps and targets in the field, and to obtain labour for tsetse suppression. The formation of "anti T and T school clubs" in ten selected schools in the STEP block-1 area generated additional awareness and support.

3.2. Baseline Data Collection and Analysis

In 1998, field operations were initiated in the 10 500 square kilometres of STEP block-1 (Fig. 1). Using 1:50 000 scale topographic maps with a standard universal transverse Mercator (UTM) grid overlay, this area and its surroundings were divided into 105 grids of 10×10 square kilometres each. This enabled the entomological, parasitological, environmental and socio-economic baseline data collection to be planned with local stakeholders and relevant national and international partner institutions, including the Faculty of Veterinary Medicine and the Departments of Geology and Biology of the Addis Ababa University (AAU), the Ethiopian Animal Health Research Institute of the EIAR, the International Livestock Research Institute (ILRI) and the IAEA.

3.2.1. Entomological Survey

These surveys were carried out in four consecutive seasons between September 1998 and October 1999 in all 105 UTM grids of block-1 at an altitude of up to 2000 metres above sea level. Between 20 and 25 grids were allocated to each of five field teams, which deployed 8-25 NG2G traps (Brightwell et al. 1987), baited with cattle urine and acetone, in suitable fly habitats in each grid for 72 hours. All trap sites were geo-referenced using hand held global positioning system (GPS) units. and information was gathered from more than 1900 trap sites in different habitat types (Vreysen 2000).

The surveys revealed the presence of *G. pallidipes* in the main valley with *G. f. fuscipes* being present in the Deme basin which has an area of 750 square kilometres and represents about 7% of the STEP block-1 area. *G. pallidipes* was sampled in 58 grids while *G. f. fuscipes* was detected in 13 grids. In eight grids both species were captured. The apparent density of the *G. pallidipes* fluctuated between 0.01 and 68.6 flies per trap per day. Most of the *G. pallidipes* flies were trapped in forests, woody grassland and bush land up to an altitude of 1990 metres above sea level (Vreysen et al. 1999, Vreysen 2000). In general fly densities were relatively low in the eastern part of block-1 and high in the thicket areas bordering Lake Abaya in the west.

3.2.2. Parasitological Survey

Based on the entomological survey and taking into account farming practices and habitat types suitable for livestock keeping, 61 geo-referenced sites were selected to collect parasitological and serological baseline data during the wet season (May-July) of 1999 and the dry season (February-April) of 2000. Blood samples were examined for the presence of trypanosomes using the buffy coat dark ground phase contrast technique (Murray et al. 1977). Sera were also collected for screening using an antibody detection enzyme linked immunosorbent assay (ELISA) (Rebeski et al. 2000) at the Animal Health Research Centre of EIAR at Sebeta.

In 35 of the 61 sampling sites, livestock were positive for one or more tsetse-transmitted *Trypanosoma* species. In most of the "positive" sites the preceding entomological surveys had confirmed the presence of tsetse flies. However, at few locations infected animals were found where no tsetse flies had previously been detected. This may be due to the tsetse density being below that which is detectable by trapping and/or because infected animals may have been moved into these sites.

3.2.3. Environmental Survey

This was conducted in March 2001-February 2002 in order to identify and, to a certain extent, quantify the potential direct or indirect risks of T and T intervention (Wilson et al. 2002). Five transects were identified representing all land use patterns in the project area.

The findings can be summarized as follows: the SIT is not expected to have any direct impact on non-target organisms. The known minor impacts of using pyrethroids are believed to be short-lived. Tsetse intervention may not be as important in influencing land

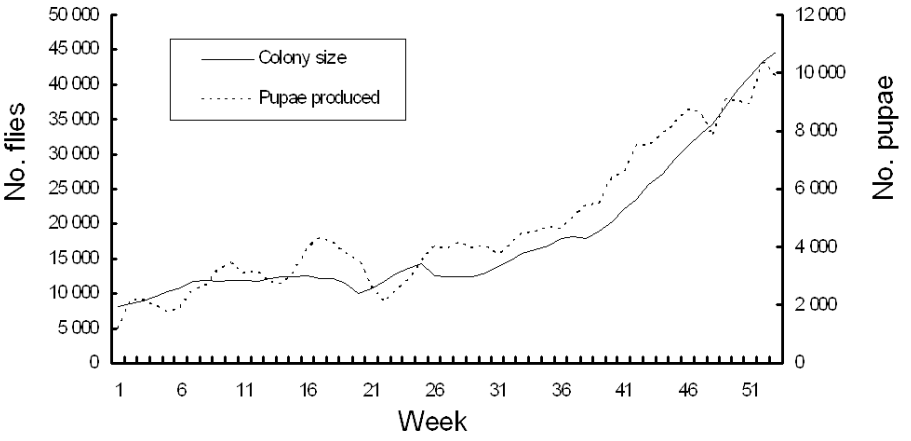

Figure 2. *Tsetse colony size and pupae production at Kaliti in 2004.*

use and land cover changes as other factors. Tsetse removal in the lowlands is expected to indirectly impact land use both in the highlands and in the lowlands. In the highlands cropland may contract as people move to the lowlands, and some native biodiversity may be regained, although not fully restored. In the lowlands, grazing, cultivation and other forms of land use are expected to expand, leading to increased pressure on riparian woodlands. Unless protective measures are taken, riparian woodland and bushland are at risk of becoming bushland and wooded grassland, respectively, bringing along losses in biodiversity. Additional "knock-on" effects will largely depend on other factors such as increased access through improved tracks and roads and migration or settlement measures. The environmental baseline data collection identified monitoring tools and indicators to help in assessing the appropriateness of land use after removal of the T and T problem, thus enabling early corrective measures to be taken. The study also highlighted the need to integrate the socio-economic and environmental assessments.

3.2.4. Socio-Economic Survey

In 2004 a socio-economic survey was carried out in the same areas/transects as described in section 3.2.3. Sixty-eight local enumerators, ten supervisors and 15 facilitators, under the supervision of a socio-economist, gathered information from 6754 households using specifically designed questionnaires (Rutebuka 2006). The data confirmed that AAT is a major obstacle for mixed crop-livestock farming systems, with 88% of the 5314 reported cattle deaths being due to trypanosomosis. According to earlier findings (Knight 2001) the break-even point for investments made for tsetse eradication in the Southern Rift Valley would be reached five to six years after eradication. The net present value over 12 years was estimated at USD million 37.4-53.8, while the internal rate of return ranged between 33 and 43%.

This information was instrumental in increasing international donor acceptance of the Southern Rift Valley as an area with high priority for T and T intervention and related sustainable agricultural and rural development, e.g. the African Development Bank (AfDB) and the United Nations Trust Fund for Human Security (UNTFHS or Japan Security Fund).

3.3. Tsetse Mass-Rearing

Activities at the Kaliti mass-rearing and irra-

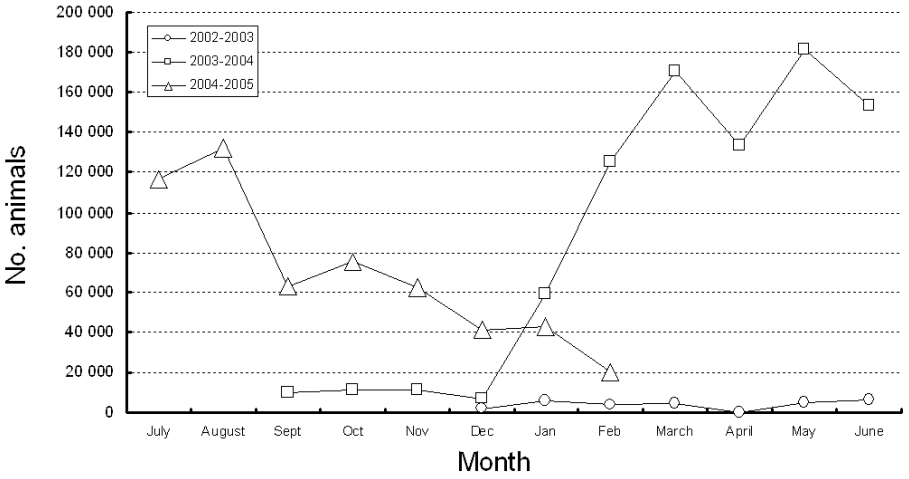

Figure 3. *Number of cattle treated with insecticides in the periods December 2002-June 2003, September 2003-June 2004, and July 2004-February 2005.*

diation facility were initiated in August 2000 following the renovation and equipping of an existing building to function as a temporary insectary. A ^{60}Co-Gammacel-220-Excel® irradiator was supplied in March 2003 for decontamination of blood and for insect sterilization.

Wild female *G. pallidipes* were shipped by air or vehicle from Arba Minch to Kaliti where the first three generations (F_1 to F_3) were kept separate, but pooled as of the 4th generation. Colony flies were held at 23-24°C and 75-80% relative humidity and under indirect low illumination (35 lux) at a 12-hour photo-scoto period. Flies were fed fresh-frozen cow blood using an *in vitro* silicon-membrane feeding system four days a week for about 15 minutes, using quality-tested blood initially imported from Austria. The availability of the ^{60}Co-Gammacel enabled locally collected blood to be processed and used for fly feeding. Despite some set-backs, by December 2004 colony size was over 44 000 females producing about 10 000 pupae per week (Fig. 2).

In parallel, work was initiated on the design and construction of a large tsetse factory at Addis Ababa-Kaliti that will consist of six rearing modules, one irradiation and sterile male module and other facilities. The Tsetse and Trypanosomiasis Research Institute at Tanga, Tanzania and the Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia, maintained seed colonies of *G. pallidipes* originating from Tororo, Uganda, which, according to field cage experiments, showed no mating barriers with *G. pallidipes* originating from Ethiopia. A colony of *G. f. fuscipes* is also being maintained at the latter institution.

In December 2000, 14 000 litres of local cow blood were collected from the Addis Ababa abattoir and stored. Following the acquisition of the gamma irradiator in early 2003 all batches of blood were decontaminated with 1.2-1.5 kGy gamma irradiation and subjected to routine microbial screening and a 25-30-day bioassay feeding test (Feldmann 1994).

3.4. Tsetse Suppression

STEP initiated community-based tsetse suppression in 2002, initially using traps and subsequently insecticide impregnated blue-black-blue targets supplemented by pour-on and

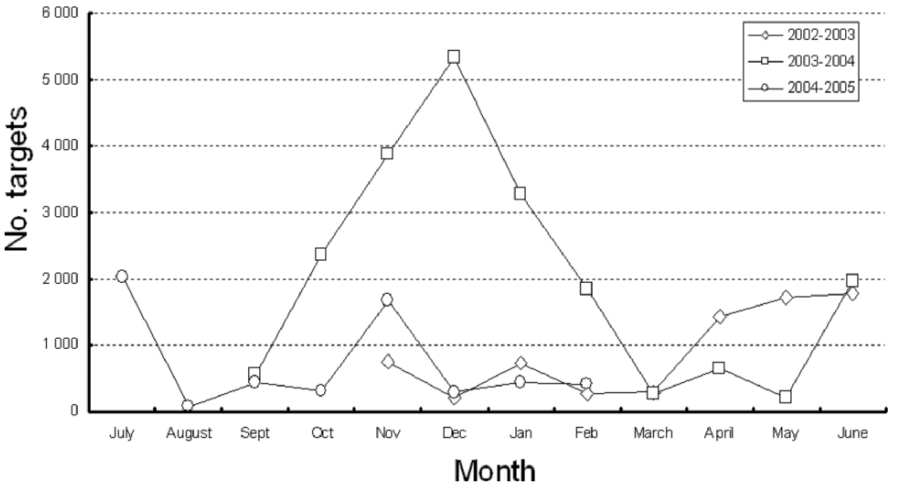

Figure 4. *Number of insecticide-impregnated targets deployed in the periods December 2002-June 2003, September 2003-June 2004, and July 2004-February 2005.*

spray formulations of insecticides on livestock. This suppression is underway in localized areas of the STEP block-1, of which about 20% is inaccessible and with additional 5-20% located in-between communal land also not being treated. The ongoing community-based tsetse control activities, therefore, are not area-wide and are insufficient to initiate the SIT phase.

3.4.1. Insecticide-Treated Cattle

Insecticide-treated cattle that move through tsetse-infested areas and attract sufficient tsetse flies for feeding can be used for tsetse suppression (Leak 1998). About one fifth of the number of cattle in a given herd was initially treated with special insecticide formulations. A 1% ready-to-use pour-on formulation of deltamethrin was applied with a T-bar along the back of the animal at a dose of approximately one millilitre per ten kilograms of body weight. This treatment requires that the herd be retreated monthly. Alternatively, e.c.-formulations of deltamethrin can be diluted to 0.005%, and animals are then sprayed with knapsack sprayers at intervals of two weeks. The number of animals treated in block 1 between December 2002 and June 2003, September 2003 and June 2004 and July 2004 through February 2005 were 26 513, 861 783, and 552 869, respectively (Fig. 3).

3.4.2. Insecticide-Impregnated Targets

Blue-black-blue targets (175 centimetres x 50 centimetres) made of polyester fabric, and impregnated with a 0.4-0.6% s.c.-formulation of deltamethrin were also used to suppress *G. pallidipes* (Leak 1998). The impregnation was done on specially designed tables using a roller brush or by soaking the target in a bucket filled with the insecticide solution. The impregnated targets were then allowed to dry and placed in a bamboo frame ready for deployment in selected sites. The numbers of insecticide-impregnated targets deployed in block 1 between December 2002 and June 2003, September 2003 and June 2004 and July 2004 through February 2005 were 7175, 20 343 and 5728, respectively (Fig. 4). This deployment was done with the assistance of specially trained community members under the supervision of STEP tsetse control personnel.

Annectodal evidence suggests that these suppression activities resulted in some

Table 1. *Results of the tsetse monitoring in each of five sites in the Dilla, Sodo and Arba Minch area (see Fig. 1 for locations).*

Area	Monitoring sites	Apparent fly density (flies/trap/day)	
		Pre-control	During suppression
Sodo	5	4.10	0.04
Arba Minch	5	4.56	0.44
Dilla	5	3.69	0.45
Total	15	4.11	0.31

improvements in the performance of their livestock as well as impacting on other ectoparasites, including ticks and nuisance flies.

3.5. Monitoring

The field teams at Arba Minch, Dilla and Wolaitha-Sodo that were supervising the suppression activities also took charge of the limited monitoring exercises.

3.5.1. Tsetse Monitoring

Each field team placed acetone and cow urine-baited NG2G traps at two monthly intervals for 72 hours at 15 identified locations. The tsetse fly apparent densities (flies per trap per day) was reduced from four to 0.3 flies per trap per day after initiating tsetse suppression.

Table 2. *Results of the parasitological monitoring.*

Area	Monitoring sites	Disease prevalence (%)	
		Pre-control	During suppression
Sodo	5	18.0	10.7
Arba Minch	5	12.0	0.0
Dilla	5	22.0	11.0
Total	15	17.3	7.2

(However, caution is needed in interpreting these data as they are from a very limited number of traps and can therefore not be considered representative for the entire project area) (Table 1).

3.5.2. Trypanosomosis Monitoring

Forty animals, randomly selected, were sampled for trypanosomosis prevalence at the same 15 locations as in section 3.5.1. Screening for trypanosomes involved blood collection from an ear vein with a heparinized capillary tube followed by centrifugation. The packed cell volume (PCV) was used to identify anaemic animals (i.e. PCV less than 25%), and the buffy coat was examined for trypanosomes using the dark ground phase contrast technique. Trypanosome positive samples were stained with Giemsa for species identification. Infected animals were then treated with a curative dose of three milligrams of Berenil® per kilogram body weight. Results (Table 2) indicate that the PCV values increased from an average of 23.2% pre-control to 27.9% during tsetse intervention. Also the prevalence of trypanosomosis declined over the same period from 17.3% to 7.2%, representing a reduction of 58%. This is a less drastic decline than that recorded for fly density, underlining the fact that tsetse flies are efficient vectors of trypanosomosis even at substantially reduced population levels. Therefore, a complete removal of the vector using the AW-IPM approach will be necessary for complete elimination of trypanosome transmission.

4. Conclusions

During the initial phase of the STEP project there has been high and consistent government commitment at national and regional levels in Ethiopia, recruitment and training of project staff in the laboratory and the field, emphasis given to strong community participation, involvement of local academia in various project activities and targeted technical and financial support from the IAEA, FAO, AfDB and the UNTFHS.

Besides expansion of tsetse suppression, mass-rearing and other activities, a flexible management system will need to be implemented based on the special needs of an AW-IPM programme with an SIT component. This will be characterized by financial flexibility in the context of a phased, conditional planning and implementation approach with the consensus of key national and international partners and stakeholders. An upscaling of the STEP operational activities in the rearing facility and the field will require additional trained and dedicated staff.

STEP has established a colony of *G. pallidipes* originating from Arba Minch, located in the STEP project area. In addition a back-up colony of this species originating from Tororo, Uganda, is maintained at the Slovak Academy of Sciences, which also maintains a colony of the second target species, *G. f. fuscipes*. Ongoing research addresses various rearing constraints encountered including pupal sexing and managing the impact of a virus on the productivity of the *G. pallidipes* colony.

Community-based tsetse suppression has been carried out in about 60%-65% of the STEP block-1 area and the very limited monitoring indicated localized reductions in the apparent fly density and the prevalence of trypanosomosis. It is considered that the use of the sequential aerosol technique (SAT) will be essential to achieve effective suppression in the 30-40% of the project area where community-based suppression is ineffective. A study will need to be conducted to assess the technical, environmental and economic feasibility of SAT and involve all relevant national stakeholders.

Other field aspects that need to be addressed include the introduction of a standardized, representative entomological monitoring system and the validation of the Access-based tsetse information recording and reporting (TIRR) database. Furthermore, areas where artificial barrier systems, consisting of a combination of insecticide-impregnated targets and insecticide-treated cattle, are required, need to be identified and validated using mark-release-recapture studies. The Deme basin is considered to be a suitable area for testing and refining these methods in the field.

The Ethiopian authorities, with the support of FAO, IAEA and African Union (AU)-Pan African Tsetse and Trypanosomiasis Eradication Campaign, have initiated efforts to generate the funding necessary for implementing all activities planned for the STEP block-1 area. USD 300 000 available through the United Nations Fund for International Partnership (UNFIP), co-financed by the USA will be used to prepare for and conduct partners' and donors' meetings.

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Don't Let Cacto Blast Us: Development of a Bi-National Plan to Stop the Spread of the Cactus Moth *Cactoblastis cactorum* in North America

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ABSTRACT The South American cactus moth *Cactoblastis cactorum* (Berg) was first detected in the continental USA on Big Pine Key in southern Florida in 1989. Although it was recognized as a potential threat to *Opuntia*-rich areas in the south-western USA and Mexico, actions were not taken to manage its spread because there are relatively few cactus plants in Florida and the moth was not known to disperse well over long distances. However, the moth has since spread along the coast of the Gulf of Mexico to the State of Alabama. In 2000, an initial meeting of assessment was held in Tampa, Florida, with subsequent planning meetings held in 2002 and 2003 to develop a strategic plan for research, detection, and control. A pheromone-based trapping system has now been developed and an area-wide management approach using the sterile insect technique (SIT) is being tested. In 2006, Mexico will begin contributing funds to help implement a bi-national plan to stop the spread of the cactus moth in North America.

KEY WORDS invasive pest, cactus moth, *Cactoblastis cactorum*, *Opuntia*, Lepidoptera, area-wide, SIT, USA

1. Introduction

Until its appearance in the continental USA as an invasive pest, the South American cactus moth *Cactoblastis cactorum* (Berg), was considered the poster child for biological control of weeds because of its role in reducing large populations of exotic *Opuntia* (prickly pear) cacti in Australia (Dodd 1940) and elsewhere around the world (Julien and Griffiths 1998). Based on this success, *C. cactorum* was intentionally introduced to the Caribbean island of Nevis in 1957 to control an unwanted complex of native *Opuntia* that were replacing grasses in overgrazed rangeland (Simmonds and Bennett 1966). The cactus moth later dispersed or was intentionally introduced to other Caribbean islands where it attacks both weedy and non-weedy native *Opuntia* species. It was reported from Montserrat and Antigua

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in 1960, Grand Cayman in 1970, St. Helena in 1971, and Ascension in 1973. The cactus moth also spread from Nevis to St. Kitts and to the US Virgin Islands (Simmonds and Bennett 1966). It was reported from Puerto Rico in 1963 (García-Tuduri et al. 1971). The cactus moth is currently also present in Haiti, the Dominican Republic, the Bahamas, and Cuba (Zimmerman et al. 2005).

2. Detection in the USA

C. cactorum was first recorded in the continental USA in 1989. Habeck and Bennett (1990) reported the discovery of cactus moth adults in the Florida Keys in October 1989. In addition, Dickle (1991) collected larvae from infested *Opuntia stricta* (Haworth) Haworth in this same area in 1989 and again in May 1990. Between May 1990 and October 1991 collections of cactus moth were made along both Florida coasts up to locations approximately 350 kilometres north of the initial detection site in the Florida Keys. By 1999, the cactus moth was reported from Cumberland Island on the southern coast of the State of Georgia. Hight et al. (2002) found infestations as far north as Folly Island near Charleston, South Carolina, and as far west as St. George Island, Florida. The current (2005) limits of cactus moth distribution along the Atlantic and Gulf coasts are at Bull Island, South Carolina, and Dauphin Island, Alabama, respectively (S. Hight, unpublished) (Fig. 1).

3. Life Cycle

C. cactorum is native to northern Argentina, Uruguay, Paraguay, and southern Brazil (Mann 1969). Larvae are phytophagous and feed on numerous species of prickly pear cacti (*Opuntia* spp.). Adult females lay eggs in a vertical chain containing 50-100 eggs that is glued to a cactus pad or cactus spine. Egg sticks take four to five weeks to develop. Upon eclosion larvae burrow into the cactus pad where they feed gregariously. A cohort of

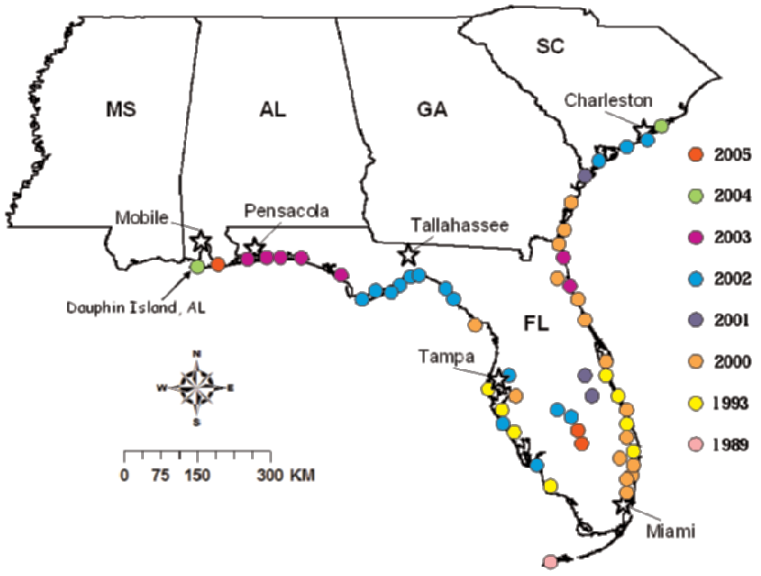

Figure 1. *Detection by year of larval infestations of* Cactoblastis cactorum *in the continental USA. Current efforts to limit the spread of the cactus moth are focused along the Gulf Coast near the western leading edge at Dauphin Island, Alabama.*

larvae typically destroys three to four cactus pads before completing development (Monro 1975). Fully developed larvae spin cocoons in the litter or between collapsed cactus pads (Pettey 1948). The cactus moth completes three generations per year in Florida (Zimmermann et al. 2004).

4. Potential Impacts

Garrett (2004) summarized the potential economic impacts from the spread of the cactus moth in the USA in a white paper for the United States Department of Agriculture-Animal Plant Health Inspection Service (USDA-APHIS). Simonson et al. (2005) prepared a preliminary assessment of impacts and risks associated with the spread of the cactus moth in the USA and Mexico for the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization of the United Nations (FAO). Currently, prickly pear cactus is of minor importance as a domestically produced food crop in the USA, although demand for both fresh and processed prickly pear pads (nopalitos) and fruit (tunas) has been increasing steadily. Production of prickly pear cactus for edible use in the USA is limited largely to California where 70-80% of the crop is produced from approximately 243 hectares of land.

Most of the commercial value of prickly pear in agriculture is in the ornamental nursery and landscape industries. In the State of Arizona this industry has been estimated to encompass 550 000 plants with a retail value of greater than USD 10 million. Prickly pear cactus is also important in the south-western USA as emergency forage for cattle during periods of drought and is considered integral to maintaining the quality of wildlife habitat for hunting-lease enterprises for animals such as the white-tail deer *Odocoileus virginianus* (Boddaert), javelina *Peccari tajacu* (L.), and bobwhite quail *Colinus virginianus* (L.).

The greatest value of *Opuntia*, however, lies in the ecological roles they play in native desert ecosystems, adding to wildlife habitat, ecosystem structure, and biodiversity in both developed and undeveloped areas. Establishment of the cactus moth in these areas could have effects far beyond a simple decrease in the number of *Opuntia* cacti. For example, in Florida, where cacti are a minor component of the native flora, there are three species of *Opuntia* that are limited to local populations in the Florida Keys and all are being attacked by the cactus moth (Pemberton 1995). These same cactus habitats are also shared by rare and endangered insects such as the Gerstaeckeria cactus weevil *Gerstaeckeria fasciata* Pierce. Other fauna associated with prickly pear cactus and affected by the loss of these host plants include the threatened gopher tortoise *Gopherus polyphemus* (Daudin) along the eastern edge of the Florida Everglades and the endangered San Salvador island rock iguana *Cyclura rileyi rileyi* Stejneger in the Bahamas (Zimmermann et al. 2004). Negative impacts such as these are expected to increase as the range of the cactus moth continues to expand. Additionally, further westward spread could lead the cactus moth into Mexico where prickly pear cactus is a major agricultural commodity and has significantly greater ecological and socio-economic importance (Hernández et al., this volume).

5. Developing an Action Plan

In order to discuss the cactus moth problem in the USA, a first meeting for assessment and planning was held in Tampa, Florida, in September 2000 with scientific experts, regulatory officials, and representatives from the conservation community from the USA, Mexico, and South Africa (Mahr et al. 2001). Meeting participants unanimously agreed that the cactus moth had the potential to be devastating to the fragile arid environments in the USA and Mexico. In July 2002, the FAO and IAEA hosted a cactus moth consultants meeting to review and evaluate the threat of *C. cactorum* to international agriculture and biodiversity (IAEA 2002). The role that the sterile insect technique (SIT) could play in addressing the cactus moth invasion as a model for invasive pests affecting not only

agriculture but the environment also was assessed at these meetings. Furthermore, FAO and IAEA agreed to support research in member states for developing the SIT. Subsequent stakeholder meetings were held in Miami, Florida, in December 2003 (www.invasivespecies.gov/profiles/cactmoth.html) and Mexico City in July 2004 (SAGARPA 2004).

In September 2004, the USDA-APHIS, Plant Protection and Quarantine (PPQ) committed to developing a strategic plan to delimit, monitor, and mitigate the spread of the cactus moth in North America. The strategic plan addresses concerns for: (1) survey and detection of infestations along and in front of the cactus moth's westward expanding range, (2) accurate identification of other Lepidoptera that feed on prickly pear cacti that may be confused with *C. cactorum*, (3) regulation of importation and domestic movement of prickly pear cacti plant material, (4) eradication and containment of known infestations using a combination of mechanical removal of infested plants (sanitation), application of insecticides, and releases of sterile insects, (5) research to refine monitoring and control protocols, (6) outreach activities to increase public awareness, and (7) cooperation with Mexico regarding cost and information sharing.

6. Researching Management Tactics

Scientists from the USDA's Agricultural Research Service (ARS) have now assembled a large body of knowledge about the cactus moths' spread in the south-eastern USA (Hight et al. 2002, Solís et al. 2004), and its behaviour and reproductive biology (Hight et al. 2003). They also have moved quickly to develop trapping protocols and evaluate both natural and synthetic lures (Bloem et al. 2003, 2005a), as well as to evaluate control strategies using different insecticides (Bloem et al. 2005b) and sterile insect releases (Carpenter et al. 2001a,b, Hight et al. 2005).

The SIT could have several applications for suppression of cactus moth populations: (1) it could provide a way to protect rare *Opuntia* cacti (such as those present in the Florida Keys) from attack, (2) it could be available as an eradication tool in new outbreak areas beyond the leading edges of current infestations, and (3) it could be used to erect a barrier to prevent or slow the expansion of the cactus moth's geographical range (Carpenter et al. 2001a). Considering the various control options available for the cactus moth, Stiling (2002) concluded that the use of SIT:

> *...offers perhaps the only realistic chance of drawing a line in the sand, literally, in Florida, and trying to prevent further spread of* C. cactorum *into the USA South-West and Mexico.*

The USDA-APHIS-PPQ, USDA-ARS, and US Geological Survey have also been working with Mississippi State University's GeoResources Institute to set up a web-based monitoring network (www.gri.msstate.edu/research/cmdmn/) for federal- and state-managed lands such as wildlife refuges, national parks and seashores, as well as lands managed by non-governmental organizations. These efforts will complement state departments of agriculture surveys of nurseries and residential properties using APHIS-PPQ's Cooperative Agriculture Pest Survey System, whose public site is at: www.ceris.purdue.edu/ napis/. APHIS-PPQ's Center for Plant Health Science and Technology (CPHST) has been using a risk zone mapping program (www.nappfast.org) to analyse cactus moth phenology data and map larval and adult activity to help predict the most appropriate times to monitor or survey for the moth's various life stages. CPHST has also been developing survey tools using handheld digital data collection systems (personal digital assistants (PDA)) and global positioning system (GPS) units to facilitate accurate data collection and the production of geospatial maps.

In 2005, APHIS initiated a cooperative research effort with ARS to validate the SIT, in combination with sanitation, as a comprehensive area-wide control and risk management strategy against *C. cactorum*, and as a

possible means of establishing a barrier that would stop the cactus moth's westward movement. The SIT evaluation involved three sites. The site along the leading edge (Dauphin Island, Alabama) received sterile insects and a sanitation procedure, a second site was only sanitized (Okaloosa Island, Florida), and a third site was left unchanged (St. George Island, Florida). Sterile cactus moths were released on Dauphin Island two to three times per week (500-5000 moths per release) during April/May, July/August, and October/November. The sanitation procedure involved the removal of cactus pads infested with *C. cactorum* larvae and the removal of all egg sticks and pupae that were encountered on a year-long basis. During this period, all three sites were monitored for adult flight activity (wild and sterile released males) using pheromone-baited sticky traps, as well as for *C. cactorum* infestation on sentinel host plants. The SIT procedure is being evaluated by comparing the magnitude of the change in larval infestations, wild moth captures, and sterile to wild overflooding ratios at each site over the course of the study.

7. Garnering Support for Action

When addressing invasive pest problems, the urgency of response is typically determined by the perceived value of the commodity at risk (i.e. the potential impact of the pest) and the level of outcry by affected stakeholders. Although alarm signals were raised by a number of scientists about the threat that the cactus moth posed to rare *Opuntia* in the Florida Keys and its likely impact should it spread (Habeck and Bennett 1990, Dickle 1991, Pemberton 1995, Johnson and Stiling 1996, 1998), very few people took notice. *Opuntia* cacti are not major components of the Florida landscape, they are of minor agricultural importance throughout the USA, the moth was not historically known to disperse rapidly, and stakeholders in the western USA where cacti are much more common have been largely silent. Not until the biological control community began to raise concerns that the cactus moth might be scrutinized as an example of non-target effects of classical biological control rather than as an invasive pest, and Mexico began to raise concerns about the cactus moth as an invasive threat to its cactus industry and the biodiversity of its desert ecosystems (Hernández et al., this volume), did regulatory officials become engaged.

Once a full assessment of the situation had been made, it became clear that significant knowledge gaps existed regarding the moth's biology and behaviour and very few tools were available to monitor and control its spread, and that these would have to be researched and developed (Mahr et al. 2001).

Rapid progress by ARS scientists in providing some of the necessary technologies has allowed APHIS to proceed with the development of a strategic plan for continued research and programme implementation. Additionally, an agreement between the USDA and Mexico's Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA) entitled, "Work Programme to Establish Management and a Containment Barrier for the Cactus Moth", through an agreement with the North American Plant Protection Organization (NAPPO), provides joint funding starting in 2006 for a broader binational implementation programme to stop/slow the spread of the cactus moth in North America.

Unfortunately, controlling the cactus moth is now a race against time. From 2001-2004, the moth moved westward along the Gulf Coast of the USA at the rate of 160 kilometres per year, limited largely to *Opuntia*-bearing barrier islands. Dauphin Island, Alabama, is the site of the current western-most infestation and it is the last barrier island with road access until Galveston Island in northern Texas. Although expanded efforts initiated in 2005 may help prevent the moth from moving further west, establishment of a true barrier and emergency response to such movement is still in its infancy. Once the moth reaches the south-western USA, the density and diversity of *Opuntia* cacti increase sharply and *Opuntia*

distribution from the coastline to the interior becomes more contiguous. Therefore, the cost of implementing an abatement programme to stop the spread of the cactus moth would also increase sharply if the infestation is allowed to move westward, and the opportunity to "draw a line in the sand" would quickly diminish.

8. Conclusions

Given the high number of exotic, potentially invasive pests entering the USA each year, and the limitations on funding and other management resources and tools available to address them, assessments of risk must be made and action priorities set. However as the cactus moth story illustrates, the perception of or actual risk may change over time. It also reinforces the fact that the window of opportunity to eradicate or contain a pest is limited. Although the potential impact of *C. cactorum* on *Opuntia* cacti in North America did not become a regulatory concern until 2000, more than ten years after its initial detection, research has since moved very quickly to provide management options. In the span of only five years, the irradiation biology of the moth has been determined, rearing and monitoring methods have been developed, and both field cage and open field trials of the SIT have been conducted. Time will now tell if these efforts and future support prove successful at managing the risk posed by this pest, or whether the line in the sand was drawn too late.

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Preventive Programme Against the Cactus Moth *Cactoblastis cactorum* in Mexico

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ABSTRACT The importance of the cactus moth *Cactoblastis cactorum* (Berg) as a successful biological control agent against ten *Opuntia* invaders in 20 countries is widely documented. Nevertheless, this species has also become a serious threat to the high diversity of both native and cultivated *Opuntia* species in many regions of the world. In particular its presence in the Caribbean islands, and its rapidly expanding range in the south-eastern USA, is an imminent threat to native *Opuntia* species in Mexico and the south-western USA. *C. cactorum*'s effective biological control role provides useful information on its expected impact should it arrive as an exotic invasive species in Mexico and the south-western USA. The cactus moth is particularly effective in the biological control of small- and medium-sized *Opuntia* weeds. Mexico and south-western USA are considered the most important centres of biodiversity for *Opuntia*. In addition the agricultural uses of *Opuntia* spp. are considerable and include forage, vegetables, fruit, cochineal and value-added medical and cosmetic products. Because of the high ecological importance and significant representation of *Opuntia* within production chains in Mexico, it is imperative to pay special attention to the *C. cactorum* threat by carrying out a preventive programme throughout the country involving: (1) monitoring and sampling in commercial and wild areas, (2) increasing awareness by the general public and establishing a regulatory system to prevent the entry and dissemination of cactus moth host materials, and (3) increasing readiness to be able to implement suppression, containment and eradication actions where and when required. In furthering these aims, the preventive programme in Mexico has counted on the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization of the United Nations (FAO) to provide financial and technical support for research and development, training in countries where the insect is established, and technology transfer. In addition, a bi-national programme between the USA and Mexico to stop the spread of the cactus moth in North America has been initiated.

KEY WORDS cactus moth, *Cactoblastis cactorum*, invasive species, *Opuntia*, prevention, monitoring, Mexico, quarantine

1. Introduction

Mexico has the highest genetic diversity of cactus. Within the Subfamily Opuntioideae alone, about 107 *Opuntia* species have been recorded (51 species of *Platyopuntia* and 56 of *Cilyndropuntia*), with 38 species being native to Mexico. It is estimated that about 150 000 hectares are cultivated for fodder (in addtion to the three million hectares grown wild), about 60 000 hectares for fruit (cactus or prickly pear) production (Cervantes-Herrera and Gallegos-Vazquez 2003), 10 500 hectares for a green vegetable called “nopalitos”, and 100 hectares for cochineal rearing, all based on a few *Opuntia* species. In addition, wild *Opuntia* cactus occur in many areas of the country, where they are used in many traditional ways by mainly rural communities. Cactus in Mexico is therefore an important food source for humans, livestock and wildlife. It is also used as a dye and to prevent and combat soil erosion. *Opuntia* species and their cultivation are a great source of employment, an important component of genetic biodiversity and a host for a wide variety of

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 345–350.

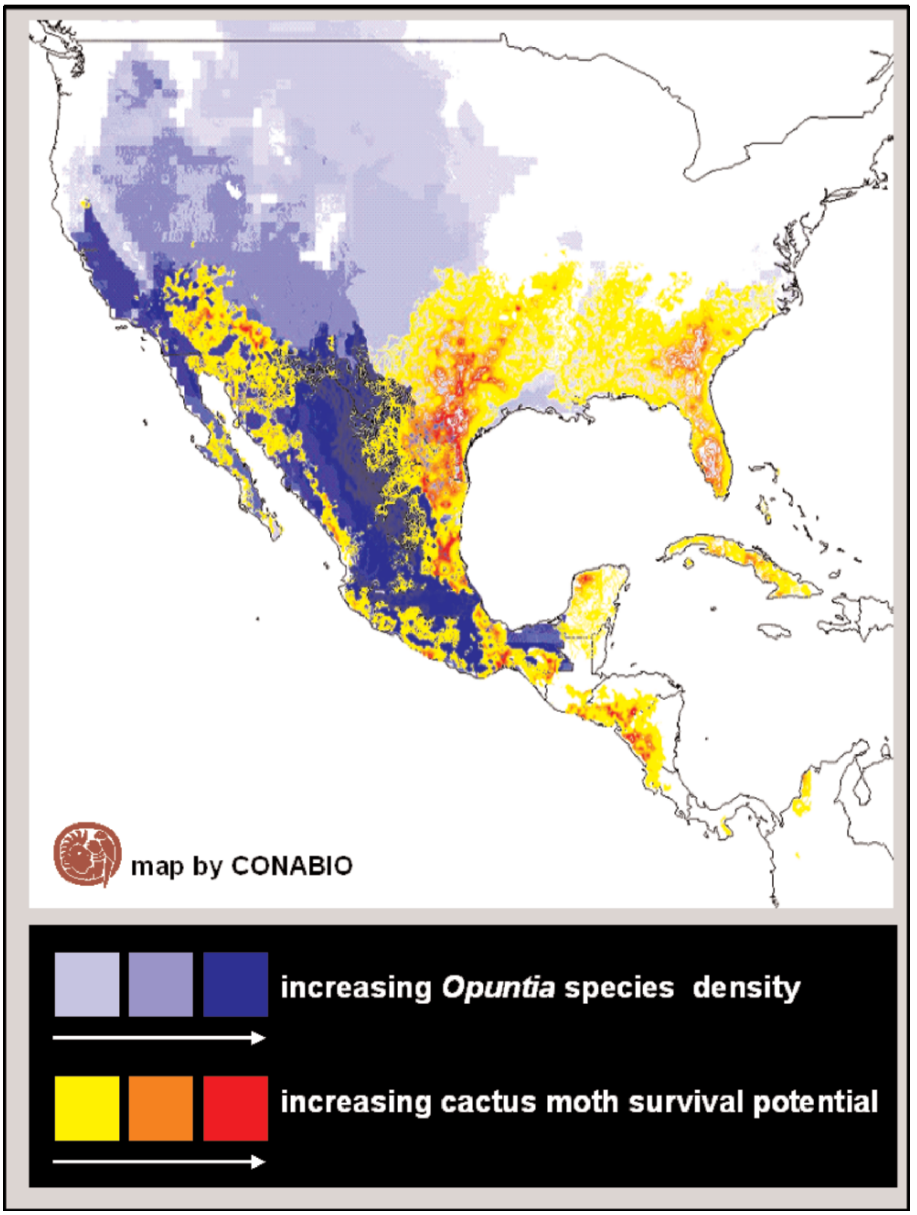

Figure 1. *Map of the USA, Mexico and Central America, indicating the density of* Opuntia *species and the potential of survival of* Cactoblastis cactorum.

wildlife.

The South American cactus moth *Cactoblastis cactorum* (Berg) in its larval stages is host-specific to *Opuntias* and it has been used as a highly effective biological control agent against invasive *Opuntia* species in

many countries including Australia (Dodd 1940, Moran and Zimmermann 1984), South Africa (Pettey 1948) and the Caribbean islands of Antigua, Grand Cayman, Monserrat and Nevis (Simmonds and Bennett 1966). It has now also spread to all other islands in the Greater Antilles and many in the Lesser Antilles where it is seriously affecting native *Opuntia* species (Zimmerman et al. 2001).

The insect was detected in Florida in 1989 (Habeck and Bennett 1990), and it is now dispersing westward in the USA towards Texas, New Mexico, and Arizona, thereby threatening vast areas where *Opuntias* are present, and with the potential to enter also Mexico (Johnson and Stiling 1998).

In response to the concern expressed by the scientific and conservation community in a meeting in Tampa, Florida in 2000 (Mahr et al. 2001), the International Atomic Energy Agency (IAEA) organized in Vienna a meeting with representatives from the Caribbean, Mexico, South Africa and the USA to raise awareness of the seriousness of the situation and to assess the potential and research and development needs of developing the sterile insect technique (SIT) to stop the advance of the pest (IAEA 2002). As a result of this and follow-up meetings with the United States Department of Agriculture (USDA), including a regional forum with national and international partners organized in Mexico City by the Plant Health General Directorate of Mexico and the IAEA (SAGARPA 2004), a strategic plan was developed to delimit, monitor and reduce the spread of the cactus moth in North America. It was also agreed to support research in monitoring, application of insecticides, the SIT, and to regulate the importation and domestic movement of cacti (K. Bloem et al., this volume).

The cactus moth is now a recognized quarantine pest in Mexico, and therefore the Mexican Government, through the Plant Health General Directorate (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria – Secretaría de Agricultura, Ganaderia, Desarrollo Rural, Pesca y Alimentación, SAGARPA), has implemented a national programme involving phytosanitary measures to prevent entry of this pest into the country.

2. The National Preventive Programme

Invasion of the cactus moth into Mexico would have irreparable ecological, economic and social effects on the country, a fact recognized by the highest authorities in the Ministry of Agriculture of Mexico (SAGARPA). Therefore, rather than waiting to respond to the pest presence once it arrives in the country, the attention of government leaders is focused on preventing the introduction of *C. cactorum*. Mandatory regulatory measures involve two action plans:

2.1. Preventing the Introduction of Cactus Moth into Mexico

This involves: (1) risk analysis of the potential ecological, economic and social impact, (2) a public awareness campaign (book, posters, brochures, calendars, articles in journals and books, magazines and newspapers, interviews in radio, broadcasting videos), (3) training on identification and detection for quarantine inspection personnel, university professors and growers (workshops, symposia, meetings), (4) establishment of a national and international advisory group, (5) permanent monitoring and host sampling in commercial and wild areas (Monitoring and sampling methods were determined by a technical advisory group of the National Preventive Programme in 2003). In commercial areas, five geo-referenced points per hectare are selected from which one plant per point is selected for checking. In wild areas, one geo-referenced point is randomly selected and all plants are checked within ten metres around the point; technicians check the points weekly), (6) surveillance of possible pathways and susceptible host areas, (7) multi-lateral cooperation with countries where cactus moth occurs, and (8) research and development projects financed by the IAEA.

Stakeholders participating in the preventive national programme include federal and state governments, national and international research institutions and regulatory organizations, commercial and subsistence growers, farmers and ranchers, and the general public.

2.2. The National Emergency Response System

This involves phytosanitary inspection at control points across Mexico, and implementation of offshore mitigation of pest populations involving an integrated pest management programme (IPM) that includes: (1) detection and monitoring surveys, (2) use of the sterile insect technique developed by the USDA in collaboration with the IAEA, (3) cultural practices, and (4) biological, chemical, and regulatory control.

For the proper operation of this system, the Mexican Official Emergency Regulation "NOM-EM-040-FITO-2003" was published on 20 May 2003 in the Official Diary of the Federation. Under this regulation, the system to prevent the entry, dissemination and establishment of the cactus moth in the national territory is enforced. However, since Mexican emergency regulations are valid for six months only, a new permanent Mexican Official Regulation "NOM-084-FITO-2004" was published in the Federal Diary and will be officially established once this regulation has been approved.

In accordance with a study carried out by the Mexico Biodiversity Commission (CONABIO; Comisión Nacional para el Uso y Aprovechamiento de la Biodiversidad), this regulation identifies susceptible states in Mexico, which are at risk of introduction and establishment of this exotic invasive species (Fig. 1) (Soberón et al. 2001, Zimmermann et al. 2003). In addition, the potential areas of greatest impact are the States of Tamaulipas, Hidalgo and Zacatecas. Besides these states, the programme is implemented in Baja California, Baja California Sur, Distrito Federal, Estado de México, Jalisco, Nuevo León, Querétaro, Puebla, San Luis Potosí, Oaxaca, Chihuahua, Coahuila, Sonora, Guanajuato, Campeche, Yucatán and Quintana Roo where many cacti and *Opuntia* species exist and there are many possibilities for the cactus moth to become established.

3. Results

Field sampling was undertaken in 20 states with the highest potential for establishment of the pest. These were chosen based on the economic, environmental and social importance and considering the probability of pest establishment based on similar prevailing climates in countries where the pest had become established. Results until now indicate that the cactus moth is not present in Mexico (28 000 commercial hectares were surveyed using 7500 sampling points). Nevertheless, it is necessary to increase efforts in research and development to help understand the biology of the insect and its host preferences, and to develop diagnostic methods for earlier detection and emergency response[1].

The operational funds provided for the preventive programme have been close to 20 million pesos (equivalent to USD 1.8 million). These came from the federal government, states and growers and have been used for monitoring and sampling, for organizing training courses for technicians and growers, workshops, symposia and expositions, and for improving public awareness through the distribution of posters, brochures, pamphlets, calendars and videos.

SAGARPA organized meetings, workshops, symposia and national and international fora jointly with universities, government and other agencies, and the FAO/IAEA. Over 3100 copies of a book entitled: "Biology, history, threat, surveillance and control of the cactus moth *Cactoblastis cactorum*" (Zimmerman et al. 2004) and 160 videos entitled: "Cactus moth: an economic, social and ecological threat" were distributed to more than 100 national and international, and public and private institutions (IAEA 2005).

Also implemented have been research and technology transfer activities supported by the

[1]*Editorial footnote: An outbreak of cactus moth was detected in 2006 on the Isla Mujeres, Yucatan Peninsula.*

IAEA and FAO. These included expert missions to the Caribbean islands to prepare risk assessment pathways, and joint research with the Universidad Nacional de Tucumán, Argentina on the biology and distribution of the cactus moth, and with USDA's Agricultural Research Service (USDA-ARS) to develop mass-rearing and to optimize pheromones for detecting and monitoring of cactus moth. This support also resulted in the development of an economic, social and ecological impact assessment for the USA and Mexico (Simonson et al. 2005), and the field-testing of contact insecticides in South Africa.

4. Conclusions

As a consequence of the described assessment, awareness and other preparatory activities, and the mutual interest of Mexico and the USA in preventing *C. cactorum* from spreading from the south-eastern USA and Caribbean Islands to vulnerable areas in the south-western USA and Mexico, SAGARPA and the USDA have agreed to cooperate in an effort to prevent introduction of the cactus moth into these areas. These agencies have prepared a bi-national plan to stop the spread of the cactus moth, and to define activities and allocate funds from both countries to implement the plan. Activities are focused on: risk assessment, public awareness campaigns, management and infrastructure improvements, surveys and detection methods, identification skills and facilities, improved regulatory efforts, control or containment methods, research and technology transfer.

The most important agreements in the bi-national plan include the need for national monitoring, regulatory and awareness strategies, as well as a large-scale field trial at the front of advance of the pest to validate the potential use of the SIT combined with sanitation and other measures within an area-wide approach for containment and eventual eradication. In the meantime the bi-national plan is being implemented with funding from both countries, including the SIT validation trials, which have been ongoing since early 2005 at four validation sites on islands in Alabama and Florida (Floyd 2006).

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Area-Wide Control Tactics for the False Codling Moth *Thaumatotibia leucotreta* in South Africa: a Potential Invasive Species

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ABSTRACT The false codling moth *Thaumatotibia leucotreta* (Meyrick) is a key pest of citrus, stone fruit, and other crops in many countries throughout continental Africa, including South Africa. There is a growing awareness that this damaging pest could soon be introduced into other countries including the USA as a direct result of increased international trade and daily direct flights from African countries. South Africa currently employs a combination of cultural, chemical, microbial and augmentative biological control to suppress false codling moth. Augmentative biological control makes use of the egg parasitoid *Trichogrammatoidea cryptophlebiae* Nagaraja. However, this integrated programme is not adequate for effective false codling moth control. The sterile insect technique (SIT) is now being developed as an additional method for false codling moth suppression in South Africa, but also as a tactic that could be rapidly integrated in an area-wide integrated pest management strategy if false codling moth were to be introduced or become established as an exotic invasive pest in other countries such as the USA. The SIT is regarded as a host-specific and environment-friendly pest control tactic that is compatible with the application of augmentative biological control. However, fully successful integration of the SIT and parasitoid releases into an effective pest management approach can occur only if the parasitoids do not negatively impact irradiated insects and their progeny more severely than they affect the wild pest population, and if the release of irradiated insects does not negatively impact the efficacy of the parasitoids. Therefore, knowledge of the compatibility of *T. cryptophlebiae* and the release of irradiated false codling moth is crucial to the evaluation of the combined use of these tactics. The development and combination of these offshore integrated pest management strategies in South Africa will develop and/or enhance scientific expertise and infrastructure in that country, reduce wild populations of false codling moth and lower the risk of its introduction into countries currently free of this pest. In addition, the development of these control tactics and the improved infrastructure (e.g. rearing/irradiation facilities in South Africa) will provide resources, technology, and strategies for eradicating invasive populations of the false codling moth should this pest be introduced into new geographical areas.

KEY WORDS false codling moth, *Thaumatotibia leucotreta*, *Cryptophlebia leucotreta, Trichogrammatoidea cryptophlebiae*, invasive species, South Africa, USA, area-wide, augmentative, SIT

1. Introduction

The false codling moth *Thaumatotibia leucotreta* (Meyrick) is indigenous to southern Africa and the Ethiopian region (Stofberg 1954, Catling and Aschenborn 1974) and has also been recorded from the islands of Madagascar, Mauritius, Reunion and St. Helena (CIBC 1984). It was first reported as a pest of citrus in Natal in 1899 and is now con-

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sidered a key pest of almost all citrus varieties in some parts of South Africa (Stofberg 1954). False codling moth is an increasingly serious pest of cotton and maize in tropical Africa (Angelini and Labonne 1970, Reed 1974). In addition, false codling moth attacks many non-commercial hosts belonging to several different plant families including Anacardiaceae, Gramineae and Euphorbiaceae (Angelini and Labonne 1970). Furthermore, false codling moth has been successfully reared from pomegranate, custard apple, guava, peach, avocado, litchi, apricot, plum, walnut, acorn, pecan, all sweet varieties of citrus (Stofberg 1954), carambola (Angelini and Labonne 1970), okra, sorghum heads (Reed 1974), macadamia (La Croix and Thindwa 1986), and olive (CIBC 1984).

The United States Port Authorities have reported intercepting false codling moth larvae from a wide variety of commercial hosts, including citrus, maize, eggplant, cayenne pepper, cola nuts and cassava in shipments arriving from 16 different African countries including South Africa (United States Department of Agriculture (USDA)-Animal and Plant Inspection Service (APHIS) Port Interception Network records).

In South Africa, false codling moth has developed some resistance against the pesticides used for its control (principally benzylureas) (Hofmeyr and Pringle 1998). Other control tactics, such as orchard sanitation (Stofberg 1954), pathogens, and biological control by parasitoids and predators, have been successful in reducing pest populations, but additional control is needed to further reduce pest populations (Newton 1989).

False codling moth typically has four to six non-discrete generations per year in South Africa (Stofberg 1954, Georgala 1969) and it has no documented true diapause (Angelini and Labonne 1970, Reed 1974). Females lay individual eggs on fruit (Catling and Aschenborn 1974) or foliage (Daiber 1978). Newly emerged larvae penetrate the fruit, where larval development is completed. Mature larvae exit the fruit and spin silken cocoons near the soil or in bark crevices (Stofberg 1954, Georgala 1969). Attack by false codling moth generally causes the fruit to drop prior to harvest (Georgala 1969). However, because larval entries take a few days to become visible, larval entries that occur close to fruit harvest might not be seen by packing house fruit graders and infested fruit can be packaged for export (Georgala 1969).

Adult false codling moths are nocturnal (Stofberg 1954) and females lay between 100-250 eggs in their lifetime (Stofberg 1954, Angelini and Labonne 1970). A sex pheromone has been identified for false codling moth (Read et al. 1968, Henderson and Warren 1970, Read et al. 1974, Persoons et al. 1976), and Hofmeyr and Burger (1995) developed a controlled release dispenser for the pheromone. Several different sticky traps have been tested successfully and are available for monitoring populations of false codling moth (Daiber 1978, Angelini et al. 1980, Newton and Mastro 1989). The adults are also attracted to mercury vapour light traps (Reed 1974).

Mass-rearing of false codling moth was originally described by Theron (1947) and modified by Schwartz (1972). Currently, a vigorous colony (about 2.5 million adults per week or ten million adults per month) is maintained at the Ceder Biocontrol insectary in Citrusdal, South Africa. The artificial diet consists of an autoclaved maize meal paste that is inoculated with *Rhizhopus* sp. fungus. Developing larvae feed on the biproducts of the fungal infection. The rearing system is labour-intensive but effective. The reason for rearing false codling moth at Citrusdal is solely to provide host material (eggs) to rear the egg parasitoid *Trichogrammatoidea cryptophlebiae* Nagaraja for augmentative releases against false codling moth populations in citrus (Newton 1988, 1989, Newton and Odendaal 1990). The current level of parasitoid production at Ceder Biocontrol is sufficient to treat 600-800 hectares of commercial citrus per month.

The development and use of the SIT for suppression of false codling moth was proposed for a number of reasons: (1) as previ-

ously mentioned, the basic infrastructure for mass-rearing false codling moth is already in place in South Africa. Apart from the utilization of the false codling moth colony for egg parasitoid rearing, improved utilization of reared false codling moth (i.e. the use of adult moths in a programme involving SIT) would improve the cost efficiency of this insectary; (2) theoretical and experimental evidence suggests that combined use of the SIT and parasitoids can provide pest control that is more effective than when either tactic is employed separately (Knipling 1992, Carpenter 1993, 2000); (3) the SIT is a species-specific environment-friendly pest control tactic that would build infrastructure, improve expertise, provide local jobs, and enhance the range of available control options; (4) the development of the SIT for false codling moth would provide resources and strategies for elimination of invasive populations should this pest be accidentally introduced into the USA, which is a major trading partner of South Africa.

2. Research Gaps and Progress to Date

2.1. Development of the SIT for False Codling Moth Thaumatotibia leucotreta

Myburgh (1963), Schwartz (1978) and Du Toit (1981) conducted preliminary studies on the effects of gamma radiation on false codling moth in South Africa. However, these studies did not address the use of inherited sterility (North 1975, Carpenter 1993, Bloem et al. 1999), which results in more competitive moths for use in programmes that employ the SIT. Additionally, no data were published on the possibility of irradiating adults rather than pupae or on fertility levels when treated males are crossed to treated females.

Bloem et al. (2003) recently published data on the radiation biology and inherited sterility of false codling moth. They examined the effect of increasing doses of gamma irradiation on the fecundity and fertility of this species. Newly emerged adults as well as mature pupae were treated with doses of radiation ranging between 0 and 350 Gy and adults were inbred or outcrossed to fertile counterparts. Results showed that fecundity was not adversely affected by dose of radiation when untreated females were mated to treated males. However, the fecundity of treated females mated to either untreated or treated males declined precipitously as the dose of radiation increased. They also found that the dose at which 100% sterility is achieved for treated false codling moth females is 200 Gy (Fig. 1). In addition, Bloem et al. (2003) examined the inherited effects resulting from treatment of parental (P) males with selected doses of radiation (0, 100, 150, 200 and 250 Gy) on the F_1 generation. Decreased F_1 fecundity (total eggs produced) and fertility (egg hatch), increased F_1 mortality during development, and a significant shift in the F_1 sex ratio in favour of males were recorded.

More recently, Hofmeyr et al. (2005) examined the effect of different release ratios of treated (T) to untreated (U) moths on the incidence of fruit damage and on the competitiveness of treated males in replicated field cage studies in South Africa. Individual navel orange trees were enclosed in large mesh cages and adult moths treated with either 150 or 200 Gy were released into the cages at ratios of 5T:1U and 10T:1U. Results showed that the number of larval entries as well as the number of F_1 progeny per cage decreased significantly as the release ratio of treated moths increased. In addition, the lowest mean number of fertile F_1 adult females and males was obtained from the treatment that combined the lower dose (150 Gy) and 10T:1U release ratio. This treatment also showed the lowest per generation rate of increase (less than 1 from the P to the F_1 generation) suggesting that growth in the wild population would have been prevented if releases of treated moths at this dose and ratio were maintained in the field.

The next step in development of the SIT for false codling moth will be a season-long validation under field conditions. A pilot study is scheduled to begin in August 2005 at the Hexrivier Blikhuis orchard north of Citrusdal, South Africa. The 36-hectare orchard is rela-

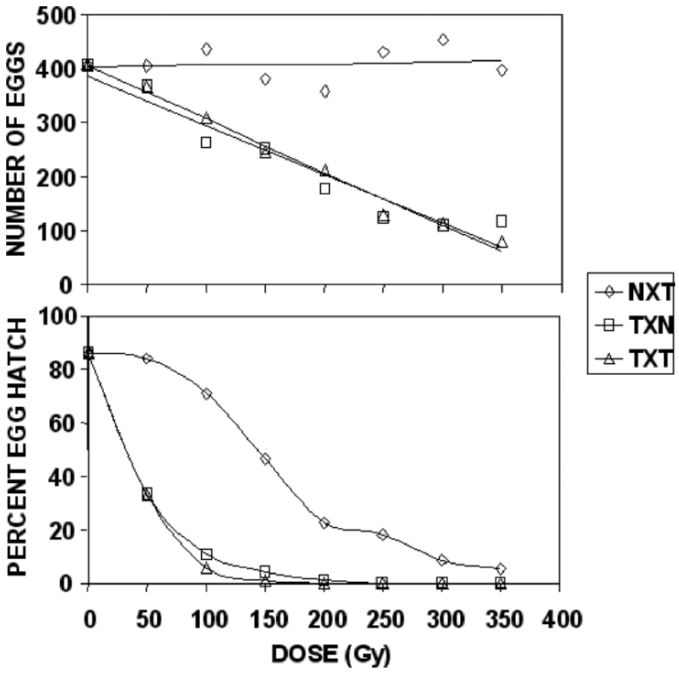

Figure 1. *Effect of radiation dose on false codling moth fecundity (mean number of eggs laid per mated female) and fertility (mean percentage of eggs that hatched). Males and females were treated (T) with 0, 50, 100, 150, 200, 250, 300 and 350 Gy and inbred (T females x T males) or outcrossed (T females x N males, N females x T males) to untreated (N) adults (Bloem et al. 2003).*

tively isolated from other orchards and, in preparation for the SIT pilot, is being managed with an aggressive sanitation programme (that removes all fallen fruit) and with monthly releases of *T. cryptophlebiae* egg parasitoids. The wild false codling moth population is being monitored weekly with pheromone-baited traps. Fruit drop is also monitored each week at 20 stations located in close proximity to trap sites.

During the SIT pilot study, bi-weekly releases of 1000 false codling moth adults per hectare will be made for 40-42 weeks. The moths will be reared at the Ceder Biocontrol insectary in Citrusdal. Newly emerged adults will be packaged, chilled, and transported to Stellenbosch, South Africa, where they will be irradiated at a dose of 150 Gy in a ^{60}Co panoramic irradiator. Treated insects will be returned to Citrusdal and released using a modified codling moth release device (Bloem and Bloem 2000) mounted on an all-terrain vehicle. Release ratios throughout the season will be monitored using pheromone-baited traps and extensive fruit sampling at midseason and at harvest will give an indication of the effectiveness of the SIT.

2.2. Combination of SIT and Parasitoids

Interest is not only in developing the SIT as an area-wide programme to suppress false codling moth in South Africa, but in examining the possibility of combining the SIT with releases of the egg parasitoid *T. cryptophlebiae*. As mentioned above, there is theoretical

and experimental evidence that suggests that combined releases of sterile insects and parasitoids can provide pest suppression that is more effective than when either technique is employed separately. In programmes involving sterile insect releases for Lepidoptera pests, both treated males and females are released in the field (Stewart 1984, Bloem and Bloem 2000). Because all matings taking place during SIT operations (including those involving treated moths) result in the production of eggs, a potentially large number of host eggs may be present in areas under the SIT. For control of the codling moth *Cydia pomonella* (L.), the combined release of irradiated insects and *Trichogramma* spp. egg parasitoids was first suggested by Nagy (1973). Field cage experiments conducted by Bloem et al. (1998) showed that an additive suppressive effect can be realized when treated codling moths are released at a 10:1 release ratio (T:U) together with *Trichogramma platneri* Nagarkatti when compared to cages receiving treated moths or egg parasitoids only.

Carpenter et al. (2004) conducted a laboratory study that examined the acceptability and suitability of false codling moth eggs to parasitization by *T. cryptophlebiae* under no choice and choice situations. Male and female false codling moth were treated with 150 or 200 Gy of gamma radiation, inbred or outcrossed to untreated counterparts, and the eggs laid by different crosses offered to *T. cryptophlebiae* as host material. Newly laid (24 hour-old) false codling moth eggs, as well as eggs that were 48 hours and 72 hours old were evaluated.

Results revealed that all treatments in the no-choice experiments were acceptable for oviposition and suitable for the development of *T. cryptophlebiae*. However, significant differences in the number of parasitized eggs were detected in the choice situations when one member of the host cross, particularly the female, was treated with gamma radiation or when the egg age was greater than 24 hours. These results suggest that *T. cryptophlebiae* would accept, successfully develop in, and emerge from false codling moth eggs laid by the different crosses that would theoretically be present in the field under a programme involving the SIT (U females by T males, T females by U males, T females by T males) and indicate that further evaluations combining releases of treated moths and parasitoids are warranted.

Field cage studies to examine the effectiveness of releasing irradiated false codling moth alone or in combination with *T. cryptophlebiae* were conducted in 2005. Four treatments were randomly assigned to 15 large-mesh cages containing individual navel orange trees with 50 fruits per tree. Treatments were: (1) U = control = 10 pairs of untreated moths, (2) U + T = untreated moths plus a 10:1 overflooding ratio of moths treated with 150 Gy = 100 pairs of treated moths, (3) U + P = untreated moths plus two releases of *T. cryptophlebiae* parasitoids (approximately 3000 parasitoids total per cage), and (4) U + T + P = untreated moths plus treated moths plus parasitoids.

Results showed that all treatments significantly reduced the number of larval entries when compared to cages receiving only untreated moths. In addition, when treated moths and parasitoids were released together, significantly more parasitoids were produced per cage than when cages received only untreated moths plus parasitoids. These results suggest that combined field releases of irradiated false codling moth and *T. cryptophlebiae* would result in rapid parasitoid population increase, which would have a positive impact on false codling moth population suppression.

3. Benefits of Developing Integrated Pest Management Strategies for South Africa and the USA

3.1. South Africa

The South African Citrus Growers Association has identified false codling moth as a top research priority. South African researchers are involved in a multi-faceted

false codling moth research programme that includes the evaluation of new insecticides and improved mating disruption products, optimization of biological control using egg parasitoids, development of a long-lasting pheromone dispenser for trap monitoring, as well as improvements in the potency and shelf-life of false codling moth granuloviruses. Because the SIT can be easily combined with all of the control tactics listed above, there is interest in integrating the SIT as an additional tactic to suppress false codling moth.

As a result of this research, co-funded by the International Atomic Energy Agency (IAEA) under project SAF5007, it is hoped that the ongoing biological control with egg parasitoids can be optimized through the synergism of combining it with the SIT as well as through the increased efficiency in false codling moth/parasitoid rearing operations at the Ceder Biocontrol insectary. It is also hoped that through the combined use of both tactics populations of false codling moth will be reduced both inside and outside citrus orchards, thereby reducing pre- and postharvest crop losses, and losses due to rejection of false codling moth infested shipment consignments.

3.2. United States of America

A significant proportion of the mission of the USDA-Agricultural Research Service (ARS) and USDA-APHIS is directed toward dealing with invasive species and potential emerging pests. USDA-APHIS research and development activities are concentrated on preventing the entry of pests into the USA. However, despite the success of these efforts, many pest species still manage to gain entry and many will become established. While early detection of an invading pest is critical, it is equally important to have a suppression/eradication plan in place before the pest becomes established and well before the geographical range of the pest begins to expand. Proactive research programmes are necessary to quickly respond to any breach in our exclusion mechanisms.

Many federal and state agencies in the USA have expressed concern that the false codling moth may soon be introduced into the USA as a direct result of increased international trade and daily direct flights from African countries. The USDA-APHIS Port Interception Network records indicate that these concerns are well founded. Since 1985, false codling moth has been intercepted more than 122 times at 19 different ports of entry from 22 host plants originating from 15 different African countries. Because false codling moth attacks so many different host plants including citrus, stone fruits, corn, cotton, and vegetables, and because it would be a quarantine issue for some crops, establishment of this pest in the USA could result in considerable economic losses.

The development of "offshore" IPM strategies (i.e. programmes in South Africa), that combine augmentative biological control with genetic control tactics (such as the SIT), would reduce pest populations in South Africa and thereby reduce the risk that false codling moth would arrive in South African shipments to the USA. Also, this project will develop an eradication tool for use against introduced false codling moth populations and improve infrastructure (e.g. rearing/irradiation facilities) and expertise in South Africa. As a result, irradiated false codling moth would be readily available for shipment to the USA for eradication purposes in the event that the pest invaded and became established in this country.

4. Conclusions

In this project it is hoped to demonstrate that this approach can serve as a model on how to prepare for other potentially invasive lepidopteran pests. A general outline of this approach would be: (1) obtain knowledge on the radiation biology of the exotic lepidopteran pest of concern, (2) assist source countries to develop integrated pest management strategies that combine augmentative biological control with genetic control tactics (which would include the ability to mass-rear the pest insect), and (3) develop partnerships between

source countries and pest free countries so that irradiated lepidopteran pests would be available for shipment and use in a programme using the SIT in the event that the pest invaded the pest free country.

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SIT for the Malaria Vector *Anopheles arabiensis* in Northern State, Sudan: an Historical Review of the Field Site

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ABSTRACT It is planned to use the sterile insect technique (SIT) as part of an area-wide integrated pest management programme to drive *Anopheles arabiensis* Patton, the major vector of malaria in Sudan, back from the northern-most edge of its distribution on the Dongola and Abri-Delgo Reaches of the Nile, close to the border with Egypt. The coincidental location of the field site with Upper and Middle Nubia provides a wealth of historical information that can, in lieu of direct data, reveal clues to past changes in vector distribution. Major environmental transitions have occurred across the region since the last glacial maximum, 18 000 years ago: from hyper-arid desert to tropical grassland, then to semi-desert and back to tropical desert today. Large wild animals as diverse as giraffe and hippopotamus emerged and receded. In parallel, human activities and settlement patterns changed markedly with the rise and fall, and rise again, of the Kingdom of Kush during the Kerma and Kushite periods. These factors will have facilitated the spread of mosquito populations and then, by 1500 years ago, contributed to their reduction, or demise. The "saqia" water wheel introduced at the start of medieval times brought an expansion of the human population along the Nile and presumably a gradual resurgence in mosquitoes, which continued with occasional setbacks to the present day. Isolation was almost complete except for limited dispersal downriver via the Abu Hamed Reach. Evidence of malaria in ancient times has been found in Egypt and neighbouring River Nile State, but as yet historical indicators in Northern State are limited to the accounts of foreign visitors and date from 1813. Entomological records are available from about 1908. From these it appears that *An. arabiensis* is the only anopheline to have been found between the Second and Fifth Cataracts and that it has remained limited to the south of Wadi Halfa over the last century with only intermittent forays into Egypt, where it caused at least two serious malaria outbreaks.

KEY WORDS *Anopheles arabiensis*, Northern State Sudan, River Nile, mosquito distribution, Gambiae Control Project

1. Introduction

Amongst the many criteria for judging a field site to be suitable for the control of mosquitoes using an area-wide integrated pest manamgent (AW-IPM) approach with an sterile insect technique (SIT) component, it is most desirable to find a single vector population that is isolated, unstructured, relatively small and at low density. Today's technology greatly facilitates the comprehensive and rapid assessment of the target population, even if starting from very little information, but the interpretation of results is not always clear-cut. An historical perspective can help corroborate present-day findings, particularly in relation to the likely origin and age of the population and its history of reproductive isola-

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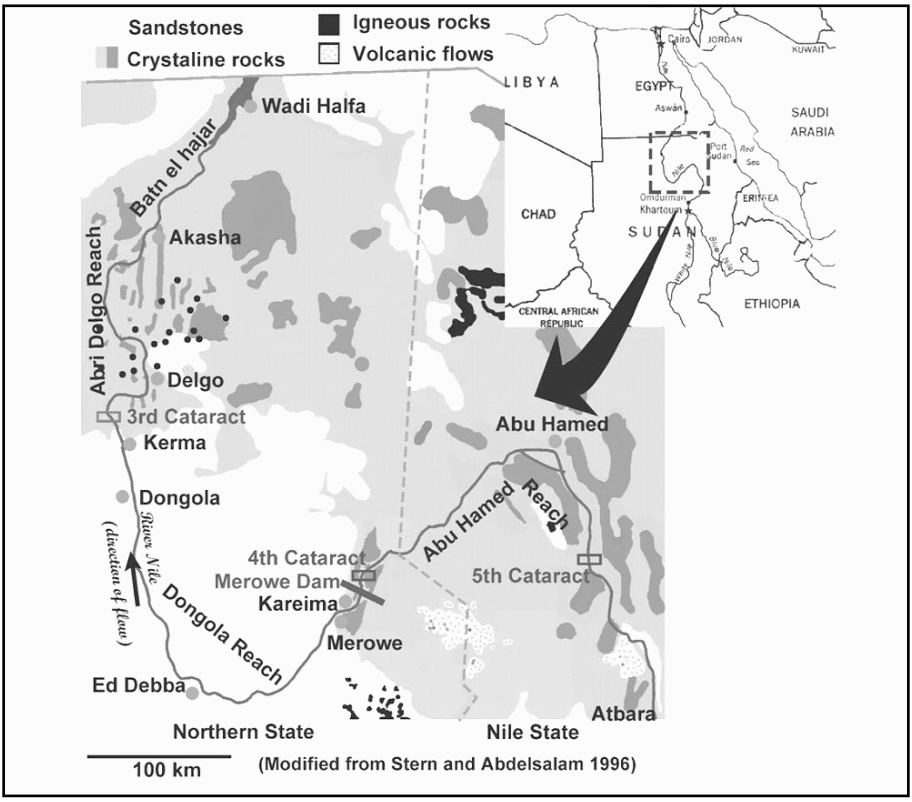

Figure 1. *Geology of Northern State, Sudan.*

tion. Other criteria may be judged with more certainty from contemporary data such as the evidence of actual or potential disease transmission and a favourable benefit/cost analysis for AW-IPM with an SIT component versus alternatives. However, even the assessment of these can be influenced by knowledge of past events, particularly since local, national and regional needs must be considered. This paper examines historical data relevant to current research to develop the SIT for control of the malaria vector *Anopheles arabiensis* Patton in Northern State, Sudan. The field site is primarily the Dongola Reach of the Nile, which extends down river from the Fourth to the Third Cataract (Fig. 1). *An. arabiensis* is restricted to within a few kilometres of the river by desert and its survival is closely associated with the human population.

Northern State encompasses Upper and Middle Nubia, the location of the Kushite civilisation that rivalled, and for at least 60 years, ruled Pharaonic Egypt. It has a very rich and ancient history with many important archaeological sites dating back before the Neolithic (7000-5000 years ago) and extending up to and including the medieval Christian and Islamic periods. This has generated information on human population movement, settlement patterns, cycles of growth and decline, land use and health, which in addition to data on climate and the environment can be used to delimit an early history of the vector population.

In the nineteenth century more directly relevant information can be gleaned from the

accounts of travellers attracted to the area by an interest in the archaeological sites and from records of military activities (Lewis 1948). In the early twentieth century there are entomological and epidemiological records from the Wellcome Tropical Research Laboratories in Khartoum and later the Sudan Medical Service. Also well documented was the invasion of *An. arabiensis* into Egypt in the early 1940s, which caused a major outbreak of malaria along about 850 kilometres of the Nile (Shousha 1948) and the building of the Aswan dam which dramatically changed the landscape at the border between Northern State and Egypt. These events prompted the creation of the Gambiae Control Project, a joint protocol between the Egyptian and Sudanese Ministries of Health to prevent an *An. arabiensis* reinvasion of Egypt. The records of their survey and control activities, along with one study in 1986 (Dukeen and Omer 1986), are about the only entomological data from Northern State in the last 50 years, so current studies are essentially starting anew.

This paper attempts to provide an overview of relevant historical data with a few highly speculative inferences on mosquito distribution. More detailed analysis, which may be possible for certain areas and time periods, is outside the intended scope and should await the outcome of current work on both archaeology and vector biology. A further caveat is that historical and archaeological studies are also limited by data paucity and subject to multiple, perhaps controversial, interpretations.

2. Present and Future

The Republic of Sudan is the largest country in Africa, covering 2.5 million square kilometres. Northern State is located in the northwestern corner, bordered by Libya and Egypt, occupying nearly one fifth of the country. It is very arid, almost entirely desert, rainfall is rare and the temperature is high throughout the year, reaching 47ºC in summer months, but can get as low as 7ºC at night in the winter.

As it travels north along the Dongola Reach, the Nile passes over sandstone and is flanked by wide alluvial flood plains that are suitable for cultivation of crops. As all agriculture is based on irrigation and the annual flood, most of the population (around 567 000) live alongside this stretch of the river, particularly within Dongola and Merowe provinces. Above the Fourth Cataract and below the Third Cataract, the bedrock is crystalline basement; there is little alluvial soil (Stern and Abdelsalam 1996), and therefore less scope for agriculture and a lower population density (Fig. 1).

Malaria in Northern State accounts for about one third of all hospital admissions. *An. arabiensis* is the only vector and apparently the only anopheline. It is a member of the *Anopheles gambiae* Giles species complex and all but relatively recent records refer to *An. gambiae*. It is, however, safe to assume that these were *An. arabiensis*, since *An. gambiae sensu stricto* is only found in the far south of the country. The vector can be found all year in Dongola and Merowe Provinces, but its population density changes temporally in relation to the Nile flood and ambient temperature. Its distribution appears to be highly correlated to human presence and it is therefore more restricted along the Abu Hamed Reach and below the Third Cataract than along the Dongola Reach. The Baiyuda desert prevents immigration from the south and so, apart from human-assisted passive transport, the only route for this mosquito into Northern State is downriver via the difficult terrain above the Fourth Cataract.

Just downriver from the Fourth Cataract, a dam is being built across the island of Merowe (Mirowy) to provide hydroelectric power and should be fully operational by 2008. An area of 711 square kilometres is expected to be under water creating a lake extending along the western Abu Hamed Reach. The lake shore will be rocky desert terrain unsuitable for agriculture and human habitation. It is therefore likely to pose a significant barrier to mosquito migration or dispersal downriver and should complete the isolation of the

Northern State vector population. However, after the dam is completed there are plans to create large irrigation channels running in parallel with the Nile all the way from Merowe to Dongola. These projects are going to dramatically transform Northern State and while the benefits are clear there is a risk they will exacerbate the malaria situation.

3. 18 000-3500 Years Ago

Given the extreme environment and near absence of human population, it is highly improbable that mosquitoes were present along the Dongola Reach 18 000 years ago, but the environment will have become progressively more suitable up to 10 000 years later. A close association with humans, such as found today, would have facilitated the presence of the vector on the Dongola Reach between 7000 and 3500 years ago. Up until the second millennium BC the population was probably not isolated and an origin in the region of the south-western frontier with present day Chad was at least as probable as one in central Sudan. By the first millennium BC the environment was becoming more similar to today, the human population had declined and it is likely that mosquito populations were not sustained. Thereafter recolonization would have to have been downriver via the Abu Hamed Reach.

The earliest known presence of humans in the north-western part of Sudan dates back at least 300 000 years, however by 18 000 years ago, the whole area was hyper-arid desert (Petit-Maire et al. 2000, Hoelzmann et al. 2001, Adams 2002) with presumably only a small population along the river margins (Fig. 2). The flow of the Nile was substantially less than today and seasonally may have been completely dry in places. As the desert receded, small pockets of humans were living close to the Second Cataract between 15 000 and 9000 years ago (Garcea 2004). About 3000 years later almost all of Northern State was tropical grassland. The Sahara started to expand again, and by the later Holocene, tropical semi-desert was encroaching on the Dongola Reach (Fig. 2) (Petit-Maire et al. 2000, Hoelzmann et al. 2001, Adams 2002). Hunter gatherers started to change to a more pastoral way of life and livestock herding had appeared by the start of the Neolithic (7000 years ago). In the northern Dongola Reach there was considerable occupation along the banks of the Nile palaeochannels that flowed to the east of the present day river, close to the Kerma and Seleim Basins and the Wadi-al-Khowi. These sites followed a loose settlement pattern across a wide area, rather than discrete densely populated centres, which developed and intensified over the next 2000 years (Fig. 3). One well-preserved settlement near Kerma was dated at 6500 years ago (Honegger 2001). Domestic livestock first appeared in the Wadi Howar around 4200 BC. Over the next 1000 years the Lower Wadi Howar was to remain fairly densely populated as settlement to the west increased, in particular along the Middle Wadi Hower and Djebel Tageru and in the West Nubian Palaeolake basin, allowing links between the Nile valley and Chad basin (Hoelzmann et al. 2001, Edwards 2004).

Environmental conditions will almost certainly have permitted migration of mosquitoes including *An. arabiensis* to the Dongola Reach and to Egypt before this time, but the more contiguous series of human settlements along the Wadi Howar and up to Kerma will have provided an even more favourable route. The source of the Wadi Howar (also known as the Yellow Nile) was in the border region of present day Chad. This could have been the origin of an early Dongola Reach *An. arabiensis* population and even of some present day mosquito populations in Egypt. Much less is known of human settlements along the Wadi el-Melik, the southern Dongola Reach and the Abu Hamed Reach, although work in the latter two areas is ongoing, so alternative or additional migration routes cannot yet be excluded.

By about 4200 years ago the lower Wadi Howar was drying up and the focus of settlement moved westwards. Cultural divergence with the northern Dongola Reach population

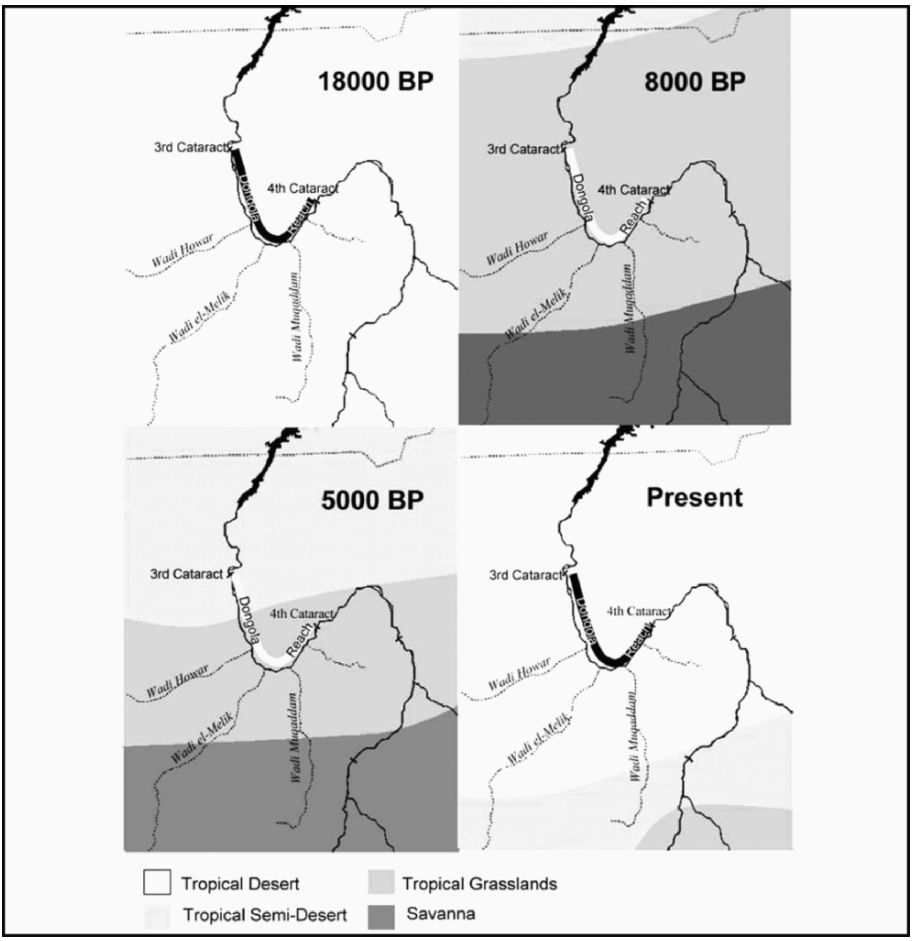

Figure 2. *Changing vegetation patterns across northern Sudan from 18 000 years ago to the present day.*

increased, indicating a marked reduction in contact (Jesse 2004). The latter was growing, primarily around a more urbanized centre at Kerma and by 4000 years ago had become the substantial Kingdom of Kush. This was probably the earliest major kingdom in sub-Saharan Africa and extended along at least 700 kilometres of the Nile to encompass both Middle and Lower Nubia. The era is known as the Kerma Period and lasted over 1000 years. The settlement patterns while still extended, became denser and mostly followed the Hawawiya, Alfreda and Seleim Nile paleochannels (Welsby 2001) (Fig. 3). The environment was mainly lightly wooded savannah; intensive agriculture had increased and there was a strong emphasis on livestock management. A wide range of large wild animals was still present including giraffe, hippopotamus, lions and antelope (Edwards 2004).

Sizable mosquito populations could easily have been sustained at this time, but probably relatively isolated from populations outside the region as the surrounding area was becoming semi-desert (Fig. 2) and the wadis to the south were no longer flowing into the Nile. The only available migration or dispersal route for mos-

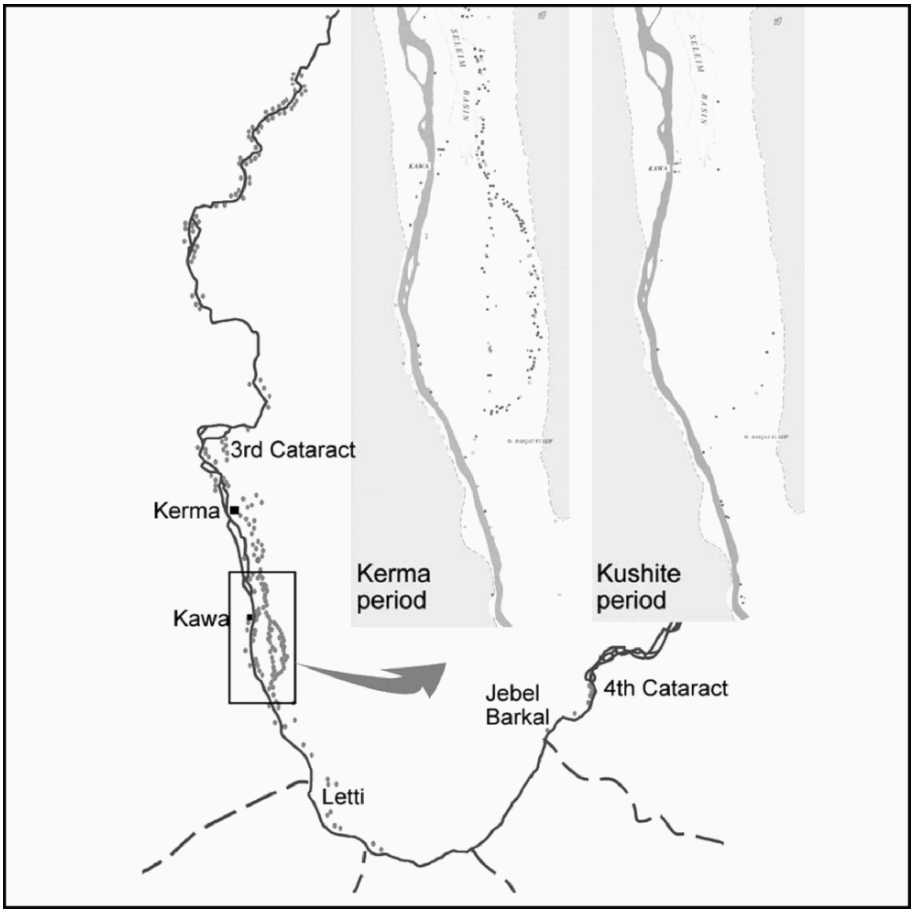

Figure 3. *Settlement patterns in northern Sudan during the Kerma and Kushite Periods (adapted from Welsby 2001 and Edwards 2004).*

quitoes from this time onwards will have been down the river Nile. There was human settlement further south in the Letti Basin and in the vicinity of the Fourth Cataract. As mentioned before, most of the Abu Hamed Reach appears inhospitable, particularly at the Fourth Cataract end, but it was nevertheless densely occupied at various times, including during the Kerma Period. Without mechanical devices to assist irrigation, the seasonally dry riverbeds between the numerous islands actually made it a more suitable location for cultivating crops than some ostensibly richer areas further downriver. The rocky terrain will also have provided protection.

4. 3500-1500 Years Ago

By about 3500 years ago the Egyptians had conquered Kerma state, with the destruction of Kerma itself and were to dominate the area for over 400 years. The expansion of the desert was by now approaching its present day boundary. Where they have recently been studied in detail, settlements adjacent to the Seleim Basin and Wadi-al-Khowi were found to have been disappearing as the palaeochannels dried up, with new sites appearing along

the bank of the present day Nile channel. The major population centre became the Pharaonic site at Kawa. Much less is known about the rural population in the period under Egyptian control and for several centuries after, but clearly the population diminished substantially and the settlement pattern changed to a small number of disconnected population centres. This pattern is still evident during the Kushite revival, which was to follow in the first millennium BC (Fig. 3) (Welsby 2001). Lower Nubia, which has been more extensively studied, had become largely depopulated and was not farmed for about 1000 years (Edwards 2004). Therefore by 2800 years ago, with a much reduced human population, located in pockets perhaps tens of kilometres apart, few if any large wild animals and a semi-desert environment, mosquito populations would have been fractured and greatly diminished, perhaps absent altogether.

After the collapse of the Egyptian New Kingdom, the Kingdom of Kush re-emerged to form an empire that survived for over 1000 years, including over half a century when Kushite Kings conquered and ruled Egypt as the 25th Dynasty. The Kushite Period can be divided into the Napatan and the Meroitic depending on the location of political power. Napata lies downriver from the Fourth Cataract, encompassing Jebel Barkal, Sanam and Abu Dom (present day Merowe and Kareima). Meroe lies between Shendi and Atbara, to the north of Khartoum. On the Dongola Reach other important sites for Napatan occupation were in the Letti Basin, Kerma, Tabo on Argo island and Kawa probably the biggest covering 40 hectares (estimated 6200-7800 people) (Fig. 3) (Edwards 2004, Welsby 2004). Below the Third Cataract there was a thin scatter of settlements, which were probably short-term and mainly existed to maintain links with Egypt (Edwards 2004). Therefore, despite a probable increase in the size of the human population it was still mainly concentrated in specific sites located far apart.

With the shift of power to Meroe in the later first millennium BC many Napatan settlements were eclipsed. Jebel Barkel remained of religious importance, but other sites contracted or disappeared. In the Northern Dongola Reach the main focus may have moved back to Kerma. Archaeological sites in the desert indicate that links with Meroe were maintained across the Baiyuda rather than upriver. Throughout the Kushite Period it seems unlikely that conditions had become any more favourable for mosquitoes along the Dongola Reach. The increasingly harsh environment will only have been partly mitigated by human activities, since despite a period of increased size the population was divided into isolated pockets. This situation probably remained into the middle of the first millennium AD only starting to change as human settlement patterns altered with the introduction of the saqia water wheel (Edwards 2004).

The end of the Kushite civilization brought a transition to two new kingdoms along the Nile in Nubia during the period 1650 to 1450 years ago: Nobadia, extending from the First to the Third Cataract, and Makuria on the Dongola Reach. The major urban centres at Kawa and Kerma had disappeared, but there was significant occupation in the Letti Basin, and especially at Old Dongola, which was to become the capital of medieval Makuria. This period also saw an expansion of settlement in the Fourth Cataract and further upstream (Edwards 2004).

5. 1500-200 Years Ago

Human population density and settlement patterns along the Dongola Reach changed dramatically in medieval times. A major factor was the introduction of the saqia water wheel, which along with changing farming practices and new multi-harvest crops contributed to the expansion of the population into areas that had been largely uninhabitable since the Kerma Period. The Kingdom of Nobadia controlled Lower and Middle Nubia between the Third and First Cataracts with a population in the order of 4000 divided into about 40 settlements, with perhaps as many as 20 people per square kilometre towards the south end of the

Batn-el-Hajar (Edwards 2004). The much better agricultural resources along the Dongola Reach will have sustained a much larger population. The Makuria Kingdom's main population centre lay at Old Dongola. Further north, settlements developed the west bank, where there had been limited human activity previously. This new settlement pattern was becoming more and more like the continuous settlement that exists along both banks today. There was also settlement along the western part of the Abu Hamed Reach above the Fourth Cataract. The occupation of the Nile banks expanded and by the early eighth century the two kingdoms unified under the King of Makuria (Edwards 2004).

From about 1500 years ago, humans were progressively creating conditions that were likely to facilitate expansion of mosquito populations along the length of the Dongola Reach, whereas for the previous 1000 years it's likely that any population would have been small and isolated, with little or no scope for recolonization from neighbouring human settlements or from outside the region. It would seem more likely that a species such as *An. arabiensis*, which is less well adapted to survival alongside humans than for example *Culex quinquefasciatus* Say, would have disappeared altogether. For perhaps the past 1700 years the only route into the Dongola Reach for mosquitoes was downriver and this remains the situation today, with the possible exception of passive transport facilitated by the railway to Wadi Halfa and Kareima, completed in the early twentieth century, and modern day transport.

In medieval times, the western Abu Hamed Reach was extensively occupied, which would have increased the likelihood of mosquito reinvasion downriver. Therefore whether or not *An. arabiensis* had disappeared previously, it was almost certainly present along the Dongola Reach for most of the last 1500 years. The biggest setbacks for both humans and mosquitoes during this time were mainly associated with floods and droughts, although major conflicts and social change were still to occur. The environmental history of the area is relatively poor for the first millennium AD, but there are records of several periods of a decade or so, when the Nile level was very low, other periods of high Nile (Adams 2001), and some years when ice formed on the Nile in Egypt, particularly in the ninth and tenth centuries. From 1300 to 1522 the Nile floods were generally good. After this and up to part way through the eighteenth century Nile levels and humidity were higher, but there were some droughts affecting the whole of Sudan in the late seventeenth century. In the eighteenth and nineteenth centuries the climate in general became more arid bringing more droughts, but interspersed with some heavy and destructive floods, with a very wet period in the 1790s (Nicholson 1978, Edwards 2004).

The data available over the last 500 years is quite detailed and could be used to make a more in-depth analysis of the likely impact on mosquito populations than the superficial account presented here. Nevertheless, it is clear that as with any mosquito at the edge of its species distribution, numbers will have fluctuated dramatically and the population structure will have been disrupted with successive contractions and expansions along parts of the Nile within Northern State, but it does not appear likely to have been eradicated from the area altogether at any point. The human population will have endured famine and conflict associated with major floods and droughts, and other major upheavals caused by political changes such as the expansion of the Funj Sultanate as far north as the Third Cataract and the expansion of the Ottoman Empire south (Edwards 2004). Nevertheless, the contiguous human settlement pattern along the Dongola Reach was maintained and became progressively more continuous, facilitating a more extended and presumably more homogenous mosquito population.

A more difficult question is the extent to which the Dongola Reach *An. arabiensis* population was isolated. The key is the Fourth Cataract and the western end of the Abu Hamed Reach, which was densely populated during the Kerma Period around 2500-1500

BC, and again in the post-Meroitic and medieval periods, between AD 350-1500. The population levels in the period in between are uncertain as work in the area is still underway. Continuity of occupation up to the present day is probable. The absence of human settlement would be a strong indication of a period of isolation. Human occupation does not however necessarily mean that mosquito migration was possible, since amongst other factors, the nature and size of the settlement and the prevailing wind and Nile level will all affect the likelihood. This again merits a more detailed analysis, but will have to await the results from the current Merowe Dam archaeological salvage project.

6. Last 200 Years

Malaria in Egypt has been confirmed from over 5000 years ago and is thought to have been widespread (Cerutti et al. 1999). Direct evidence for malaria in Northern State before the last 200 years does not yet exist. In neighbouring River Nile State, a Kushite, post-Meroitic and medieval cemetery at Gabati near Atbara, shows mortality patterns associated with anaemia that suggest malaria was present there (Judd 2005). Similar studies are in progress within Northern State. Lewis (1948) provided evidence for malaria-like illness in Dongola in the early nineteenth century gleaned from the literary accounts of travellers to the area. The earliest was 1813 described by Burkhardt (1819) in the Dongola area of a fever which occurred in epidemics, but not every year and which was often fatal. Entomological records confirming the presence of *An. arabiensis* first became available after the founding of the Wellcome Tropical Research Laboratories at Gordon Memorial College, Khartoum, in 1902 under the first director Dr Andrew Balfour. In the beginning medical, entomological and sanitation work in Khartoum took priority and then expeditions to the south. Many of the difficulties encountered in this early field work were overcome in 1907, by outfitting a boat as a floating laboratory (D'Arcy 1999), and this approach has also been adopted by the Gambiae Control Project. King (1908) was perhaps the first to record *An. arabiensis* in Northern State in 1906 and 1907.

From around 1918, outbreaks of malaria in Egypt at Nag Hammadi, Armant and Kom-Ombo were associated with the introduction of sugarcane. Few deaths were recorded and the incidence progressively declined with little or no intervention. This could have been due to *Anopheles pharoensis* Theobald, but a malaria epidemic in 1919-20 was thought more likely to be due to *An. arabiensis*. This followed heavy rains in the area around Ed Derr about 80 kilometres into Egypt (Fig. 4) and resulted in about 1000 deaths. The most detailed documented invasion of Egypt by the vector started in 1942 reaching Asyut, 850 kilometres downriver from the border. It caused a malaria epidemic estimated to have resulted in over 10 000 deaths within two years. An extensive and well-executed control programme successfully eradicated it from Egypt by early 1945 (Shousha 1948). Further control activities removed it from the Wadi Halfa area between Saras and Faras by early 1996 (Lewis 1948).

An. arabiensis was not controlled again in the vicinity of Wadi Halfa or further south until 1951, and was not found north of Ferka (120 kilometres south from the border) until late 1950, when it appeared at Aneiba in Egypt and then in neighbouring villages. It was again controlled with DDT and larviciding oil. Thereafter, it was decided to maintain a control programme using oil between Saras and Aneiba. In 1954, the Sudanese and Egyptian Ministries of Health agreed a joint programme to control *An. arabiensis* south of Wadi Halfa (Shousha 1948, Lewis 1956). This was to be the forerunner to the Gambiae Control Project, which started in 1970. The objectives of this control programme were to monitor and maintain an *An. arabiensis*-free zone, the "Red Zone", from Aswan in Egypt upriver to a point well inside Sudan and through control activities further upriver (the "Yellow" and "Green" Zones) to try and extend it. In 1970 the northern-most limit of

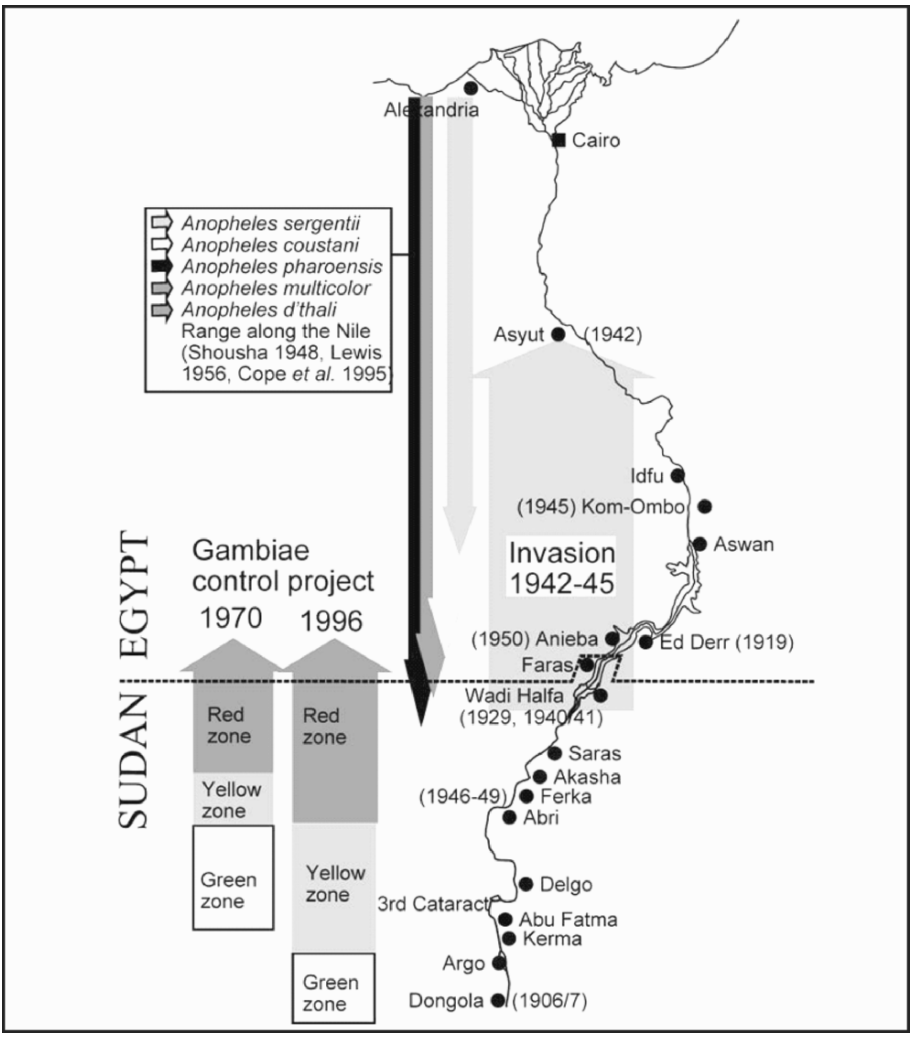

Figure 4. *Northern distribution of* Anopheles arabiensis *over the last 100 years.*

An. arabiensis was judged to be Akasha and by 1996 this had been pushed back close to Abri (Fig. 4).

All historical entomological records for Northern State indicate *An. arabiensis* is the only anopheline, and therefore the only malaria vector present, with the exception of reports of *An. pharoensis* just south of Wadi Halfa at Abka and *Anopheles multicolor* Cambouliu and *Anopheles d'thali* Patton a little further north at Faras. Two others, *Anopheles sergentii* (Theobald) and *Anopheles coustani* Laveran merit attention since they have come close to the border. An interesting observation is that four of these species are found in other parts of Sudan; *An. pharoensis* in particular is relatively common south of Khartoum (Shousha 1948, Lewis 1956, Cope et al. 1995). If these species are indeed absent from Northern State, or present only periodically in small numbers, it would be of interest to determine the degree of genetic differentiation

between the Egyptian and Sudanese populations, since it is conceivable that their separation dates back to the Kerma Period or before, when the environment was much more favourable for migration along the Nile.

Two important components of research and development for the use of the SIT are population studies on the target and an understanding of its relationship with other species likely to be affected by its suppression or eradication. Other potential vectors are the most immediate concern. The fact that they appear to be absent is a major advantage to the project, but there is clearly some merit in knowing why they are absent and how easily that situation might change.

Present day population studies increasingly rely on the use of molecular markers, but as with most approaches the data can often have multiple interpretations. Preliminary studies based on analysis of microsatellite DNA data suggest that the Northern State *An. arabiensis* population is isolated by the desert and partly by the western stretch of the Abu Hamed Reach. Analysis of mitochondrial DNA, however gives a different picture. This is easily resolved if it is assumed that the isolation is sufficiently recent to only show up with the more rapidly-evolving microsatellite DNA. This interpretation appears therefore to be consistent with the inferences made from the historical data. Despite being highly speculative, the scenario proposed for past changes in the vector population, does provide an interesting perspective that may prove more useful as further analysis of the present day population becomes available and as archaeological studies continue in Northern State, particularly at the present time around the Fourth Cataract.

7. Conclusions

It is suggested that *An. arabiensis* was absent from Northern State 18 000 years ago, but between 8000 and 3500 years ago it migrated from any of several diverse locations to establish a presence in the Dongola Reach and spread further into Egypt. It then receded from Egypt and most probably also the Dongola Reach. Other mosquitoes found today in both countries may also have receded from the Northern State area and some, such as *An. pharoensis*, may never have re-established a significant presence. Within the last 1500 years *An. arabiensis* became established again in Northern State, almost certainly from upriver, and despite many bottlenecks maintained a continuous presence reaching as far as the Second Cataract, with only intermittent incursions into Egypt. A more detailed investigation may throw more light on the factors, frequency and duration of likely bottlenecks during the last 1500 years and the extent to which the population has been supplemented by migration or dispersal downriver along the Abu Hamed Reach.

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Integrated Management of Rice Stem Borers in the Yangtze Delta, China

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ABSTRACT The rice striped stem borer *Chilo suppressalis* (Walker), the yellow stem borer *Scirpophaga incertulas* (Walker), and the pink stem borer *Sesamia inferens* (Walker) are the most injurious insect pests of rice in the Yangtze Delta, one of the country's rice "bowls" and a region undergoing rapid economic development. In recent years, the population densities of *C. suppressalis* and *S. inferens*, and associated yield losses and costs of control have reached the highest recorded levels. This is attributed to the combination of a large-scale shift from pure double cropping to single-double mixed cropping, the increased yield potential of new varieties, and the development of high levels of resistance of stem borers to dimehypo, triazophos, and other insecticides. The risk of resistance developing to the few current very effective insecticides (e.g. fipronil), coupled with the harmful effects of such insecticides on freshwater shrimps, crabs and honey bees makes it imperative that researchers and farmers explore and implement alternative approaches to relying on insecticides. The indigenous nature of the agroecosystem and the characteristics of local dispersal between habitats suggest that an area-wide approach is probably the best way to achieve the objectives of managing rice stem borers in such a way that their density is kept below economic injury thresholds, while protecting the rural environment and the health of farmers. Components of the current integrated management approach include: (1) ploughing and irrigating the fallow rice paddy in early spring to kill overwintering larvae and pupae, (2) postponing and synchronizing seeding and transplanting dates of rice to reduce the opportunity of oviposition by overwintered moths, (3) use of stem borer mid-resistant varieties of rice, (4) use of *C. suppressalis* pheromone and highly effective light traps to trap and kill moths, (5) not spraying of insecticides during the first 30 days after transplanting, and using microbial insecticides in mildly damaged fields and fipronil in highly damaged fields, and (6) providing better communication and information to farmers for using control techniques properly. This IPM approach is very promising, and if implemented on an area-wide basis will lead to much improved control of the stem borers.

KEY WORDS integrated pest management, area-wide, rice, stem borers, Yangtze Delta

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 373–382.

1. Introduction

The Yangtze Delta of China is one of the largest rice-producing regions in the world. It has been called the "big rice bowl" for many years. Many biological constraints limit the attainment of potential yields. Rice stem borers have a long history of being the most injurious insect pests of rice, with the rice striped stem borer *Chilo suppressalis* (Walker), the yellow stem borer *Scirpophaga incertulas* (Walker), and the pink stem borer *Sesamia inferens* Walker, being the main species involved (Khan et al. 1991, Cheng et al. 1998).

The Yangtze Delta is also one of most rapidly developing regions of China. Agricultural land is being converted to industrial uses, and farmers are becoming factory employees since the opening-to-the-world policy started in the 1980s. Meanwhile, the costs of agricultural production, including material and labour inputs, are rising. However, as one of the most densely populated countries in the world, rice production for food security is still the national policy in China. Therefore, maintaining and increasing rice unit yields remain the main objective of the agricultural sector, and management of stem borer pests is one of the most important measures to achieve this.

This article summarizes the current situation, general strategy, the main measures to control stem borers in the Yangtze Delta, and proposes improvements towards an area-wide approach.

2. Occurrence of Rice Stem Borers and Main Causes

In recent years, population densities of *C. suppressalis* and *S. inferens*, crop losses and costs of control have reached highest recorded lev-

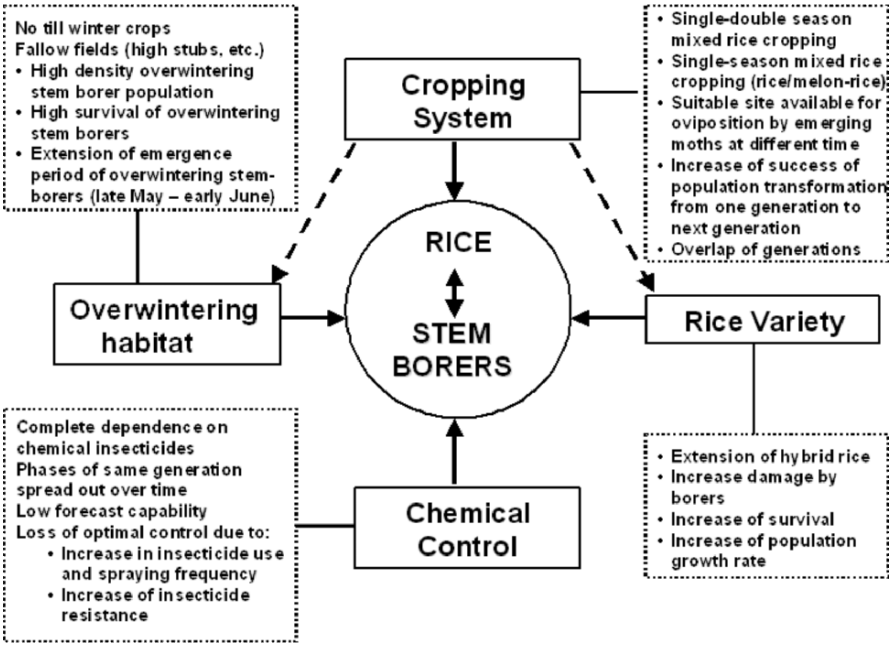

Figure 1. *Diagram indicating the main factors causing outbreaks of the rice stem borers in the Yangtze Delta in China.*

els. Most likely, the reasons for such high population densities can be attributed to one or a combination of the factors shown in Fig.1.

2.1. Diversification and Enhancement of Overwintering Habitats for Rice Stem Borers

Since industrialization of the Yangtze Delta region, cultivation of winter crops has fallen, and the proportion of fallow lands has increased significantly. Meanwhile, the types of winter crops have also diversified and now include rape, green manure, wheat or barley, fish-feeding grasses, vegetables, and others. The survival of overwintering stem borer larvae is normally significantly higher in fallow and wheat fields and lower in manured fields due to early ploughing during the pupation period from April to mid May (Jiang et al. 2002a,b). The tools used for harvesting the rice crop are also an important factor influencing stem borer larval survival during winter. Normally, the higher the rice stubble remaining over winter, the higher the larval survival. In addition, winter temperatures in recent years in the region were frequently higher than the average for the last 50 years, and minimum winter temperatures in the aforementioned habitats were higher than the super-cooling points of stem borer larvae. This implies that survival of overwintering larvae, even the younger larvae, was little affected. These are some of the main factors increasing the number of overwintering larvae in the region.

2.2. Shift from Double to Single-Double Mixed Cropping Systems

Since the 1980s, the cropping system in the Yangtze Delta has shifted significantly from only double-cropping to a single-double mixed cropping system (Cheng et al. 1998). In such a mixed system, the sowing date of rice varies from late April to early June, and the transplanting date therefore varies from early May to late June. Directly-sown rice is planted from early May to mid June. Thus almost all newly emerged adult moths from different overwintering periods can find rice fields at a suitable growth stage for oviposition.

2.3. Increased Yield Potential of New Varieties

Hybrid japonica rice, the super high-yield hybrid and conventional indica rices are all relatively tall plants, have a large stem diameter and inner antrum, and high ratios of nitrogen to carbon. All of these encourage stem borers to bore, grow, survive, and reproduce (Zhu et al. 2002). Hybrid japonica rice (e.g. Hanyou xiangchen, Mingyou 55 and Bayou 161) is more susceptible to damage by stem borers (Fig. 2), probably because of the longer heading period (average seven days) compared with indica varieties (average around five days), which increases the possibility of boring by early-stage stem borers.

Stem borer-tolerant high-yielding hybrid and conventional rice varieties are widely grown in the Yangtze Delta and yield reductions by stem borers in tolerant varieties are lower. However, on a regional scale, this helps stem borer populations to accumulate, and therefore increases the risk of damage to other types of rice and other crops, e.g. the water oat (wild rice) *Zizania* spp., an aquatic vegetable which serves as a host plant for stem borers, and likewise increases the pressure to apply chemical insecticides. However, given the important role of high-yield rice varieties for national food security, their large-scale cultivation is unavoidable, and hence development of effective measures for management of stem borers is a matter of urgency.

2.4. Development of High Levels of Resistance to Insecticides by Stem Borers

The resistance of *S. incertulas* to the two neurotoxic insecticides monomehypo (monosultap) and dimehypo, has been reported to be as high as at 40-243 fold, i.e. the need to increase 40-243 times the amount of insecticides to get the same 50% kill of insects (Su et al. 1996, Zhu et al. 1987, Li et al. 2001, Jiang et al.

2002a, Jiang 2004). The lowest level was detected as 21.4 fold in the mulberry area of Jiaxing, North Zhejiang, where the neurotoxic insecticides are not recommended for use due to their high toxicity to mulberry-raised silkworms *Bombyx mori* (L.). These data confirm the fact that the two insecticides have very low effectiveness for controlling stem borers in the Yangtze Delta region.

Triazophos, an organophosphate insecticide, has been one of the main insecticides used for large-scale control of rice stem borers in China since the early 1990s. Recently, it was reported that the field efficacy of this insecticide had decreased significantly, and that the rice stem borer had developed resistance. Thus, Peng et al. (2001) reported that rice stem borers in Gaochun, Jiangsu Province had developed 23-fold resistance to triazophos. Although the level of resistance of *C. suppressalis* to triazophos in the central Yangtze Delta region is still low (around 5-fold), the level in the southern fringe of the region (i.e. on the east coast of Zhejiang Province) is 40-fold (Jiang 2004), and may be as high as 203-fold (Qu et al. 2003).

Fipronil is one of the most effective insecticides developed in the last decade to control rice stem borers. Nevertheless, there is a high risk of resistance to this compound also developing in rice stem borers. The resistant levels of some striped stem borer populations in locations in the Zhejiang Province was detected as high as 3.1-fold. Also, there are strong possibilities that this insecticide has harmful effects on fresh-water shrimps, crabs, and honey bees, which are the other main sources of income for Zhejiang farmers. Additionally, the high price of these insecticides increases the costs of rice production for farmers. All of these factors require researchers, local technical extension staff and farmers to explore and implement alternative approaches to the current reliance on insecticides.

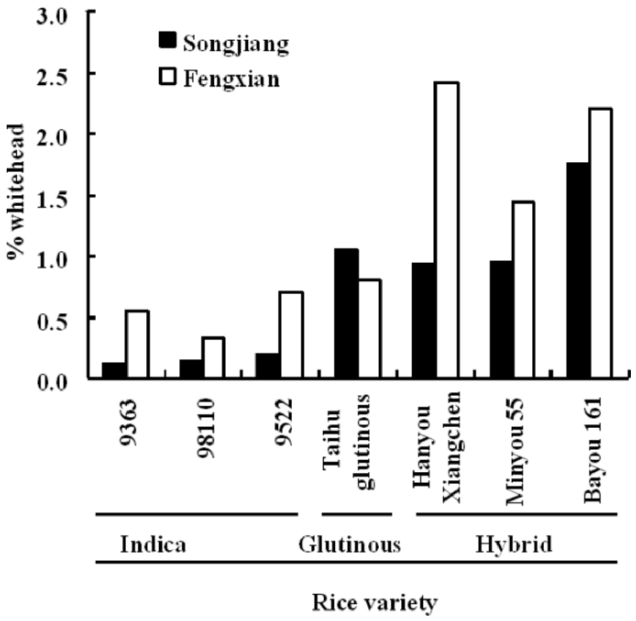

Figure 2. *Comparison of different damage levels (whitehead) caused by stem borers to three varieties of rice in fields of Songjiang and Fengxian counties, Shanghai, China.*

3. Area-Wide Strategy for Management of Rice Stem Borers

3.1. Rationale for an Area-Wide Strategy

The nature of the local agro-ecosystems and the characteristics of dispersal of rice stem borers among habitats suggest that an area-wide approach is probably the best way to achieve the objectives of managing these pests in ways that reduce their densities to below economically damaging thresholds while maintaining the rural environment and safeguarding the health of farmers. Also, the national policy of improving food safety requires an area-wide approach to the design and implementation of rice pest management programmes. Addressing large-scale multiple-source pollution caused by agricultural chemicals (fertilizers and pesticides), is the main challenge for restarting clean-food production in the Yangtze Delta region. Effective control of stem borers and reductions in chemical insecticide applications are the main ways of addressing this challenge.

3.2. Components of the Area-Wide Management Approach

The main components of the area-wide rice stem borer management approach are outlined in Fig. 3.

3.2.1. Rice Paddy Management to Increase Pupal Mortality in Early Spring

Field investigations have shown that the survival of larvae during winter (November-February) was very high, and that most mortality occurred during the pupation period around late April to May. Thus, the mortality of overwintering larvae of *S. incertulas* and *C. suppressalis* from January to April in the period 2001 to 2004 in Nanhui County, Shanghai, ranged from nil to 33% and from 0.9 to 16.7%, respectively while the values increased abruptly to 95 and 89%, respectively during the pupation period in late April. Therefore, flooding and ploughing during late April and May to kill the overwintering stem borer was recommended to farmers and local extension stations.

Fields growing the first-season rice should

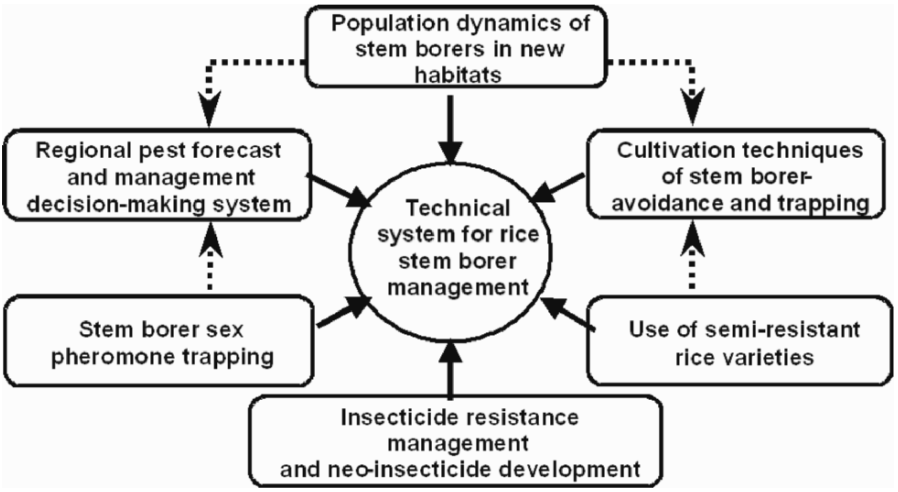

Figure 3. *Diagram showing the main technical components of the area-wide rice stem borer management approach.*

be ploughed and flooded immediately, particularly between the peak period of pupation and the beginning of emergence of the first generation of moths. However, fields planned for growing single-season rice or winter crops or fields left fallow and planned as seedling nurseries of second season rice, should be flooded as deeply as possible to ensure submergence of all stubs, and kept so for at least three days to kill the overwintering stem borers.

Additionally, straw with a high density of active overwintering stem borers should be used as livestock feed, bedding, or other purposes before emergence of moths takes place.

3.2.2. Improved Rice Cultivation Practices

Normally, overwintered stem borer moths in the Yangtze Delta emerge around early to mid May. If they cannot find suitable host plants for oviposition, populations of these moths would certainly be reduced in the following generation.

Transplanted rice: In the single-season rice cropping area, a 4-year field experiment in Changshu, Jiangsu Province indicated that postponing the sowing date from 10 May to 20 May could reduce the oviposition of first generation stem borers by as much as 80% in the seedling bed, as well as the level of damage in transplanted fields. A similar experiment during 2002 in Shanghai, showed corresponding reductions of 81 and 70%, respectively, and grain yield increases of 27% when sowing and transplanting were carried out on 20 May and 20 June, compared with sowing on 5 May. Such cultivation techniques could be extended to the area of single-season rice cropping with no effect on grain yield potential due to the possible negative effect of lower photosynthesis in late season.

The densities of *S. incertulas* and *C. suppressalis* in seedlings sown in seedbeds at 7-day intervals on different dates between 5 May and 26 May, decreased gradually with each delay of sowing. Similar trends were found in the percentages of plants damaged by stem borers. The percentage of whitehead in the late sown/transplanted rice, however, was not lower than early sown/transplanted rice. The highest yield was harvested from the rice sown on 25 May. Therefore, sowing on 20 May and transplanting on 16 June were recommended to avoid damage by rice stem borers.

Directly-sown rice: The experiment with different sowing dates for directly-sown rice (hybrid japonica rice: Hanyou Xiangqing) showed that with delayed sowing, the percentage deadheart caused by the first generation of all three species of stem borers decreased (sampled on 3 July). However, the percentage deadheart caused by second-generation stem borers generally increased with the postponement of the sowing date, with the lowest percentage deadheart occurring around 20 May; the lowest percentage of whitehead by *S. incertulas* of the third generation was also around 20 May. This implies that the optimal sowing date for this type of cultivation could be 20-25 May.

3.2.3. Use of Stem Borer Semi-Resistant Rice Germ-Plasm

Semi-resistant rice germ-plasm and varieties can reduce populations of rice stem borers through non-preference for oviposition, reduction of boring success rate, larval and pupal survival and emergence. Such semi-resistant rice germ-plasm or varieties have quantitative trait loci for stem borer resistance and the characteristic is normally stable (Papst et al. 2004). For long-term use, introgression of quantitative resistance into high yielding rice varieties is particularly necessary.

3.2.4. Trapping with Pheromone-Baited and Light Traps

There is evidence of significant positive correlations between the numbers of *C. suppressalis* moths caught in pheromone traps and the density of egg masses in seedling beds, the percentage of damaged sheaths, and the percentage of whitehead (Jiao et al. 2002). The percentages of brownish leaf sheath, deadheart and whitehead decreased respectively by 71, 57 and 44% in plots with the *C. suppressalis* pheromone baited traps at a density of 25 traps per hectare as compared with the chem-

ical control plots in a large-scale field experiment in Lujiang County of the Anhui Province located in the mid Yangtze River region (Su et al. 2003). Additionally, in the future, the synergistic effect of stem borer sex pheromone and rice plant volatiles may be one practical way to interrupt stem borer mating and host-finding behaviour.

Other studies have also shown that certain types of light traps can reduce damage to rice plants by as much as 80% (Yang et al. 2004). This resulted from the lower density of ovipositing female moths and eggs and hence, of damaging larvae. In paddy fields treated with frequency-vibration light traps, the number of insecticide applications was reduced to two and the quantity of insecticide applied was reduced to 2.1 kg/ha, thereby reducing the cost of pest control over the whole rice growing season by USD 17/ha.

3.2.5. Improved Insecticide Management

In the double-cropping rice region, cessation of the practice of spraying insecticide within 30 days of transplanting rice can be highly effective for conserving the local non-harmful invertebrates in rice fields and thereby increasing the food supply for and populations of predators. Similar effects were also found for egg and larval parasitoids of stem borers (Jiang et al. 1999) and planthoppers (Zhu et al. 2004b, c), and predators of planthoppers (Zhu et al. 2004a).

To reduce the development of resistance to fipronil in *C. suppressalis*, the number of fipronil applications in a rice-growing season should be restricted to two, while applications of triazophos should be restricted to between two and three. Alternatively, the application of different types of insecticides is recommended. Because of its high toxicity to shrimps, crabs and honeybees, the use of fipronil in rice fields near shrimp- and crab-raising pools and honey-source crops should be restricted.

3.2.6. Improved Public Information

Access to information on the dynamics of emergence of stem borers in the diverse overwintering habitats, and on changes in oviposition and population dynamics in rice paddy fields due to unsynchronized sowing and transplanting dates should be improved. After collection and processing of the necessary information, the occurrence, timing, density and damage forecasts, as well as recommendations for controlling stem borers should be announced immediately through proper and effective channels for the different types of cropping systems. After interventions, the relics of stem borer populations should be monitored so that any needed secondary action can be taken.

There are several pathways and delivery systems to disseminate pest warnings and management recommendations. Printed pest information notes, local newspapers, cable broadcasts, etc. have been the main ones employed since the establishment of pest forecast stations at district and county levels in the 1960s. Due to the popularization of television in the early 1990s and particularly of cable TV since the late 1990s, the amount of visual and active information presented in TV programmes has increased, and the efficiency of delivery improved. Information boards, posters distributed by pesticide companies and vendors are also very common and effective.

In a comparison of different information delivery tools in Jiaxing in North Zhejiang Province, announcements of application timing and types of insecticides through cable broadcasts plus a paper note were the most effective, while broadcasts or paper notes alone made no significant difference (Zhu and Cheng 1998).

Recommendations for the control of rice stem borers should vary according to the geographical distribution of the pests, in order to avoid overuse of insecticides in low-risk sites and loss of control in high-risk sites. Therefore, fine scale spatial maps for information dissemination through TV programmes or web sites are important and they can be easily understood by farmers.

Based on the current outbreaks and analysis of the causes of rice stem borer populations in the Yangtze Delta, an interprovincial col-

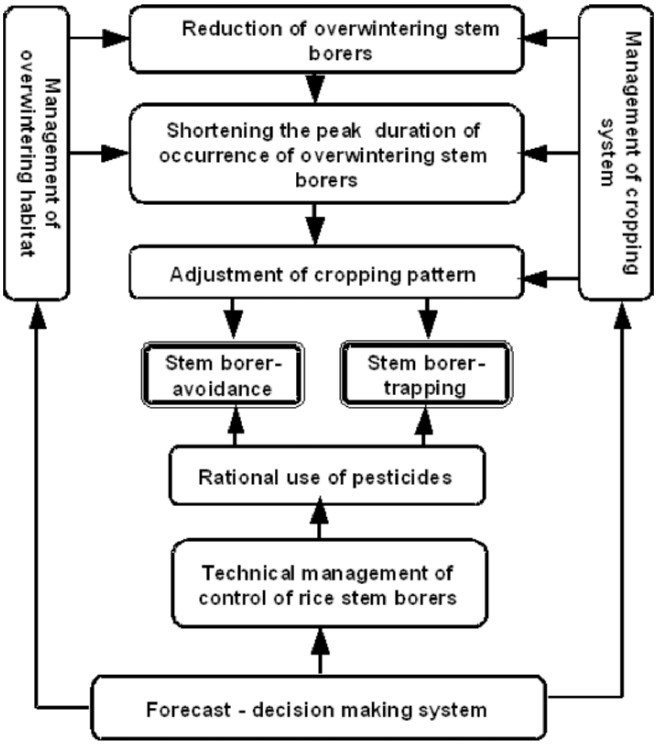

Figure 4. *Flow chart of techniques used in the area-wide integrated management of rice stem borers in China.*

laborative project was set up for the regional ecological management of rice stem borers. The main measures for managing these pests effectively involve three strategies: (1) management of overwintering habitats, (2) management of cropping systems, and (3) a forecast-decision making system (Fig. 4). Results from the first year of the project (which will be reported elsewhere) were very promising, and if implemented on an area-wide basis will lead to much improved control of the stem borers.

4. Discussion

For managing rice stem borers in the Yangtze Delta region, an area-wide pest management approach is both necessary and potentially effective. The main components of the approach, including overwintering habitat management, cropping systems management and a forecast-decision making system, are interacting and targeting the same objectives, i.e. improving rice production through control of stem borers. Enhancing rice varieties and adjusting cropping patterns can assist in avoiding damage by stem borers, while the use of pheromone and light traps can reduce their populations. Such a "push and pull" approach conducted area-wide would certainly be highly effective in controlling stem borers.

Organization of this approach should be improved. Distribution of rice varieties with moderate levels of resistance to stem borers and with high quality and yield potential should be coordinated through the seed companies. Decisions on optimal ploughing times,

the allocation of irrigation water for flooding to increase the mortality of overwintered larvae and pupae, sowing and transplanting dates, etc., have been made by local extension agencies at the township level. Farmers usually buy suitable insecticides from dealers and sales representatives located in villages. In this way, local dealers' recommendations for insecticides strongly influence farmers' decisions. However, both the way that information is delivered and the type of information provided should be improved. Information on the occurrence and control of rice stem borers could be both improved and simplified by releasing group messages through mobile phone companies. Additionally, farmer training on the essential knowledge needed for pest recognition and on the techniques for pest management would further encourage farmers to use new techniques within the framework of area-wide pest management. Such technical and organizational steps have now been designed and will be emphasized in future implementation of the project.

5. Conclusions

The combination of a regional-scale shift from pure double cropping to single-double mixed cropping, the large-scale planting of new varieties with high yield potential and increased levels of resistance to stem borers to the main insecticides have contributed to increasing the occurrence of stem borers since the 1990s.

Area-wide pest management approaches are both necessary and most likely would be most cost-effective for controlling rice stem borers in China. The area-wide management approach could include combinations of the following: (1) early spring ploughing and irrigating the fallow rice paddy, (2) postponing and synchronizing seeding and transplanting dates of rice, (3) use of stem borer semi-resistant rice varieties, (4) use of stem borer pheromone and highly effective light traps to trap and kill moths, (5) not spraying insecticides during the first month after transplanting, (6) optimal use of microbial and chemical insecticides, and (7) providing better communication and information to farmers for using control techniques properly. These components belong to three interacting categories: (1) overwintering habitat management, (2) cropping system management, and (3) a forecast-decision making system. The implementation of this approach is ongoing and early results attest to its effectiveness, although further information delivery and technical refinements are required.

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Management of Cotton Insect Pests in Tajikistan

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ABSTRACT Cotton is a major crop in many irrigated areas in Tajikistan and its cultivation is severely hampered by several species of cutworms, especially the turnip moth *Agrotis segetum* Schiff and the cotton bollworm *Helicoverpa armigera* (Hübner). The population dynamics of these two species is described as it relates to their impact on cotton cultivation and the importance of other crops, as well as weeds, is also described. The paper indicates ways that an understanding of phenology can be used to manage the insect pests. Based on this understanding an area-wide approach is suggested that will lead to improved control using less insecticide.

KEY WORDS *Helicoverpa armigera*, *Agrotis segetum*, phenology, planting times, cotton, irrigation, Tajikistan

1. Introduction

The total area of Tajikistan is 143 100 square kilometres, of which only 780 000 hectares are suitable for irrigation. Most of the irrigated land is concentrated in river valleys. One such valley is the Vakhsh Valley, with a total area of 1 479 000 hectares, of which 243 100 hectares are irrigated. More than 30% of the country's irrigated land is located in this valley. During the period covered by the research reported here, industrial crops occupied 153 116 hectares of this land, of which cotton was cultivated on 84 149 hectares.

Ninety two different species of cutworm (Noctuidae) belonging to nine subfamilies have been found in this area. As virgin land is reclaimed for agricultural crops on the floor of the valley, overwintering and propagation sites shrink, especially for monovoltine species, and these are therefore now on the verge of extinction. However, for polyvoltine species of cutworm, which produce three to seven generations over the spring-summer and autumn periods, oases and irrigated land are the main sites for breeding and development since it is only at these locations that the plants on which they feed grow in sufficient quantities. These species of cutworm account for 23.9% of the total number. They are playing a central role in determining the interspecies relationships and interdependencies between the main and diverse groups of organisms on irrigated land that feed on insects due to their high number of generations and diversity of their developmental periods which serve as prey for useful insects. Despite their species diversity only four species of cutworms are pests causing economic damage to agricultural crops, including cotton, and of these, the turnip moth *Agrotis segetum* Schiff and the cotton bollworm *Helicoverpa armigera* (Hübner) cause most damage to cotton and other crops on irrigated land in Tajikistan.

2. Dynamics and Control of *Agrotis segetum* in Cotton

First generation *A. segetum* larvae attack the seedlings of cotton plants. However, in many cases the control measures that were devel-

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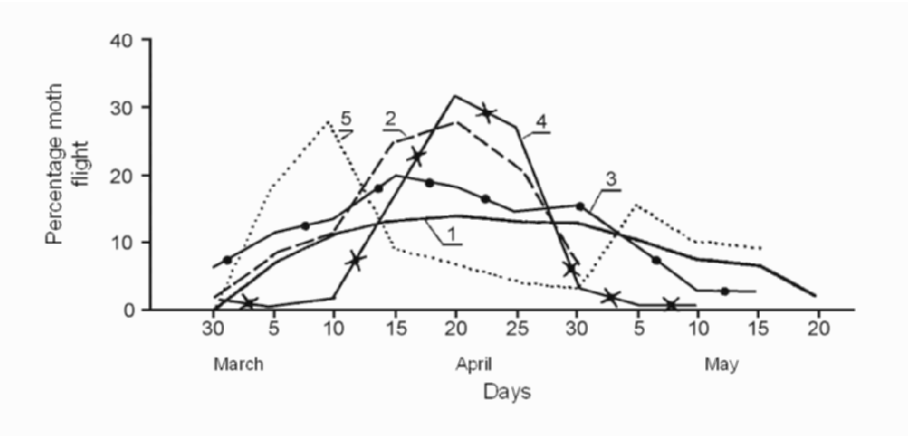

Figure 1. *Dynamics of the flight of moths from overwintering generations of winter cutworm at five-day intervals according to (1) a computer simulation, and as sampled (2) on cotton plants, (3) on vegetables, (4) in orchards and vegetables, and (5) in gardens.*

oped – namely seed dusting or soil applications with pesticides before sowing – were based primarily on the application of pesticides over large areas of cotton-growing land without taking into account the biology of the pest and related agroecological characteristics. However, studies of the ecology of this species of cutworm have shown that, under the conditions in the Vakhsh Valley, the flight of moths from overwintered generations of this species begins 10-15 days before cotton plant seedlings appear (Fig. 1).

The females' intensive egg-laying period occurs in April and continues for more than 10 days. During this period, weeds and cotton plants are beginning to sprout in most fields. The females lay their eggs on the soil, guided by a response to substances secreted by plants during seed development and by the root system of perennial weeds.

The population dynamics and density distribution of first generation *A. segetum* larvae in cotton fields depend on the extent to which cotton sowing coincides with the mass-flight of moths and their egg-laying, and by the number of broad-leaved weeds that attract these pests.

Due to their low resistance to damage by larvae, the critical period for cotton plants is between the appearance of seedlings and the formation of 6-8 leaves. This period can be divided into two stages as regards the nature of the damage inflicted. In the first stage, the larvae gnaw right through the seedlings' root collar and the plants die. This leads to crop destruction and reduced yields. As the root collar grows tougher and 4-5 true leaves appear, larvae in weed-free fields feed on cotton plant leaves and sometimes gnaw off the growing point and the root system; however, this does not affect the final harvest under normal plant development conditions.

The most dangerous period for damage to cotton crops by *A. segetum* larvae begins during the last 10 days of May when the larvae mature "en masse". This period lasts a maximum of 10 days during which the actual damage to cotton plants depends on many factors including: the stage of plant development, the degree of weed infestation and the proximity of cotton plantations to alfalfa fields. Therefore, when inspecting fields, account must be taken of the number of damaged cotton plants since even when the density of larvae is high, they do not always inflict economic damage, especially if weeds are present (Table 1).

With an average monthly temperature of

Table 1. *Population dynamics and damage caused by winter cutworms in relation to crops preceding cotton.*

Inspection date	Preceding crop	Area[1]	Sowing date	Total cotton plants per square metre	Damage (%)	Larvae per square metre (no.)	Total weeds per square metre	Damage (%)
25-May	Cotton	33	3 April	13.0 ± 2.0	0.0	0.4 ± 0.1	0.8 ± 0.2	100
	Alfalfa	32	3 April	12.0 ± 1.5	0.0	0.8 ± 0.2	5.0 ± 0.9	37.4
	Rape	14	6 April	14.0 ± 1.9	0.0	7.2 ± 1.3	18.0 ± 2.0	39.0
30-May	Cotton	16	8 April	15.0 ± 1.4	0.0	0.8 ± 0.2	2.8 ± 0.6	33.3
	Rape	10	9 April	18.0 ± 1.1	5.5	8.4 ± 1.2	14.0 ± 4.0	50.0
	Rape	12	11 April	17.0 ± 1.5	5.8	12.0 ± 2.0	24.0 ± 5.0	58.3

[1]Hectares

over 7°C in January, 10°C in February, and 12°C in March, the development of first generation cutworms outpaces that of the cotton plants, especially if temperatures during the sowing period in the first 10 days of April do not exceed 13°C. Under such conditions, damage to crop seedlings from more mature larvae is over 50% if the plants are at the stage where they have grown two or three true leaves in the last 10 days of May. In weed-infested fields with high *A. segetum* larval populations and after weeding in the last 10 days of May, the number of damaged cotton plants can increase by a factor of 15 to 35, especially in fields that are heterogeneous or where plants are underdeveloped. Therefore, the level of development of larvae on each plot must also be determined before weeding.

To reduce the damage caused by winter cutworm larvae, the following adjustments to

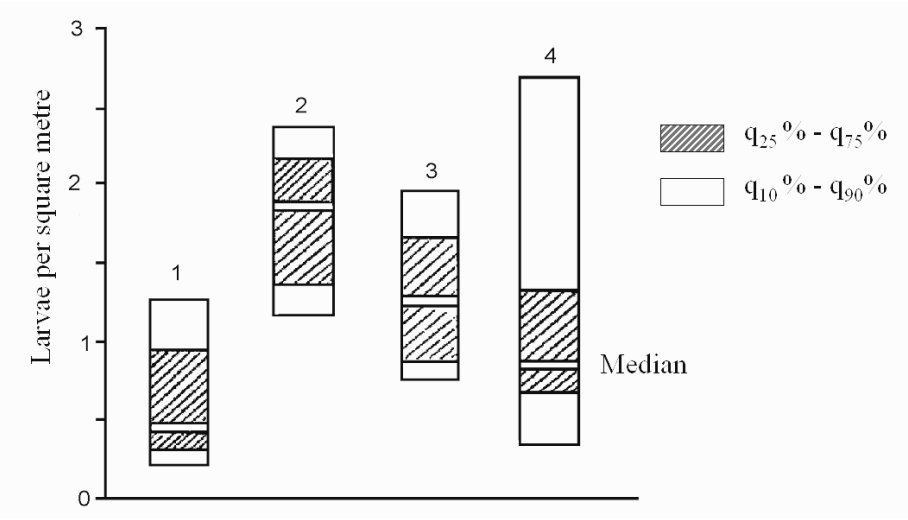

Figure 2. *Variations in overwintering larval populations of the Turnip moth in various parts of Tajikistan's cotton zone. (1) cotton, (2) vegetables, (3) alfalfa, and (4) corn.*

the technique and schedule for tending cotton crops are recommended: (1) when thinning out seedlings, first remove underdeveloped plants, and (2) if the field has not been weeded before the last 10 days of May and there is a high density of larvae, weeding should be delayed for several days to allow most of the more mature larvae to finish feeding on the weeds and enter the pupal stage.

The ecology and the economic damage thresholds of the pest should also be taken into account when implementing any control or eradication measure. The threshold for economic damage by *A. segetum* in early cotton sowings in the last 10 days of May (weed-free) when more mature larvae predominate is approximately one larvae per square metre, while it is three to seven larvae per square metre in fields with weeds, and 0.3 to 0.5 larvae per square metre in sown fields.

The times proposed in the recommendations for applying soil pesticides before sowing are not ecologically viable for controlling first generation larvae in cotton crops. This is because 45-50 days elapse from the time the pesticides are applied to the time when the pests begin to inflict heavy damage and their toxicity to the larvae falls over this time period.

In summary, the system used to control damage from the winter cutworm must take into account the developmental period of first generation pests, the sowing schedule and developmental stage of the cotton, pest-attracting weeds and thresholds of economic damage. When these factors are taken into account, the damage caused by larvae in fields can be controlled with minimal pesticide use. It has been established that fields sown with vegetables and alfalfa are areas where overwintering cutworm populations are abundant (Fig. 2).

3. Dynamics and Control of *Helicoverpa armigera* in Cotton

In managing the damage caused by *H. armigera*, it is important to take account of the agroecological factors which affect their reproduction and development during the season in cotton-based agrosystems. Reproduction of cotton bollworm moths in cotton fields is crucially dependent on the availability of food plants, and on the pests' initial development prior to the budding of cotton plants. Certain types of weeds act as significant resources during initial land reclamation along the verges of irrigation canals, reservoirs and vacant lands, and in the cotton fields. Depending on the technique used to cultivate the crop, these plants, played a major role in regulating larval populations and the damage they inflicted in cotton fields. Until the mid 1960s, the borders on each side of open reservoirs were several tens of metres wide and each reservoir extended for tens of kilometres through various farms. Gradually, with the use of intensive cotton cultivation techniques, these areas were used for cultivation of the crop and on newly reclaimed land, these structures are now non-existent. As a result, weed growth and development now coincide with those of cotton seedlings in these areas.

During the egg-laying period, *H. armigera* are attracted to fully-formed plants that are 30-40 centimetres high, and not to seedlings. Weeds begin to sprout during mass-flight of moths from overwintered generations of bollworms and their egg-laying in the fields in the last 10 days of April and at the beginning of May. The developing stages of weeds do not attract moths for egg-laying and therefore the significance of weeds as a source of food for *H. armigera* gradually decreases. In addition, remaining small patches of weeds along irrigation canals are generally destroyed in April-May by agronomic and chemical measures, so that by the time the cotton bollworm lays its eggs, the broad-leaved weeds have completely disappeared. Therefore, weeds have long since lost their significance for the reproduction and development of first-generation *H. armigera* in the Vakhsh Valley.

Chickpeas also have no practical significance for the development and reproduction of the cotton bollworm in this zone. Melons and gourds, particularly pumpkin and marrow,

which were previously considered as development sites for first-generation *H. armigera*, have also lost their significance owing to the increased cultivation of other crops in the cotton agroecosystem. Nowadays, even if pumpkin and marrow are grown in kitchen gardens or on specialized vegetable farms, they are planted late and therefore begin to flower when the flight of moths from overwintered generations is coming to an end.

Of the other host plants that occupy second place in terms of area after cotton, alfalfa is important. This crop begins to grow in the Vakhsh Valley at the end of February, beginning of March, and many cotton pests and their predators develop on it. In the spring, by the time of mass-flight and egg-laying of moths from overwintered generations, the alfalfa is harvested for green fodder for livestock and, during harvesting the equipment pulverizes all the green material. As a result, almost all members of the species are killed and removed from the fields.

Of all of the plants cultivated in crop rotations with cotton during development of the first generation, the cotton bollworm currently finds partial refuge only in maize and tomato fields. At the time of mass-flight of the moths of overwintered generations, the maize has not yet formed cobs and therefore the pests lay their eggs on the leaves near the growing point. The hatched larvae then penetrate into the channels and continue to develop but their numbers in April and May are never very significant. Depending on a farm's desirable crop rotation, maize is sown over an area of 80-200 hectares and tomatoes over 6-7 hectares. Generally speaking, the area of small patches with first generation bollworms for all farm crops does not exceed 8-10% of the entire cotton field agroecosystem.

Thus, the majority of moths from the pupae of overwintered generations emerge and realize their potential before the cotton plants bud. However, a proportion of this generation emerges late and can therefore fully realize its reproductive potential in cotton crops at the end of May. Quantitatively, this proportion of the population is not significant and does not lead to a large pest population in cotton fields. High pest populations on cotton plants can only arise from the offspring of first generation moths, no earlier than mid June. Therefore, it can be assumed that cotton plants are colonized by moths that emerged from pupae of overwintered generations which ended up 30-40 centimetres deep in the soil as a result of autumn ploughing and that completed their development at a relatively low temperature. However, it is also possible that they migrated from other foothills or mountainous areas of the country where their populations developed on maize, tobacco and tomatoes and, in the spring, their reactivation began much later.

Although there is a major *H. armigera* flight during the period of mass-budding of cotton plants from 16 to 25 June, this is not due to overwintered populations since temperatures in the Vakhsh Valley in April-June exclude the possibility of pupae diapausing for so long. Also, since their reactivation (according to the data in the literature) begins in mid March, the greatest risk for cotton crops is from moths emerging in the second half of June. At this time, the scale of crop colonization and the density of the larvae are several times greater than in the first half of the month. It is precisely at this time that serious damage to the reproductive structures by larvae must be prevented. The fourth five-day period in June is a particularly critical time because first and second instar larvae, which are the most sensitive to control measures, predominate in most fields.

The distribution of and damage caused by pest larvae are also linked to the topography of the area (Fig. 3).

On farms where the fields are bowl-shaped, bollworm eggs and larvae are found in large numbers in the strip that is 20-30 metres from the beginning of the rows from the edge, decreasing sharply beyond that. In these areas, the physiological state of the cotton plants is better due to the more intense accumulation of organic matter, the bushes are more developed and much higher than those growing below this strip. In level fields, the

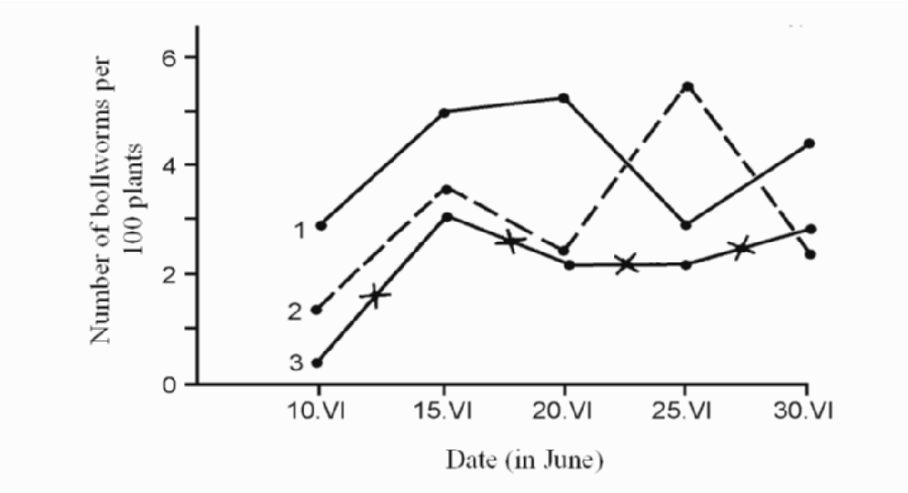

Figure 3. *Population dynamics of the first generation of cotton bollworms on various terraces of variety 6445 cotton crops. (1) Kalandarov State Farm, (2) Guliston State Farm, and (3) Turkmenistan State Farm.*

plants develop uniformly and the accumulation of reproductive structures proceeds almost identically over the entire field. Accordingly, moths in these areas lay their eggs uniformly on plants over the entire field. During egg-laying fine staple varieties of cotton attract moths more than medium staple varieties. Since the latter are sown much later than the former or when replanting instead of the former, their reproductive structures appear after mass egg-laying of first generation pests.

In the Vakhsh Valley, the population dynamics of second generation *H. armigera* in cotton plantations varies, with the greatest number of bollworms being found in fields on the fifth terrace where the density of the pest in unfavourable years is almost the same as in years of mass-reproduction on other terraces. In years of mass-reproduction, the pest population within each farm is 3-6 times greater than in less favourable years. A characteristic feature of flights with a high number of second generation bollworms is that population increases are already noticeable in the first ten days of July, and on the basis of such observations it can be predicted that populations everywhere will increase subsequently. In such years, females may simultaneously lay eggs at the plants' growing point on high-, low-, and medium-height cotton bushes alike. This generation reproduces intensively in the second third of July and mass-hatching of larvae follows from 17 to 22 July. It is during this period that the bollworms are most sensitive to the effects of pesticide control measures.

As mentioned earlier, fine staple varieties are more attractive to egg-laying females than medium staple varieties. However, this process can be affected by various agronomic measures carried out at different times in fields with different varieties during the second generation's development period. For example, the quality with which the top foliage of the cotton plants is removed has a significant effect on reducing the numbers of and damage caused by this and subsequent generations of cotton bollworm. When cotton sowing times are optimal and the set of reproductive structures is good on each node of the bush in fine staple varieties, removal of top foliage begins on 15 July and is finished within 6-8 days. By adopting a judicious approach to this practice, the harmful activity of the

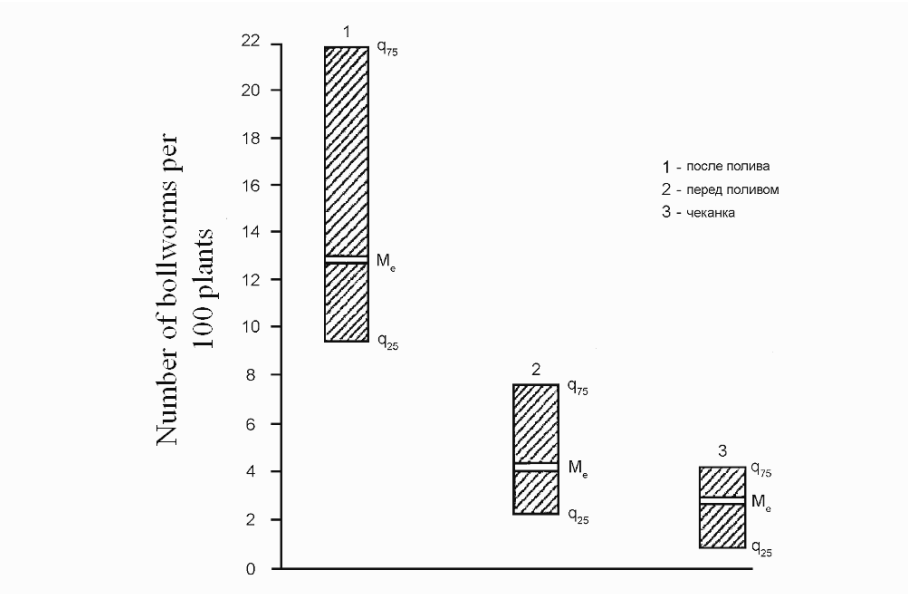

Figure 4. *The influence of agricultural techniques on the second-generation cotton bollworm population. (1) after irrigation, (2) before irrigations, and (3) with top foliage removal.*

bollworm can be averted and one pesticide treatment can be eliminated in most fields (Fig. 4). Top foliage removal can also be beneficial in fine staple varieties if it is carried out when the larvae have not yet gone more than 2-4 centimetres down the plant from where the eggs were laid. This usually coincides with the onset of the mass-appearance of first instar larvae and the occurrence of a single second instar larvae. If larvae begin to transform "en masse" into second and third instars, then the effectiveness of this technique in reducing cotton bollworm damage and numbers is practically zero. In the Vakhsh Valley, 16 to 22 July is considered to be the most effective time to remove top foliage in crops that were sown early or at the optimal time.

Irrigation of the vegetation also significantly influences the development of first and second generations of the pest (Fig. 4).

Increasing irrigation times accelerates the metabolic activity of plants which in turn leads to intensive secretion of various aromatic substances that attract moths, even from other habitats, to lay eggs. Therefore, in order to prevent increased numbers of and damage to cotton caused by cotton bollworms during the intensive egg-laying period, lengthy irrigation over large areas should be avoided. In fields without irrigation, all parts of the plant, especially the leaves and the buds at the growing point, become limp. This slows down oviposition of eggs on those plants and, in most cases, the eggs that are laid and the larvae that hatch, die.

In order to cluster the pests and take timely measures to control them, irrigation in large cotton fields should be carried out in small patches during the period of intense egg-laying.

Based on a comprehensive study of the life cycle of cutworms and the development of cotton plants, a precise area-wide pest control plan has been developed and implemented (Fig. 5).

In this plan, instead of the traditional measures and schedules for protecting crops that had no ecological basis, it is recommended to implement basic control measures during the periods of vulnerability to cutworm damage.

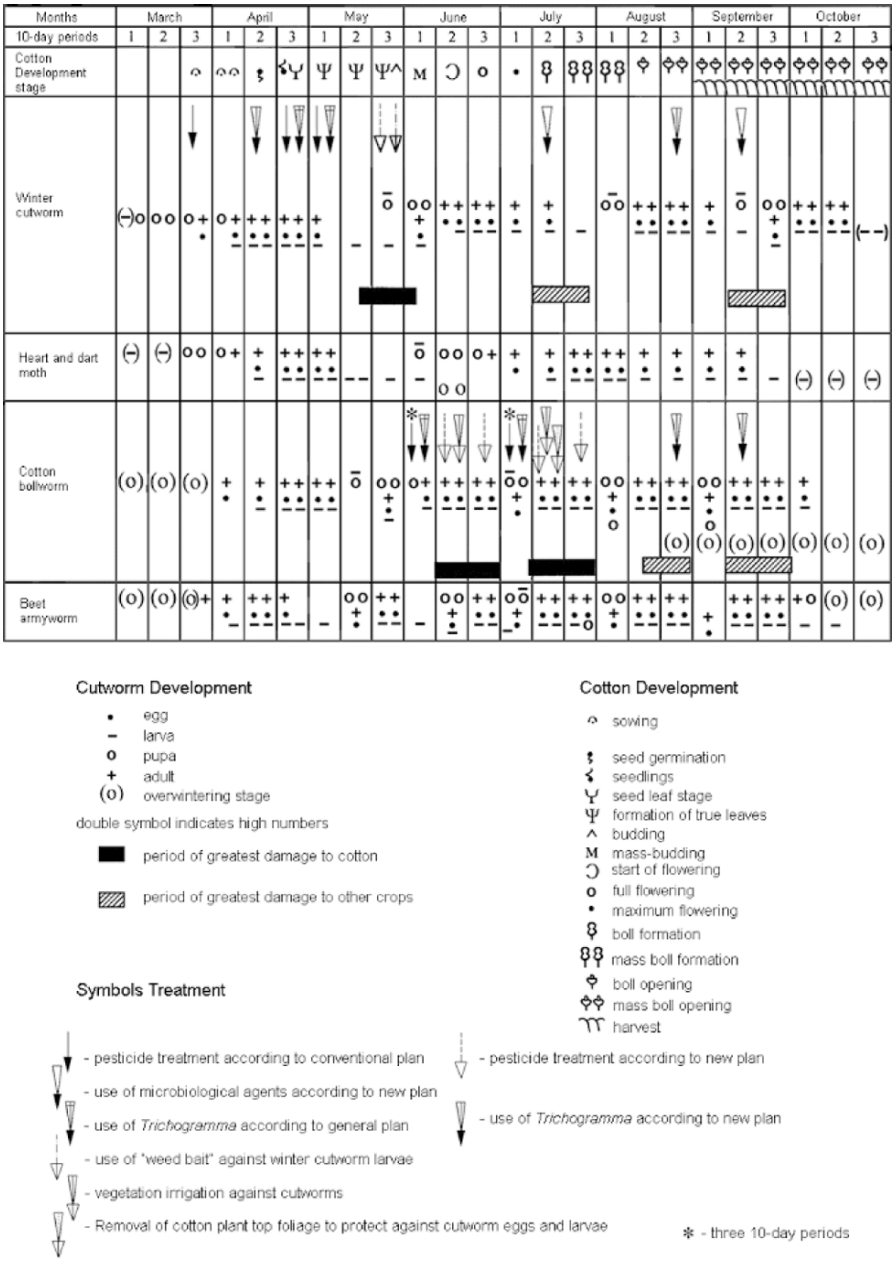

Figure 5. *Diagram displaying the different development phases of cotton and cutworms and proposed schedules for integrated control.*

4. Conclusions

Through the judicious use of several cultural and phenological approaches implemented on an area-wide basis it will be possible to substantially reduce the use of insecticides for cotton growing in the Vakhsh Valley. The major cotton pests in this valley are *A. segetum* and the *H. armigera*. Cultural control involves the removal of the top foliage at the appropriate times and this together with the use of the right varieties and seeding at the right times can substantially reduce damage caused by these pests.

Insecticidal Wound Treatment of Livestock on Isla de la Juventud, Cuba: an Efficient Suppression Method of New World Screwworm *Cochliomyia hominivorax* Prior to the Release of Sterile Insects

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ABSTRACT A two-year pilot trial was conducted on the Isla de la Juventud, Cuba to assess the effect of insecticidal treatment of wounds of livestock on the wild New World screwworm *Cochliomyia hominivorax* (Coquerel) population. On average, 338 921 and 369 622 animals were inspected every month in 2001 and 2002, respectively out of a total livestock population of 86 000. The average monthly infestation rate (number of positive screwworm myiasis cases as a proportion of total animals inspected) declined from 0.058 during the first quarter of the programme to 0.005 in the last quarter, i.e. a statistically significant reduction of 92%. The trial demonstrated that a systematic animal inspection programme coupled with insecticidal wound treatment can effectively suppress New World screwworm. The impact of the suppression programme on the adult fly population, sampled using vertical sticky traps, was less clear. Fly sampling data from the southern part of the island – an area with very few livestock – indicated little impact and the dynamics of the fly population showed a seasonal pattern. In the north, the fly population remained stable and fairly low in 2001 due to the insecticidal treatment of wounds, but increased in 2002, possibly as a result of migration from the south in the aftermath of a hurricane that created unfavourable conditions in the south. The paper argues that a systematic wound treatment programme, possibly combined with a dense adult fly trapping network should be implemented in screwworm area-wide integrated pest management (AW-IPM) programmes well before the release of sterile insects. This will enable the sterile males to compete effectively with the wild males and exploit the inverse-density dependence of the sterile insect technique (SIT). In countries with low labour costs, this strategy will make AW-IPM programmes with an SIT component more cost-effective.

KEY WORDS New World screwworm, *Cochliomyia hominivorax*, insecticides, wound treatment, vertical sticky trap, myiasis, Isla de la Juventud, Cuba

1. Introduction

The New World screwworm *Cochliomyia hominivorax* (Coquerel) is an obligate parasite of warm blooded animals including humans, and causes primary myiasis in pre-existing wounds. This larval habitat is obligatory and in the field, larvae can only develop on living animals (Spradbery 1994). The larvae from a single oviposition can kill smaller animals and

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multiple infections can kill mature cattle (Krafsur et al. 1987), e.g. 1.3 million cattle died in 1934 in the south-eastern USA as a result of screwworm infestation (Dove 1937). The fly has a very high reproductive rate and females can oviposit up to 400-450 eggs at 3-4 days intervals on the dry edge of wounds or body orifices. The eggs hatch after 12-20 hours, the larvae migrate immediately to the wound and start feeding superficially on the wound fluids. The second and third instar larvae burrow deeply into the host tissue for feeding. Mature larvae leave the animal and pupate in the soil and the entire life cycle can be a short as 21 days under optimal temperature conditions (Spradbery 1994).

The New World screwworm has been eliminated from the southern USA, Mexico, Central America and Panama, using an area-wide integrated pest management (AW-IPM) approach that included the release of sterile insects. In the Darien gap of Panama, a buffer zone of 30 000 square kilometres was created through the weekly release of 40-50 million sterile males to protect the screwworm-free countries from reinvasion from South America. The annual direct benefits of this campaign to the livestock industry were estimated at USD 896 million, USD 328 million and USD 87.8 million for the USA, Mexico and Central America, respectively (Wyss 2000).

As well as in South America, New World screwworm continues to be a significant animal and human health problem in Cuba, Hispaniola (Haiti and Dominican Republic), Jamaica and Trinidad-Tobago. Since 1996, efforts have been undertaken to eradicate the pest from Jamaica, but the programme was beset by various difficulties and little progress was made until 2005 (Vreysen et al., this volume). In view of their geographical location and increased global trade, these countries continue to pose a significant threat to the screwworm-free countries in the region.

Cuba is the largest screwworm-infested territory in the Caribbean and from 1995 to 2003, 88 985 New World screwworm cases were reported in cattle, swine, sheep, goats, horses and humans. Ninety two percent of all myiasis cases were due to New World screwworm. The Government of Cuba signed an agreement with the Food and Agriculture Organization of the United Nations (FAO) to develop a national eradication programme which would be initiated with a pilot trial on the Isla de la Juventud followed by an island-wide effort (Vargas-Terán et al. 2005). The estimated cost to eradicate the New World screwworm from Cuba was USD 62.5 million over a period of four years.

In 2000, a suppression trial was initiated on the Isla de la Juventud with the objective of assessing the effects of systematic insecticidal wound treatments and adult fly trapping on native New World screwworm. Economic losses on this small island were estimated at USD 4.3 per animal per year, or close to USD 400 000 for the entire island (FAO 1998). The Instituto de Medicina Veterinaria implemented the programme and the International Atomic Energy Agency (IAEA) provided technical assistance through a Technical Cooperation project. The data from this two-year suppression trial are presented in this paper.

2. Materials and Methods

2.1. Study Area

The Isla de la Juventud, called Isla de Pinos until 1978, is the largest and most western island in the Archipiélago de los Canarreos. It has a surface area of 2419 square kilometres, a maximum elevation of 303 metres and is separated from Cuba by the 100 kilometres-wide Batabanó's gulf (Gort et al. 1994). The northern part of the island contains mainly pine groves and savannah areas whereas the most prominent feature in the south is the Ciénaga de Lanier marshland, a protected area that contains a high number of endemic plant species, and constitutes an important nesting site for various chelonian, amphibian, crustacean and fish species.

According to a livestock census in 2001, there were 86 124 domestic livestock of which

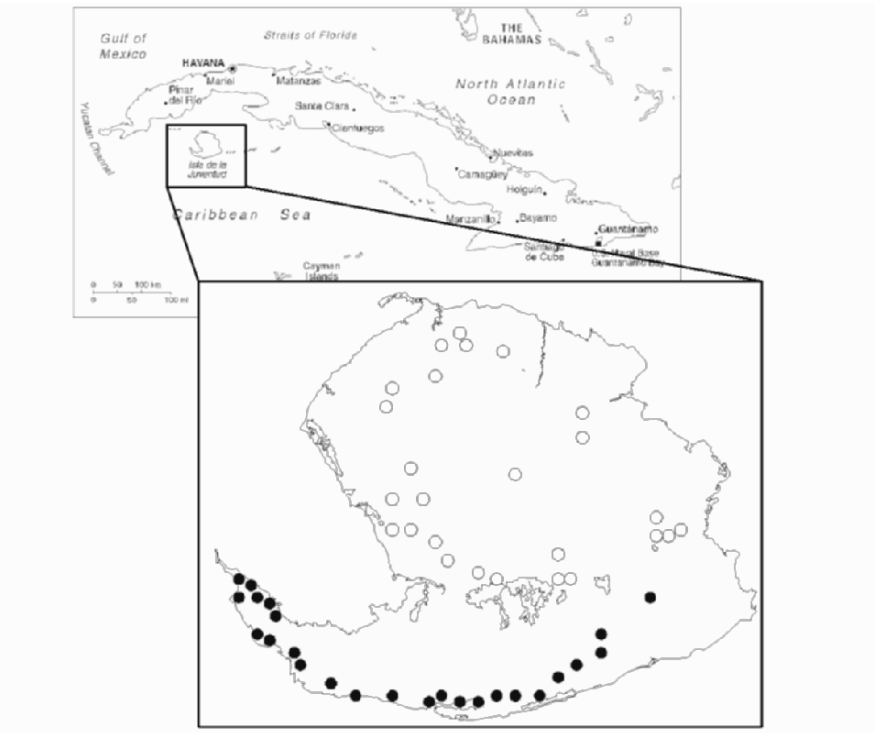

Figure 1. *Map of Cuba and the Isla de la Juventud, indicating the trap locations in the north (open points) and in the south (solid points) (Map of Cuba reproduced with permission from www.worldatlas.com).*

the majority were cattle (34 770), followed by pigs (21 161), sheep and goats (9883), and horses (1364). Wildlife such as deer (2500) and jutia conga *Capromys pilorides* (Say) are likewise abundant whereas wild pigs, monkeys and feral domestic animals are common in the protected areas (Rivero 1999).

Taking into consideration the distribution and abundance of livestock, the existing network of roads, accessibility, and topographical features, the entire island was divided into four operational blocks, each subdivided into ten zones. Four laboratories were established on the island to ensure correct and timely identification of the collected samples.

2.2. Logistics

There were 50 veterinarians and 103 technicians on the island and 21 of these staff were engaged full-time in the pilot project. The protected areas in the south were managed by the National Company for the Protection of Flora and Fauna and its personnel collaborated with the project and reported any myiasis case immediately.

2.3. Implementation of the Trial

The efficient collaboration of all stakeholders, including the livestock farmers and animal and pet owners ensured the smooth implementation of the pilot trial. Project personnel distributed more than 15 000 sampling kits to the livestock owners and to personnel of the collaborating organizations. Each kit contained forceps for the removal of larvae from the wounds, plastic vials with alcohol to pre-

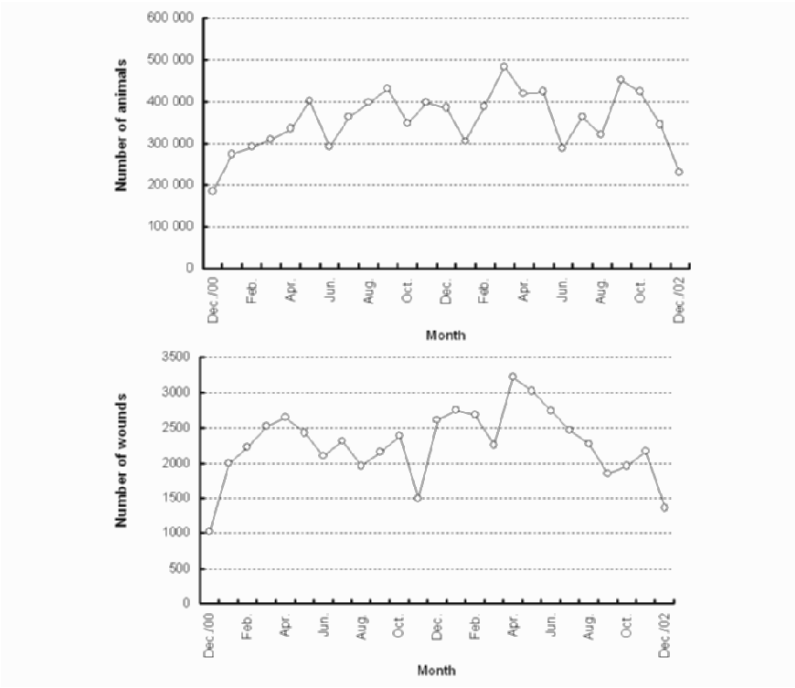

Figure 2. *(upper) The monthly number of animals inspected for wounds and New World screwworm infestations on the Isla de la Juventud (December 2000-December 2002), and (lower), the monthly number of wounds treated with insecticides.*

serve the larvae, a sachet of insecticide powder (coumaphos), an instruction leaflet and a data sheet. All samples were sent to one of the four laboratories for species identification and the results compiled in a database Epi Info 6 (CDC 2005).

Daily visits were made to sites which were known to have high infestation levels and all animals in each site were inspected, larvae removed from wounds and all wounds (infested and non-infested) treated with insecticides. The correct treatment of a wound will kill for seven to ten days any ovipositing female. Most of the inspection sites were located in the northern half of the island.

Adult sampling was done with triangular vertical sticky traps (dimensions 30 centimetres x 30 centimetres x 42.5 centimetres) (Welch 1994, Welch and Garcia 1997) baited with the chemical attractant swormlure-4 (Mackley and Brown 1984). Each week, 25 to 125 traps were deployed in easily accessible areas (i.e. mainly along existing roads) in the northern half of the island and between 25 to 175 traps in the southern half. The traps were checked at least every two days, depending on the location. The traps were used to monitor the adult population and acted as an additional suppression tool.

2.4. Data Analysis

The percentage infestation was defined as the number of animals infested expressed as a proportion of the total animals inspected. Data on monthly percentage infestations were analysed per quarter, and data on weekly fly catches of the northern and southern part of the island were analysed per month. To normalize the data, monthly percentage infesta-

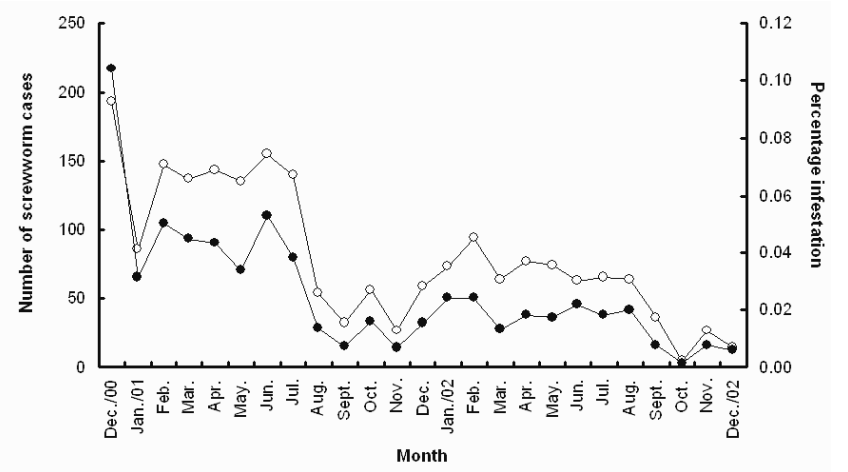

Figure 3. *The monthly number of screwworm cases (open points) and the percentage infestation (solid points) on the Isla de la Juventud from December 2000 to December 2002.*

tion data were arcsine transformed and weekly fly catch data log (n + 0.01) transformed. All data were analysed by single factor ANOVA and Tukey's HSD test was used to separate the means at $P < 0.05$. Comparison of pairs of data sets of number of animals inspected and wounds treated were analysed by a paired *t*-test.

3. Results

3.1. Myiasis and Percentage Infestation

Over a period of 25 months (December 2000 to December 2002), a total of 8 841 432 animals were inspected, on average 338 921 and 369 622 per month in 2001 and 2002, respectively ($t = 0.35$, d.f. = 11, $P = 2.57$) (Fig. 2, upper). During the same period, a total of 56 525 wounds were treated with insecticides, which represents an average of 2261 wounds treated per month (Fig. 2, lower). The number of wounds treated in the first year did not change significantly from that in the second year (n = 2141 for 2001 and n = 2390 for 2002) ($t = 0.53$, d.f. = 11, $P = 2.20$). Larvae were most frequently found in the navel area (24.8%), in wounds caused by barbed wire (23.8%), from bites (16.5%), in the vulva area post-parturition (11.0%), by ear tags (8.7%) and branding (6.8%) and others (less than 2%). Cattle were mostly affected (59.8%) followed by pigs (31.1%), whereas birds, lambs, dogs, goats and horses were much less infested.

From December 2000 to December 2002, a total of 2022 New World screwworm myiasis cases were found (i.e. an average of 80.8 ± 49.8 cases per month). Most of the cases (99%) were found in the northern half of the island, where livestock was abundant. The percentage infestation was 0.104% in the first month, December 2000 (Fig. 3). The ANOVA analysis showed significant temporal differences in the average quarterly percentage infestation ($F = 6.48$, d.f. = 17, $P < 0.001$), with significantly less animals infested in quarters 3 and 4 of 2001 and quarters 3 and 4 of 2002 ($P < 0.05$, Tukey HSD) as compared with the first quarter (data for December 2000 were included in the first quarter 2001 for the analysis) of the project. The percentage infestation was not significantly different in the first and second quarters of 2002 than in the first quarter. This increase in screwworm myiasis cases was likewise reflected in the adult trap data. The average percentage infestation in the last quarter of 2002 was 0.005%, which

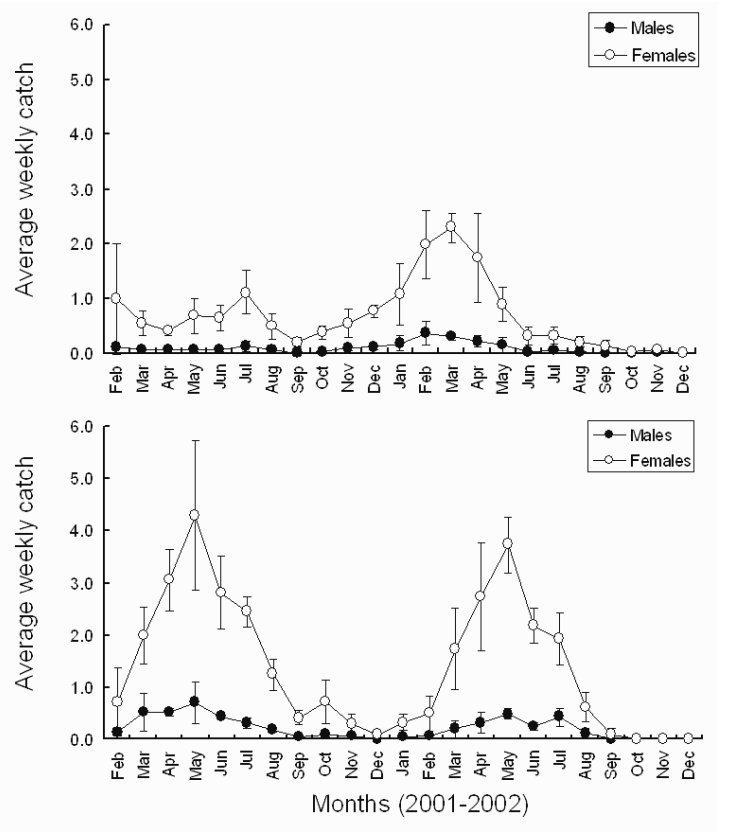

Figure 4. *The average weekly catch (male and female flies/trap/week) of New World screwworm in the northern (upper graph) and southern (lower graph) half of the Isla de la Juventud from February 2001 to December 2002.*

represents a 92% reduction as compared with the first quarter of 2001.

3.2. Sampling Adult New World Screwworm Cochliomyia hominivorax *Flies*

During the pilot trial, a total of 7143 and 11 241 adult New World screwworm flies were trapped in the northern and southern parts of the island, respectively. In 2001, average weekly male and female catches were significantly higher in the south than in the north, i.e. 0.28 males/trap/week (south) versus 0.07 males/trap/week (north) ($F = 9.93$, d.f. = 92, $P < 0.01$), and 1.7 females/trap/week (south) versus 0.57 females/trap/week (north) ($F = 12.18$, d.f. = 92, $P < 0.001$). In 2002, however, average weekly male and female catches were similar in the north and south, i.e. 0.18 males/trap/week (south) versus 0.12 males/trap/week (north) ($F = 0.0002$, d.f. = 90, $P > 0.05$), and 1.31 females/trap/week (south) versus 0.85 females/trap/week (north) ($F = 0.11$, d.f. = 90, $P > 0.05$) (Fig. 4). In both parts of the island, the sticky traps caught significantly more females than males, i.e. 88.1% and 87.1%, respectively (χ^2, $P < 0.001$).

The temporal fluctuations in the average weekly catches of male and female flies were considerable, especially in the south. Weekly

catches in the northern part of the island were not significantly correlated with those of the south (for females: r = 0.24, d.f. = 21, $P > 0.05$ and for males: r = 0.04, d.f. = 21, $P > 0.05$) and therefore the data were analysed and presented separately (Fig. 4).

The average weekly female catch varied significantly with time, both in the south (F = 37.92, d.f. = 22, $P < 0.001$) and the north (F = 15.79, d.f. = 22, $P < 0.001$). In the south, the highest female catches were in April-June 2001 (range 2.80-4.29 females/trap/week) and 2002 (range 2.17-3.73 females/trap/week). In the north, the highest average female catches were in May-July 2001 (range 0.68-1.11 females/trap/week) and February-April 2002 (range 1.74-2.29 females/trap/week). The vertical sticky traps caught the least females in September 2001 and October-December 2002 in the north, and in December 2001 and October-December 2002 in the south (Fig.4).

The temporal fluctuations in the average weekly trap catch of the males were less than in the females but the ANOVA showed significant temporal changes, both in the south (F = 17.47, d.f. = 22, $P < 0.001$) and the north (F = 8.28, d.f. = 22, $P < 0.001$).

The weekly catch of males was highly significantly correlated with those of females, both in the north (r = 0.89, d.f. = 18, $P < 0.001$) and in the south (r = 0.93, d.f. = 18, $P < 0.001$), indicating a similar trap response.

4. Discussion

The SIT was an essential part of the AW-IPM programme that eradicated the New World screwworm from the USA, Mexico and Central America (Wyss 2000). This programme emphasized IPM, which is a sustainable approach to managing pests by the integration of biological, cultural, physical and chemical tools in a way that minimizes economic, health, and environmental risks (National IPM Network 2001), and AW, which is the application of control tactics against an entire pest population within a delimited geographical area, with a minimum size large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area (Klassen 2005).

Even in the early days of the screwworm programme in the USA, it became rapidly clear that:

> *...an effective surveillance programme carried out well in advance of the release of sterile flies, was a vital part of a successful eradication programme (Meyer 1994).*

This need prompted the development of an effective adult screwworm suppression tool that could reduce populations to levels where sterile males could effectively compete with the native male flies. The final result was the screwworm adult suppression system (SWASS), which contained a powerful attractant (swormlure) (Mackley and Brown 1984), a food-bait as a short range attractant, and an insecticide to kill the adult flies feeding on the mixture (Snow et al. 1982). In dry areas of the southern USA and northern Mexico, the SWASS pellets were dispersed by aircraft for four to ten weeks at a rate of 0.18 kg/km^2 (Snow et al. 1982). This short period was sufficient to reduce screwworm populations by up to 90% (Coppedge et al. 1978). As the eradication programme progressed towards the more humid, tropical areas of southern Mexico and Central America, the swormlure lost much of its attractiveness and the SWASS became less effective (Spradbery 1994). Together with the high cost (Snow et al. 1982) and concerns about environmental pollution (Spradbery 1994), SWASS was finally abandoned and intensive treatment of animal wounds with insecticides that killed ovipositing females became the prime suppression tactic.

This two-year pilot trial, demonstrated that a systematic, properly executed animal inspection programme, (on average more than 330 000 animals were inspected each month for a total livestock population of 86 000 animals), where every wound detected was consistently treated with insecticides, could significantly reduce screwworm infestations in livestock. The 92% reduction of the average

monthly percentage infestation from the first to the last quarter is testimony to the efficacy of the approach. However, it should be noted that the hurricanes that passed over the island could have contributed to some reduction of the population.

Many screwworm eradication programmes have in the past relied on "the number of screwworm cases" to monitor programme progress. Vreysen (2005) and Vreysen et al. (this volume) have cautioned this approach especially in those programmes where farmers were requested to inspect their animals and report cases to the programme (passive sampling). The fluctuations in reported cases were likely more related to farmer collaboration and reporting efficiency than actual variations in the pest population density. Only when the same number of animals are inspected in each time unit, does the number of screwworm cases measure progress. The data presented in this paper corroborate the above observations, as exemplified by the following case in point: from February to May 2001, the number of screwworm cases declined from 147 to 135 cases, which is a reduction of only 8%, whereas the percentage infestation was reduced from 0.05 to 0.034%, i.e. a reduction of 32%. This again demonstrates the importance of presenting and analysing screwworm case data as a proportion of inspected animals to allow accurate data interpretation.

Progress was also monitored through the trapping of adult flies. However, the effect of the suppression programme was less clear from the fly trapping data than from the percentage myiasis infestation (monthly total fly catches were nevertheless, significantly correlated with the monthly percentage infestation ($r = 0.55$ d.f. $= 21$, $P < 0.05$)). This was most likely related to the spatial characteristics of the suppression programme, i.e. most of the livestock were present in the north, where most of the cases (99%) were also found. Livestock were largely absent in the south with the only animals present being wildlife and small pets belonging to fishermen and the staff of the national park. Consequently, the fly population in the south was little affected by the insecticidal wound treatment programme, and this is illustrated in the seasonal, natural dynamics of the screwworm population (Fig. 4).

In the north, adult screwworm catches remained fairly low in 2001 as a result of the insecticidal wound treatment, with average weekly catches not exceeding 1.11 females/trap/week and 0.12 males/trap/week. In November 2001, a category four hurricane (Michelle) passed over the island which destroyed much of the vegetation in the south and left large areas flooded for several weeks. This created unfavourable conditions for the screwworm population and most likely stimulated fly migration to the north, which was much less affected by the hurricane. This might explain the increase in average weekly fly catches in the north between December 2001 and March 2002, whereas in the south, the fly population only recovered from the impact of the hurricane as of March 2002. On 19 and 30 September 2002, two hurricanes (category one and two) passed over the island, affecting the north and south equally. Average weekly fly catches remained extremely low in the following months as did the number of screwworm cases.

The traps were not deployed along a regular grid, and hence the trapping data might have been biased. Nevertheless, the data collected were an important indicator of programme progress and provided valuable complementary information on screwworm population dynamics (Vreysen 2005). The spatial complexities of the often highly aggregated screwworm populations could be more efficiently analysed both in space and time using geo-referenced data collection (using Global Position System (GPS)) and geographic information systems (GIS)-based analyses (Cox and Vreysen 2005, Cox, this volume).

On average, 75 and 84 sticky traps were deployed every week in the southern and northern parts of the island, respectively. These sticky traps caught more than 16 000 females during the trial. Although no data were available on absolute wild fly densities, this high number of trapped females seems to

indicate that these traps (Welch and Garcia 1997) show potential as an additional suppression tactic for New World screwworm. The traps are cheap, easy to handle and deploy and the odour bait (swormlure-4), while not very effective for sampling gravid females (Guillot et al. 1977a,b) is a good attractant for young females (Coppedge et al. 1977). The vertical sticky trap (baited with swormlure-4) is a new design, and field tests in Costa Rica indicated its superior performance for catching male and female flies (871 flies trapped) as compared with the standard wind-oriented-trap (Broce et al. 1977) (11 flies trapped) (Welch 1994). Sterile screwworm flies released around the mass-rearing facility in Tuxtla Guttièrez, Mexico (as part of biosecurity measures) were sampled up to five kilometres from the rearing facility with the wind-oriented-trap but up to 30 kilometres with the vertical sticky trap (R. Garcia, unpublished). Although the deployment of many traps over large surface areas is labour intensive and logistically demanding, it will probably be cost-effective in those countries with low labour costs. Each young wild female fly that is trapped will be equal to three to five wounds not becoming infested, preventing the potential development of 800 to 1200 larvae.

5. Conclusions

In most operational screwworm programmes, suppression using insecticidal wound treatment was usually initiated simultaneously with the release of sterile insects, masking the relative contribution of each control tactic to the progress of the campaign. The data from this pilot trial have shown that a properly implemented insecticidal wound treatment programme can reduce New World screwworm myiasis considerably. Due to the lack of livestock there was no suppression of the screwworm population in the south, which in view of the high mobility of the fly and the small size of the island must have created the possibility of flies migrating to the north. Suppression would have been improved had the control tactics been applied on the entire screwworm population, following an AW-IPM approach.

The pilot trial was carried out in preparation of the release of sterile insects on the island. The data from the suppression phase of the project, which was planned to last only six months but was implemented for two years, indicate that the release of sterile insects would have been the next logical step in order to achieve eradication. Due to a variety of reasons, some political, the programme never received the necessary follow-up and the release of sterile insects was never implemented. This is very unfortunate as the pilot suppression programme succeeded in creating optimal conditions for the sterile males to compete effectively with the native males. Initiating systematic wound treatment possibly combined with a dense trapping network before initiating the release of sterile males, would make the SIT component of screwworm AW-IPM programmes more cost-effective, especially in countries with low labour costs.

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Section 5

Commercialization and Regulation

Area-Wide Integrated Pest Management Programmes and Agricultural Trade: Challenges and Opportunities for Regulatory Plant Protection

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ABSTRACT The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) entered into force for all member countries in 2000. It states that measures to protect human, animal and plant health or life shall be based on international standards where possible. These measures shall be based on a scientific risk assessment and should be implemented only to the extent necessary to achieve an appropriate level of protection. The International Plant Protection Convention (IPPC) is the international standard setting body for protecting plant health identified in the SPS Agreement. Both international treaties make provision for control of pests at regional levels (regionalization) and for identification of pest free areas. The IPPC provides guidance to countries, in the form of international standards, on the implementation of pest free areas and pest risk analysis (including systems approaches and other risk management measures). These standards can contribute to area-wide integrated pest management (AW-IPM) programmes for two main reasons. First, when AW-IPM programmes are implemented according to IPPC standards, trading partners should be prepared to recognize the results of a successful AW-IPM programme as meeting requirements, for example, of a pest free area or an area of low pest prevalence. Second, these standards provide scientific and technical guidance for the design and operation of key components of AW-IPM programmes. Therefore, countries that implement AW-IPM programmes that are in accordance with IPPC standards are better positioned to take advantage of liberalized trade while maintaining their phytosanitary security.

KEY WORDS agricultural trade, sanitary and phytosanitary measures, area-wide integrated pest management, International Plant Protection Convention

1. Introduction

The purpose of this paper is to address the relationship between area-wide integrated pest management (AW-IPM) programmes, agricultural trade and the application of phytosanitary measures by importing and exporting countries. AW-IPM programmes may be differentiated from more conventional pest control programmes (e.g. localized integrated pest management programmes) in that they incorporate systematically applied pest management strategies to reduce pest populations. In the context of this paper, the term "pest" refers to:

> *Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products (FAO 1997a).*

Such programmes often are applied over large geographic areas often for extended periods of time, but may also be applied on smaller scales (e.g. greenhouses, production units, etc.). The goal of such programmes may be suppression, prevention, containment or eradication of a particular pest (Hendrichs et al. 2005). While many other considerations

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(economic, biological, environmental, social, political, etc.) will affect the decision to use, and the ultimate success of AW-IPM programmes, the focus of this paper is to discuss their role in relation to agricultural trade.

2. Area-Wide Pest Management

The decision to undertake an AW-IPM programme may be influenced by many factors, including feasibility, economics, biology of the organism, the area over which management may occur and the demand for and relative benefits of undertaking such a programme. Benefits of area-wide pest management are linked to efficiency and effectiveness gains that are possible when applying similar phytosanitary measures over large, usually environmentally similar areas. The nature of the benefits is in turn linked to epidemiological and/or economic factors. Such benefits may include reduction in pesticide use, reduced impacts on the environment, increased production and quality, increased food security, increased income for producers and reduced costs over time for managing serious pests. An additional benefit of area-wide pest management may be increased opportunities for trade from areas where pest populations are drastically reduced, contained or eradicated leading to areas of low pest prevalence or pest free areas. The role of area-wide pest management in agricultural trade is poised to grow as the volume of agricultural trade and the awareness of the risks of accompanying pests moving to new areas continue to increase (Griffin 2000).

3. Agricultural Trade

Agricultural trade has increased steadily since the World Trade Organization (WTO) provided for a global, liberalized trade framework in the 1990s. As of 2000, the global value of agricultural trade exports was approximately USD 275 000 million (USDA 2002). Developed countries such as Australia, Canada, countries of the European Community, Japan and the USA account for the majority of trade, while developing countries have had mixed results taking advantage of the liberalized trade environment. One potential barrier to agricultural trade for many countries are measures imposed by importing countries to protect human, animal or plant life and health – or sanitary and phytosanitary measures (Henson and Loader 2001, Huang 2004).

3.1. The Agreement on the Application of Sanitary and Phytosanitary Measures

The Agreement on the Application of Sanitary and Phytosanitary Measures, or SPS Agreement, is a subsidiary agreement of the WTO. It sets out rights and obligations for members of the WTO with respect to sanitary and phytosanitary measures that may be implemented for protecting human, animal or plant life and health. The SPS Agreement provides a framework to ensure that measures are applied only to the extent necessary to protect health and that the measures are technically justified. In the same vein, the agreement maintains that such measures should not be implemented arbitrarily or as disguised barriers to trade. Annex A of the SPS Agreement defines the scope of SPS measures (Fig. 1) (WTO 1994).

3.1.1. Provisions of the Sanitary and Phytosanitary Agreement

The SPS Agreement contains several key provisions that define rights, obligations and responsibilities of members in designating SPS measures at the national level. Provisions that are particularly applicable to AW-IPM programmes include: (1) risk assessment, (2) harmonization, (3) equivalence, (4) least trade restrictive (minimal impact), (5) appropriate level of protection, (6) regionalization, (7) area freedom, and (8) low prevalence.

Article 2 (Basic Rights and Obligations) of the SPS Agreement states that members shall ensure that any sanitary or phytosanitary measure is applied only to the extent necessary to protect human, animal or plant life or health, is based on scientific principles and is not maintained without sufficient scientific evidence.

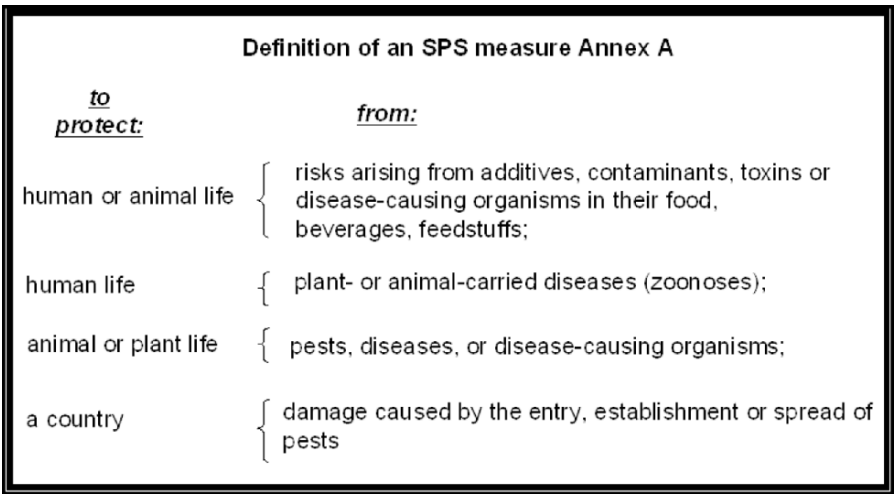

Figure 1. *Definition of a Sanitary and Phytosanitary (SPS) measure from Annex A of the Agreement on the Application of Sanitary and Phytosanitary Measures (WTO 1994).*

This means that measures should be technically justified and based on available scientific information (see also Articles 3 and 5) (WTO 1994).

Article 3 (Harmonization) of the SPS Agreement states that measures should be based on international standards or that measures that deviate from standards should be technically justified. Members can use international standards as the basis for their national regulations and know that those measures cannot be challenged under the SPS Agreement. The agreement identifies the internationally recognized standard-setting bodies as: (1) the Codex Alimentarius Commission for food safety and human health, (2) the Office Internationale des Epizooties (OIE) for animal health, and (3) the International Plant Protection Convention (IPPC) for plant health.

International standards developed by these organizations incorporate available scientific information and assess the risks associated with a given situation. As such, any measure based on a standard is by default considered to be technically justified (WTO 1994).

Article 4 (Equivalence) of the SPS Agreement states that members should accept as equivalent alternative measures that achieve the same level of protection that differ from their own measures (WTO 1994).

Article 5 (Assessment of Risk and Determination of the Appropriate Level of Sanitary or Phytosanitary Protection) addresses the application of risk assessment in determining appropriate SPS measures. Importantly, it states that risk assessments used for determining SPS measures should be based on methods developed by relevant international organizations and take into account available scientific evidence. It is also important to note that measures, which deviate from standards, as defined in Article 3, should be based on risk assessment. Furthermore, Article 5 states that members should implement measures only to achieve an appropriate level of protection, and that these measures should be the "least trade restrictive" possible. The SPS Agreement allows for measures that are more stringent than those defined by standards, but these measures should be fully justified by a risk assessment (WTO 1994).

Article 6 (Adaptation to Regional Conditions, Including Pest or Disease-Free Areas and Areas of Low Pest or Disease

Prevalence) discusses the provisions for regionalization, pest free areas and areas of low pest prevalence. These concepts are significant for AW-IPM programmes as they provide for the recognition of pest free areas and areas of low pest prevalence – the goal of AW-IPM programmes (WTO 1994).

3.2. The International Plant Protection Convention

As stated above, the SPS Agreement identifies the IPPC as the international standard setting body for plant health. The IPPC is an international treaty with its own signatories, or contracting parties. It originally entered into force in 1952; it was amended in 1979 and the 1979 text entered into force in 1991. More recently, in 1997, the text was revised again largely to meet expectations set forth in the SPS Agreement. The 1997 amendments created a secretariat, the Commission on Phytosanitary Measures and formalized standard-setting as part of the IPPC's mission. The 1997 text will enter into force after the amendments have been accepted by two-thirds of the contracting parties to the IPPC (currently 137 countries). Until this happens, the Commission on Phytosanitary Measures operates as the Interim Commission on Phytosanitary Measures (ICPM).

The purpose of the IPPC is to secure common and effective action to prevent the spread of plant pests and to promote measures for their control. Although the IPPC has a clear relationship to the SPS Agreement and to agricultural trade, its scope is not limited to trade. The scope of the IPPC applies to protecting all plants including wild flora from plant pests. Plant pests include any organism that may affect plant health, including diseases and weeds (FAO 1997a). To achieve this goal of protecting plant health, the text of the IPPC sets forth rights, obligations and responsibilities of contracting parties, including pest risk analysis, harmonization, equivalence, minimal impact, regionalization, pest free areas and areas of low pest prevalence. According to the IPPC, contracting parties should make provision for a national plant protection service that is responsible for performing certain key functions including: (1) phytosanitary certification, (2) establishment of an official contact point, (3) surveillance, (4) implementation of appropriate phytosanitary measures, (5) conducting treatments and certifying exports, (6) exchanging scientific and technical information, (7) developing and observing standards, (8) recognition of equivalence, (9) conducting eradication programmes, and (10) recognition of pest free areas and areas of low pest prevalence (FAO 1997a).

3.2.1. International Standards for Phytosanitary Measures

Contracting parties receive guidance on meeting these provisions through the use of international standards developed under the auspices of the IPPC. International Standards for Phytosanitary Measures (ISPMs) are developed under the guidance of the ICPM. The ICPM (comprised of contracting parties to the IPPC and members of the Food and Agriculture Organization of the United Nations (FAO)) decides which ISPMs should be developed as part of the work programme. An expert panel is formed to draft the standard, ensuring that the best scientific expertise is incorporated into the standard. The Standards Committee reviews and revises the draft standard, which is then sent to all members of the ICPM for comment. After another review by the Standards Committee, the draft standard can be submitted to the ICPM for adoption. To date there have been over 20 standards adopted. It should be noted that although priorities for new standards are determined by the ICPM by consensus, topics may be suggested by individual members (countries), regional plant protection organizations, international organizations (e.g. International Atomic Energy Agency (IAEA), WTO) or other organizations such as international non-governmental organizations.

There are three general types of standards: reference, concept and specific. Reference standards include the ISPM No. 5 (Glossary of Phytosanitary Terms) (FAO 2001) and

ISPM No. 1 (Principles of Plant Quarantine as Related to International Trade) (FAO 1995). Concept standards include ISPMs such as ISPM No. 4 (Requirements for the Establishment of Pest Free Areas) and ISPM No. 6 (Guidelines for Surveillance). The pest risk analysis standards (ISPMs No. 2 Guidelines for Pest Risk Analysis, No. 11 Guidelines for Pest Risk Analysis for Quarantine Pests Including Analysis of Environmental Risks and Living Modified Organisms) are also concept standards. Specific standards address specific pests or commodities. Currently, ISPM No. 15 (Guidelines for Regulating Wood Packaging Material in International Trade) (FAO 2002b) is the only specific standard; however, several commodity and pest specific standards are in various stages of development.

3.2.2. Standards and Area-Wide Pest Management

Of the standards that have been developed to date, several have direct implications for area-wide pest management programmes. These include: (1) ISPM No. 3 Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and other Beneficial Organisms (Revised) (FAO 2005a), (2) ISPM No. 6 Guidelines for Surveillance, (3) ISPM No. 4 Requirements for the Establishment of Pest Free Areas, (4) ISPM No. 22 Guidelines for Areas of Low Pest Prevalence, (5) ISPM No. 9 Guidelines for Pest Eradication Programmes, (6) ISPM No. 10 Guidelines for Pest Free Places of Production and Pest Free Production Sites, (7) ISPM No. 2 Guidelines for Pest Risk Analysis, (8) ISPM No. 11 Guidelines for Pest Risk Analysis for Quarantine Pests Including Analysis of Environmental Risks and Living Modified Organisms, and (9) ISPM No. 14 The Use of Integrated Measures in a Systems Approach for Pest Risk Management.

These standards can contribute to AW-IPM programmes for two main reasons. First, when AW-IPM programmes are implemented according to these standards, trading partners should be prepared to recognize the results of a successful AW-IPM programme as meeting requirements, for example, of a pest free area or an area of low pest prevalence. This means that such programmes will meet criteria for pest risk management as defined in the pest risk analysis standards (ISPM Nos. 2, 11 and 14) so that exporting countries can more fully benefit from their AW-IPM programmes through enhanced trade opportunities. At the same time, importing countries should be prepared to consider and recognize such programmes as pest risk management options, according to the principles of harmonization and equivalence (WTO 1994, FAO 1997a).

Second, these standards provide scientific and technical guidance for the design and operation of key components of AW-IPM programmes. This guidance covers many of the key elements that are integral components of all AW-IPM programmes. For example, ISPM No. 6 (Guidelines for Surveillance) provides valuable information on how surveillance programmes should be designed and executed, and covers basic sampling techniques (FAO 1997b). The pest risk analysis standards address how biological information on pests should be gathered and analysed. Although the design of AW-IPM programmes does not necessarily include a risk assessment component, there is considerable overlap in the type of information needed to make accurate judgements (FAO 1996a, FAO 2004). ISPM No. 14 provides extensive guidance on the integrated use of different types of pest risk management options to reduce pest risk. A national plant protection organization could accept the use of an AW-IPM approach for a specific pest to implement a phytosanitary measure, either independently (if it reduced risk to an acceptable level) or combined with other phytosanitary measures as part of a systems approach (e.g. treatments, seasonal shipping, etc.) as necessary (FAO 2002a). Finally, several standards discuss eradication, pest freedom and low pest prevalence – all potential outcomes of AW-IPM. They provide both scientific and technical guidance as to how such programmes may be developed, the types of information that should be gathered and analysed, the requirements for certain

procedures (e.g. surveillance) and how pest freedom can be officially recognized by other countries (FAO 1996b, 1998, 1999, 2005b). Each of these standards can contribute significantly to AW-IPM programmes. However, it is important to note that these standards are not meant to be used alone; rather, each one of these standards builds upon others to form a comprehensive system for plant protection. Similarly, the successful implementation of standards relies heavily on all countries actively participating in the standards process. All countries – when exporting and importing – should accept phytosanitary measures that are based on standards, including AW-IPM programmes where appropriate. More to the point, all countries should also actively participate in the development of new standards. For instance, a country, or group of countries, can recommend priorities for the development of new standards to the ICPM. This is especially important for certain pests that are the target of AW-IPM programmes, where the development of a specific international standard could add scientific and technical guidance and provide valuable impetus to a given programme.

4. Other Trade Considerations

It should also be noted that other factors related to agricultural trade could play a pivotal role in whether a country decides to invest its resources in AW-IPM programmes. The adoption of the Montreal Protocol, requiring the reduction in use or elimination of ozone depleting substances, may lead to decreased future availability of methyl bromide, an important quarantine treatment (UNEP 2000). Although the use of methyl bromide for quarantine purposes is exempted from the protocol, there is still a desire on the part of many countries to scale back their use of methyl bromide. In the absence of suitable alternatives, the application of AW-IPM approach for a specific pest, involving the area-wide rather than local integration of phytosanitary measures, will become increasingly important to countries wishing to trade agricultural products. Likewise, market forces and food safety standards are leading to acceptance of lower and lower levels of pesticide residues (maximum residue limits) in food, with a concomitant reduction in the reliance on certain pesticides in the field.

Concurrently, it is becoming widely recognized that the operational standard of "*Probit-9*" for quarantine treatments is not a technically justifiable requirement for many, if not most, pests. *Probit-9* security refers to the level of efficacy of a phytosanitary treatment and converts to 32 surviving individuals for every one million individuals treated (Follet, this volume). For more than 50 years, it has been assumed that this level of security was sufficiently protective, especially for fruit fly pests. However, as pest risk analysis continues to evolve, it has become evident that for many pests, *Probit-9* may be too stringent of a requirement; in some cases, *Probit-9* may not afford enough security. In either case, national plant protection organizations are re-evaluating the need for *Probit-9* security and AW-IPM programmes may prove to be suitable alternatives to long used point of origin-, in transit- or post-entry quarantine treatments (Liquido et al. 1997).

5. Implications for AW-IPM Programmes

Evidence exists that countries can benefit significantly when AW-IPM programmes are implemented for the purpose of enhancing trade opportunities, thus meeting requirements of standards. Chile eradicated Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) from most of its territory integrating the sterile insect technique (SIT) and benefited through increased trading opportunities with the USA and many other countries (Liquido et al. 1997, Mumford 2002). Other countries that have initiated AW-IPM programmes for the purpose of increasing trade opportunities (and reducing phytosanitary requirements on exports) include Argentina, Australia, Brazil, Mexico and South Africa (Liquido et al. 1997, Mumford 2002). Most of

these programmes are for fruit fly pest suppression, containment or eradication, but other major quarantine pests, such as codling moth *Cydia pomonella* (L.) have also been targeted (IAEA 2004).

Nonetheless, it should be noted that not all pests are suitable targets for AW-IPM programmes (and in particular where the objective is eradication), even when there could be clear benefits with regard to trade. As mentioned at the beginning, many factors will affect the decision to undertake such a programme. Importantly, social, political, biological, physical and economic considerations, in addition to trade opportunities, must be taken into account before deciding to aim for area-wide pest management. These factors must also be considered in determining whether the goal of a programme might be suppression, prevention, containment or eradication (Myers et al. 1998, Hendrichs et al. 2005).

AW-IPM programmes may be resource-intense, require long-term commitment from a wide range of interest groups (growers, exporters, governments, researchers, etc.) and may run for several years or more. Even highly successful programmes can be expensive for long periods of time. Costs of eradication of Mediterranean fruit fly from California in 1975 are estimated at USD 328 million, with continuing costs for prevention, survey and detection (Mumford 2002). The present Mediterranean Fruit Fly Preventive Release Programme in California's Los Angeles basin, is the result of a conversion from a reactive to a proactive approach with a significant reduction of cost, is another example of an AW-IPM programme using the SIT. Myers et al. (1998) examined historical eradication and suppression programmes, including benefit/cost analyses. It was determined that, in some cases, eradication was an expensive choice and the relative benefits of some eradication programmes were not worth the costs. However, in others cases the benefit/cost ratio of eradication programmes is highly favourable (Dyck et al. 2005).

Over the past 50 years, a wealth of information has been accumulated on AW-IPM programmes. There is a growing understanding of the biological and epidemiological aspects of such programmes. Likewise, there is a better understanding of the importance of evaluating the economics and socio-political implications of these programmes (Dyck et al. 2005). As our experience continues to grow, we will develop a clearer understanding of the costs and benefits of such programmes. This will lead to improved decision-making with regard to when, and under what circumstances, to undertake AW-IPM programmes. These programmes represent a significant commitment of resources in the form of time, money and expertise; in some cases, however, this commitment of resources may prove to be a valuable and wise investment.

6. Conclusions

All of the factors identified above – increased agricultural trade, increased risks for the movement of pests, requirements for reduced pesticides residues and for international harmonization, evolving science – are leading national plant protection organizations to rethink phytosanitary requirements and seek alternative solutions to reducing pest risk. This means the need for AW-IPM programmes, rather than local IPM approaches, will likely increase in the future. However, complacency is not an option for anyone involved in these programmes. Scientists and researchers must understand the political, social and economic factors that may negatively or positively affect AW-IPM programmes. At the same time, decision makers and regulatory officials need to be open to alternative pest risk management strategies, including the wider application of AW-IPM programmes as stand-alone measures or as parts of systems approaches. By looking toward the horizon, instead of business as usual, all countries can benefit from enhanced agricultural trade while assuring phytosanitary security. The judicious implementation of AW-IPM programmes will play a vital and continuing role in this process.

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Systems Approaches as Phytosanitary Measures: Techniques and Case Studies

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ABSTRACT The development of export strategies for horticultural products considered to be hosts of quarantine pests follows a logical series of steps. The foundation for the process is the completion of a commodity plant pest risk assessment that satisfies the needs of the national plant protection organization of the importing country. After identifying the significant pests, a set of proposed mitigation measures is designed. Regulatory officials are increasingly using systems approaches to supplement or substitute for direct postharvest treatments in developing export strategies. The components of systems approaches can be divided into a series of five categories of measures: field and production measures, preharvest measures, postharvest measures, inspection and certification measures, and shipping and distribution measures. The United States Department of Agriculture (USDA), working with the national plant protection organizations of other countries, has used the systems approach concept to develop quarantine strategies for both the domestic movement and the importation of fruit fly host commodities. The Mexican fruit fly *Anastrepha ludens* (Loew) programme in the Texas citrus production area of the lower Rio Grande River Valley is an example of a domestic systems approach. USDA allows the importation of papayas from Central America when produced in accordance with a systems approach that primarily targets the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). Systems approaches are groups of integrated pest risk management measures designed to provide importing countries with adequate phytosanitary security while facilitating trade in situations where direct postharvest commodity treatment is undesirable, not feasible or non-existent, or imported products are marginal hosts of the quarantine pest produced in a low-pest prevalence area.

KEY WORDS systems approach, quarantine, pest free areas, import and export, *Ceratitis capitata*, *Anastrepha ludens*

1. Introduction

The development of export strategies for horticultural products considered to be hosts of quarantine pest hosts follows a logical series of steps. The foundation for the process is the completion of a commodity plant pest risk assessment (PRA) that satisfies the needs of the national plant protection organization (NPPO) of the importing country. The PRA should assess the risk of all pests that may be quarantine significant including "hitchhiker" or contaminating pests, i.e. pests that are carried by a commodity and, in the case of plants and plant products, does not infest those plants or plant products (FAO 2002b). After identifying the significant pests, a set of proposed mitigation measures is designed. The efficacy of the proposed measures should be documented and a benefit/cost analysis (formal or informal) should be conducted. The end product is a written proposal or workplan that clearly indicates pests of concern, the proposed measures and the respective roles and responsibilities of the exporting and importing NPPOs, growers and other concerned parties.

It is the responsibility of the importing NPPO to ensure that proposed pest risk management measures provide them with phytosanitary security to their satisfaction. This usually requires that the exporting NPPO provides documentation as to the efficacy of the

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proposed risk management measures.

Documentation typically includes some or all of the following: (1) trapping data, usually at least one year's worth of data; for those systems where a pest free area, production site freedom or low pest prevalence is employed as a component of the systems approach, (2) research reports and/or published research papers providing evidence for the efficacy of the systems approach components, and (3) empirical data, e.g. from field-test or pilot studies of the systems approach.

2. Systems Approaches

The mitigation measures used to facilitate exports of commodities, particularly quarantine-significant fruit fly hosts, have traditionally involved postharvest direct treatments such as cold treatment or fumigation. Regulatory officials are increasingly using systems approaches to supplement or substitute for direct postharvest treatments in developing export strategies. Systems approaches are defined as:

> *The integration of different pest risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of phytosanitary protection (FAO 2002a).*

The concept of systems approaches evolved from the need to consider pre- and postharvest biological factors affecting the level of infestation of commodities before, during and after production, processing and shipping (Jang and Moffit 1994, NPB 2002).

Jang and Moffit (1994) proposed dividing the components of systems approaches into a series of five categories of measures: (1) field or production: measures to reduce the pest population in the field, (2) preharvest: measures that rely on the infestation biology of the pest and or commodity pest phenology to reduce the likelihood of pest infestation of the commodity prior to shipment, (3) postharvest: measures that not only include traditional direct treatments, but also handling procedures such as culling, sorting, grading and packing applied as the name implies, postharvest, (4) inspection: these measures include inspections for the pest as well as certification that the commodity has undergone all required treatments and may include both preshipment and port of entry inspection, and (5) shipping/distribution: these measures again rely on the pest biology and host commodity phenology, but in this case they are applied in the importing country.

What follows in the next several sections of this paper is by no means an exhaustive list of all of the possible measures to be used in designing an export systems approach for fruit fly host material. Rather it is intended as a sampling, to give the reader an idea of the breadth of techniques available. Indeed, the strength of the systems approach is in its flexibility and the ability to adapt and adopt a broad variety of measures as available and as required.

3. Field and Production Measures

Among the field measures are trapping for purposes of detection, delimiting and for monitoring (IAEA 2003, USDA 2003). Trapping can be combined with capture thresholds which when reached, trigger a pest control response. These trapping and trigger measures are critical for the establishment and maintenance of pest free areas, pest free production sites and areas of low pest prevalence (i.e. an area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subjected to effective surveillance, control or eradication measures) (FAO 1996, 1999, and 2002a). Similar monitoring and management measures may be required in designated buffer zones surrounding free areas and production sites. Fruit cutting may be used to supplement trapping by detecting fruit fly larvae (USDA 2003). In the event that fruit cutting reveals a larval find, fruit may be stripped and ground treatments applied.

Trapping and accompanying triggers may be used inside and outside pest free growing structures such as screenhouses and glasshous-

es. USDA has prescribed this approach for several commodities, for example, screenhouse-grown tomatoes from Central and South America.

Field treatments may include bait or cover sprays applied to the commodity during the growing season, either as a routine production practice or in response to trap captures. These treatments may also be applied outside the production field to preferred wild hosts or in shade trees where fruit flies might rest. Lure-and-kill devices (i.e. bait stations), may be used in the production fields or in buffer zones surrounding production areas.

The sterile insect technique (SIT) may be used before or during the growing season or in response to trap captures (USDA 2003). Biological control activities from classical biological control to augmentation or conservation approaches may also be employed (Hopper 2003).

Other, perhaps less commonly used fruit fly management measures include the aforementioned use of pest free growing structures, such as screenhouses or glasshouses; limiting production sites to a certain altitude above which fruit flies are not known to occur; requiring quarantine road stations and signage to prevent the movement of infested host material into free areas; fruit bagging; and, requiring that export production sites register with the exporting NPPO.

4. Preharvest Measures

Examples of preharvest measures include: (1) exclusion of other fruit fly hosts from the production areas and buffer zones, (2) removal of mature or overripe fruit, especially fallen fruit from the production areas, (3) removal of shade trees that act as fruit fly resting sites during the heat of the day from production areas and buffer zones, (4) requiring the exclusive use of resistant or less susceptible varieties of the commodity, (5) prohibiting harvest after a specific date or when trapping triggers indicate unacceptably high populations of fruit flies, and (6) prohibiting, through regulation, the movement of fruit fly host material into the production area.

5. Postharvest Measures

Direct commodity treatments are the most obvious form of postharvest measures. Examples of direct commodity treatments include fumigation, pesticides, irradiation, cold treatment, hot water immersion, heated air or radio frequency, to name a few (Sharp and Hallman 1994).

Other measures that may be considered postharvest measures include requiring that all packinghouse openings be covered with insect-proof screening. The exporter may be required to safeguard the fruit to prevent infestation during movement from the field to the packinghouse to the export port. Packinghouse activities may be limited to daylight hours. This measure is actually directed not toward fruit flies, but rather against hitchhiker or contaminating pests. Culling or grading of export fruit may be required to remove fruit with defects (Jang and Moffit 1994). For example, bananas imported into the continental USA must be free from cuts and cracks (CFR 2005a). Export fruit may be limited to a certain stage of maturity that is either not susceptible or less susceptible to fruit flies (Armstrong 1994).

6. Shipping and Distribution Measures

The export season may be limited to coincide with times when fruit fly hosts are either unavailable or not susceptible in the importing region. Similarly, distribution of the commodity may be limited geographically in the importing country or region to reduce the likelihood that fruit flies could find suitable host plants. Shipment size may be limited to reduce the likelihood that fruit flies will enter in sufficient numbers for pairs to mate. Shipments may be limited to refrigerated containers.

7. Inspection and Certification Measures

Inspection and certification measures include the obvious postharvest and port of entry

inspections by the exporting and importing NPPOs, respectively, as well as postharvest and port of entry fruit cutting. Other inspection and certification measures include requiring a phytosanitary certificate with an additional declaration stating, for example, shipment or production area freedom from fruit flies, or production of the commodity in accordance with specific regulations or agreements.

The importing and exporting NPPOs may agree to a pre-clearance programme where all phytosanitary inspections are conducted prior to export. For the importing NPPO, this has the advantage of keeping risk offshore. For the export growers, the advantage is in knowing the phytosanitary status of their product before paying shipping costs.

Other measures include audit inspection visits by the importing NPPO, requiring packinghouse registration, labeling or box colour requirements for limited distribution programmes, compliance agreements between packinghouses and the export NPPOs and workplans signed by both the exporting and importing NPPOs.

8. Case Studies

8.1. Mexican Fruit Fly Anastrepha ludens *Programme in the Lower Rio Grande River Valley, Texas*

The domestic fruit fly quarantine systems approach in the lower Rio Grande River Valley is at once both a domestic and an international area-wide fruit fly control programme. The systems approach measures are designed to impact the domestic movement of citrus fruit in the USA, but at least some of the measures are applied on both sides of the Texas-Mexico border. The primary target pest of this programme is the Mexican fruit fly *Anastrepha ludens* (Loew). The protocol area includes five production areas in three counties of the lower Rio Grande River Valley, Hidalgo, Cameron, and Willacy counties. These three counties comprise the major citrus production area in Texas.

The five production areas are under federal regulation that requires that once fruit fly trapping triggers are reached, the fruit must be treated, most commonly by fumigation, before it can be shipped out of regulated areas to other citrus producing states in the USA (CFR 2005b). The objective is to conduct an area-wide management programme to suppress wild Mexican fruit fly populations to capture levels below the quarantine trigger in these five production areas The target commodity for this programme is citrus, but a lengthy list of commodities is regulated including apple, apricot, avocado, calamondin orange, cherimoya, citron, custard apple, grapefruit, guava, Japanese plum, lemon (except Eureka, Lisbon, and Villa Franka cultivars), lime (except sour limes), mamey, mandarin orange (tangerine), mango, nectarine, peach, pear, plum, pomegranate, prune, plum, pummelo, quince, rose apple, sour orange, sapote, sapodilla, yellow chapote, Spanish plum, ciruela, and sweet orange. There are three major components of the Texas programme: (1) a preventive sterile fly release programme, (2) a detection and delimitation trapping programme, and (3) bait spray treatments around wild fly detections.

The sterile fly release programme currently consists of the release of 30 million flies per week. The release is scheduled to increase to 150 million flies per week this year. The current release rate translates to about 240 flies per hectare per week and that will quadruple this year to about 1000 flies per hectare per week.

Detection trapping is conducted at a density of two traps per square kilometre. Traps are placed according to an overlaying grid. Those sections of a grid that do not contain host material, rangeland for example, are not trapped. When a wild fly is detected in a trap sprays are applied in a 250-metre radius around the detection. The spray treatments consist of two options: either a spinosad bait spray applied every seven to ten days for 60 days or a malathion bait spray applied every ten to 14 days for 60 days. Fruit cutting is initiated for 60 days within that 250-metre

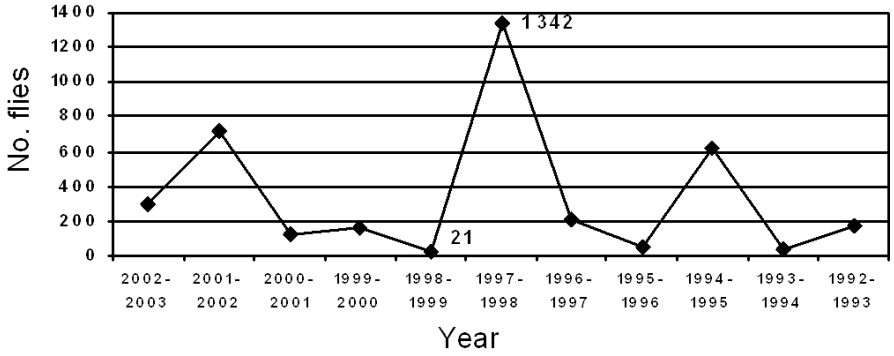

Figure 1. *Mexican fruit fly* Anastrepha ludens *captures in Texas by year (R. Vlasik, unpublished).*

radius, and finally a delimiting survey is conducted by placing an additional six traps around the detection.

Over the ten-year period from 1992 to 2002, fruit fly captures ranged from a low of 21 flies to a high of 1342 (R. Vlasik, unpublished). As illustrated in Fig. 1, fruit fly captures varied considerably in the ten-year period from 1992 to 2002.

Despite these fluctuations, quarantine trigger levels of wild Mexican fruit fly captures are reached in some or all of the five production areas in most seasons (R. Vlasik, personal communication). Although quarantine triggers are reached in most seasons, the Mexican fruit fly programme is still considered a success since, in most years, fruit fly populations are kept below quarantine triggers until late spring, which is after most of the commercial fruit has been harvested. Delaying triggers until the bulk of the harvest is completed reduces mandatory fumigations and the costs incurred by growers.

8.2. Papayas from Central America

A second example of a systems approach used to facilitate export of fruit fly host material is the case of fresh papaya fruit imported into the USA from Central America (CFR 2005c). The primary target pest for this programme is the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). The major components of this systems approach are the poor host status of the commodity, low prevalence of the pest in export production areas, the requirement for specific resistant cultivars of the Solo type, limiting export fruit to specific stages of maturity and a hot water dip treatment.

In addition to those measures, the systems approach calls for trapping at a rate of one trap per hectare beginning at least one year prior to harvest and continuing through completion of harvest. The traps are serviced weekly and if the capture rate exceeds seven Mediterranean fruit flies per trap per week, then remedial action must be taken to reduce the fly population. The national ministry of agriculture must keep records of fruit fly finds for each trap. If the average trap catch exceeds 14 Mediterranean fruit flies per trap per week, importations of papayas from that production area must be halted until the rate of capture drops to an average of seven or fewer flies per trap per week.

Cultural components of the systems approach include removal, burial or destruction of any culled, fallen or greater than half ripe fruit from the production area beginning at least 30 days prior to harvest. Half ripe is defined as more than one quarter of the fruit surface having turned yellow.

Prior to packing, fruit is treated by hot water immersion at 49 °C for 20 minutes When packed, papayas must be less than half ripe and appear to be free of all injurious

insect pests. Papayas must be safeguarded from exposure to fruit flies from harvest to export, including fly-proof packaging. The package containing the papayas must not contain any other fruit, including papayas not qualified for importation into the USA. The papayas are prohibited from entering Hawaii and the shipping cartons must be clearly marked to indicate this restriction. All activities must be supervised by plant health officials of the national ministry of agriculture. All shipments must be accompanied by a phytosanitary certificate issued by the national ministry of agriculture stating that the papayas were grown, packed, and shipped in accordance with these conditions.

9. Conclusions

Systems approaches are groups of integrated pest risk management measures designed to provide importing countries with adequate phytosanitary security while facilitating trade in situations where direct postharvest commodity treatment is undesirable, not feasible or non-existent or imported products are marginal hosts of the quarantine pest produced in a low-pest prevalence area. The components of systems approaches may be grouped into a series of five categories of measures (Jang and Moffit 1994): (1) field or production measures, (2) preharvest measures, (3) postharvest measures, (4) inspection, and (5) shipping and distribution measures. These measures may take various forms from traditional field and postharvest treatments to SIT, to less commonly used measures like pest free growing structures and restricted shipping seasons. Whatever measures are chosen, the successful design and implementation of systems approaches as phytosanitary measures requires close cooperation between the NPPOs of the importing and exporting countries. The USA, in cooperation with its trading partners, has successfully employed systems approaches to facilitate the safe movement of quarantine pest host commodities both internationally and domestically as evidenced by the examples discussed above, as well as an increasing number of other import programmes.

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Postharvest Phytosanitary Radiation Treatments: Less-Than-*Probit 9*, Generic Dose, and High Dose Applications

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ABSTRACT With world trade in agricultural commodities increasing, the introduction of exotic insects into new areas where they become pests will increase. Interest in the use of irradiation as a phytosanitary treatment for agricultural commodities is growing worldwide, particularly since International Plant Protection Convention (IPPC) and Codex Alimentarius standards now endorse and facilitate trade based on this disinfestation method. Irradiation is broadly effective against insects and mites at doses that do not compromise quality of most commodities. Unlike other disinfestation techniques, irradiation does not need to kill the pest immediately to provide quarantine security, and therefore live – but not viable or sterile – insects may occur with the exported commodity making inspection for the target pests redundant. Generic irradiation treatments are being developed to control broad groups of insects or insects in all commodities, and will expedite new trade in agricultural products. High dose or default dose treatments are also being used by determining an irradiation dose at the upper limit of what is believed to control the insect groups that infest a commodity without specific data for the quarantine species of concern. For quarantine insects on poor or rarely infested hosts, or that are effectively managed as a result of effective preharvest area-wide pest management for export under a systems approach, the *probit 9* standard for quarantine treatment efficacy – 99.9968% mortality – can be replaced by risk-based less-than-*probit 9* approaches that reduce the severity of the quarantine treatment and the number of insects required for testing during treatment development. The availability of generic and high dose treatments makes irradiation an attractive option compared with other quarantine treatments.

KEY WORDS irradiation, postharvest, quarantine, phytosanitary treatments, agricultural trade, IPPC, *probit 9*, generic dose, high dose

1. Introduction

World trade in agricultural commodities continues to grow. As agricultural trade is increasing, the risk of introducing exotic insects into new areas where they may become plant pests will increase. The establishment of new pests can be costly due to increased crop damage, control programmes, and quarantine restrictions on trade. Quarantine treatments or systems eliminate, sterilize, or kill regulatory pests in exported commodities to prevent their introduction and establishment into new areas. As exclusion is the goal for quarantine pests, the tolerance for the pest in the commodity is essentially zero (Follett and Neven 2006). Quarantine or phytosanitary treatments such as heat, cold, irradiation, and fumigation disinfest host commodities of insect pests before they are exported to areas where the pests do not occur. Whereas development of heat, cold, and fumigation treatments involves generating data for each commodity and pest combination, irradiation treatments are developed for a pest species irrespective of fruit (or vegetable) host. This is possible because ionizing radiation penetrates commodities quickly, and most commodities can tolerate irradiation at doses that kill the pest (Morris and Jessup 1994, Thomas 2001). The development of

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heat, cold and fumigation treatments, on the other hand, involves finding a balance between killing the pest and minimizing the adverse effects of the treatment process on quality of each commodity (Paull 1994).

Unlike other disinfestation techniques, irradiation does not need to kill the pest immediately to provide quarantine security, and therefore live (but not viable or sterile) insects may occur with the exported commodity making inspection for the target pests redundant as a confirmation of treatment application and efficacy. This places an added level of importance on the certification procedures for irradiation facilities and proper documentation accompanying export shipments confirming treatment at approved doses. It also places an added responsibility on researchers to ensure that the minimum absorbed dose approved for each quarantine pest has an adequate margin of safety. Irradiation technology is not universally accepted as a phytosanitary treatment (Follett and Neven 2006). However, irradiation as a phytosanitary measure is now approved internationally by the International Plant Protection Convention (IPPC) (FAO 2003) and Codex Alimentarius and can no longer be used as a non-tariff trade barrier by any importing country. Herein, several recent developments in the application of irradiation and risk management are discussed that should expand the use of the technology worldwide and facilitate trade in agricultural commodities, particularly fresh commodities.

2. Less-Than-*Probit 9* Treatments

2.1. Probit 9 *Treatments*

Postharvest commodity treatments for pests requiring a high degree of quarantine security are commonly referred to as *probit 9* treatments. The reference originates from the statistical method (probit analysis) used for deriving the dose response relationship. A response at the *probit 9* level results in 99.9968% efficacy. The required response may be mortality, sterility, or prevention of maturity. The United States Department of Agriculture (USDA) has used 99.9968% efficacy as the basis for approving many quarantine treatments, particularly for tephritid fruit flies. A *probit 9* treatment usually provides adequate quarantine security, and developing the treatment frequently proves to be the quickest and most easily accepted method for overcoming phytosanitary restrictions (Follett and Neven 2006).

To achieve *probit 9* mortality at the 95% confidence level, a minimum of 93 613 insects must be tested with no survivors after exposure to the treatment. Quantitative methods have been developed to calculate the number of test insects and confidence limits for other levels of precision and treatment efficacy, with and without survivors (Couey and Chew 1986). Although *probit 9* testing seems like a comfortable level of safety, given a highly-infested commodity or a high enough volume of infested commodity imports, even *probit 9* security could be overwhelmed (Mangan et al. 1997, Powell 2003). Other countries (Japan, Australia, New Zealand) accept quarantine treatment efficacy at 99.99% (at the 95% confidence level), which is obtained by treating 29 956 insects with no survivors (Couey and Chew 1986). Japan requires a total of 30 000 individuals in three to four trials (Sproul 1976), New Zealand requires three replicates of 10 000 test insects, and Australia accepts a cumulative total of 30 000 treated insects with no survivors (Heather and Corcoran 1992).

2.2. Alternative Approaches

In certain cases, less-than-*probit 9* numbers of insects may be acceptable during quarantine treatment development if the potential economic and environmental impact of the pest should it be introduced is low. For example, irradiation treatment with a dose of 300 Gy was accepted for the mango seed weevil *Sternochetus mangiferae* (F.) (USDA/APHIS 2002), a monophagous pest of mangos, based on evidence for the weevil's limited potential impact on US agriculture (Follett and

Gabbard 2000), and cumulative data from several studies with a few thousand insects showing prevention of adult emergence from the fruit at this dose and sterilization at lower doses (Seo et al. 1973, Heather and Corcoran 1992, Follett 2001).

Irradiation negatively affects commodity quality in some cases. Lowering the irradiation dose may reduce the undesirable effects on the commodity. Landolt et al. (1984) pointed out that the *probit 9* standard may be too stringent for commodities that are rarely infested or are poor hosts, and hence a less severe postharvest treatment might still provide quarantine security. The less-than-*probit 9* or alternative treatment efficacy approach measures risk as the probability of a mating pair or reproductive individual surviving in a shipment. This will be a function of many biological, operational, and environmental factors (Vail et al. 1993, Yamamura and Katsumata 1999, Follett and Neven 2006). The main quantitative argument for deviating from *probit 9* treatment efficacy is low infestation rate of the commodity, resulting from poor host status, early harvesting, or effective preharvest pest suppression.

A number of quarantine pest commodity systems are amenable to the less-than-*probit 9* approach (Liquido et al. 1995a, Follett and McQuate 2001). For example, nectarines are an inherently poor host for the codling moth *Cydia pomonella* (L.). Only three live codling moths (larvae) were found infesting 326 625 packed nectarines sampled from packinghouses in the San Joaquin Valley of California for an infestation rate of 9.2×10^{-6} (Curtis et al. 1991). In an average shipment of 16 000 kilograms (89 600 fruits), the probability of one or more mating pairs surviving after a *probit 9*-level quarantine treatment is 1.7×10^{-10}. The actual mortality level required from a quarantine treatment to prevent a mating pair of codling moths in a single shipment of nectarines with 95% confidence is 77.74% (probit 5.65). Hypothetically, if 100 shipments arrived at the same location the probability of one or more codling moth mating pairs surviving in nectarines after a *probit 9*-level quarantine treatment is still extremely small (1.7×10^{-6}). In this case, a *probit 9* treatment provides a high level of overkill and a less severe treatment might be developed that provides adequate quarantine security while minimizing any negative effects of the treatment on commodity quality. Low infestation rate at harvest can also be the result of effective pest management before harvest and/or the harvest of climacteric fruit (those that continue to ripen after harvest) at a less susceptible or non-preferred maturity stage. An additional advantage to using the less-than-*probit 9* approach is that fewer insects may be needed during research to develop quarantine treatments, which would make new treatments available on a more timely basis (Follett and McQuate 2001).

The less-than-*probit 9* approach fits with the systems approach where multiple procedures are used to cumulatively provide quarantine security (Jang and Moffitt 1994). For example, in Hawaii the main quarantine pest of avocados is the oriental fruit fly *Bactrocera dorsalis* (Hendel), although avocado is a poor host (Liquido et al. 1995b). Use of irradiation alone to provide quarantine security for oriental fruit fly requires a dose of 150 Gy for *probit 9* mortality (Follett and Armstrong 2004), and this irradiation dose causes dark vascular streaking of the fruit flesh. A systems approach against oriental fruit fly might be developed for avocados, that includes a less-than-*probit 9* irradiation dose if quarantine security is maintained and fruit quality problems are reduced. An irradiation dose of 80 Gy will kill more than 99% of oriental fruit flies (Follett and Armstrong 2004), and darkening of the avocado flesh is less severe at this dose. This treatment dose could be integrated with other components for sequential mortality providing quarantine security. Other components of the systems approach for avocados might include effective pest suppression in orchards and surroundings through the integration of monitoring and control measures including protein bait sprays, male annihilation, etc., as well as poor host status, early harvest, fruit cutting and inspection, limited dis-

Table 1. *Range of radiation doses predicted to control various arthropod pest groups*[1].

Pest group	Required response	Dose range (Gy)
Hemiptera	Sterilize adult or prevent generation turnover	50-250
Thrips	Sterilize actively reproducing adult	150-350
Tephritid fruit flies	Prevent adult emergence from eggs/larva	50-150
Bruchid seed weevils	Sterilize actively reproducing adult	70-300
Curculionid weevils	Sterilize actively reproducing adult	80-150
Scarab beetles	Sterilize actively reproducing adult	50-150
Stored product beetles	Sterilize actively reproducing adult	50-250
Stored product moths	Sterilize actively reproducing adult	100-600
Lepidopteran borers	Prevent adult emergence from eggs/larva	100-250
	Sterilize adults from late pupae	200-400
Mites	Sterilize actively reproducing adult	200-400

[1]Modified from Hallman (2001) and FAO (2003)

tribution period (winter months), and limited geographic area for distribution (i.e. to non-fruit fly supporting area) (Follett and Neven 2006).

3. Generic Doses

A "generic" quarantine treatment is one that provides quarantine security for a broad group of pests. From a regulatory standpoint, generic can also refer to a treatment for a pest on all commodities it infests. A generic treatment for a group of insects could be applied at many taxonomic levels, e.g. to all Diptera (flies), or to flies in the family Tephritidae (fruit flies), or to tephritid fruit flies in the genus *Bactrocera*. A generic irradiation dose is recommended after information has accumulated on effective quarantine irradiation doses for a wide range of insects within the taxon or for the important economic pests within the taxon (Bakri and Hendrichs 2004, Follett and Neven 2006). Irradiation is the ideal technology for developing generic treatments because it is effective against most insects and mites at dose levels that do not affect the quality of most commodities (Bakri and Hendrichs 2004).

Arthropod groups vary in their tolerance to irradiation (Table 1). Among insects, Diptera (flies), Coleoptera (beetles), Hemiptera (true bugs) tend to be less radiotolerant than Lepidoptera (moths and butterflies), although there is considerable variation among the species that have been tested within these groups (Bakri et al. 2005). Estimates for Hemiptera (scales, mealybugs, aphids and whiteflies) and Thysanoptera (thrips) are based on a small number of studies. Two of the most radiotolerant insects are the Indian meal moth *Plodia interpunctella* (Hübner) and the Angoumois grain moth *Sitrotroga cerealella* (Olivier), both stored products pests (Ignatowicz 2004). The Angoumois grain moth reproduced at 500 but not at 600 Gy (Ignatowicz 2004). Most insects are controlled at doses below 300 Gy. Several species of mites have been tested and they appear to be relatively tolerant of ionizing radiation. Theoretically, the high dose in the range could be used as a generic dose for each pest group in Table 1. However, the dose ranges are often estimates from limited data or from a limited sample of species within the group.

Initially, development of the generic dose concept has focused on tephritid fruit flies. The International Consultative Group on Food Irradiation (ICGFI) was the first group to formalize a recommendation for generic irradiation treatments (ICGFI 1991). In 1986, based

on irradiation data for several tephritid fruit fly species and a limited number of other insect pests, they proposed a dose of 150 Gy for fruit flies and 300 Gy for other insects. Adoption of the 150 Gy dose for fruit flies was blocked by research suggesting three tephritid fruit fly species in Hawaii required higher irradiation doses to prevent adult emergence from infested fruit (Seo et al. 1973, Hallman and Loaharanu 2002). Based on the data presented by Seo et al. (1973), the USDA-Animal Plant Health Inspection Service (APHIS) approved irradiation doses of 210, 225 and 250 Gy, respectively for the melon fly *Bactrocera cucurbitae* (Coquillet), the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), and the oriental fruit fly *B. dorsalis,* for exporting fruits and vegetables from Hawaii (USDA/APHIS 1997).

The generic dose concept has been applied only on a limited scale to irradiation treatment for fruits exported from Hawaii to the US mainland. After a rambutan shipment from Hawaii to California was interrupted in 1997 due to the presence of surface pests (thrips, mites, and mealybugs), the California Department of Food and Agriculture reviewed studies from Japan (Dohino et al. 1994, Kumagai and Dohino 1995, Dohino et al. 1996) on surface pests and established a generic treatment dose of 400 Gy for all surface pests. In 2001, the USDA-APHIS convened a meeting to establish treatment protocols for a new commercial irradiation facility in Hawaii, and approved generic irradiation doses of 250 Gy for any species of Tephritidae (fruit flies) and Thysanoptera (thrips); and 400 Gy for any species of Coccidae (soft scales), Pseudococcidae (mealybugs), and immature Lepidoptera (moths) infesting eight fruits being exported to the US mainland. In this case, the doses for non-fruit fly pests were established based on information from studies in Japan and Hawaii on a limited number of species within each taxa (Dohino et al. 1994, 1996, 1997, Follett and Lower 2000, Hara et al. 2001, Yalemar et al. 2001, Jacobsen and Hara 2003). This was the first time the regulatory authority of any country allowed use of generic irradiation treatments for a broad group of insects. Similarly, New Zealand prepared a rule to allow import of tropical fruits from Australia using generic irradiation treatments of 150 Gy for fruit flies, 250 Gy for other insects, and 300 Gy for mites (Corcoran and Waddell 2003).

These regulatory actions paved the way for formal adoption of generic irradiation doses by USDA-APHIS. Research was conducted demonstrating that 150 Gy was sufficient to control melon fly, Mediterranean fruit fly, and oriental fruit fly and that the approved doses could be lowered (Follett and Armstrong 2004). Subsequently, a generic dose for tephritid fruit flies of 150 Gy was proposed to USDA-APHIS based on data for 17 species of *Anastrepha*, *Bactrocera*, *Ceratitis*, and *Rhagoletis* fruit flies (Follett and Hallman, unpublished). In 2005, USDA-APHIS published a proposed rule recommending generic doses of 150 Gy for tephritid fruit flies and 400 Gy for all insects except pupa and adult Lepidoptera and mites (USDA/APHIS 2005). This rule by the US Government is a watershed event for promoting the use of irradiation as a phytosanitary treatment in international agricultural trade. Previously, the International Plant Protection Convention had adopted and published guidelines for the use of irradiation as a phytosanitary treatment, establishing a standard under which countries can negiotiate trade in irradiated commodities (FAO 2003). Adoption of generic irradiation doses for tephritid fruit flies and other insect groups by other countries is anticipated.

4. High Dose Approach

Use of an irradiation dose at the upper limit of what is believed to control the insect groups that infest a commodity without specific data for the quarantine species of concern is termed the “high dose” or “default dose” approach. The high dose approach is a conservative variation on generic doses. For most types of quarantine treatments, an ultra-severe regimen could be devised that is universally lethal to insects. In many cases, this treatment would

also likely be detrimental to commodity quality. A high dose approach is practical for irradiation because most commodities do not suffer significant loss of quality at doses that control phytosanitary insect pests.

Recently, the high dose approach was used to expedite trade in sweet potatoes from Hawaii to the US mainland. Sweet-potato growers in Hawaii are unable to ship their product to California and the US mainland without a quarantine treatment because of the presence of three quarantine pests. West Indian sweet potato weevil *Euscepes postfasciatus* (Fairmaire), and sweet potato vine borer *Omphisa anastomosalis* (Guenee), are federal quarantine pests, and sweet potato weevil *Cylas formicarius elegantulus* (Summers) is a quarantine pest for California. An irradiation treatment of 400 Gy for sweet potatoes was published based on preliminary data on radiation tolerance of the insect pests and a recommendation for a high dose based on the literature for insects related to the sweet potato pests (USDA/APHIS 2004). The 400 Gy dose is believed to control most species of Coleoptera and Lepidoptera. This was the first time APHIS considered the high dose approach for controlling a pest complex until research is completed to confirm a lower dose. Recent research indicates the dose to control the sweet potato pests can be reduced to 150 Gy (Follett 2006).

Theoretically, a universal irradiation dose could be set for all insects. As reported above, the most radiation-tolerant insect species known is the Angoumois grain moth that successfully reproduced after irradiation treatment at a dose of 500 Gy but not at 600 Gy (Ignatowicz 2004). If this is indeed the most tolerant insect, irradiation treatment with a minimum absorbed dose of 600 Gy should control any insect.

A limiting factor for the practical use of a generic treatment at 600 Gy in the USA is the 1000 Gy (1 kGy) maximum allowed dose for fresh produce set by the US Food and Drug Administration. With typical dose uniformity ratios at commercial irradiation facilities of 1.5-3.0, treatment to achieve a minimum absorbed dose of 600 Gy without exceeding 1 kGy would be difficult. Also, doses above 600 Gy adversely affect the quality of many fresh commodities (Kader 1986, Morris and Jessup 1994).

Another approach would be to set the generic dose for insects at a dose lower than 600 Gy and exclude any species or insect groups found to tolerate a dose above this level (Follett and Armstrong 2004, Follett and Griffin 2006). USDA-APHIS used this approach in its proposed rule recommending a generic irradiation dose of 400 Gy for insects excluding Lepidoptera pupae and adults, and mites (USDA/APHIS 2005). The 400 Gy generic dose reduces the problem of exceeding the 1 kGy limit during commercial treatment.

5. Conclusions

The availability of generic and high dose treatments makes irradiation an attractive option compared with other quarantine treatments. Developing irradiation treatments for taxonomic groups or guilds of insects and groups of commodities rather than for individual pests and commodities helps avoid unnecessary research, and regulatory and trade bottlenecks. An International Database of Insect Disinfestation and Sterilization (IDIDAS) developed by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations (Bakri et al. 2005, IAEA/FAO 2005) contains radiotolerance information for many Coleoptera (79 species, mainly curculionids), Lepidoptera (72 species, mainly pyralids and tortricids), and other pest groups. Information could be gleaned from the database to set additional generic doses although the majority of the studies referenced in the database for individual species were not designed for quarantine purposes and lack the large-scale tests needed to confirm the efficacy of an irradiation dose. Data for other important regulatory arthropod groups such as Thysanoptera, Hemiptera, and Acari is limited. Before generic treatments below 400 Gy can be recommended for a

wider range of insect groups, information from coordinated research projects and large-scale tests is needed on effective irradiation doses for key pests and under-represented taxa.

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Tools for the Trade: the International Business of the SIT

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ABSTRACT Historically the sterile insect technique (SIT) as part of area-wide integrated pest management (AW-IPM) programmes has been developed and implemented primarily by the public sector. With rising demand for the SIT, government production facilities have sold sterile insects to other governments to use in their own programmes. Although this trend is not conducive to commercial approaches, gradually new private production and markets are emerging. Commercial considerations, such as protection of intellectual property and elimination of below real-cost sterile insect supplies, are necessary for the SIT to thrive in the new model of using the technique for other than emergency programmes funded by governments. Furthermore, as the range of stakeholders involved in decisions about pesticide use and alternatives expands, benefit/cost analysis can be employed as a persuasive means for calculating the indirect benefits of using sterile insects, such as reduced environmental impacts. Recently, a model business plan for sterile insect production facilities was developed, drawing particularly on international experience with one pest species (the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann)). The plan includes a financial spreadsheet and other decision-making tools for managers to evaluate various options in the context of their specific location and potential markets. Progress also has been made in the area of harmonization of regulation of production, shipment and release of sterile insects through guidance from the International Plant Protection Convention (IPPC). Such global agreements are important as the SIT evolves into an increasingly international business.

KEY WORDS SIT, commercial, business plan, benefit/cost analysis, contingent valuation, IPPC, Mediterranean fruit fly, sterile insect production facilities

1. Decision-Making for the SIT: Early Experiences

The sterile insect technique (SIT) has been part of area-wide integrated pest management programmes (AW-IPM) in more than 25 countries since the concept was developed in the 1950s (Dyck et al. 2005). The insect pests controlled in some large-scale programmes include New World screwworm *Cochliomyia hominivorax* (Coquerel), codling moth *Cydia pomonella* (L.), pink bollworm *Pectinophora gossypiella* (Saunders), onion fly *Delia antiqua* (Meigen), one species of tsetse fly *Glossina austeni* Newstead and at least seven species of fruit flies with significant economic impact. However, unlike the use of pesticides, the "business" (both in terms of research and development and field application) of the SIT has remained in public hands for much of the history of this approach to pest control, and this has influenced decision-making to date.

Decisions about investment, for example, have been overwhelmingly governmental or intergovernmental, albeit always influenced by the "pay off" to the private sector or, especially in the case of controlling animal and human disease vectors, to society at large. Many programmes have also responded to emergency situations (e.g. a new incursion of a quarantine pest) and were aimed at eradicat-

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ing a population of an exotic pest species (Hernandez et al., this volume, K. Bloem et al., this volume, Suckling et al., this volume). Even when attempting to eradicate a long-established species, programmes were designed with a particular time frame for completion. The use of continual releases of sterile insects for the purpose of suppressing a population, or for exclusion as a preventive measure, is relatively new (Hendrichs et al. 2005). This huge market potential for using sterile insects as a replacement for insecticides requires a different approach to investment decisions. The range of decisions required for selection of and implementation of the SIT is examined below.

1.1. Technical Decisions

Technical decisions arise in all phases: the selection of a target species, the production of sterile insects, genetic traits that can be linked to sex selection, development of economical artificial diets, levels of mating compatibility and quality control, etc. This is also true for packaging and shipping sterile insects, storing and releasing them, monitoring the results of release, and demonstrating the outcome in population control.

When sterile insects have not previously been considered as part of an AW-IPM programme for a particular pest insect, information about the biology of the pest will determine the outcome on the initial decision of whether to further consider the technique. A list of characteristics that need to be assessed has recently been developed (IAEA 2007).

The scientific community working on these technical issues has matured and grown over the past decades. Hendrichs (1996) noted that the community of fruit fly researchers had increased from no more than 30 individuals in the 1950s and 1960s to over 300 in the 1990s. While these professionals are not all working on research directly supporting the SIT, a clear trend is emerging. As an example, the Tephritid Workers Database (www. tephritid.org) shows at time of publication over 700 fruit fly experts from more than 65 countries. However, much remains to be done since even among the relatively well-studied fruit fly pests, there are a number of injurious species that have hardly been addressed (ole-MoiYoi and Lux 2004). While international experience provides a key contribution to these technical decisions, in general many decisions must be both species- and location-specific and therefore considered on a case-by-case basis.

1.2. Operational Decisions

Many operational and technical decisions are closely linked. The decision whether to eradicate or suppress a population is one example. Insights into the pathways of introduction of the target pest will influence decisions about control strategies (Hendrichs et al. 2005). If new populations are repeatedly introduced, eradication efforts could seem futile until there is a parallel improvement in quarantine measures. If despite improvements in quarantine measures, pest introductions still repeatedly occur, then preventive releases could be the preferred strategy.

On decisions regarding eradication and suppression, Geier (1970) expressed the frustrating conflict between technical and operational objectives by stating that:

> *...as specialists [our] course is determined for us, not by the guidelines of our trade, but by the terms of reference that govern our operations.*

In some cases, projects that began with eradication as the goal had to adjust midstream to a strategy of suppression due to operational challenges and the cost of simply demonstrating that eradication had been achieved. There is a growing literature on operational decisions, both successes and failures, and international symposia on fruit flies of economic importance and conferences on area-wide control have contributed significantly to getting these issues documented (Tan 2000) as well as recent books (this volume, Dyck et al. 2005).

1.3. Political Decisions

The political decisions related to applying the

SIT in an AW-IPM programme sometimes reflect necessary judgements regarding the socio-economic situation of the country or region, rather than simply "politics". Mumford (2000) distinguishes the subjective components of these decisions by explaining why the same data may not always result in the same decision. Some of these subjective issues are related to the variability inherent in the perception of risk. The country's stated – or implied – acceptable level of risk should influence decisions regarding pest control. Choice of limits for externalities to be taken into account, and preferences on time horizon for return on investment may reflect attitudes of society at large more than a quarantine perspective. In fact, there are few purely objective elements (e.g. numbers of the target pest found in traps) to form the basis of sound decision-making. One can well imagine that this subjectivity with respect to so many phytosanitary measures explains why it has taken the contracting parties to the International Plant Protection Convention (IPPC) some years to develop and gain endorsement for an international standard on the efficacy of phytosanitary measures (FAO 2004, 2005a).

The political dimension often blurs into investment decisions. For example, after a successful eradication of the New World screwworm on the island of Curaçao, the USA began a lengthy programme to eliminate the pest from its territory. In 1966, the Secretary of Agriculture of the USA was criticized for bending to political pressure when, as no screwworms were found in the USA for several months, he declared the pest eradicated. This led to a change in its status to an "exotic pest", so that future control was financed entirely by the federal government rather than by the industry and state governments. In fact, numerous cases requiring control occurred from 1966 until 1982, calling in question the earlier declaration of eradication (Klassen 2000).

Today, eradication "by fiat" rather than by fact would be impossible due to scrutiny from trade partners, if nothing else. The same political pressures, however, are at play. The choice between suppression and eradication may therefore become a political decision related to the impact on trade. However, technical and operational realities will ultimately dictate the outcome.

1.4. Investment Decisions

With increased use, and by benefiting from technological advances and research, the SIT has become more efficient and operational costs have decreased. However, the technique remains management intensive and complex (Vreysen et al., this volume). When applied on an area-wide basis, it must deal with adjoining urban and non-commercial areas, besides those with direct commercial benefits. It requires complex coordination among numerous stakeholders including growers and growers' organizations, public authorities (regional, national and international), private companies, and teams of experts (Vreysen et al., this volume). Finally, and despite the decrease in costs over the past years, AW-IPM programmes using sterile insects still require a high initial capital investment for the mass-production component unless an existing source of sterile insects can be secured.

In the earlier conceptual model, the initial capital investment for sterile insect production, therefore, has been drawn almost entirely from public funds. Gardiner (2005) indeed argues that the initial capital costs must be at least partially supported by a grant of public funds in order for such an endeavour to survive the delay in accrual of benefits.

More recently, the demand for supplies of sterile insects for commercial operations, especially in the case of plant pests such as fruit flies, has led several groups to consider private financing of new production facilities. However, it is impossible for decisions to be taken on investment in this sector until government-run facilities reconsider their own pricing of sterile insects that have been provided *ad hoc* to numerous external projects to date. Otherwise, a commercial operation may find itself either directly "under bid" or simply facing the perception that costs, and therefore prices, are lower than what is actually the

case (Bassi et al., this volume).

Beyond the production phase, basic research will continue to be the domain of government-based research agencies and universities. Applied research, however, can attract a broader range of partners (Enkerlin 1996). Also, the present policy of some international and national organizations to leave intellectual property unprotected may not encourage innovation by others, facilitate control over the use of the developments, or protect from pirating of intellectual property. Some United Nations agencies have taken a different approach that secures protection of intellectual property, while encouraging commercialization under terms that satisfy the basic principles of that body (IAEA 2007).

1.5. Information Needs for Decision-Making

Technical decisions for operational programmes need to be supported by various types of data including: (1) information about the existing population (delimitation, density, seasonal fluctuations, etc.), (2) information about the host population, including non-commercial hosts that could serve as reservoirs, (3) predictions from simulation modelling using field data, and (4) information about the quality and performance of the sterile insects.

However, for investment decisions these need to be linked to market studies. During the preparation of a model business plan (IAEA 2007) for a sterile insect production facility, the collection of market information was hampered by the lack of published data and variation in reporting. Information on trapping regimes and counts, initial efforts to reduce the population, ratio of sterile to wild insects and other issues affecting release numbers is often not directly available. Also, many programmes appear to be optimistic in estimates without reporting on known limitations such as upcoming change in governments. Furthermore, unknown limitations can alter market estimates from eradication campaigns – lasting years and requiring millions of sterile insects – down to pilot programmes, smaller scale suppression efforts or no application of the SIT at all.

The experience of preparing the model business plan indicates that much useful information regarding actual costs of production of sterile insects is either not routinely collected from, or possibly not even recorded by, government-run production facilities. There has been no reason in the past for government-run facilities to share this information beyond what is required for internal budgetary reporting. Therefore, investment decisions are particularly challenging whether considering only sterile insect production or the development of a "full service" SIT management company.

The Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) are beginning to coordinate this type of information through their Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture (FAO/IAEA 2000).

1.6. Challenges to Informed Decision-Making in the International Business of the SIT

The range of decisions to be made in the international business of sterile insect production poses a special challenge. Access to timely, comprehensive and accurate information is key to the success of emerging business models. Some of this information may be available in the near future, but only if the operators of those facilities are informed about what type of data will be useful and choose to record and share it. As discussed above, those facilities that were set up for one purpose, such as eradication of a national threat, may not even take capital costs into account when estimating production costs, which *de facto* turn into market prices when excess production is sold externally. Exceptions will be those government-run facilities that are moving towards full-cost reporting and recovery, or possibly, single project facilities for which all costs must be captured in a publicly available budget.

There may be no motivation for private

business to share information about actual production levels, costs, sales, pricing, etc. Therefore, increasing involvement of private businesses for profit suggests that this type of information, so useful for verification or development of new tools, will be even harder to come by in the future.

It would seem that there are two options for addressing this lack of information for use by the entire community. If the issue of protecting intellectual property for an international public good is resolved, the international bodies supporting countries using the SIT may contractually require this exchange of information as a component of any technical assistance or support provided. Alternatively, users/buyers of sterile insects – rather than the producers – may be asked to contribute their information on actual numbers of sterile insects delivered and released, pricing, etc., thus allowing an analysis of the most critical final outcome data. This makes sense, as it will be the users who can most benefit from access to such information. This source also should provide more true market projections, as the users are the markets. A proposed set of data for users to provide annually, to reflect what is actually released, is outlined in Table 1.

Regardless of the approach taken, the actual requirements for information should not be onerous. Some data, such as that under "type of insect", may be available elsewhere. The directory of facilities within the International Database on Insect Disinfestation and Sterilization (IDIDAS) continues to be developed (www-ididas.iaea.org).

Other information worth collating for future research and analysis includes: actions taken by the national plant protection organizations, such as pre-release verification of sterility or post-release monitoring; and feasibility studies, benefit/cost analyses or other policy decision materials that lend themselves to ex-post review. Also, some of these issues were discussed at the 7th Session of the

Table 1. *Proposed data for annual reporting by users of sterile insects*

Nature of project	time scale (short term, multiple year, on going), purpose (ie. eradication, suppression, preventative, pest free area), target host(s) (animal, crop and/or wild hosts), target area (distinguish primary and buffer areas), and participants (ie. private association, public sector, mixed, etc)
Type of insect	scientific name, strain (e.g. genetically-linked traits), sex (male or mixed sexes), life stage shipped
Source	production facility supplying, any alternative suppliers
Total number and method of shipping	whether imported or supplied domestically, noting the circumstances for any loss of an entire shipment
Total number, timing and method for release	frequency and duration of releases (e.g. weekly numbers by season, or on going); aerial, ground releases – with which equipment, etc.
Costs	including any contractual parameters regarding quality assurance or other factors that affect the true cost of the sterile insects released
Treatments prior to release	e.g. pesticide applications to lower initial population

Interim Commission on Phytosanitary Measures (ICPM) in relation to producing a uniform certificate for movement of all living organisms entering a country for the purpose of pest control (i.e. those covered by the revised International Standards for Phytosanitary Measures 3 (FAO 2005b)), as a more targeted document than the phytosanitary certificate. If such a template is developed, it should reflect these information needs to avoid duplication and facilitate sharing (in that case, mandatory) information through public channels.

2. Financial Decision-Making Tools

One of the primary outcomes of the model business plan (IAEA 2007) was the development of a generic financial spreadsheet to support decisions on pricing of the product, reasonable profit objectives, and the impact of local labour costs related to production of sterile Mediterranean fruit flies. However, the overall framework of the spreadsheet could be adapted to any species with adequate information.

The model reflected a ten-year time frame, beginning with the construction of the production facility. Capital outlay was based on a regression of all the real examples of the past decade (described below). Fixed and variable costs were based on available data from operating facilities. Decision makers may alter the capacity of the facility, the sales target proportion of that capacity, and time from the start of construction until full capacity is reached. Other variables to set include interest rates, labour inflation rate, planned profit (in percentage of sales rate) and opportunity cost as well as the discount rate. The model can either accept a set maximum competitive price (USD per million sterile males), or generate the proposed price based on the other parameters.

The model is quick to use and permits application of stochastic software (in this case, the Crystal BallTM program). Although it operates in Excel® without capturing the variability, the addition of this element is generally appreciated by any seasoned investor who wants to know the risks as well as the best-case scenario.

Other financial tools include a regression of construction costs for Mediterranean fruit fly facilities (Fig. 1), a graph of cash versus loan options for financing, and a demonstration of the impact of estimating costs for sterile insect production based on weekly versus quarterly production runs. The regression of capital costs is an approximate guide for the cost of new facilities based on production

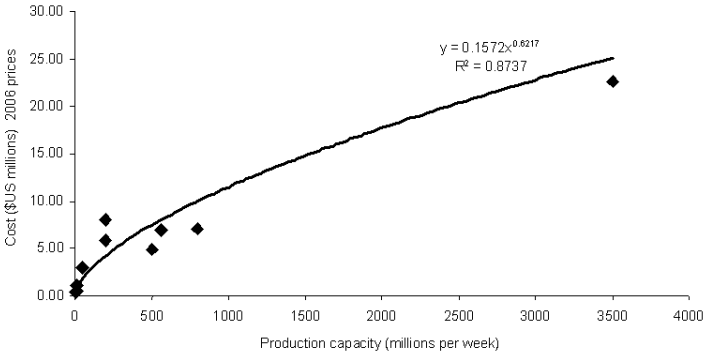

Figure 1. *Capital costs of sterile Mediterranean fruit fly facilities built between 1991 and 2006. Adjusted for inflation using GDP (chained) price index (US Treasury, May 2006). If not adjusted for inflation,* $R^2 = 0.8752$.

capacity. As with the financial spreadsheet, this is only to indicate an international norm; diversion from the regression line may be legitimate but warrants explanation since a number of components for construction of such a facility will have similar costs, regardless of location. Since there is still a scarcity of data for such a tool to be reliable, it should be recalculated with each new facility.

2.1. Projections and Evaluation of Assumptions

The financial model was tested on a proposed facility for production of Mediterranean fruit flies in Slovakia. This was to be a joint venture between a private company and the Government of Slovakia, which generously shared the business proposal. The model made it easy to compare various assumptions. The four scenarios tested resulted in breakeven points ranging from year three to year six. More importantly, the average cost per million sterile male Mediterranean fruit flies varied from USD 222 to USD 309, as compared to an estimated USD 344 in the model business plan (IAEA 2007). While this entire range of costs falls below the likely acceptable price per million (USD 375 per million sterile males was used), clearly the profit will vary enormously among these four scenarios.

The first scenario is essentially what the original proposal concluded. The third and fourth scenarios impose a series of assumptions from the model business plan onto the Slovakian situation, with the fourth scenario using a lower capital cost for construction based on the international data (Table 2). For full information on assumptions tested, see Annex 1 in IAEA (2007).

One factor that was easily tested was the cost of labour (scenario 2, which included other assumptions from the generic model). In the model business plan, cost of labour was found to be one of the most sensitive parameters in terms of affecting the total cost, second only to the cost of insect diet and well ahead of sales target as a proportion of production capacity, the third most important factor affecting cost. Much of the labour will be minimum wage earners who are trained in the many low skill level jobs associated with sterile insect production. The Slovakian proposal showed the cost of this labour as static (no increase in minimum wage) over the time projected. In scenario 2, the cost of labour was set to match the level of Portugal, simply as another member of the European Union with lower wages, which required an increase of the rate of over 19%/year. In fact, in the four

Table 2. *Summary of assumptions applied and results of various scenarios in application of the financial model to the case of Slovakia.*

Assumptions applied	Capacity/ production (million per week)	Labour inflator	Profit level	Average cost[1]	Net present value	10-year cumulative profit
				USD million		
Scenario 1	1000/1000	–	69%	222	42.858	68.091
Scenario 2	1000/1000	~20%	44%	260	33.027	51.063
Scenario 3	1132/1000	~20%	11%	337	8.849	16.135
Scenario 4	1132/1000	~20%	22%	309	18.024	28.736
Model Business Plan	1100/ 972	6%	10%	344	6.535	12.856

[1]Average cost (in USD million) is shown per one million male Mediterranean fruit flies sold. Cost should not be confused with price charged.

years to date since running that scenario, the increase was closer to 9%. This new data now allows the manager to alter the assumption with accurate historic data and to see the financial impacts of that change.

2.2. Application of the Financial Spreadsheet

The spreadsheet was also employed in the business plan for a new sterile Mediterranean fruit fly production facility that is being built in Bahia, Brazil. The projected cost for this facility is approximately USD 4.5 million, with USD 1.34 million committed by the national Ministry of Science and Technology and other resources from the State Governments of Bahia and Pernambuco (Wonghon 2005, Malavasi et al., this volume). The facility will produce 100 million sterile males per week for release over 11 000 hectares of mango produced for export to the USA and Japan. Much credit for the success of this fund-raising was given to the model (A. Malavasi, personal communication). The purpose of the model is to provide transparency and an international context, and not to promote sterile insect production that cannot be sustained.

The tools described above are intended to support decision-making regarding investment in the production of sterile insects. Decisions about the investment necessary for an entire SIT operation – whether this includes construction of a production facility or purchase of sterile insects – can be supported by the use of benefit/cost analysis.

3. Benefit/Cost Analysis as a Tool

3.1. Use of Benefit/Cost Analysis for SIT

To further assist public entities and/or private organizations and companies that are considering investment in the production or release of insects, benefit/cost analyses represent a valuable quantification and planning tool (Enkerlin and Mumford 1997, Mumford 1997, Larcher-Carvalho et al. 2001, Mumford et al. 2001). Whether applied to eradication or suppression programmes, such analyses provide consistency, objectivity, flexibility, inclusiveness and comprehensiveness at the decision-making stage.

Consistency and objectivity are ensured by permitting the same approach to be taken when comparing the overall balance of benefits and costs of the SIT integrated in different approaches, including conventional ones. The direct and indirect benefits and costs of each option can be quantified and compared using the same methodology.

When analysing the potential of the use of sterile insects within an AW-IPM programme, decision makers and stakeholders are faced, as demonstrated above, not simply with the choice of whether to proceed or not but several scenarios from which to select. Benefit/cost analyses provide the flexibility required to evaluate these options. For example, whether to use imported sterile flies, to use imported eggs for insect production or to build a local rearing facility. Different time horizons can also be analysed and returns calculated for different periods. Costs of sterile insects, programme areas, host ranges, host maturation times, intensity of damage, and discount rates can all be varied to analyse the viability of projects.

Different economic indices, such as net benefits and net present value can also be used to estimate likely economic success. Additionally, recent economic analyses have been developed using probabilistic models, with Monte Carlo simulation, to account for the uncertainty in many elements. These models provide information on the likelihood of success of a project given, for instance, uncertainty about the intensity of damage or control levels achieved (Larcher-Carvalho and Mumford 2002).

3.2. Stakeholder Model to Enhance the Analysis

Incorporating a stakeholder model provides useful information for programme planning. By identifying the key stakeholders (public

authorities, private organizations, farmers, consumers, civil society), and analysing their interests and the potential impact the programme may have on these, the study of the programme's viability is strengthened (Lindquist 2000). It indicates potential supporters (governmental authorities and research institutes) and potential opponents (pesticide industry, farmers outside programme area). It also becomes possible to apportion the different classes of benefits to different stakeholders, and thus the inclusion of different interests in the decision-making process. Such a model would assists, for example, in identifying possible sources of funding and partnerships that could be put in place. A further advantage of benefit/cost analyses is that they are comprehensive, i.e. technical, commercial, regulatory, social and ecological factors can be taken into account.

Costs of the SIT in AW-IPM are becoming more clearly defined as the number of programmes around the world increases, but still need to be evaluated in relation to the operational choices made. Amongst the direct benefits, the key elements include higher control effectiveness, reduction in residual losses (i.e. losses despite control), access to new markets and increased market value of commodities.

3.3. Indirect Benefits as Part of a Comprehensive Economic Analysis

Direct benefits do not provide the full picture, and therefore the more recent inclusion of indirect benefits in the analyses permits those interested in investing to understand the full potential. While difficult to estimate accurately, their inclusion allows a better understanding of the overall costs and benefits and the relative distribution of public and private returns to be made. Indirect benefits consist mainly of reduced environmental and health costs, but can also include more intangible benefits, such as the societal value of scientific capacity-building and of nature conservation.

For example, in analysing the SIT programme for Mediterranean fruit fly suppression on the Madeira Islands, the health and environmental costs of using organophosphate pesticides were also calculated (IAEA 2005). These costs correspond, to a great extent, to the indirect benefits, which derive mostly from the reduction in pesticide use. Therefore, quantifying the indirect benefits corresponds to quantifying the indirect costs of pesticide otherwise known as pesticide externalities.

Pesticides are inputs for an economic activity – agriculture – and use the environment as a sink for pollution. The costs of using the environment in this way are called externalities since they represent a cost of an economic activity, which is not included in the price of the product (Pimentel, this volume). It is very important to quantify these externalities to ensure that activities that are costly to society as a whole are not encouraged even if the private benefits are high (Pretty et al. 2000). For that reason, several methods have been used to quantify pesticide externalities. Since all have advantages and disadvantages, for the Madeira economic analysis two of these approaches were followed (IAEA 2005). The first (the financial method) attributed a financial cost to the externalities of pesticides, while the second estimated people's willingness to pay for food without pesticides using the contingent valuation method.

3.3.1. Financial Method

The financial method consisted of extensive interviewing of local experts and farmers to collect data on the costs incurred by society when dealing with the externalities caused by pesticides. This approach does not value the externality but uses society's expenditure in dealing with that externality as a proxy (Pretty et al. 2000).

Using this method, the indirect benefits quantified included the costs incurred by public and private authorities in monitoring pesticides in the environment (water and soil) and in food, and the treatment or prevention costs incurred in restoring the environment and human health. One important element was the cost of managing waste, such as pesticide

container recycling programmes. The results of such analysis can assist public authorities not directly involved with projects, such as environmental ministries, in deciding how much the use of sterile insects would enhance their environmental policies. They also inform water management authorities on potential benefits for their own operations. Since the tightening of European Union legislation regarding pesticides in drinking water has increased monitoring expenses, the indirect benefits analysis informs water authorities of the large savings that could be made through reduced pesticide use.

Alongside the reduction (or elimination) of these costs on the Madeira Islands were added the benefits arising from the opportunities created for organic farming (or integrated pest management), and for preserving the traditional agricultural landscapes. Quantification of these benefits assists ministries of agriculture and European institutions, which finance the preservation of traditional agricultural infrastructures, in establishing whether the SIT is worthwhile. This quantification may also be relevant to environmental organizations, which, in Madeira, advocate the establishment of agricultural reserves.

In terms of health costs, the acute effects of pesticides on farmers and their household members, their impact on consumers' health, as well as the costs incurred to restore public health, were also considered in the Madeira case study. These data will be important for decision-making by health authorities and consumer associations.

Social benefits of know-how and capacity building in the scientific technical arenas brought about by the programme are another important class of benefits. These benefits may be captured in the form of added prestige by education ministries and science and technology institutions.

3.3.2. Contingent Valuation

This method is based on the assumption that what people want should be the basis for benefit measurement. One way to identify what people want is to analyse how they respond when presented with choices regarding goods and services. A positive preference will show in the form of a willingness to pay for them. This is one way of representing the value of goods and services and thus allows an estimation of benefits. Based on this method, a survey was conducted to quantify the maximum amount respondents would be willing to pay for organic and locally produced fruits and targeted two groups of people: the local community and tourists.

The willingness to pay for organic products represents the value people are willing to pay for fruits with lower pesticide residues and for more environmentally friendly production systems. The values of the willingness to pay for fruit produced locally, reflects the value given to fruit quality (respondents associated locally produced fruit with better quality), and also to the social, environmental and economic values attributed to fruit production on Madeira.

Consumers' willingness to pay for organic and locally produced fruit may be used by national authorities to inform policy and decision-making regarding, for instance, support for local production. Tourists' willingness to pay for these products provides information to the tourist industry on the benefits they could collect by supporting agricultural development projects. This is particularly important in Madeira due to the large role the tourist industry plays in the regional economy.

As highlighted by Mumford (2004), the uncertainty associated with the calculation of indirect benefits is high. However, the combination of techniques, rigorous data collection methods and probabilistic analysis, increases confidence in the results. Benefit/cost analyses incorporating the valuation of indirect benefits constitute an important instrument for project appraisal. They are equally important for developing partnerships and raising contributions from public and private institutions and organizations, which are indirect beneficiaries. The values obtained also provide an important guideline to establish cost

recovery schemes for such projects.

4. New Tools/New Trade: International Standards to Facilitate SIT Application

The use of sterile insects in AW-IPM for pest species of fruit flies will be facilitated by two endorsed and one proposed International Standards for Phytosanitary Measures (ISPMs), which are developed and endorsed by contracting parties to the IPPC: (1) ISPM No. 22 Requirements for the Establishment of Areas of Low Pest Prevalence (FAO 2005c), (2) ISPM no. 26 Requirements for the Establishment and Maintenance of Pest Free Areas for Tephritid Fruit Flies (FAO 2006), and (3) Guidelines for the Establishment of Areas of Low Pest Prevalence for Fruit Flies (Tephridtidae) (Draft ISPM submitted in 2007 for adoption by the Commission of Phytosanitary Measures).

Guidance for the use of sterile insects is now included under ISPM No. 3, Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms (FAO 2005b). This formerly excluded any organism that was not self-replicating and was directed at "exotic" species rather than those that had become established. Although this standard does not enter into much detail in relation to sterile insects, many countries have based their national legislation on the original ISPM No. 3, which began as an FAO Code of Conduct. The revision of ISPM No. 3 to extend international harmonization of rules around shipment of beneficial organisms to include sterile insects should reduce the possibility of barriers to transport and transit of sterile insects due to individual country legislation or regulation variability. This ISPM also helps to address the issue faced by many countries of having legislation and/or regulations written explicitly for biological control agents that subsequently were applied inappropriately to sterile insects (e.g. the need to maintain an organism in quarantine for more than one generation).

These new ISPMs should be important new tools in furthering the international business of the SIT because of their legal status and wide application. They complement existing and upcoming manuals for more specific standard operating procedures, e.g. the manual for product quality control and shipping procedures for sterile mass-reared tephritid fruit flies (FAO/IAEA/USDA 2003).

5. Conclusions

Increased opportunities for using sterile insects in AW-IPM programmes have been sparked by increasingly stringent regulatory constraints on pesticide use, greater demand for high quality, safe food, rigorous phytosanitary requirements, and higher levels of awareness of environmental issues. These programmes provide a viable alternative to continual pesticide use in the control of particular plant or animal insect pests. There is a trend towards more commercial demand for sterile insects for the purpose of suppression rather than eradication, and thereby as a replacement for pesticide use. The final decision to integrate sterile insects depends on numerous technical, operational, political and financial decisions that rely on timely and accurate information. While information resources have improved, there is still a dearth of detailed data. The SIT has remained publicly controlled for much of its history, despite private sector involvement in all aspects of the approach. As a result, most government-run production facilities do not have, or are not accustomed to sharing, the historic financial information that could contribute to a generic model.

Nevertheless, a model business plan (IAEA 2007) does provide some simple tools for decision-making, primarily regarding sterile insect production. Based on sterile Mediterranean fruit fly production, a financial planning model is available to support full cost recovery, appropriate pricing and other management decisions. The simple tools presented in that model business plan can contribute to the success of sterile insect production and budgeting for AW-IPM programmes.

Benefit/cost analysis is another useful tool that can predict the likelihood of success given the time horizon and range of stakeholders selected by the analyst. Indirect benefits such as human health and environmental impacts, maintenance of agricultural landscapes or tourism, the value of non-commercial fruit consumption or other factors may be included.

Some new international phytosanitary standards for the use of sterile insects are appearing from the IPPC. Barriers will be avoided with harmonized guidance for issues ranging from establishment of a pest free area for tephritid pests to the paperwork required for transport of beneficial organisms, including sterile insects. These new international standards complement more detailed standard operating procedures that hold no legal status, but are crucial to efficient production and release of sterile insects.

The tools presented in this paper all require assumptions that were based on international information. These assumptions should be considered when applying the tools to a specific programme or project to avoid false conclusions. The SIT has become an international business with commercial considerations making these decision-making tools an increasingly valuable resource.

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Privatizing the SIT: a Conflict Between Business and Technology?

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ABSTRACT A programme to suppress the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) using the sterile insect technique (SIT) has been in operation in South Africa since 1999. After a difficult start, the Hex River Valley SIT Pilot Project covering 10 000 hectares of table grapes has been regarded as a success. Two other fruit production areas have since joined the area-wide integrated pest management programme (AW-IPM) that includes an SIT component. There is wide acceptance in the fruit industry that integrating the SIT for the development of fruit fly-free areas or fruit fly-low prevalence areas under a systems approach, are essential to remain competitive on the international fruit export market. Due to insufficient government funding to fully sustain this AW-IPM programme, and in the absence of capital investment for the production of sterile insects, economic realities ultimately compelled the AW-IPM programme to privatize its sterile fruit fly production and distribution operations in 2003. SIT Africa (Pty) Ltd thereby became the first commercial Mediterranean fruit fly sterile insect production company in the world, albeit a very small one. This development, at a time when the existence of the entire programme was seriously threatened by economic considerations, probably saved the programme from an early demise. However, economic, operational and cultural factors existing in the programme present a number of dilemmas with respect to the long-term success and expansion of the SIT in South Africa. Without economies of scale, the quest for commercial survival of the small production facility has the potential to create a conflict between what is good for business and what is good for the SIT. In order to sustain the SIT in South Africa, a middle road needs to be found where neither the sterile insect technology nor business viability is compromised.

KEY WORDS sterile insect technique, Mediterranean fruit fly, *Ceratitis capitata*, privatization, funding, economic factors, operational factors, cultural factors, conflicts of interest

1. Introduction

South Africa's main deciduous fruit-producing area, the Western Cape, is host to two species of tephritid fruit flies of economic importance, the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and the Natal fruit fly *Ceratitis rosa* Karsch. Mediterranean fruit fly tends to dominate in many fruit production areas, with Natal fruit fly more predominant in the milder, coastal areas. The use of the sterile insect technique (SIT) in the establishment of fruit fly-free areas has been well documented in the literature (e.g. Dowell et al. 2000, Reyes et al. 2000, Dyck 2004, Nigg et al. 2004, Dyck et al. 2005). In 1999 the Hex River Valley SIT Pilot Project, covering 10 000 hectares of table grapes, was initiated with the goal of cost-effectively suppressing Mediterranean fruit fly in the area. Despite a number of constraints, the pilot project achieved its goal. Further details are provided by Barnes et al. (2004).

A small rearing facility in Stellenbosch produces 8.5 million sterile Mediterranean fruit fly males per week. These flies are released by ground every week in three distinct fruit production areas – the Hex River Valley, the Elgin/Grabouw/Vyeboom/Villiersdorp area, and the Riebeek Valley area. The total area of fruit in the SIT areas is approximately 18 000 hectares. However, sterile flies

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are released almost exclusively in home gardens and backyards on farms and in the urban areas at a density of 2000 to 4000 flies per hectare, with the intention of reducing wild fly populations before they move to the commercial orchards and vineyards towards ripening and harvest.

Although the current AW-IPM programme focuses exclusively on suppression of Mediterranean fruit flies, there is increasing acceptance in the deciduous fruit industry that to remain competitive on international fruit export markets, the SIT is a necessity in order to create fruit fly-free areas. The intention in South Africa is to expand the AW-IPM programme to the point where the area covered by the SIT is large enough to justify the implementation of quarantine measures, which are necessary for the creation of Mediterranean fruit fly-free areas. Wider area freedom including from the Natal fruit fly will only be possible once certain mass-rearing issues for this species have been addressed. In the meantime, SIT pilot projects for two other key fruit pests, codling moth *Cydia pomonella* (L.), and false codling moth *Thaumatotibia leucotreta* (Meyrick), have recently been started.

The SIT is becoming an increasingly important facet in fruit production in South Africa. However, the key constraint in the fruit fly AW-IPM programme has been inadequate funding. After appeals for sustained government funding for the programme were unsuccessful, other funding sources had to be investigated. The unsustainability of a subsequent funding partnership ultimately gave rise to the privatization of sterile fruit fly production and distribution.

A number of economic, operational and cultural factors are impacting on the success of the SIT and on the business of producing and distributing sterile Mediterranean fruit flies in South Africa. These factors can give rise to potential conflicts of interest between business and technology in this young, small, privatized AW-IPM programme. While circumstances will not always give rise to such conflicts, other AW-IPM programmes considering privatization should bear these issues in mind.

Before discussing the economic, operational and cultural factors, it is necessary to outline the funding of the Mediterranean fruit fly AW-IPM programme in South Africa.

2. Funding the Programme

From 1999 to 2003, the Hex River Valley SIT Pilot Project was funded by a combination of government and non-governmental organizations (Barnes et al. 2004). The main project contributor was the International Atomic Energy Agency (IAEA) by way of the provision of equipment, training and technical support. A formal SIT partnership between the Agricultural Research Council (ARC) (a parastatal organization) and the Deciduous Fruit Producer's Trust covered the costs of producing sterile Mediterranean fruit flies. The Hex River Valley growers purchased sterile flies at a subsidized rate and financed all field operations including the release of sterile flies. The contribution of the national government was through involvement of ARC Infruitec-Nietvoorbij in which management of the project was vested. Limited and irregular grants were made available by the Western Cape Department of Agriculture.

The pilot project was always under severe financial constraints. The aforementioned organizations were therefore unable to financially sustain the programme. In an unfavourable economic climate, capital investment was also not forthcoming. The continuation of the programme was thus in jeopardy. Privatization was seen as the only means of maintaining it, and so in 2003 the production and distribution of sterile male Mediterranean fruit flies was commercialized with the formation of a private company, SIT Africa (Pty) Ltd This step probably saved the programme from demise.

Up to the time of writing the government has not financially supported SIT Africa (Pty) Ltd. The full costs of the SIT are currently borne by the fruit growers. Growers purchase the sterile Mediterranean fruit flies from SIT Africa (Pty) Ltd under contract, and SIT area coordinators, contracted by the company, facilitate sterile fly release, trapping, and ster-

ile/wild fly identification. Growers also purchase and apply their own fruit fly bait. They are supplied with trap catch data for the whole production area and are advised on baiting and orchard/vineyard sanitation interventions on their particular farms. SIT Africa (Pty) Ltd contracts technical expertise in the SIT from the ARC, and an ongoing promotional campaign is aimed at bringing more areas into the programme.

3. Economic Factors Influencing the Programme

A number of factors have impacted on the economic viability of the Mediterranean fruit fly AW-IPM programme in South Africa.

3.1. Absence of Government Investment

The SIT is implemented using the AW-IPM concept. With certain exceptions such as the onion fly *Delia antiqua* Meigen, which is released over small plots of up to six hectares (Loosjes 2000), the SIT is usually most effectively conducted over thousands rather than hundreds of hectares. An AW-IPM programme with an SIT component is a management- and cost-intensive operation, which needs to be wholly or largely administered by a financially-sound agent that can drive the programme and assume overall responsibility for its funding and management. Costly quarantine measures, which are a government responsibility, are also often associated with a large-scale programme.

Export fruit industries are high value operations. Multiplier effects ensure that a thriving fruit industry brings prosperity to the region. Successful AW-IPM programmes involving the SIT therefore benefit not only the growers in any fruit production area, but also the economic well-being of everyone in the region and thus the country at large. As a result such programmes around the world are typically driven by government and have a large component of government funding – protection of high income-generating agricultural industries is invariably an excellent investment. These programmes are usually co-funded by local and national governments together with the fruit industry, ensuring a viable and sustainable funding base.

Notwithstanding a certain amount of national and provincial government assistance during the pilot project phase, insufficient government support after commercialization has significantly limited the success of the programme, and restricted its much-needed roll-out to other production areas.

3.2. Lack of Private Investment

Efforts to raise private venture capital within South Africa to support a commercial SIT company have so far been unsuccessful. The SIT is largely unknown outside of the deciduous fruit industry. It is a very young technology in South Africa, and there are no local, economically successful models that can be used to entice potential investors and to demonstrate that they will get the required return on investment. The economic environment in South Africa has been harsh during the past 5-10 years, further discouraging potential investors from risking capital in a largely unknown technology.

3.3. Fruit Industry Economics

The South African deciduous fruit industry has suffered a series of negative economic influences over the past few years: (1) consecutive years of adverse climatic conditions (e.g. drought, warm winters) have taken their toll on the size and quality of the crop, (2) a strong South African rand which has appreciated in value relative to major international currencies, (3) volatile and difficult marketing conditions, (4) shrinking traditional markets, (5) rising fuel prices and input costs, and (6) stiff competition from competing fruit-producing countries. All these factors have negatively affected the export crop size and quality and fruit sales on export markets, thus negatively affecting producer income (Faure 2003, Lombard 2005).

The past few years have thus been a very

bad time to sell the SIT to fruit growers. The aforementioned factors have also reduced the ability of the deciduous fruit industry to fund research and development projects, and to contribute more widely to the implementation of larger initiatives such as the AW-IPM programme. In these circumstances it is difficult to persuade growers to adopt a new and little-understood technology that may initially be more expensive even if more cost effective in the long term. The money to buy relatively expensive sterile fruit flies, and to appoint people to dedicated fruit fly SIT posts, is in short supply.

3.4. Lack of Economies of Scale in the Rearing Facility

The SIT Africa Medfly Rearing Facility, located in Stellenbosch, was commissioned in 1999 as a pilot facility to produce sterile Mediterranean fruit flies for the Hex River Valley SIT Pilot Project. Its estimated maximum output was judged at the time to be eight million sterile males per week, though demand was only five million per week. In reality this target was never met due to a variety of circumstances (Barnes et al. 2004). Since then, due to improved quality management and improved genetic sexing strains of the Mediterranean fruit fly, the current demand for seven million sterile males per week can be comfortably and routinely met. Maximum output is now estimated to be 10-12 million sterile males per week.

The small size of the rearing facility resulted in very poor economies of scale and very expensive sterile Mediterranean fruit flies. Growers paid between USD 1150-1300/million flies (Rand 7100-8100) at the time of writing. The lower price is in effect the production cost, and applies to the Hex River Valley where farmers pay for the collection of the sterile pupae from the SIT Africa (Pty) Ltd facility and release them after emergence. The higher price applies to the Elgin/Grabouw/Vyeboom/Villiersdorp and Riebeek Valley areas, for which the sterile flies are emerged and transported to the release areas by the facility. The difference in price equates to the cost of eclosion and delivery. Sterile Mediterranean fruit flies in South Africa therefore cost about four times as much as sterile flies offered by larger Mediterranean fruit fly facilities around the world.

Until such time as demand rises above about ten million sterile males per week, thus requiring a larger facility, production costs will remain high due to a lack of economies of scale. However, it is unrealistic to think that demand for sterile Mediterranean fruit flies in South Africa will reach levels where significant economies of scale will be realized. The maximum demand in South Africa has been estimated at between 69 million and 76 million per week (Badenhorst 2001), whereas it is generally accepted that favourable economies of scale are realized from approximately 100 million sterile males per week.

In order to successfully implement the SIT in any area, the cost should be no more than the cost of conventional control. Locally-produced sterile Mediterranean fruit flies will likely remain relatively expensive in South Africa unless a much larger market for sterile flies (including foreign markets) justifies a larger facility. Expensive flies are difficult to sell during the best of economic times, and even more so in a harsh economic climate.

4. Operational Factors Influencing the Programme

In this context, operational factors refer to implementation of the SIT in the field, as well as to certain business aspects concerned with the production and sale of sterile flies to clients.

4.1. Fragmented Expansion of the SIT Suppression Areas

Following on the Hex River Valley Pilot Project, it was originally planned to expand the area covered by the release of sterile insects in a logical, systematic manner by taking the SIT to adjacent production areas and thus increasing the contiguous area. In the absence of greater government sponsorship

whereby growers do not have to pay the full cost of the SIT, the commercial programme can only be expanded to production areas where there are sufficient growers in a contiguous area that are prepared to pay for the SIT. As a result, and with the economic factors mentioned above, use of the SIT in the Western Cape has evolved in a fragmented patchwork of relatively small areas receiving sterile fruit flies. Commercialization in South Africa has not benefited from economies of scale in the production of sterile insects, and also not from the application of the SIT over a single, large area.

4.2. Different Funding Mechanisms

Partly due to cultural differences between groups of growers in different production areas (see below), growers tend to have different views on the mechanisms by which they are prepared to pay for the various components of SIT implementation. In one area the necessary funds are raised through a levy on all growers in the area and are managed on their behalf by a grower trust organization. In other areas the involvement of all growers has not been achieved and participating growers have signed individual contracts with SIT Africa (Pty) Ltd. Privatization has thus resulted in differential payment mechanisms, which are not in the best interests of an AW-IPM approach.

4.3. Need to Maximize Sales of Sterile Flies

Any private company needs to ensure a positive cash flow and ultimately show a profit. In a private sterile insect production facility, business reasoning dictates that sales of sterile flies need to be maximized. In the drive to get more clients, there can be a temptation to start releasing sterile flies in areas that are unsuitable geographically for SIT integration, where fruit production practices are not ideal for its application, or where prerequisites for starting the SIT have not been met. This will most likely happen in a small company with cash flow under pressure. This is exacerbated when growers without adequate knowledge of the sterile insect technology state that they "want sterile flies" as a "quick-fix" to solve their fruit fly problem.

4.4. Difficulties with the Management of Small Areas

With the fragmented manner in which the use of the SIT has evolved in South Africa, there are a number of relatively small areas under sterile insect releases, in most cases each being further subdivided into a number of smaller areas. For example, the Elgin/Grabouw/Vyeboom/Villiersdorp area is divided up into three smaller sub-areas, and the Riebeek Valley area into four such sub-areas. While each area has its own coordinator and field staff, management and communication within a group of smaller sub-areas is more complex and responsibilities also become fragmented. There is a greater risk of certain key procedures being overlooked, with a consequent negative impact on the success of the programme.

5. Cultural Factors Influencing the Programme

The Western Cape deciduous fruit industry is spread over an area of approximately 50 000 square kilometres, and incorporates at least 15 separate fruit growing areas of varying sizes. Fruit growers in these areas come from different backgrounds, have different cultures and home languages, grow many different kinds of fruit crops, and have a diverse outlook on fruit farming practices. On the other hand, businessmen are also necessarily involved with a commercial AW-IPM programme, and understandably they tend to look at a programme using the SIT through other eyes. All these factors also have an impact on the ultimate success of SIT application.

5.1. Lack of Coordination between Growers

Being an area-wide technology, the SIT requires a significant degree of grower coop-

eration and coordination within the area. However, fruit growers normally tend to act independently in pest management activities and seldom if ever cooperate on or coordinate these activities on an area-wide basis. This contributes to the fragmentation of areas where the SIT is established, and presents a risk to the area-wide strategy.

5.2. Lack of Technical Knowledge on the SIT

Most growers lack knowledge on the more technical aspects of the SIT and often have unrealistic expectations of the conditions under which it works and what it can deliver. The result is often suboptimal or even total lack of implementation of necessary procedures to the detriment of the SIT. This can be addressed by adequate technology transfer and public relations exercises, but with a resource-poor programme these interventions are often inadequate.

Likewise, businessmen involved in a commercial company will in most cases not have technical knowledge or training in pest management practices, and even less so in SIT technology. They may not appreciate the technical requirements and complexity involved, nor the need to follow certain essential and well-established SIT procedures. Their attention tends to be focused more on business management and financial matters, and making the books balance.

5.3. Different Expectations of Different Growers

Some fruit growers produce fruit for the export market, some for the canning and dried fruit markets, and an increasing number for the organic market. Some have farms that are fairly isolated from other orchards or vineyards. Some grow fruit that is less susceptible to fruit fly (e.g. apples) than other growers with more susceptible fruit (e.g. stone fruit). This has led to a call for differential prices for sterile fruit flies, e.g. "Why should I pay the same for my isolated apple orchard as others who grow stone fruit within a large production area?". This tends to retard the acceptance of SIT where growers in effect have to fund the programme.

6. The Conflicts and Dangers

The biggest potential conflict in a private company in this field may be a temptation to make decisions that will benefit the business, but which do not take into account the technology underpinning the SIT. For example, a drive by the company to market sterile insects to new clients mainly with the intention of boosting income, but without considering the technical requirements essential for successful application, would be a real threat to the success of the programme. The same would apply to any decision to cut expenditure, which would, for example, compromise sterile insect production. Company managers may be tempted to play down or ignore unpopular advice from technical experts. Requests for sterile insects as a quick-fix for their problems by growers with little knowledge of these technical requirements will exacerbate this threat. If sterile insects are released without considering the technical requirements for the SIT, there is a serious danger of the technology failing in that area. This will lead to growers losing faith in the technique and proclaiming that it does not work. This could be the death knell for the SIT in the whole region.

A further danger is that without a large, financially sound organization driving the SIT, growers will have to fully fund the operation. If the sterile insects are as or more expensive than conventional control, this is likely to result in resistance by many growers to implement the SIT, promoting the development of a patchwork of small areas. Implementation on an area-wide basis will be slowed, with detrimental effects on the efficacy of the SIT, its reputation, and the goal of creating pest free areas.

7. Conclusions

In South Africa, insufficient government funding to fully sustain an AW-IPM pro-

gramme with an SIT component, the absence of government investment in the commercial company SIT Africa (Pty) Ltd, together with an economic crisis in the deciduous fruit industry has necessitated a radical departure from the manner in which such programmes are normally funded and operated. In effect, responsibility for funding the programme has fallen entirely on deciduous fruit growers. In addition, economic, operational and cultural factors in South Africa have significantly reduced the speed and extent to which the SIT for Mediterranean fruit fly suppression has developed and expanded.

Under privatization, potential conflicts of interest between what is good for business and what is good for the technology can easily arise, and more so in a resource-poor programme. The causes of these potential conflicts should be addressed and managed before they exert an influence on the programme. It is essential that company businessmen seriously consider the advice of technical experts.

In the context of the South African programme, a sound financial basis is essential. If government is to be approached again to more fully support the SIT and the creation of fruit fly-free areas, the total, inclusive cost of fruit flies to the country should first be calculated to emphasize the importance of these pests. This should include the value of markets that may be lost due to the presence of fruit flies in South Africa, and the value of new markets that will be gained by having a fruit fly-free area.

All role-players and beneficiaries, including SIT Africa (Pty) Ltd, the fruit industry, exporters and government, should adopt a long-term vision for the AW-IPM using sterile insects in South Africa, especially with regard to the ultimate objective and benefits of the SIT and its expansion to new areas. If a successful commercial enterprise is to be sustained over time, the technical integrity must not be compromised. Failure of the technique in any area because, for example, of marketing sterile flies to areas or growers where this was not technically justified, will likely lead to the failure of the SIT in that area. This in turn will unjustifiably give the technique a bad name. Equally, training of SIT coordinators and advisors in business principles will likely promote innovation.

Company decision-makers should understand the area-wide concept of pest management in general, and the SIT principles in particular, and acknowledge that certain technical requirements and procedures are essential to success.

A change in mindset amongst fruit growers is needed, specifically to embrace the area-wide concept of pest management. This will necessitate greater grower cooperation and coordination within production areas. The manner in which growers pay for the SIT components should also be reviewed. In an area-wide programme it is essential that all growers in specified areas buy into the programme. Payment for sterile flies should be through a single agency (e.g. a grower organization), preferably through a levy on each and every grower in that area, and not between the sterile fly production company and individual growers.

The conflicts that have been discussed are likely to be more of a problem in small, resource-poor programmes and less likely to occur in larger, better-funded programmes.

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Private Sector Investment in Mediterranean Fruit Fly Mass-Production and SIT Operations – The "Sheep" of the Private Sector Among the "Wolves" of the Public Good?

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ABSTRACT Since the first large-scale use of the sterile insect technique (SIT) against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in southern Mexico in the 1970s, the fruit industry has been the major beneficiary and sometimes a financial contributor to the technological development of Mediterranean fruit fly SIT. Until recently, the involvement of the private sector in the "SIT package" was limited to the commercialization of traps, lures, attractants and insecticides, and occasionally the provision of specific services such as complementary suppression or monitoring, or the transport, emergence, feeding and release of sterilized flies, however not the mass-production of sterile males. Assessment in 2000 of the potential market for supplying sterile male Mediterranean fruit flies in the Mediterranean basin, and more recently the development of a model business plan for insect rearing facilities, generated some interest from the private sector to invest in the mass-production of the Mediterranean fruit fly. Since then, three private companies have been established namely InSecta Ltd in the UK, SIT Africa Ltd in South Africa, and Bio-Fly Ltd in Israel. Bio-Fly Ltd was established as the result of a bottom-up approach in which all stakeholders, i.e. grower communities, government institutions, supranational organizations and the private sector were consulted to quantify the needs for sterile flies, to identify the most suitable private partner, and to construct the facility with all the technical support available from the various stakeholders. Based upon these recent experiences, it was found that the sustainability and expansion of these companies in the near future will depend largely on: (1) the plans and capabilities of competing government-funded facilities to supply sterile flies at semi-subsidized prices to meet a growing demand in the Mediterranean region, (2) an increasing demand for environment-friendly pest control methods due to consumer pressure and more stringent laws regulating pest levels and insecticide residues, (3) the awareness of the end-users and beneficiaries of the availability of a cost-effective SIT technology for integration with other environment-friendly technologies available on the market, (4) national policies promoting and facilitating an area-wide approach to pest control and the transfer of SIT know-how, and (5) to some extent, the licensing of technology and the outsourcing of the management of existing mass-rearing infrastructure from government institutions to the private sector.

KEY WORDS commercialization, Mediterranean fruit fly, *Ceratitis capitata*, SIT package, rearing, private sector, public good

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1. Introduction

Since the concept of the sterile insect technique (SIT) was developed in the 1950s (Knipling 1955), it has been applied against several key insect pests, mainly Diptera and Lepidoptera that cause damage to crops or transmit diseases to animals and humans (Dyck et al. 2005).

Commercial losses have always been the major driving force behind the technical development of the SIT for a given insect pest. The largest SIT programme ever implemented worldwide was the New World screwworm *Cochliomyia hominivorax* (Coquerel) eradication programme, which over five decades eradicated this pest from North and Central America (Wyss 2000), reaching Panama in 2001 (Vargas-Terán et al. 2005). This programme was launched following lobbying by the cattle industry in Florida, which was facing yearly losses of about USD 20 million due to this pest (Klassen and Curtis 2005).

Tephritid fruit flies account for major economic losses in the most valuable crops worldwide. The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is undoubtedly the most important tephritid pest of economic significance in most temperate, subtropical and tropical countries, where it attacks over 300 wild and cultivated host plants (Liquido et al. 1991). Since the first large-scale use of the SIT against the Mediterranean fruit fly in southern Mexico (Hendrichs et al. 1983), the technology has been further developed and fine-tuned to address the concerns of the fruit industry worldwide. To date the SIT for Mediterranean fruit fly has reached a stage of development and knowledge of insect ecology, biology, genetics and behaviour rarely encountered for any other insect pest species. However, although the private sector has been the major beneficiary and sometimes a financial contributor to the technological development of Mediterranean fruit fly SIT, its commercial involvement in the implementation of the various components of the "SIT package" (rearing, transport, release, monitoring) has been, until recently, focused on limited aspects of the technique.

The present paper reviews the involvement of the private sector in the commercialization of the SIT package for Mediterranean fruit fly control and analyses the factors that led to the establishment of Israel's first commercial Mediterranean fruit fly rearing facility, Bio-Fly Ltd.

2. Fruit and Vegetable Industry and the Funding of SIT Operations

A sustainable source of funding for SIT activities as part of area-wide integrated pest management (AW-IPM) programmes for fruit flies is obviously a key to their success. Though the fruit and vegetable industries (Fig. 1, lower, right) are the major beneficiaries, the governments or supranational authorities have funded the vast majority of these programmes in the past. These often powerful industries (for example in the USA) lobby successfully for governmental involvement and stress that area-wide interventions are the responsibility of the government and are fully covered by their taxes. However, in a few cases the fruit and vegetable industries (i.e. local grower communities) have partially or entirely funded the cost of the operations. In the Hex River Valley of South Africa, the Mediterranean fruit fly suppression programme has been largely funded from the very first day by the growers (Barnes et al. 2004, Barnes, this volume). The AW-IPM programme in Patagonia, Argentina, which resulted in the region recently being declared free of the Mediterranean fruit fly (USDA 2005), has been partially funded through taxes imposed on fruit exports from the region (Guillén and Sánchez, this volume). In the Arava Valley of Israel, the local grower association has been increasingly involved in the funding of the Mediterranean fruit fly suppression programme since its inception in 1998 and now cover large parts of its operating costs. The very successful programme to eradicate the

Figure 1. *(upper, left) Servicing of a Multilure® trap baited with Biolure®, Aqaba in Jordan, (upper, right) adult flies ready for ground release produced by Bio-Fly Ltd, Sde Eliyahu in Israel, (lower, left) preparing for loading a chilled adult release container aboard the aircraft, Arava Valley in Israel, and (lower, right) citrus ready for export to Europe displayed by the owner of a commercial packing house, Batra region in Israel. (Photos from Ilan Mizrahi for the IAEA, reproduced with permission).*

Mexican fruit fly *Anastrepha ludens* (Loew) and the West Indian fruit fly *Anastrepha obliqua* (Macquart) from various states in northwestern Mexico (Reyes et al. 2000) has been funded equally by growers' associations, state governments, and the federal government. This formula is increasingly being used for other programmes (Enkerlin 2005).

3. Commercialization of the Mediterranean Fruit Fly *Ceratitis capitata* SIT Package

The involvement and contribution of the private sector in the commercialization of the SIT package has been guided by some basic business rules such as the need for minimum initial investment in infrastructure and research and development, a prompt return on investment and maximum profits generated in the long term. While the fruit and vegetable industries quickly identified the potential benefits that could be obtained by integrating the SIT in large AW-IPM fruit fly programmes launched by national or supranational authorities, they also realized that some components of the implementation of the SIT package were more amenable for private sector involvement than others, and these have been part of many SIT operations (Table 1). In this respect, the outcomes of Coordinated Research Projects (CRP), a mechanism used by the Joint Food and Agriculture Organization of the United Nations/International Atomic Energy Agency (FAO/IAEA) Division of Nuclear Techniques in Food and Agriculture to research and develop components of the SIT (Klassen et al. 1994), as well as FAO/IAEA consultants' meetings, have contributed to raising the private sector's interest (FAO/IAEA 2000). In addition, some large area-wide fruit fly control programmes, such as the Programa Moscamed in Mexico,

Table 1. *Overview of the involvement of the private sector in the various components of SIT operations.*

Component of the SIT package	Involvement of the private sector	References
Monitoring operations	Outsourcing of field monitoring activities to individual contractors with their own vehicles	Y. Roessler, personal communication
	Off-the-shelf GIS equipment (GPS units, GIS software, bar code readers, data loggers)	Cox and Vreysen 2005
Trapping	Commercialization of new traps	Ros et al. 2000, FAO/IAEA 2003
Lures, attractants and insecticides	Commercialization of new attractant	Heath et al. 1997, Epsky et al. 1999, Katsoyannos et al. 1999
	Commercialization of trimedlure, food attractants and dichlorvos (DDVP)	FAO/IAEA 2003
Complementary suppression methods	Commercialization of organically certified product for bait spray applications	Peck and McQuate 2000, Revis et al. 2004
	Outsourcing of aerial bait applications to specialized pesticide treatment companies	Roessler 1989
Production, sterilization and shipment	Establishment of commercial Mediterranean fruit fly rearing facilities (InSecta Ltd, SIT Africa Ltd, Bio-Fly Ltd)	Barnes et al. 2004, Vreysen et al. 2006, Barnes et al., this volume
	Outsourcing of Mediterranean fruit fly pupae sterilization	R. Argiles, personal communication
	Outsourcing of shipment of Mediterranean fruit fly pupae to air cargo companies	Cayol et al. 2004
	Outsourcing of shipment of Mediterranean fruit fly eggs to other AW-IPM programmes	Cáceres et al. 2007
Fly emergence, feeding and packing	Outsourcing operations of *Anastrepha* spp. at release centres or emergence facilities	Leal Mubarqui 2005
	Outsourcing emergence, feeding and packing for ground and/or aerial release operations	Cayol et al. 2004
Sterile insect release	Development of new chilled adult release machines	Tween 2004, Tween and Rendón, this volume
	Outsourcing of aerial release operations to air-companies	Dowell et al. 2000, Cayol et al. 2004

have adopted an action plan to involve the private sector in all stages of the programme (Enkerlin 1996). The Programa Moscamed in Guatemala has also attempted to privatize part of its operations (see section 3.5).

3.1. Monitoring

Monitoring pest populations in the field (Fig. 1, upper, left) to obtain feedback on the effectiveness of the various suppressive activities and SIT operations is one of the major components of all AW-IPM programmes (Vreysen 2005). In most of these operations the personnel carrying out the trapping and fruit sampling are government employees; however, in some cases these activities have been outsourced to the private sector. In the

Mediterranean fruit fly suppression programme in Israel, for example, only the field supervisors are government staff, assuring the quality of field monitoring activities carried out by many individual contractors all working with their own vehicles (Y. Roessler, personal communication). In other programmes, the maintenance of the fleet of government-owned field vehicles is outsourced, or these are even rented. Since the mid 1990s, AW-IPM programmes that integrate the release of sterile insects are increasingly making use of geographic information systems (GIS) as a decision support tool for the planning and day-to-day implementation of their field operations, for the spatial analysis and management of the field data, and for assessing programme progress (Cox and Vreysen 2005). Although specific data management systems were developed for the field operations, the main items of equipment (global positioning units (GPS), GIS software, bar code readers, data loggers) are available commercially and do not warrant specific investment by the private sector.

3.2. Traps

During the early days of applying the SIT against the Mediterranean fruit fly, it was not rare for programmes to develop their own locally-made trap components such as inserts or wire hangers of Jackson traps, or modified traps, as illustrated by the Maghreb-Med-type trap used in northern Africa in the early 1990s (FAO/IAEA 1993, Katsoyannos 1994). The research and development efforts that were initiated in the late 1980s to compare and standardize trap types and trapping materials for Mediterranean fruit fly AW-IPM programmes, (Katsoyannos 1994, IAEA 1996), contributed to making the market attractive for investment by the private sector considering the number of potential customers for the same trap type. Through these efforts, traps such as the Tephri® (Ros et al. 2000) and the MultiLure® traps (Fig. 1, upper, left) were developed and since then, became commercially available. Furthermore, the publication of trapping guidelines for fruit fly AW-IPM programmes (IAEA 2003) facilitated the international harmonization and recognition of trapping results, but also the acceptance of fewer but standardized traps worldwide, hence supporting the involvement of the private sector.

3.3. Lures, Attractants and Insecticides

Lures, attractants and insecticides are also of major importance for fruit fly AW-IPM programmes. Since the development of some of these products, the agrochemical industry has provided and further developed various formulations of lures such as trimedlure (liquid, silicon-plug type), a Mediterranean fruit fly male attractant, as well as of the fruit fly food attractants such as NuLure used in liquid bait traps, and insecticides such as dichlorvos (2,2-dichlorovinyl dimethyl phosphate or DDVP).

A major breakthrough, complementing the development of genetic sexing strains that allowed the release of only sterile males was the development and validation through an FAO/IAEA coordinated research project of a Mediterranean fruit fly female attractant (Heath et al. 1997, IAEA 1998). This synthetic attractant was tested and validated through this international research network (Epsky et al. 1999, Katsoyannos et al. 1999) and has been commercialized under the name Biolure®. It is now utilized in most Mediterranean fruit fly AW-IPM programmes that use sterile males from a genetic sexing strain (Franz 2005) such as the Arava/Araba project in Israel/Jordan (Cayol et al. 2004).

3.4. Suppression Methods

In many cases, Mediterranean fruit fly AW-IPM programmes require the use of complementary control tactics such as the bait application technique to suppress the original wild population to levels low enough for the sterile males to effectively compete and/or to control major outbreaks. In some programmes that include aerial operations, only the aircraft services are provided by private companies to carry out aerial bait applications; in other

cases companies specialized in aerial pesticide applications provide all components of the suppression package (Roessler 1989).

The increasingly stringent restrictions on the use of organophosphate insecticides such as malathion and the need for environment-friendly control tactics for integration with the SIT (Peck and McQuate 2000) has encouraged the agrochemical industry to develop and commercialize an organically certified product named GF-120® based on spinosad that replaces the traditional fruit fly bait formulation (protein and organophosphate) (Revis et al. 2004). Since its commercialization, this product, which is compatible with an environment-friendly programme, has been registered for fruit fly control in an increasing number of countries notably through the lobbying of users of sterile Mediterranean fruit flies.

3.5. Production and Sterilization

The mass-rearing of sterile male Mediterranean fruit flies that meet internationally agreed quality control standards (FAO/IAEA/USDA 2003) is obviously at the core of Mediterranean fruit fly AW-IPM with an SIT component. Most of the large Mediterranean fruit fly rearing facilities worldwide have been financed and are operated by government or supranational institutions (IDIDAS 2004), although construction and some of the related services, for example the processing of agricultural subproducts to be used as bulk ingredients in the larval diet, the recycling of spent diet, or the disposal of waste, have been outsourced to the private sector. The first attempt to involve the private sector in mass-rearing operations took place in the Programa Moscamed in Guatemala, where the management of the fly production facility in El Pino was outsourced in 1996 to the company Bio-Systems (C. Cáceres, personal communication). Unfortunately, the attempt was not successful and the management of the facility returned once more to the government.

Assessment of the potential needs for supplying sterile male Mediterranean fruit flies in the Mediterranean basin (FAO/IAEA 2000), as well as the development of a model business plan for insect rearing facilities (IAEA 2007), generated renewed interest from the private sector in this field. In 2000, InSecta Ltd was established in the UK. This company offers assessment services for fruit fly control, sterile Mediterranean fruit flies, and integrated pest control programmes for several other insect pests. In 2003, SIT Africa Ltd was established in South Africa to produce and distribute sterile male Mediterranean fruit flies (Vreysen et al. 2006, Barnes, this volume). This company took over an existing small rearing facility that was originally established by the government with IAEA support for the Mediterranean fruit fly suppression programme in the Hex River Valley (Barnes et al. 2004). The facility was handed over to the SIT partnership formed by the Deciduous Fruit Producer's Trust and the Agricultural Research Council and the original capital investment by SIT Africa Ltd was therefore limited. In 2004, Bio-Bee Sde Eliyahu Ltd, the main producer of biological control agents in Israel, decided to construct the first Mediterranean fruit fly mass-rearing facility in the eastern Mediterranean region under the name of Bio-Fly Ltd (see section 4).

Sterilization of male pupae requires investing in the purchase, accreditation and maintenance of a gamma irradiator or, as recently proposed for some programmes, an X-ray machine. Considering the additional costs involved and the regulatory aspects of the accreditation process, programmes such as the Mediterranean fruit fly suppression programme in Valencia, Spain, are outsourcing the irradiation of pupae to specialized private companies (R. Argiles, personal communication). In Israel, in order to limit its initial investment, Bio-Fly Ltd outsourced the irradiation process to the Hadassa Hospital during 2005-2006. However, in view of the negative impact on fly quality of the extra handling of pupae between the rearing facility and the hospital, as well as problems associated with the limited access to the irradiator, Bio-Fly

Ltd acquired its own Gammacell-220® at the end of 2006.

3.6. Shipment

Shipment of Mediterranean fruit fly pupae from the production to the adult emergence sites or release centres is a critical aspect of area-wide control programmes as quality control standards need to be respected to preserve the quality of the sterile insects (FAO/IAEA/USDA 2003, FAO 2007). Most sterile fruit fly transboundary shipments are carried out by air cargo companies as was the case for the weekly provision of sterile male Mediterranean fruit fly pupae from the El Pino facility in Guatemala to the Arava/Araba project during 1997-2005 (Cayol et al. 2004). However, some mass-rearing facilities directly transport the sterile insects to their customers; for example, the El Pino facility uses its own ground and air transport fleet to deliver eggs to the Moscamed mass-rearing and sterilization facility in Mexico and to transport pupae to the various release centres (Cáceres et al. 2007). Since 2005, Bio-Fly Ltd delivers its weekly shipments directly to the various projects and sites that release sterile males in Israel.

3.7. Fly Emergence, Feeding and Packing

The emergence, feeding and packing of sterile male Mediterranean fruit fly adults prior to their release (Fig. 1, upper, right and lower, left) is done at release centres or emergence facilities which must operate strictly in accordance with specific quality control standards (FAO/IAEA/USDA 2003, FAO 2007) to ensure the field efficiency of the released adults. These release centres usually operate independently, though in close collaboration with the mass-rearing operations to avoid a potential conflict of interest between the producer and the recipient. Considering the additional infrastructure and manpower required for adult emergence, some programmes have opted to outsource these operations completely to the private sector, e.g. the Mexican National Fruit Fly Campaign has outsourced the operation of five Mexican fruit fly *Anastrepha ludens* (Loew) and West Indian fruit fly *Anastrepha obliqua* (Macquart) release centres in Mexico (Leal Mubarqui 2005). For some small- to medium-scale programmes, the additional construction and operation costs of a dedicated emergence facility would represent, in the short term, an unbearable financial investment. In Israel, the small-scale programmes launched in 2005 in the Batra and Besor regions (see section 4) outsource the emergence, feeding and packing for ground releases of about three million sterile males weekly to Bio-Fly Ltd. Since its initiation in 1997, the Araba Valley project in Jordan has outsourced all emergence and aerial release operations to the Arava Medfly Eradication Programme (AMEP-Israel) (Cayol et al. 2004).

3.8. Release of Sterile Insects

The release of sterile insects, a major component of SIT application as part of Mediterranean fruit fly AW-IPM, can be carried out from the ground or aerially, and requires release equipment, release vehicles (ground or aircraft), GPS guidance equipment, as well as the services to carry out the release operations (FAO 2007). All or part of these components have often been outsourced to the private sector.

For example the chilled adult release machine originally used for sterile Mediterranean fruit fly release in southern California in 1975-76 was a modified version of a system developed by the United States Department of Agriculture (USDA) for the US-Central America Screwworm Eradication Programme (FAO 2007). Research has focused on various ways to improve release systems and operations (Villaseñor 1985, Vargas et al. 1995, Salvato et al. 2003), or through new designs for the chilled adult release machine using frozen carbon dioxide rather than mechanical refrigeration (Tween 2004, Tween and Rendón, this volume). These efforts have resulted in several different sys-

tems (such as bags, various boxes, and free adult release), as well as designs of machines for chilled adult release being commercially available. Since the release machines are often not patented and need to be adapted to fit the specific type of aircraft used in the different programmes, private companies are often reluctant to market the machines unless they are also awarded the contract for the releases. While some large programmes like the Programa Moscamed lease a dedicated air fleet for releases, some others such as the Los Angeles Basin Preventative Release Programme (Dowell et al. 2000) outsource the entire aerial release operations to private companies that have permanently modified aircrafts to fit the chilled adult release machines. The limited scale of operations of smaller programmes such as Madeira-Med (Dantas et al. 2004) and originally the Arava/Araba programme in Israel/Jordan (Cayol et al. 2004) often does not justify the purchase of an aircraft hence the outsourcing of aerial release operations. In Israel, following a few years of outsourced operations, this proved not to be cost-effective in the long term and AMEP-Israel purchased an already-modified aircraft in 2005. In South Africa's Hex Valley, in view of the small areas involved, releases are no longer carried out by air; instead they are carried out only by ground, concentrating on hot spot and host areas in the surroundings of the grape fields.

4. Establishment of a Commercial Mediterranean Fruit Fly Facility in Israel

The establishment of the first commercial Mediterranean fruit fly production facility in the Middle East was the result of a thorough process in which all the actors, i.e. growers' communities, government institutions, supranational organizations and private sector were consulted. Throughout the process, a bottom-up approach was adopted to quantify the needs, to identify the most suitable private partner, and to build the facility with all the technical support available from the various national and international actors.

4.1. Growing Market for Sterile Mediterranean Fruit Flies in the Middle East: an Historical Perspective

In 1994, a panel of experts evaluated the potential of the SIT as an additional control tactic to suppress and/or eradicate the Mediterranean fruit fly in the Middle East, including countries such as Cyprus, Syria, Lebanon, Israel, Jordan and the Territories Under the Jurisdiction of the Palestinian Authority (TUJPA) (FAO/IAEA 1995). In 1995-1996, an economic evaluation was conducted of three alternative strategies (bait application technique for suppression, SIT for suppression, and SIT for eradication) for the control of the Mediterranean fruit fly in the Middle East. This study concerned Israel, Jordan, the TUJPA (Enkerlin and Mumford 1997), Lebanon and Syria (IAEA 2001). In 1997, the IAEA supported a pilot study to eradicate the Mediterranean fruit fly from the Arava/Araba Valley in Israel/Jordan (Roessler et al. 2000, Cayol et al. 2004) and in 2001, Israel, Jordan and the TUJPA were awarded a USD 2.5 million United States Agency for International Development-Middle East Regional Cooperation (USAID-MERC) grant to control the Mediterranean fruit fly (Cayol et al. 2004). From 1997 to 2005 the project relied on the import of sterile male pupae from the El Pino facility in Guatemala. However, the increasing shipping duration between Guatemala and Israel, notably following additional restrictions after 11th September 2001, resulted in lower sterile to wild male ratios in the Arava/Araba Valley that reduced the effectiveness of the SIT operations.

4.2. Seeking and Convincing a Reliable Commercial Partner

In view of the increased shipment duration from Guatemala in 2001-2002, the increasing loss of shipments, as well as the potential increasing sterile fly demand in the region, the USAID-MERC project management commit-

tee concluded that the only way to ensure a sustainable supply of sterile flies to the projects in the long term was to seek private investment to establish a commercial rearing facility in the region. It was agreed that the private partner should have a good knowledge of the regional limitations and constraints, and preferably with experience in biological control methods.

Bio-Bee Sde Eliyahu Ltd, a company that had mass-produced beneficial arthropods for biological pest control since 1983, and bumblebees *Bombus* spp. for natural pollination since 1991, was soon identified as the most suitable business partner in the region. IAEA, with the financial support of the USAID, provided assistance to Bio-Bee Sde Eliyahu Ltd through the Israeli Plant Protection and Inspection Services to better understand the technology involved, to visit other rearing facilities and operational programmes in Latin America and in Southern Europe, to prepare its own business plan, and finally to design its future rearing facility.

In 2004, the management of Bio-Bee Sde Eliyahu Ltd established Bio-Fly Ltd, a daughter company that aimed at a weekly production of ca 150-200 million sterile male Mediterranean fruit fly pupae in the medium term. From a business perspective, the decision to establish Bio-Fly Ltd was founded on the foreseen shortage of sterile Mediterranean fruit fly pupae in the Mediterranean region due to the expected shift from pesticide-based control methods to integrated area-wide SIT application caused by the ban of organophosphates in the European Union (IAEA 2007).

4.3. Construction and Operation of the Bio-Fly Ltd Rearing Facility: a Success Story in Record Time

The establishment of Bio-Fly Ltd facilities with an initial weekly production capacity of ca 20 million male pupae was completed in record time. While the groundbreaking took place in November 2004, the facility opened in March 2005 and the first sterile flies were delivered to the Arava programme in May 2005. In November 2005, the facility was certified ISO-9001 for the production of Mediterranean fruit fly.

Since 2005, Bio-Fly Ltd has supplied sterile male pupae to the Arava/Araba Mediterranean fruit fly suppression project in Israel/Jordan, as well as ready-for-ground-release sterile male adults to the Bata pilot project (220 hectares of mainly mangos and avocado orchards) next to the Sea of Galilee, and to the Besor pilot project (1000 hectares of citrus) in the Western Negev.

Compared with the other commercial Mediterranean fruit fly rearing facilities, Bio-Fly Ltd remains, at the time of publication, the first facility established in response to a demand from existing operational SIT programmes and which was not originally based on existing facilities.

5. Commercial Mediterranean Fruit Fly Rearing Facilities: Is the Private Sector Here to Stay?

Two decades passed between the development of the concept of the SIT and its first large-scale use against the Mediterranean fruit fly in the 1970s in southern Mexico (Klassen and Curtis 2005). The next two decades experienced an increasing demand for sterile Mediterranean fruit flies but the private sector's interest in mass-rearing operations was only manifested in 2000 with the establishment of the first private company, InSecta Ltd in the UK. In only five years, three private companies decided to invest in this field. Their sustained interest and long-term expansion in the market will largely be influenced by: (1) the plans and capabilities of competing government-funded facilities to supply sterile flies at semi-subsidized prices to meet a growing demand in the Mediterranean Region, (2) an increasing demand for environment-friendly pest control methods due to consumer pressure and more stringent laws regulating pest levels and insecticide residues, (3) the awareness of the end-users and beneficiaries of the availability of a cost-effective SIT technology for integration with other environment-friend-

ly technologies available on the market, (4) national policies promoting and facilitating an area-wide approach to pest control and the transfer of SIT know-how, and (5) to some extent, the licensing of technology and the outsourcing of the management of existing mass-rearing infrastructure from government institutions to the private sector.

5.1. Commercializing Sterile Insects versus Pesticides: a Radically Different Approach

Conventional Mediterranean fruit fly control based on a farm-by-farm approach relies on the use of pesticides that can be purchased directly by growers from agrochemical companies. Bio-Bee Sde Eliyahu Ltd is using the same business approach for the commercialization of beneficial arthropods for biological pest control, which in Israel are often sold to individual growers for use in greenhouses.

The commercialization of sterile Mediterranean fruit fly pupae/adults for use in AW-IPM programmes is a radically different approach. The sterile insect pupae could be partially seen as a "public good" that cannot or will not be sold to individual growers since they will resist paying for the large indirect benefits. Within an AW-IPM approach and considering the dispersal capability of the pest, it would be extremely difficult and not technically sound for the growers to purchase flies individually. Hence a new kind of partnership is needed to get individual growers organized into associations to identify and raise funds for the purchase and use of the sterile insects.

5.2. Is Public Sector Investment in Mass-Rearing Beneficial to the End-Users?

When most large-scale Mediterranean fruit fly control programmes were launched, commercial rearing facilities were absent and the rearing facilities were built using funding from the government for the initial capital investment and often for the recurrent running costs. This situation still prevails in the Western Hemisphere and in southern Europe as demonstrated by the recent establishment of a government-funded rearing facility in Valencia, Spain.

Rearing facilities owned by the public sector have the obvious advantage for the end-user that the pupae can be sold at cost or at a very low subsidized price, which does not reflect the capital cost. Consequently, maintaining the price of the sterilized pupae artificially low through government subsidies will encourage few, if any, private companies to face a governmental competitor which is not obliged to secure a return-on-investment from the price of the final product (for production from Bio-Fly Ltd, the concealed capital cost represents ca 22% of the price per million sterilized pupae). As a result, as long as no commercial facilities are established that can provide sterile male pupae on demand, some small to medium AW-IPM programmes, aiming at controlling the Mediterranean fruit fly in a single valley for example, will never be launched. Thus, in some cases, the current situation perpetuates the use of conventional control methods with their known detrimental environmental impact.

5.3. Would Sterile Insect Availability Stimulate a Wider SIT Application?

There has been a "chicken and egg" situation in relation to the establishment of mass-rearing facilities: should the offer (i.e. sterile male pupae commercially available) be driven by the demand (i.e. needs of operational programmes) or vice-versa (i.e. would the availability of sterile flies encourage a wider initiation of SIT applications)? For some years, this dilemma has delayed private sector investment in mass-rearing operations, leading to radically different business approaches. Some potential investors considered that future customers (i.e. existing/future operational programmes) must commit financial resources prior to the establishment of the commercial rearing facility, thus limiting the original risk from the investor's side. The major difficulty with this approach is that the

future customers would rightly request to assess the capability of the company to produce Mediterranean fruit fly pupae in line with the international quality control standards (FAO/IAEA/USDA 2003) prior to commit financial resources. Other investors accepted that the facilities should first be established, preferably in the vicinity of existing operational programmes, and that, in the medium term, the demonstrated capability of the new company to produce pupae in line with quality control standards and its capacity to adjust to a growing demand would suffice to generate the establishment of new or expanded operational field programmes in the region concerned.

The trend towards increased application of the SIT as part of a suppression strategy (Hendrichs et al. 1995), which entails the continuing release of sterile insects and thus a permanent demand for sterile insects, has reduced the investment risk that has been inherent in eradication programmes. This changed framework is starting to tip the balance towards encouraging, to a certain degree, the commercialization of the mass-production of sterile insects.

5.4. Can Commercial Rearing Facilities Withstand Competition from the Public Sector?

Large government-funded rearing facilities such as El Pino in Guatemala, which export their production at low cost across continents, represent a major threat or deterrent to private investment into sterile insect production. For obvious reasons, if a subsidized mass-rearing facility were to be established by the public sector in the Middle East, the current customers of Bio-Fly Ltd would preferably purchase a cheaper product of equal quality rather than a relatively expensive product, a situation that would eventually destroy the original investment of Bio-Fly Ltd Should the private sector be here to stay in commercial sterile Mediterranean fruit fly production, a fair market competition would need to be established by including the capital cost in the price of sterile pupae sold by government-funded facilities outside their domestic SIT-based programme.

5.5. Sustainability of Commercial Rearing Facilities: the Need for Market Diversification

In order to generate the required return-on-investment to ensure their long-term sustainability, commercial rearing facilities need to diversify their market. This can be done by providing services other than only producing sterile pupae. In that respect and as mentioned earlier, Bio-Fly Ltd is providing ready-for-ground-release sterile male adults, vertically integrating the product with services, and saving the customers the need to establish an adult emergence facility. Commercial rearing facilities should build-in the capacity to provide general expertise in the field of Mediterranean fruit fly AW-IPM. This approach was already adopted by the fruit and vegetable industries in some countries where exporters support the farmers with technical advice and transfer of more efficient technologies to increase quality and yields of the products that they buy and commercialize.

Targeting other potential customers in the region is another option. Bio-Fly Ltd is ideally located in the Mediterranean basin to be able to supply sterile flies and services to Mediterranean fruit fly AW-IPM programmes in southern Europe and in northern Africa. In order to allow the business of the SIT to operate confidently in a wide range of markets, it is imperative to have harmonized international regulations for trade and transport of sterile insects as well as quality (FAO/IAEA/USDA 2003, FAO 2005).

A diversification of the pest species that are mass-reared can result from some investment in research and development to develop new products. For example, it is obvious that the first commercial company to master the mass-rearing of the olive fruit fly *Bactrocera oleae* (Gmelin), a major pest in the Mediterranean basin, will generate maximum profits. Bio-Fly Ltd is also considering pro-

viding research and development services to the Israeli Plant Protection and Inspection Services on exotic fruit fly pests which could in the near future threaten the national fruit and vegetable industries, such as the polyphagous peach fruit fly *Bactrocera zonata* (Saunders) and the Asian fruit fly *Bactrocera invadens* Drew, Tsuruta, and White, recently introduced into sub-Saharan Africa and rapidly expanding its geographical distribution (Lux et al. 2003, Mwatawala et al. 2004, Drew et al. 2005).

6. Conclusions

During the past five decades, the private sector has invested in nearly all components of the Mediterranean fruit fly SIT package. However, the development of commercial mass-rearing facilities remains the major cornerstone for larger privatized SIT operations and for wider SIT use by small to medium AW-IPM programmes. This requires a gradual change in the mindset of potential AW-IPM programme managers and government institutions to first investigate the availability or establishment of commercial suppliers before investing in a new public sector infrastructure. In turn, commercial companies need to adjust their business strategy to better address local/subregional/national representatives of the fruit and vegetable industries rather than the individual grower. It will also depend on government implementing policies that support the commercialization of SIT.

In the long term, it is in the interest of all the main actors in the fruit and vegetable industries, as well as consumers and local communities, to support efforts by the private sector to commercialize the SIT package through ensuring fair competition and a sustainable market for its products.

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Section 6

Pilot Programmes

Assessment of the Sterile Insect Technique to Manage Red Palm Weevil *Rhynchophorus ferrugineus* in Coconut

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ABSTRACT The red palm weevil *Rhynchophorus ferrugineus* Olivier is one of the most destructive internal borers of coconut palms in India, Sri Lanka, Indonesia and the Philippines. Recently, this pest has spread to countries in the Persian Gulf and some areas of the Mediterranean basin where it is a serious menace to date palms. Management of this pest using conventional methods is not effective due to the difficulty of detecting early infestations. Trials were therefore conducted to assess the possibility of eventually including the sterile insect technique (SIT) to target populations at low densities as part of future integrated management of this pest. The steps taken included the adoption of a new mass-rearing method for weevils using coconut petioles, determination of the sterilization dose at 15 Gy, a new relative method for estimating population levels of red palm weevils, and finally field release and recapture studies using pheromone traps. This paper deals with the results of these attempts to develop the SIT for use against the red palm weevil on Poothuruth Island near Dalavapuram Island in Kerala.

KEY WORDS red palm weevil, *Rhynchophorus ferrugineus*, integrated management, sterile insect technique, coconut palm, date palm, release-recapture studies, Kerala

1. Introduction

The red palm weevil *Rhynchophorus ferrugineus* Olivier is one of the most destructive pests of palms and particularly of coconut in the world (Ghosh 1911, Brand 1917, Leefmans 1920, Viado and Bigornia 1949). The pest invades palms by making holes on the palm, tunnels inside and feeds on the inner contents. Since it remains inside the palms, the detection of an infestation is very difficult, and the inevitable delay in detection of weevil attack leads to permanent debility of the palms. It has therefore become important to develop alternative and economic methods of pest management to control this pest. Consideration was given to using the sterile insect technique (SIT) for eventual inclusion into an area-wide integrated pest management (AW-IPM) approach to counteract attacks of the red palm weevil.

The entire study was conducted in two phases: (1) through initial laboratory studies to determine the optimal dose of radiation for sterilizing insects, and (2) trial releases of sterile male weevils in a coconut garden to ascertain the effectiveness of the method in the field. The laboratory studies were conducted at the Department of Entomology, College of Agriculture, Vellayani, Kerala, while the field trials took place on Poothuruth Island in Dalavapuram, Kollam District, Kerala, India.

2. Mass-Rearing of Red Palm Weevil

Substantial numbers of adult weevils need to be reared to develop and apply the SIT, and mass-rearing of red palm weevils requires specific techniques due to the internal feeding habit of the pest and its long life cycle.

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Cannibalism is another major obstacle in obtaining the required numbers of insects for sterilization. The insect is routinely reared in the laboratory on coconut petioles or on the stems of coconut, sugarcane (Rahalkar et al. 1972) and other palms (Rananavare et al. 1975). The insect can also be successfully reared on an artificial diet (Rahalkar et al. 1978). However, in all these cases, the percentage of adults recovered from eggs is very low, and both the mechanical injury and the cost involved are high. Hence, these methods were not suitable for the rearing component of the SIT trial. Steps were therefore taken to develop suitable culturing and rearing techniques for *R. ferrugineus*, and the individual rearing method in cells proved satisfactory for mass-rearing (Krishnakumar and Maheswari 2003).

3. Laboratory Studies

Male red palm weevils were irradiated immediately after their emergence from cocoons, since their sperm remains immature and vulnerable to dominant lethal mutations when exposed to gamma radiation (Ramachandran 1998). Irradiation was carried out in a gamma radiation chamber (model 900) with a capacity of one litre and at a dose rate of 1 Gy/16 seconds, which was ascertained by Fricke dosimetry. Ten adult weevils were each placed in a plastic container and irradiated at doses of 15, 16, 17, 18 and 20 Gy. Further studies revealed that 15 Gy was the optimal sterilizing dose (Krishnakumar and Maheswari 2004).

4. Population Estimation Model for Red Palm Weevil

4.1. Survey of Red Palm Weevil-Infested Coconut Plantations

A survey was conducted during 2000-2001 in red palm weevil-infested coconut plantations in four selected districts of Kerala, i.e. Thiruvananthapuram, Kollam, Kottayam and Alappuzha. This involved both interviews with farmers and direct observation. The sur-

Table 1. *The number of red palm weevil individuals of each life stage present in three types of infested palms (n = 25 for each type) that were dissected from different red palm weevil infested-coconut plantations of Kerala during 2000-2001.*

Type of infestation	Development stage										
	I Instar	II Instar	III Instar	IV Instar	V Instar	VI Instar	VII Instar	VIII Instar	IX Instar	Prepupae and pupae	Adults
Crown infestation	2.22 (1.79)[1]	1.09 (1.45)	9.96 (3.31)	1.78 (1.67)	1.72 (1.65)	3.93 (2.22)	14.29 (3.91)	14.29 (3.91)	19.97 (4.58)	29.91 (5.56)	2.96 (1.99)
Stem infestation	0.72 (1.31)	2.50 (1.88)	2.57 (1.89)	0.56 (1.25)	6.73 (2.78)	9.76 (3.28)	13.83 (3.85)	12.10 (3.62)	19.34 (4.51)	19.97 (4.58)	3.75 (2.18)
Bole infestation	4.48 (2.34)	4.36 (2.32)	3.75 (2.18)	1.28 (1.51)	8.98 (3.16)	10.56 (3.40)	12.54 (3.68)	26.56 (5.25)	9.96 (3.31)	12.91 (3.73)	6.18 (2.68)
Critical difference	0.55	0.68	0.55	0.39	0.69	0.65	0.63	0.91	1.05	0.43	0.28

[1]The figures in brackets indicate the √x +1 transformed value

Table 2. *The estimated number of red palm weevils for each of the different life stages in Poothuruth Island near Dalavapuram, Ashtamudi Lake in the Kollam district of Kerala, India.*

Type of infestation	Number of palms infested	Estimated number of weevils for each of the different life stages									
		Adult	Prepupae and pupae	IX Instar	VIII Instar	VII Instar	VI Instar	V Instar	IV Instar	III Instar	II Instar
Crown infestation	6	18	180	120	84	84	24	12	12	60	6
Stem infestation	5	20	100	100	60	56	40	42	5	12	12
Bole infestation	5	30	65	50	135	52	55	54	10	20	25
Total	16	68	345	270	279	192	119	108	27	92	43

vey revealed the presence of three different types of infestation by red palm weevil, namely: (1) crown infestation, (2) stem infestation, and (3) bole infestation. The crown-infested palms showed specific signs of attack by red palm weevil like yellowing or browning of their inner leaves, holes with chewed-up fibres and toppling of crowns. However, in these palms, there were no signs of attack on the trunk or in the bole. In the case of stem and bole infestations, holes with oozing of red fluids were seen only on the trunk and bole respectively.

To count the number of life stages present in the three types of infested palms, 25 plants from each type were dissected from different regions of the surveyed plantations. Mean values for the number of different life stages present in the infested palms were determined, rounded to a whole number and kept as ratings for the number of specific life stages of the weevil. The average values recorded are shown in Table 1.

4.2. Estimation of Populations of Red Palm Weevil

The red palm weevil is capable of flying about 900 metres in one flight (Kalshoven 1951). For the sterile insect release trial, an area with natural boundaries of at least one kilometre was needed to prevent migration of weevils from released spots to the surrounding areas and vice versa. For this experiment, an island named "Poothuruth Island" near Dalavapuram, was selected. This island is separated by three kilometres from the mainland in all directions. It is a typical island on the Ashtamudi Lake in the Kollam district of Kerala, having an area of two hectares and a total of 460 palms. A field survey of the entire area of the island showed that 16 palms were infested with red palm weevil, of which six were crown infested and five each were stem and bole infested.

The estimated number of different life stages of red palm weevil in the island was calculated by multiplying the number of palms infested by the rating corresponding to that life stage (Table 2).

5. Release of Sterile Insects

For the sterile insect release trial, the number of adults and the possible number of weevils to emerge from pupae were taken into account. At the beginning of the releases the total numbers of adults together with the num-

Table 3. *Number of sterile male red palm weevils (first generation) released based upon the estimated population density of wild weevils in Poothuruth Island near Dalavapuram, Ashtamudi Lake in the Kollam district of Kerala.*

Number of release	Estimated wild weevil population			Number of sterile males released
	Total	Females	Males	
1st	68+345= 413	206.5	206.5	2065
2nd	270	135.0	135.0	1350
3rd	279	139.5	139.5	1395
4th	192	96.0	96.0	960
5th	119	59.5	59.5	595
6th	108+27 = 135	67.5	67.5	675
7th	92+43 = 135	67.5	67.5	675
Total	1543	771.5	771.5	7715

ber estimated to emerge from pupae were found to be 413. Since the sex ratio in red palm weevil is 1:1 (Ramachandran 1998), the possible number of males at the initial stage of release was assumed to be 206. Since earlier studies had shown that the optimal sterile to wild male ratio was 10:1 (Maheswari et al. 2003), 2065 sterile red palm weevil males were initially released.

The prepupal and pupal periods of the red palm weevil are respectively 13 ± 1.5 and 17.5 ± 1.0 days and adult weevils attain sexual maturity as soon as they emerge from cocoons. Since the total life cycle of the insect is 92-127 days, an average of 15 days was selected as the release interval. In this time interval, each individual will have developed into the next larval instar. This will maximize the possibility that newly emerged females would mate with released sterile males. Sterile male weevils were released in the field after making a 'V'-shaped notch in the right elytra for identification. Table 3 shows the numbers of sterile males released in the first generation.

6. Trapping of Weevils

Before releasing the sterile insects, five pheromone traps, baited with the aggregation pheromone Ferrolure, were set in the field randomly. After release, female weevils were collected in the traps along with the different types of males (i.e. wild and sterilized males).

Table 4. *Average number of female red palm weevils captured per trap together with native, sterilized males, or both during each 20-day period after release.*

Females trapped with	Days after release						
	0-20	21-40	41-60	61-80	81-100	101-120	121-140
Native males	1.8	1.4	0.2	0.2	0.0	0.0	0.0
Sterilized males	0.0	0.8	1.4	1.6	3.6	2.4	2.0
Native and sterilized males	4.2	4.8	2.4	2.2	1.4	1.0	0.6
Total	6.0	7.0	4.0	4.0	5.0	3.4	2.6

Table 5a. *Number of eggs oviposited by native female palm weevils, before and after the release of sterile insects in each 20-day period after release.*

Females trapped	Number of eggs oviposited during indicated periods (days) after release						
	0-20	21-40	41-60	61-80	81-100	101-120	121-140
Before release of sterile males	176.9	161.3	128.8	102.3	121	154	121
With native males	162.1	159.7	131.4	104.9	---	---	---
With sterilized males	---	161.5	129.6	103.3	116	96	102
With native and sterilized males	158.6	161.1	129.8	105.8	149	122	112
Critical difference	---	2.1	1.8	3.5	---	---	---

Table 5b. *The rate of sterility induced in the native female palm weevil population (as indicated by the percentage egg hatch) before and after the release of sterile insects in each 20-day period after release.*

Females trapped	Percentage egg hatch during indicated periods (days) after release						
	0-20	21-40	41-60	61-80	81-100	101-120	121-140
Before release of sterile males	78.5	74.6	71.4	73.4	78.8	79.1	65.0
With native males	76.2	70.9	68.3	68.4	---	---	---
With sterilized males	---	26.4	18.9	2.1	---	---	---
With native and sterilized males	61.3	45.3	39.7	34.5	39.6	35.2	32.5
Critical difference	3.1	3.9	3.7	5.4	---	---	---

Insects were observed on alternate days and the data were pooled at 20-day intervals for assessment. Sterile insects collected in the traps were re-released while wild males were killed and wild females were brought to the laboratory for studies on egg viability. Table 4 shows the number of female red palm weevils that were trapped with different categories of males after the first generation release of sterile males. Observations at 20 days after release indicated that out of 30 female weevils trapped, 21 were trapped with both types of

Table 6. *Estimated number of female red palm weevil present on the island as indicated by mating status (with native or sterile males) on indicated days after release of sterile males.*

Females mated with	Estimated number of females on indicated days after release of sterile males						
	0-20	21-40	41-60	61-80	81-100	101-120	121-140
Native males	61.8	27.0	7.0	4.8	---	---	---
Sterilized males	---	15.4	48.8	38.4	42.8	47.6	51.9
Native males and sterilized males	144.5	92.6	83.7	52.8	32.7	17.5	15.6

Table 7. *Age specific life table and reproductive rate[1] of red palm weevil (values are derived from ten pairs of adults).*

X	lx	dxf	dx	100 qx	Sx	Surviving number
Expected eggs	1852	Mortality	296	15.98	0.84	1556[2]
I instar	1556	Cannibalism	793	50.96	0.49	763
II instar	763	Cannibalism	212	27.78	0.72	551
III instar	551	Cannibalism	131	23.59	0.78	430
IV instar	430	Cannibalism	114	26.51	0.72	306
V instar	306	Cannibalism	97	31.69	0.68	209
VI instar	209	Cannibalism	82	39.23	0.60	127
VII instar	127	Cannibalism	11	8.66	0.91	116
VIII instar	116	Cannibalism	5	4.31	0.95	111
IX instar	111	Cannibalism	3	2.70	0.97	108
Prepupae	108	Fungus and virus	7	15.42	84.58	91
Pupae	91	Fungus and virus	8	8.80	81.20	75
Adult	75	Fungus and virus	---	---	1.00	75

[1]Ten pairs produce a progeny of 75, one pair produces 7.5 progeny (multiplication rate can be rounded to 8)
[2]Viable eggs

male weevils, nine were trapped with normal males alone and there were no females trapped with sterilized males alone. As the study progressed, with increasing total numbers of released sterile insects in the field, the proportion of females trapped with normal males decreased while the numbers trapped with sterilized males increased steadily.

7. Egg Viability of Females Trapped with Different Categories of Males

The female weevils trapped were brought to the laboratory and kept separately in screw-capped glass bottles to assess the fecundity and viability of eggs. Pooled data from seven observations were considered as seven replicates and the data were subjected to statistical analysis. Table 5 shows the percentage egg viability from females trapped with different categories of males. The results indicated that up to 100 days after release, the viability of eggs oviposited by the weevils trapped with wild males alone was similar to that with normal males before field release. At 100 days after release, the viability of eggs oviposited by females trapped with sterilized males alone was nil. However, those trapped with both categories of males had nearly a 50% egg hatch. From this study, it can be inferred that females trapped with native males possibly only mated with native males, those trapped with sterilized males mated only with sterilized males, and females trapped with both categories may have mated with both type of males.

From these results, the proportion of females in the whole population that mated with a particular type of male could be derived from:

> *Number of females of the whole population mated with a particular type of male insect of the whole population = (Number of females trapped with particular type of male / Total number of females trapped) X (Total female population at the time of release)*

Using this formula, the different proportions of female weevils on Poothuruth Island that mated with different categories of males were calculated. The results are shown in

Table 8. *Population development as revealed by trap catches of female palm weevils after seven release sessions (first generation release).*

Females mated with	Trap catches on day 100 after the release of sterile males						
	1st release	2nd release	3rd release	4th release	5th release	6th release	7th release
Native males (x 8)	494	216	56	38	---	---	---
Sterilized males (x 0)	---	---	---	---	---	---	---
Native and sterilized males (x 4)	578	370	335	211	130	70	62
Total	578	370	335	211	130	70	62

Table 6.

8. Assessment of Reproductive Rate

Every insect has a particular innate capacity to multiply. The number of progeny produced by a particular insect species depends on its reproductive rate. To determine the reproductive rate of the red palm weevil, a laboratory study was conducted involving ten pairs of freshly emerged adult beetles. Each pair was kept in a screw-capped glass bottle and scrapings of coconut petiole provided as an egg laying medium. The number of eggs oviposited each day was counted and the total number of eggs oviposited by all ten pairs recorded. The eggs were kept inside moistened filter paper on petri dishes, hatched and their mortality recorded. The hatched grubs were transferred to fresh coconut petioles (10 x 5 centimetres) at a density of 20 grubs per petiole by making small holes on the petiole using a sharp-pointed auger. This was carried out to create artificially the field conditions of cannibalism. Feed changes were carried out each three days. The number of larvae surviving at each feed change and subsequently between each instar was also observed. Based on these studies, the reproductive rate of weevils was calculated using the indices according to Morris and Miller (1954):

Table 9. *Number of sterile male red palm weevils (second generation) released based upon the estimated population density of wild weevils in Poothuruth Island near Dalavapuram, Ashtamudi Lake in the Kollam district of Kerala.*

Number of release	Estimated wild weevil population			Number of sterile males released
	Total	Females	Males	
8th	1072.4	536.2	536.2	5362
9th	586.4	293.2	293.2	2932
10th	390.8	195.4	195.4	1954
11th	249.6	124.8	124.8	1248
12th	130.8	150.4	150.4	1504
13th	70.0	35.0	35.0	350
14th	62.4	31.2	31.2	312
Total	2562.4	1366.2	1366.2	13 662

Table 10. *Average number of female red palm weevils captured per trap together with native, sterilized males, or both during each 20-day period after release (second generation release).*

Females trapped with	Days after release						
	0-20	21-40	41-60	61-80	81-100	101-120	121-140
Native males	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sterilized males	5.2	4.4	4.8	3.0	4.0	0.8	0.6
Native and sterilized males	0.8	0.6	0.2	0.2	0.2	0.4	0.4
Total	6.0	5.0	5.0	3.2	4.2	1.2	1.0

x = the age interval; lx = the number surviving at the beginning of the stage noted in the x column; dx = the number dying within the age interval stated in the x column; dxf = mortality factor; 100 qx = percentage mortality; and Sx = survival rate within the stage mentioned in the x column

The age-specific life table of red palm weevil is presented in Table 7. From ten pairs of adult weevils, a progeny of 75 was produced. Therefore, one pair of weevils was able to produce 7.5 weevils and hence the reproductive rate can be fixed as eight. Since the sex ratio is 1:1, it can be inferred that one pair of weevils can produce a progeny of four males and four females.

9. Expected Population Growth in the Next Generation

Expected population growth in the next generation was calculated by multplying the number of females by the reproductive rate. The

Table 11a. *Number of eggs oviposited by native female palm weevils, before and after the release of sterile insects in each 20-day period after release (second generation release).*

Females trapped	Number of eggs oviposited during indicated periods (days) after release					
	0-20	21-40	41-60	61-80	81-100	101-120
With native males	0.0	0.0	---	---	---	---
With sterilized males	156.2	151.5	148.7	156.3	124.0	129.3
With native and sterilized males	161.6	139.4	137.7	---	---	---

Table 11b. *The rate of sterility induced in the native female palm weevil population (as indicated by the percentage egg hatch) before and after the release of sterile insects in each 20-day period after release (second generation release).*

Females trapped	Percentage egg hatch during indicated periods (days) after release					
	0-20	21-40	41-60	61-80	81-100	101-120
With native males	---	---	---	---	---	---
With sterilized males	---	---	---	---	---	---
With native and sterilized males	38.9	37.5	32.4	---	---	---

Table 12. *Estimated number of female red palm weevils present on the island as indicated by mating status (with native or sterile males) on indicated days after release of sterile males (second generation release).*

Females mated with	Estimated number of females on indicated days after release of sterile males						
	0-20	21-40	41-60	61-80	81-100	101-120	121-140
Native males	---	---	---	---	---	---	---
Sterilized males	464.7	258.0	187.6	117.0	143.2	23.3	18.7
Native males and sterilized males	71.5	35.2	7.8	7.8	7.2	11.7	12.5

reproductive rate was eight for the females mated with wild males alone, but it was zero for females mated with sterilized males alone because the hatching percentage became nil after 100 days. For females mated with both types of males it was four, because the hatching percentage was 50% in females mated with both types of males.

The expected population growth on the island in the second generation is shown in Table 8. Based on these assessments, releases of sterile insects were carried out for the subsequent generations and the observations were repeated as in the case of first generation release. The results of release-recapture studies on sterile red palm weevils on the island during second and third generations are shown in Tables 9-14.

10. Conclusions

Using the new relative method of population estimation and lifespan assessment discussed above to guide the release of sterile insects, led to a reduced red palm weevil population. This new method has definite advantages over the old mark-release method. In the mark-release method, the population of adult weevils could only be assessed, and the method does not facilitate the assessment of the exact time interval between two releases; also, it is impossible to calculate the number of insects to be used for further releases.

The progress of the release programme could be more accurately predicted with the new method, whereas only egg viability assessment is possible by the existing meth-

Table 13. *Population development as revealed by trap catches of female palm weevils after seven release sessions (second generation release).*

Females mated with	Trap catches on day 100 after the release of sterile males						
	8th release	9th release	10th release	11th release	12th release	13th release	14th release
Native males (x 8)	---	---	---	---	---	---	---
Sterilized males (x 0)	---	---	---	---	---	---	---
Native and sterilized males (x 4)	286	35.2	7.82	---	---	---	---
Total	286	35.2	7.82	---	---	---	---

Table 14. *Number of sterile male red palm weevils (third generation) released based upon the estimated population density of wild weevils in Poothuruth Island near Dalavapuram, Ashtamudi Lake in the Kollam district of Kerala.*

Number of release	Estimated wild weevil population			Number of sterile males released
	Total	Females	Males	
15th	286.0	143.0	143.0	1430
16th	35.2	17.6	17.6	176
17th	7.8	3.9	3.9	39
Total	329.0	164.5	164.5	1645

ods. The number of generations of releases required in an infested area can also be estimated in the relative method. The release and recapture studies are continuing on the island, enabling further validation and subsequent standardization of the results.

In this experimental case, only the SIT was used against the red palm weevil. Nevertheless, in view of the cost of mass-rearing weevils, it most likely will be a component of an AW-IPM strategy. When the weevil population is low, the SIT can be an effective method of management of the pest. However, with higher weevil populations, suppression methods of pest management such as pheromone traps and chemical control measures should be carried out to reduce pest population before initiating SIT releases.

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Area-Wide Suppression of Invasive Fire Ant *Solenopsis* spp. Populations

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ABSTRACT The fire ants *Solenopsis invicta* Buren and *Solenopsis richteri* Forel were inadvertently introduced into the USA early in the 1900s and currently inhabit over 150 million hectares in Puerto Rico and twelve southern states from Texas to Virginia. Imported fire ants have also become established in isolated sites in Arizona, California, Maryland, and New Mexico. The large numbers and potent sting of fire ants have resulted in significant medical, agricultural, and environmental impacts. The population densities in the USA are five to ten times higher than in South America, most likely due to their escape from natural enemies. Recently, biological control agents have become available in the USA, e.g. *Pseudacteon* spp. decapitating fly parasitoids and a microsporidian pathogen *Thelohania solenopsae* Knell, Allen & Hazard, setting the stage for integrated fire ant management. An area-wide fire ant management project proposal was funded by United States Department of Agriculture-Agricultural Research Services headquarters to demonstrate control of fire ant populations over large areas using commercially available insecticide baits and self-sustaining biological control agents. Untreated, bait control and integrated pest management (IPM) demonstration sites (120 hectares and periphery) were set up in each of five states (Florida, Mississippi, Oklahoma, South Carolina and Texas). The control and IPM sites both had bait (hydramethylnon and methoprene) applications, but only the IPM site had biological control agents released around the periphery. After 3.5 years, decapitating fly parasites have been established at the demonstration sites. The microsporidian pathogen is established in all sites except Mississippi. Fire ant populations have been reduced by 85-99% in the IPM demonstration sites as compared to untreated areas of the same sites. Environmental assessment has demonstrated that bait toxicants do affect non-target ant species but do not affect the arthropod species richness. Educational outreach activities resulted in informative brochures, the establishment of a programme web site, videos, and general information on fire ants. The research component has responded with additional biological control organisms and better pathogen detection methods.

KEY WORDS fire ants, *Solenopsis invicta*, *Solenopsis richteri*, baits, *Pseudacteon* spp., *Thelohania solenopsae*, area-wide, integrated pest management

1. Introduction

The fire ants *Solenopsis invicta* Buren and *Solenopsis richteri* Forel were inadvertently introduced into the USA in the early 1900s and currently inhabit over 150 million hectares in Puerto Rico and twelve southern states from Texas to Virginia. Imported fire ants have also become established in isolated sites in Arizona, California, Maryland, and New Mexico (Fig. 1) (Callcott and Collins 1996, CFR 2001). They have recently widened their invasive character through accidental importation and establishment in Australia, China, Hong Kong, and Taiwan.

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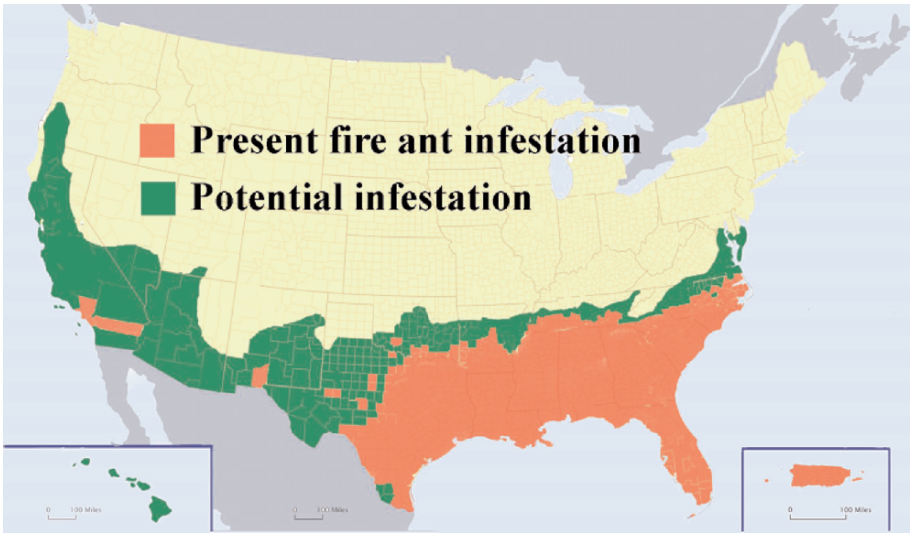

Figure 1. *Present fire ant infestation in the USA and potential infestation based on an ecological model using temperature as limiting factor for fire ant development.*

1.1. Economic Impact

Fire ant colonies (single queen), can contain up to 250 000 workers and reach infestation rates of over 130 mounds per hectare (Tschinkel 1988). More recently, multiple-queen colonies have proliferated in the southern states with even greater nest and population densities (Macom and Porter 1996). The fire ants' large numbers, resource requirements, aggressive behaviour, and potent sting have resulted in many negative interactions with humans and the ecosystem. The fire ant is an opportunistic omnivore that affects agriculture in many ways. The ant damages germinating seeds and root systems of row crops (Shatters and Vander Meer 2000), negatively affects citrus (Adams 1986), and causes USD 67 million in annual losses to cattle producers in Texas alone (Barr and Drees 1996). More than 30% of the human population in infested areas is stung each year and because venom hypersensitivity occurs in 1% of the population, hundreds of thousands of people may require medical attention each year (deShazo et al. 1999). To date around 80 people have died in the USA due to fire ant stings. This highly aggressive ant reduces populations of native ants, other insects, and destroys many small animals including endangered species (Porter and Savignano 1990). The economic impact of fire ants to the US economy is estimated at more than USD 5500 million annually in damage, medical treatments, and control (Fig. 2) (Pereira et al. 2002).

1.2. Chemical Control

Several mound drenches have been developed for fire ant control, but are impractical on a large scale. The most effective and environmentally safe method of control is the use of toxic baits. Toxic bait development by the chemical industry has primarily focused on the lucrative urban/homeowner market, thus few companies have pursued registration of baits for use in agricultural settings. Even when available, toxic baits are expensive and their non-specific nature adversely impacts non-target native ant species, as well as the environment. Chemical treatment strategies alone are not a viable option for large tracts of land (pastures).

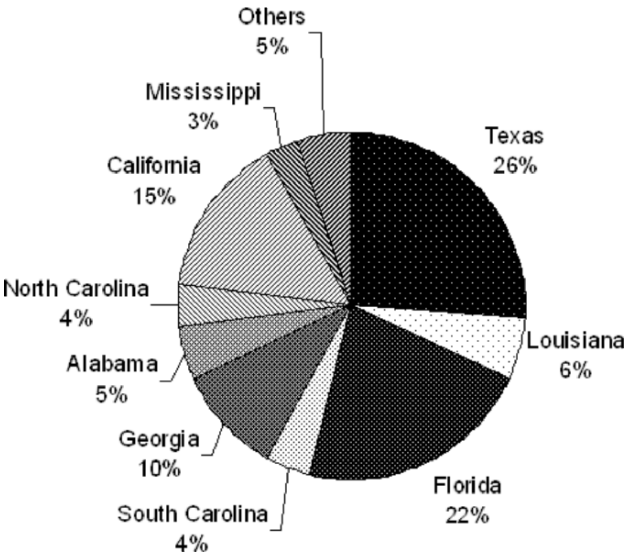

Figure 2. *Pie chart showing the percentage of annual fire ant economic impact on the US economy by state. Total impact is estimated at more than USD 5500 million per year for cost of treatment, damage to property, medical treatments, and all other costs.*

1.3. Biological Control

Fire ant densities in the USA are five to ten times those normally found in their native South American homeland. Thus, it appears that fire ants in the USA have escaped the effects of numerous natural enemies that were left behind in South America (Porter et al. 1992, Porter et al. 1997). Over the past two decades the United States Department of Agriculture (USDA) has actively pursued the identification, importation, and release of selected biological control agents (Williams et al. 2003), such as microsporidian pathogens and decapitating *Pseudacteon* spp. flies (Porter et al. 2004, Vazquez et al. 2006). These biological control agents are important stressors to fire ant populations and can impact them over wide areas. *Pseudacteon* spp. flies are very host-specific (Porter and Gilbert 2004), completing their development within the fire ant heads and causing them to fall off. The flies cause direct mortality, but most important may be the reduction of foraging and consequent weakening of fire ant colonies (Mehdiabadi and Gilbert 2002). Microsporidian pathogens *Thelohania solenopsae* Knell, Allen & Hazard and *Varimorpha invictae* Jouvenaz & Ellis are also very host-specific. They cause chronic diseases in workers and reproductives. *T. solenopsae* causes slow colony death (Williams et al. 1999), and infected ants are more susceptible to the insecticide hydramethylnon (Valles and Pereira 2003).

2. Area-Wide Suppression of Fire Ants

With the availability of self-sustaining biological control agents and effective toxic baits, the Fire Ant Unit applied for and received competitive funding for development of an integrated pest management (IPM) system for area-wide suppression of fire ants through a USDA-

Agricultural Research Service (ARS) headquarters-funded programme entitled "Crop Protection and Quarantine". The objective of this programme is to promote the integration of biological, genetic, cultural, physical, and chemical control technologies into effective, economical, and sustainable IPM systems and area-wide suppression programmes so that they can be transferred to customers as effective management programmes to insect and mite problems.

2.1. Goal and Potential Benefits

The overall goal of the project is to maintain low fire ant populations with reduced need for bait toxicants by using available self-sustaining fire ant biological control agents in conjunction with bait toxicants (Pereira 2004). Anticipated benefits include: (1) demonstration of practical and long-term area-wide control of fire ant populations, (2) quantification of regional differences in treatment frequency to maintain fire ant control, (3) development of decision-making tools for efficient timing of bait toxicant treatments, (4) reduced pesticide risk, (5) sustained fire ant population reduction, (6) increased farm worker safety, (7) lower livestock production costs, (8) establishment of ongoing partnerships with land managers that foster even broader area-wide fire ant management in the future, (9) transfer of area-wide management technology to state agencies and to federal, state, and private land managers, (10) providing a model area-wide fire ant management programme that can be utilized beyond pastures and the cattle industry, (11) restoring the ecological balance toward the native fauna, and (12) providing a better understanding of the economics of fire ants in the cattle industry. To determine if any of these potential benefits are being realized requires measurement of fire ant populations, monitoring the environmental impact of fire ants and the effects of their control on native ant fauna, developing educational materials on fire ant control options, and assessing the economic impact associated with fire ants and the benefits of fire ant control.

2.2. Programme Management Plan

The area-wide project for fire ant population suppression in pastures is coordinated by a management team composed of scientists from the USDA-ARS, the USDA-Animal Plant Health Inspection Service (APHIS) (Gulfport Plant Protection Station, Gulfport, MS), land grant universities, and state agencies. USDA-ARS scientists provide the central leadership in directing major activities associated with executing the project in the five diverse locations chosen for this demonstration. State members of the management team are charged with the development of the within-state infrastructure needed to carry out the complex pre- and post-treatment assessments required for evaluation of project success.

2.3. Demonstration Site Methodology

Demonstration sites were established in pastures in Florida, Mississippi, Oklahoma, South Carolina, and Texas. All sites were established in improved pastures with multiple queen fire ant populations (*S. invicta*), except in Mississippi where single queen black (*S. richteri*) and hybrid (Vander Meer et al. 1985) fire ant populations were present. Each demonstration site consisted of a central area of between 60-120 hectares where fire ant populations were controlled with the bait combination mentioned below, and a surrounding area that received no bait treatment. The surrounding areas served as negative controls and as the sites for establishment of biological control agents. Thus, two types of sites were established in each state: (1) the IPM site where biological control agents were established and the bait combination was used (Fig. 3), and (2) the control site where only the bait combination was used.

At each of the sites, sampling plots were marked both in the bait-treated area and in the surrounding untreated area. These plots consisted of 500 square metre circular areas, with the centre permanently marked on the soil and geo-referenced using a global positioning sys-

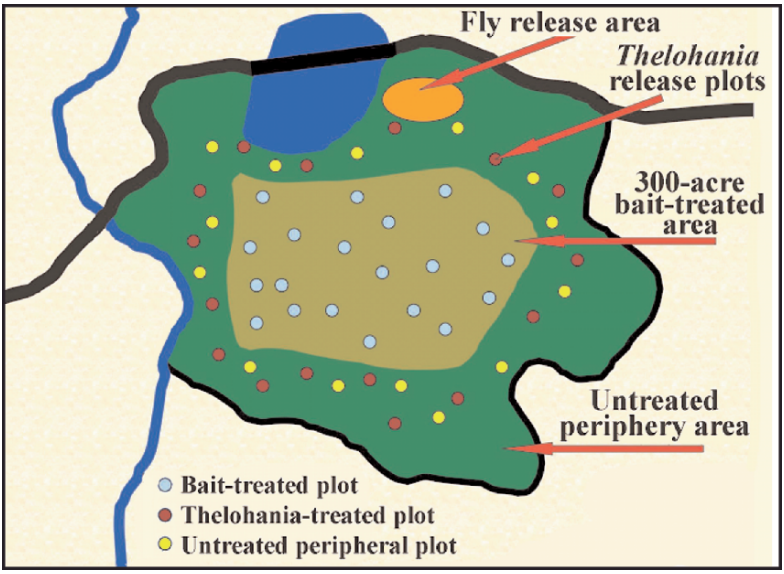

Figure 3. *Schematic drawing (not to scale) of IPM demonstration site with its different components. All bait applications were done by aircraft.*

tem. Several measurements were taken in these plots in order to monitor the fire ant population, the presence and spread of the fire ant pathogen *T. solenopsae*, the decapitating flies, and the non-target arthropod population.

2.3.1. Population Assessment

Fire ant population monitoring was done using two techniques: (1) mound counts and rating, and (2) ant activity monitoring lures. Active fire ant mounds found in the sampling plots were scored using a population index rating system (Lofgren and Williams 1982). The population index rating varied from one to ten. A pre-established bait treatment threshold of 50 mounds per hectare with a population index higher than seven was used to trigger application of the bait combination. Ant activity was monitored by deploying ten pieces of processed meat sausage in each of the sampling plots. After 30-60 minutes, these food lures were observed for the presence of fire ants or other ants. The fire ant population was estimated using the number of ants per lure and the percentage of lures with fire ants.

2.3.2. Bait Treatments

A combination of baits containing hydramethylnon and methoprene (1:1) was chosen for use in the demonstration sites to take advantage of two different modes of action. This combination has been tested extensively with excellent results in Texas (Barr 2002). Hydramethylnon offers a much quicker effect on the fire ant population with mortality occurring within a week or so but without much residual effect. Methoprene causes a decrease in reproductive potential without immediate effect on the fire ant population. The combination of the two active ingredients provides for a quick but lasting effect on the fire ant population. Importantly, these products are registered for grazed cattle pastures and could be later adopted by farmers. They also have little impact on the non-target fauna in the demonstration sites, which is important since the rebound of the natural population of arthropods, including competing native ant populations, is an expected result of the fire ant management programme.

All bait applications were done using aircraft (Fig. 3). After the initial treatment no fur-

ther applications were done until the population threshold of 50 mounds per hectare was reached or surpassed. Baits were applied at a rate of 1.7 kilogram of 1:1 hydramethylnon and methoprene mixture per hectare. Four weeks after bait application, the fire ant populations were assessed to determine the effectiveness of the bait application.

2.3.3. Biological Control Establishment

The decapitating flies were released at the IPM sites either as adult flies (*Pseudacteon tricuspis* Borgmeier) distributed over partially excavated mounds or in laboratory-parasitized ants returned to their mother colonies (*Pseudacteon curvatus* Borgmeier, *Pseudacteon litoralis* Borgmeier). The total numbers of parasitized ants released at each site were approximately 4000-6000. Decapitating flies were considered established at a location when active flies were observed hovering over disturbed fire ant mounds in the spring or summer after surviving a winter in the field. Initially, fly presence was monitored just in the release areas, but after clear indication of establishment, fly presence was monitored at increasing distances from the release sites.

T. solenopsae inoculations were made within active fire ant mounds by introducing approximately three grams of live, *T. solenopsae*-infected brood per mound in areas where the microsporidian was not yet established at the start of the demonstration project (Mississippi and South Carolina). Worker ant samples were subsequently returned to the laboratory, macerated, and examined for *T. solenopsae* spores using a phase contrast microscope (Williams et al. 1999). *T. solenopsae* was considered established when spores were found in samples more than six months after inoculation.

2.3.4. Environmental Assessment

The environmental effect of the fire ant control programme was assessed by monitoring the surface-active arthropod population using pitfall traps. In the spring and fall of each year, four traps were deployed in each sampling plot for 48-72 hours, and then processed in the laboratory. Of special interest was the presence and number of native ants that could impact fire ant reinfestation rates. Insects, arthropods and other small animals that fell into the pitfall traps were tabulated by morphospecies.

3. Results

3.1. Research Component

The research component of the area-wide project is not critical to the project's success, but if successfully implemented the results would facilitate the attainment of project goals. One example is given on improved pathogen detection.

A major component of the red imported fire ant (*S. invicta*) area-wide project was augmentation with the microsporidian pathogen, *T. solenopsae*. Evaluations to detect successful spread of this pathogen were limited to microscopic examination of macerated ants for the presence of the characteristic spore stage. Unfortunately, this method is labour intensive and cannot detect incipient infections because of the absence of spores. In order to improve detection of all stages of infection of *T. solenopsae* in fire ants, a multiplex polymerase chain reaction (PCR) method was developed. Oligonucleotide primers were designed to unique areas of the 16S rDNA gene of *T. solenopsae* and a region of the *Gp-9* gene of *S. invicta*. Multiplex PCR resulted in sensitive and specific detection of *T. solenopsae* infection of *S. invicta*. The *T. solenopsae*-specific primer pair only amplified DNA from *T. solenopsae* and did not result in amplification products from DNA preparations from uninfected *S. invicta*. The *Gp-9* specific primers recognized and amplified DNA from monogyne and polygyne *S. invicta* social forms, but not from *T. solenopsae*, and, as such, served as a positive control verifying successful DNA preparation. Multiplex PCR detected *T. solenopsae* in all *S. invicta* life stages. *T. solenopsae* could be detected in workers with only ten spores if the *T. solenopsae*-specific primer was used. Multiplex PCR detection of *T. solenopsae* offers the advantages of a posi-

tive control, a single PCR amplification, detection of all developmental stages of *T. solenopsae*, and increased sensitivity and specificity compared with microscopy (Valles et al. 2003).

3.2. Educational Activities

The objective of the educational component is to educate the public on fire ant biology, impact and control, including chemical and biological control agents. A web site (http://www.ars.usda.gov/fireant/) for the project was created and updated continuously with new information. Educational videos describing the fire ant disease caused by *T. solenopsae* and the decapitating flies were produced and distributed both via the web site and in a compact disc playable on any computer or other players attached to a television monitor. Also brochures explaining the project concept and objectives, as well as the biological control agents were produced and distributed by direct mailing, insertion in trade magazines, and direct distribution to the public on several occasions, including state agricultural fairs. All these materials have been used in conjunction with slide shows and other public presentations on the project and its various components.

Public interest in all these materials has been enormous, and their response to the information on the biological control agents, especially the decapitating flies, has been very good. Part of the video describing the parasitic decapitating fly was the subject of a very positive article by a nationally syndicated columnist. This article caused a huge influx of requests to the project web site, requiring it to be moved to a more robust server. At agricultural fairs where the decapitating flies were displayed, the public showed great curiosity and support for the research and use of these flies to control fire ants. It is anticipated that the web site will continue to be maintained after the project formally ends, in order to provide continuity to current and future users of the technology. The educational component of the area-wide project has been very successful as indicated by the high level of public knowledge of fire ants, the individual projects, and by high use of the web site.

3.3. Environmental Impact

Pitfall traps were used to evaluate the environmental impacts of treatments at the demonstration sites on the abundance of non-target ants and the species richness of other surface-active arthropods. For the Florida sites the results were mixed concerning non-target ant abundance. The IPM site usually had more native ants in the plots treated with fire ant baits than in the untreated surrounding plots, while at the control site it was generally the reverse, but this was not consistent over time. The number of non-target ant species in the areas treated by baits was about 20% lower at both Florida IPM and control sites, but again this trend was not consistent over time. The species richness of non-ant arthropods collected in the pitfalls did not differ between plots treated with baits and surrounding untreated plots.

Preliminary evaluation based on these data indicates that the bait treatments did not affect the overall richness of arthropod morphospecies, although there is evidence, though not always consistent, for a modest reduction in the richness and abundance of non-target ants. In the 2005-2006 season, the data for all five sites will be assembled in order to compare sites with and without fire ant biological control agents.

3.4. Economic Assessment

Economic surveys were prepared by an agricultural economic team from the Texas A&M University and sent to the farmers involved in the demonstration sites as well as the researchers in each state. These surveys assessed the impact of the fire ant pests on farm activities, as well as the costs and benefits of the area-wide IPM project. These surveys are being analysed, and the data obtained so far have been used to estimate the economic impact of fire ants on the US agriculture and on the economy of individual states. Estimated impacts in Texas and Florida represent approximately 50% of the impact of fire ants in the

USA, with the rest divided among the other infected states. The estimated value for California assumes the infestation is not eradicated.

4. Measures of Success

Evaluation of the following expected outcomes three years after project implementation can serve as a measure of project success:

4.1. Release and Spread of Biological Control Agents

Decapitating flies have been released and established in all states where the area-wide project has been implemented, and in several other locations throughout the USA. Three decapitating fly species have been released and others will be soon.

4.2. Sustained Fire Ant Control

Fire ant populations at the demonstration sites have been maintained at less than 20% of pretreatment levels throughout the project duration, and farmers are aware of a noticeable decrease in the fire ant population. Results in Florida and Texas indicate that the IPM approach provides advantages over the pesticide-only approach. In Florida, fire ant control averaged 88% where the IPM approach was used as compared to only 71% where fire ants were controlled only by chemical pesticides. In Texas, plots with high decapitating fly populations had decreased fire ant populations as compared to plots with low or no decapitating fly presence.

A simplified method for evaluation of fire ant populations has been developed using food lures. This method makes fire ant control over an area easier, less costly, and more efficient.

4.3. Lower Livestock Production Costs

Area-wide project economists have not yet been able to estimate benefit/cost ratios for the project, mainly due to the short duration of the project.

4.4. Restored Ecological Balance among Native Ants, Birds, and Wildlife

No effects have been observed on the abundance of other arthropods. However, this may change after a longer period with low fire ant populations.

The current demonstration sites will continue to be monitored, but under a less intensive regime. This is possible because of the information gathered from the intense monitoring of the sites during the first three years of the project.

4.5. Increased Farm Worker Safety and Reduced Pesticide Risk

Decreased fire ant populations and the use of low-risk bait toxicants increases farm worker safety and decreases pesticide risk; however, direct assessment has not been made.

5. The Future

The area-wide project has entered the last three years of its expected duration. A new protocol has been developed to expand the project from the initial demonstration sites to other smaller sites in areas under different land use. Current sites were all established on improved, grazed pastures under cattle production. New demonstration sites will be established on “high value” properties where fire ant control is highly desirable and represents a high economic, environmental, and/or aesthetic value (e.g. parks, poultry farms, hunting clubs, natural areas, military facilities, urban horticulture, etc.). The objective is to expand the area-wide concept to other customers besides cattle farmers and to demonstrate that the concept of using biological controls in combination with toxic bait applications can be used in many different situations. This will take what has been learned from the large-scale area-wide programme on pastures to properties and owners that have a high probability of continuing the fire ant IPM programme after project funding expires. It is expected that these properties will serve as examples for neighbouring property

owners, and thus create a knowledge base on fire ant management and biological control that will provide for continuing expansion of interest in fire ant IPM in different regions in the USA.

6. Conclusions

This demonstration has enabled the implementation of IPM for fire ants over large areas, over a sustained length of time, and in diverse areas of the USA. A significant part of fire ant IPM has been the dissemination of self-sustaining parasites and pathogens in the infested areas. For the most part these biological control agents have become established and spread as anticipated or at an even greater rate and population density. In South America, fire ant populations are five to ten times lower than in the USA without the use of pesticides. If introduced natural enemies of the fire ant are half as effective in the USA as in South America, this would lead to a reduction of fire ant populations in the USA by 40-45%. This would significantly reduce pesticide use for fire ant control and diminish the human impact of fire ants, as well as their negative effects on agriculture and the environment. While at this point in time results with biological control agents are not dramatic, they are very encouraging for the long-term future (10-20 years), as additional biological control agents are released.

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A Cultural Method for the Area-Wide Control of Tarnished Plant Bug *Lygus lineolaris* in Cotton

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ABSTRACT In the mid-southern region of the USA, a method involving one application to marginal areas near roads, fields, and ditches of an herbicide that selectively kills key spring broadleaf hosts of the tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois) was developed and implemented in four 23 square kilometre areas. Overall mean numbers of tarnished plant bug adults and nymphs were significantly lower in treated-area cotton. The average reductions in overall mean numbers of plant bugs in the treated areas were 45.5 and 47% for adults and nymphs, respectively, from 1999-2001. Economists at Mississippi State University conducted an analysis of the programme used on over 8400 hectares of cotton in 1999-2001, and demonstrated that the technology produced savings of USD 14.59/ha in insecticide costs (herbicide application included). An environmental impact study conducted by Louisiana State University, detected no to extremely low levels of herbicide residue in run-off water from conducting the programme. Research is currently being conducted to investigate the use of a fungal entomopathogen, sterile males, and parasitoids to augment or replace the use of herbicides.

KEY WORDS tarnished plant bug, *Lygus lineolaris*, cotton, marginal areas, early season wild host plant, area-wide suppression, herbicides

1. Introduction

The tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois) is a serious pest of cotton that is becoming more resistant to insecticides, requiring growers to use increasingly higher levels of chemicals to achieve the same level of control. Populations in the delta of Arkansas, Louisiana, and Mississippi, have become resistant to pyrethroid insecticides, with lower levels of resistance to a cyclodiene and several organophosphate insecticides (Snodgrass and Elzen 1995, Pankey et al. 1996, Snodgrass 1996, Hollingsworth et al. 1997). Within a few years, insecticides may no longer be effective against this pest. When eradication of the boll weevil *Anthonomus grandis* Boheman is completed in the mid-southern region of the USA, plant bugs will likely remain as the primary pest of cotton in this area. Additional insecticide applications to control plant bugs will reduce benefits that growers have derived from boll weevil eradication and control of lepidopteran pests with transgenic cotton. Insecticides are damaging to the environment and make other control tactics, e.g. biological control using natural enemies, more difficult. For these reasons, a non-insecticidal control method for this insect is urgent.

The delta region of the mid-southern USA is intensively farmed and only a small area of the land is undisturbed by agricultural practices. Snodgrass et al. (1991) estimated that marginal areas near roads, fields, and ditches undisturbed by agriculture comprised only 2.4% of the land in a 6.4 square kilometre area of Washington County, Mississippi. In these

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marginal areas, broadleaf weeds are abundant and are utilized for food and reproduction by tarnished plant bugs in the winter and spring. Snodgrass et al. (1984) identified 169 host species representing 36 plant families in the mid-southern USA. As these weeds senesce, adult plant bugs move into cotton and other crops (Tugwell et al. 1976, Snodgrass et al. 1984).

Management of wild hosts in marginal areas with herbicides could be economically feasible because of the small acreage involved. In addition, farmers in the mid-southern USA in the mid 1990s widely adopted a weed control programme in which winter and spring weeds are controlled in commercial fields with herbicides, mainly in February. This farming practice further restricts plant bugs in early season to the wild host plants available in the marginal areas not treated by the growers.

A large experiment was conducted in 1999, 2000, and 2001 to determine whether numbers of tarnished plant bugs found in cotton could be reduced by suppression of early season broadleaf wild host plants found in marginal areas near the cotton fields with a single herbicide application. The herbicide application used in the experiment was found to be very effective in reducing numbers of wild host plants and plant bug populations in marginal areas (Snodgrass et al. 2005). Results from the experiment showing the effect of the herbicide treatment on plant bugs found in cotton grown within treated areas are reported herein.

2. Materials and Methods

The experiment was conducted during each of three years using four, 23 square kilometre areas that were approximately square in shape. In two of the test sites each year, a single application of Trimec® (PBI/Gordon Corp., Kansas City, MO.) in 1999 or Strike 3™ (Agriliance, LLC, St. Paul, MN.) in 2000 and 2001 was applied to most marginal areas with wild host plants during the first two weeks of April 1999 and first two weeks of March 2000 and 2001. These herbicides both contain mecoprop, 2,4-D, and dicamba and are effective in killing broadleaf weeds, thereby reducing reproduction of tarnished plant bugs in treated marginal areas (Snodgrass et al. 2005). These herbicides do not have activity on graminaceous weeds. The marginal areas of the remaining two test sites (controls) did not receive the early-season herbicide application each year. In 1999 and 2000, the same treated and control test sites were used. The two treated test sites were located near Tribbett and Hollandale in Washington County, Mississippi, while the two control test sites were near Holly Ridge in Washington County and Kenlock in Sunflower County. The site near Tribbett was used as a control site in 2001, while the second control site was located near Choctaw in Bolivar County. The two treated test sites in 2001 were located near Arcola and Holly Ridge in Washington County. The chosen treated and control areas had similar environments, e.g. size and composition of marginal areas, cropping systems, farming practices used, etc.

Cotton fields in all four test areas were identified in May of each year and their location marked on aerial maps of the test areas obtained from the Geographic Information Satellite Center at the Delta Research and Extension Center, Stoneville, Mississippi. Each of the test sites was divided into quadrants for sampling purposes. Approximate field size was established by determining row width and number in each field and by measuring field length with a vehicle odometer. Sample fields were chosen at random each week from those found in each quadrant of each test area. Each week 15 to 20 fields were sampled from each of the four test sites. A total of 157, 185, and 212 fields were available in the four test sites for sampling in 1999, 2000, and 2001, respectively.

Sampling was by sweep net, and each sample was ten sweeps with a standard (38 centimetre) sweep net that was swept back and forth across a single row of cotton. The number of samples taken per field was determined by field size and varied from ten in small

Table 1. *Weekly mean numbers of tarnished plant bug adults and nymphs found in cotton grown over three years in 23 square kilometre areas of the Mississippi Delta in which broad leaf weeds in marginal areas were either left untreated or treated with a herbicide in March or April.*

	Mean[1] (± SE)/10 sweeps						
June	Untreated	Treated	Cv	Trt F	*P* > F	Trt x Yr F	*P* > F
1st Week	0.23 ± 0.06	0.14 ± 0.05	6.48	1.33	0.27	0.97	0.39
2nd Week	0.13 ± 0.03	0.08 ± 0.03	4.39	1.82	0.25	0.56	0.59
3rd Week	0.13 ± 0.03	0.10 ± 0.02	3.61	0.58	0.46	3.28	0.05
4th Week	0.20 ± 0.04	0.08 ± 0.05	6.48	3.18	0.16	1.36	0.34
July							
1st Week	0.17 ± 0.04	0.09 ± 0.04	4.68	3.27	0.13	1.00	0.43
2nd Week	0.25 ± 0.06	0.17 ± 0.06	8.27	1.06	0.37	0.32	0.76
3rd Week	0.35 ± 0.09	0.18 ± 0.09	10.52	2.29	0.21	0.31	0.76
4th Week	0.57 ± 0.17	0.27 ± 0.17	15.11	2.50	0.15	0.60	0.58

[1]Means are data from 1999, 2000, and 2001 combined over years and treatment by sample week. The means are based on samples from 30 or more fields for each treatment in each week of each year.

fields to 100 in large fields. Numbers of tarnished plant bug adults and nymphs captured were recorded in the field. Sampling began during the first week in June and ended during the last week in July.

An agricultural economist from the Delta Branch Experiment Station, Mississippi State University, Stoneville, Mississippi, compiled insecticide use and cost data for plant bug control using information obtained each growing season from growers in the treated and untreated sites. These data were used to calculate the average per hectare costs for plant bug control in cotton grown in the control and treated sites. The authors kept records of the amount of herbicide used, application equipment used, and labour costs. The number of hectares of marginal areas treated was calculated based on the amount of herbicide used and the application rate. The total cost of the herbicide treatment was calculated by the agricultural economist each year, using the Mississippi State Budget Generator (Laughlin 1999).

Experimental design in each year was completely random with two replicates per treatment and several levels of subsampling. The following three analyses of variance (ANOVA) were performed: (1) data for each year were analysed separately by sample week and year, (2) data for all three years were combined by sample week using years as additional replication, and (3) data were combined by treatment over all sample weeks and years. All analyses were performed with PROC MIXED (SAS Institute 1999). In the data analyses by sample week and year, comparisons of means for plant bugs found in the treated and control areas used a *P* value based on the error estimate from the ANOVA. Declaring significance at $P \# 0.05$ is equivalent to using a least significant difference comparison of the means.

3. Results

In 1999, the mean number of plant bugs for all sample weeks found in cotton grown in the treated test sites (0.06 per sample) was threefold lower than the overall mean number found for plant bugs in cotton grown in the control test sites (0.18 per sample), although significantly higher numbers of plant bugs were found in the control sites in the third and

fourth weeks of July. In 2000, although the overall mean number of plant bugs found in cotton grown in the treated sites (0.17 per sample) was 1.5-fold lower than in the control sites (0.25 per sample), no significant differences were found in any week between numbers of plant bugs found in cotton grown in treated sites as compared to numbers of plant bugs found in cotton grown in control sites. In 2001, significantly higher mean numbers of plant bugs were found in cotton grown in the control sites during three weeks of July. However, mean numbers of plant bugs for all sample weeks in 2001 were similar in the cotton grown in the treated (0.31 per sample) and control (0.39 per sample) sites.

Results from analysis of data combined over years by treatment (Table 1) showed no significant differences in mean numbers of plant bugs from cotton in the treated and control test areas among sample weeks. However, in every case, the mean number found in the cotton from the treated areas was lower than the mean number from cotton in the control areas. The coefficient of variation (CV) (Table 1), which indicated the degree of precision with which the treatments were compared (it expresses experimental error as a percentage of the mean), was consistent and less than 7% through the first week in July. In the remainder of July, it increased to its highest percentage (15.1%) in the fourth sample week of July. The year-by-treatment interaction was only significant for one week (the third sample week in June), indicating that mean numbers of plant bugs found each week in cotton in the treated and control areas were consistent from year to year (Table 1).

Analysis of data combined by treatment over all sample weeks in all three years showed that the mean numbers of nymphs, adults, and total plant bugs collected in cotton were significantly lower in the treated test sites than the control test sites (Table 2). Adults, nymphs, and total plant bugs averaged 45.5, 47.0, and 46.1% lower per sample, respectively, in the cotton from the treated areas.

Total costs for the herbicide applications were USD 6469, USD 6206 and USD 6411 in 1999, 2000, and 2001, respectively (Table 3). Totals of 314, 273, and 202 hectares of marginal areas with wild hosts were estimated to have been treated to protect an estimated 2409, 3320, and 2702 hectares of cotton grown in the treated sites during the three years. Expressed as a percentage of the 4664 hectares found in two 23 square kilometres treated areas, 6.7, 5.9, and 4.3% of the total areas were treated in 1999, 2000, and 2001, respectively.

The average costs per hectare for tarnished plant bug control with insecticides were lower for cotton growers in the treated sites in all three years (Table 4). Growers in the treated sites spent USD 15.98, USD 19.29, and USD 8.50 less per hectare in 1999, 2000, and 2001, respectively, than did growers in the control sites. The net savings in plant bug control costs in the treated sites were USD 32 027,

Table 2. *Overall mean numbers of tarnished plant bugs found in cotton grown over three years in 23 square kilometre areas of the Mississippi Delta in which broad leaf weeds in marginal areas were either left untreated or treated with a herbicide in March or April.*

	Mean[1] (± SE)/10 sweeps			
	Untreated	Treated	F	$P > F$
Adults	0.20 ± 0.03	0.11 ± 0.03	66.82	0.01
Nymphs	0.04 ± 0.01	0.02 ± 0.01	13.35	0.05
Both	0.25 ± 0.03	0.13 ± 0.03	56.23	0.02

[1]The means are for all sample weeks by treatment in 1999, 2000, and 2001

Table 3. *Cost in USD for treatment of marginal areas near fields, roads, and ditches in the Mississippi Delta with a single herbicide application in March or April. The totals in the table are for treatment of the marginal areas found in two 23 square kilometre areas in each year.*

Year	Labour cost[1]	Herbicide cost[2]	Equipment cost[3]	Total cost	Marginal areas treated (hectares)	Cotton areas protected (hectares)	Treatment cost per hectare cotton
1999	2009	3589	871	6469	314	2409	2.69
2000	2217	3173	816	6206	273	3320	1.87
2001	2560	3214	637	6411	202	2702	2.37

[1]The areas were treated each year using two permanent and one or two temporary employees of the Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS. Labour costs varied from USD 40 to USD 54 per hour.

[2] Trimec® (1999) or Strike 3™ (2000 and 2001) were the herbicides used

[3] Calculated using the Mississippi State Budget Generator for development of cost of production estimates (Laughlin 1999). A description of the application equipment and herbicide rates used is found in Snodgrass et al. (2005).

USD 57 847, and USD 16 556 in the three years of the study.

4. Discussion

The herbicide treatment greatly reduced numbers of wild hosts and the opportunity for short-range migration of adult plant bugs produced on them into cotton in the treated sites in June and July. This reduction helped to lower numbers of plant bugs found in cotton in the treated sites, and indicated that short-range migration from wild hosts is important in infestation of cotton by plant bugs. Several authors (Tugwell et al. 1976, Cleveland 1982, Anderson and Schuster 1983, Snodgrass et al. 1984, Fleischer and Gaylor 1987) have listed wild hosts on which plant bugs can build up and be available to move into cotton in the south-eastern USA. However, movement studies among wild hosts, or between wild hosts and crops have not been done.

The lower numbers of tarnished plant bugs found in cotton in the treated test sites was reflected in insecticide control costs. These costs were lower and fewer applications were made in cotton grown in the treated test sites in all three years (Table 4). This is important because it showed that the lower numbers of plant bugs found in cotton in the treated test sites could have been the result of the herbicide treatment, not higher insecticide use in the treated sites. Growers in the treated sites spent an average of USD 14.59 less per hectare on plant bug control over the three years of the study as compared to growers in the control sites. This was a considerable savings in costs, because an estimated average of USD 35 477 in net savings (herbicide application included) in the treated sites was found per year over the three years of the study.

The treatment of marginal areas with a herbicide, combined with the control of winter and spring weeds in fields by growers, produced relatively large areas where few wild hosts were available to tarnished plant bugs during March-June. However, the presence of other crops that flowered prior to cotton and were plant bug reproductive hosts may have influenced the results of the current study. Laboratory experiments and field observations by agricultural consultants suggest that corn is an important reproductive host during June (Abel and Snodgrass 2003). However, only one small field of corn was grown in the test sites during the three years of the study. In all three years, about equal amounts of soybeans were grown in the treated and untreated sites. The importance of soybeans as a tarnished plant bug host is not known and no estimates

Table 4. *Grower costs and savings in USD for tarnished plant bug control with insecticides in cotton grown in 23 square kilometre areas of the Mississippi Delta in which broad leaf weeds in marginal areas were controlled with a herbicide in March or April, as compared to the cost of plant bug control in cotton grown in untreated 23 square kilometre areas.*

	Treated areas			Untreated areas					
Year	No. growers	Mean no. applications	Mean cost per hectare[1]	No. growers	Mean no. applications	Mean cost per hectare	Cost per hectare difference	Insecticide cost savings[2]	Net savings[3]
1999	6	0.9	22.50	14	1.7	38.48	15.98	38 496	32 027
2000	18	3.1	57.95	4	3.8	77.24	19.29	64 053	57 847
2001	11	5.5	93.09	9	7.0	101.59	8.50	22 967	16 556
Mean	12	3.2	57.85	9	4.2	72.44	14.59	41 839	35 477

[1]Includes insecticide application and material costs

[2]Cost per hectare difference in insecticides used in cotton for plant bug control in the check areas as compared to the treated areas multiplied by the number of hectares of cotton protected

[3]Insecticide cost savings in the treated areas minus total cost of the early season herbicide treatment

of their possible contribution to the overall populations found in cotton can be made. Additional research is therefore needed to understand the impact of corn and soybean on the population dynamics of the pest within the treated areas.

The authors are currently working with scientists in the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Southern Insect Management Research Unit, Stoneville, Mississippi on the development of the use of the sterile insect technique (SIT), parasitoids, and entomopathogens that could be applied to augment or replace the use of herbicides for the area-wide programme. With respect to the first, tarnished plant bugs were administered dosages of 0, 50, 100, 150, 200, and 400 Gy of gamma radiation from a ^{137}Cs source. Reductions in egg hatch and egg-to-adult development in both irradiated parents and their F_1 progeny were proportional to the dosage received. Egg hatch averaged 58.9% in untreated parents and fell to 35.5 and 4.6% when males were treated with 150 and 400 Gy, respectively. Egg-to-adult development was more severely curtailed than egg hatch, with untreated groups averaging 36.0% development compared with 6.8% at 150 Gy and 0% at 400 Gy. Eggs from untreated females mated with F_1 male progeny averaged 58.9% eclosion for the untreated control compared with 18.5 and 0.9% for the 100 and 200 Gy groups, respectively. Egg-to-adult development in those groups averaged 38.4, 5.4, and 0.1%, respectively. Females were more susceptible to the effects of radiation than males. Hatch of eggs from F_1 female progeny mated to untreated males averaged 61.8, 21.0, 3.7, and 0% for dosages of 0, 50, 100, and 200 Gy, respectively, and egg-to-adult development averaged 39.4, 5.5, 0.1, and 0% for those treatments, respectively. Mortality in the 50 and 100 Gy groups was similar to the untreated control, but fell abruptly at 150 to 200 Gy and again at 400 Gy. The effectiveness of the lower dosages in lowering egg hatch and egg-to-adult development has stimulated further study into the competitiveness of irradiated tarnished plant bugs and their potential efficacy in the field.

Preliminary work on the tarnished plant bug parasitoid *Anaphes iole* Girault, has examined parasitism rates of plant bug eggs in a dozen host plants (L. Williams, personal communication). Parasitism rates in spring weed hosts ranged from 60 to 85%, while parasitism

in cotton was around 90%. These results suggest that *A. iole* has the potential to suppress plant bugs in the spring and summer, especially as part of an area-wide programme. Augmentative releases of *A. iole* might increase suppression of plant bugs on spring hosts that remain after burn-down, as well as in cotton.

Other research (J. Leland, personal communication), may provide the most promising method to augment or replace the use of herbicides in the area-wide programme. Leland and Snodgrass (2004) described the prevalence and distribution of natural *Beauveria bassiana* (Balsamo) Vuillemin infections in tarnished plant bug populations from wild host plants in Mississippi. This work led to the discovery of 20 new *B. bassiana* isolates that were characterized and compared to the commercial *B. bassiana* isolate (GHA) for pathogenicity to plant bugs and beneficial insects, *in vitro* spore production, tolerance to solar radiation, and germination at high temperature. Ten of the new isolates were significantly more pathogenic than *B. bassiana* (GHA), and several were over ten times more pathogenic. One isolate was better able to germinate at high temperatures. Thus far, some isolates have shown high pathogenicity to tarnished plant bug and *Lygus hesperus* Knight, have low pathogenicity to some non-target organisms, are prolific spore producers, produce low mycotoxins levels, and are more tolerant to artificial sunlight and high temperatures. Laboratory production of spores was sufficient to initiate field trials in 2004 and will be expanded to conduct field trials in Mississippi, California, and Arkansas in 2005. In the future, this pathogen may be useful for tarnished plant bug control in the area-wide programme.

5. Conclusions

The area-wide approach developed and tested, proactively removing key spring broadleaf hosts during the critical spring period, effectively suppressed the build-up of tarnished plant bug populations before they move into cotton growing areas, and resulted in average cost savings for tarnished plant bug control of USD 14.59 per hectare. Future research will attempt to improve the efficiency of the programme. Research will be conducted to determine the influence of non-cotton crop hosts on the population dynamics of the tarnished plant bug. This research will help determine the impact that non-cotton hosts have on the effectiveness of the area-wide programme. Research will also be conducted to develop other methods, e.g. the sterile insect technique and biological control, to augment or replace the use of herbicides in the programme.

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Use of the Sterile Insect Technique Against *Aedes albopictus* in Italy: First Results of a Pilot Trial

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ABSTRACT In Europe, the mosquito *Aedes albopictus* (Skuse) is widespread in Italy, Albania and most probably in neighbouring Montenegro. Recent introductions have also been reported in France, Spain and southern Switzerland. In Italy, the species is currently recognized as the most noxious mosquito, thus requiring the implementation of intensive control programmes. *Ae. albopictus* is also a potential vector of human diseases, which has raised the issue of whether eradication campaigns are called for. This species is particularly suitable for application of the sterile insect technique (SIT) because of its urban-related distribution, recent introduction, low active dispersal potential, low population density which may be maintained by conventional control measures, and ease of mass-rearing. In 1999, a programme was initiated that focused on the application of the SIT against *Ae. albopictus*. A pilot rearing facility, targeted at the production of up to 20 000 male pupae per week has been established. Blood feeding is performed with a thermostatically controlled device using defibrinated bovine blood, egg hatching is stimulated with a nutrient broth culture, and egg counts conducted automatically. Larval density and larval diet are still being investigated in order to improve productivity, the separation of males is currently conducted at the pupal stage using calibrated metal sieves, and irradiation studies are performed at the ^{60}Co plant Calliope, Italian National Agency for New Technologies, Energy and the Environment in Rome. During the summer of 2004, eight weekly sterile male pupal releases were organized in Rimini to evaluate sterile male performances in the field against a natural population. A significant difference was observed in the release area compared with the control area when the effects on egg fertility and egg density were cumulated. It is planned to continue the programme on a larger scale to improve rearing efficiency and obtain a preliminary benefit/cost evaluation.

KEY WORDS *Aedes albopictus*, Asian tiger mosquito, SIT, pilot release, Italy, rearing

1. Introduction

The Asian tiger mosquito *Aedes albopictus* (Skuse) has invaded several countries in recent years, mainly due to passive transportation in used tyres (Reiter and Sprenger 1987). In Europe, the species was first recorded in Albania in 1979 (Adhami and Murati 1987), in Italy in 1990 (Sabatini et al. 1990), in France in 1999 (Schaffner and Karch 1999), in Belgium in 2000 (Schaffner et al. 2004), in Montenegro in 2001 (Petric et al. 2003) and in Switzerland in 2003 (Flacio et al. 2004). Other countries have already been invaded or are about to be so in the Middle East, Africa and the Americas.

In Italy, establishment appears to have been rapid, mainly due to passive transporta-

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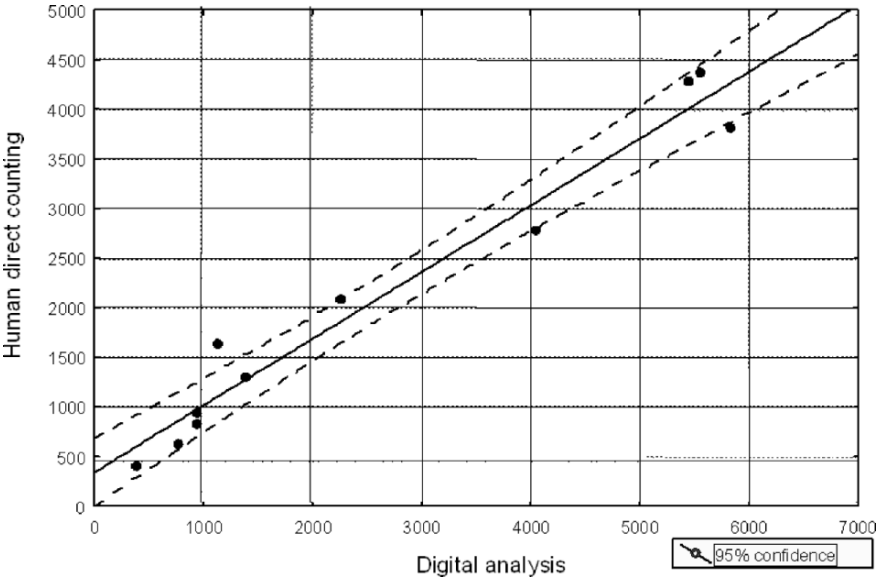

Figure 1. *The correlation between automatic egg counting versus human direct counting (Equation: Human direct counting = 345.5 + 0.67Digital analysis, r = 0.979).*

tion of adults inside vehicles, and the species is currently found in twelve regions (Urbanelli et al. 2000, Romi 2001).

In its area of origin (Asia), *Ae. albopictus* is known to be an important vector of many arboviruses including yellow fever and dengue. Moreover, it is also capable of transmitting indigenous arboviruses in newly invaded areas (Shroyer 1986), as well as filariasis (*Dirofilaria immitis* Leidy and *Dirofilaria repens* Railleiet and Henry), and other arboviruses like Sindbis, Chikungunya, West Nile and Rift Valley (Cancrini et al. 1992, Mitchell 1995). Finally, this species can also be a serious nuisance because of its high anthropophily and painful bite.

The species is mainly found in urban and peri-urban areas where it develops in man-

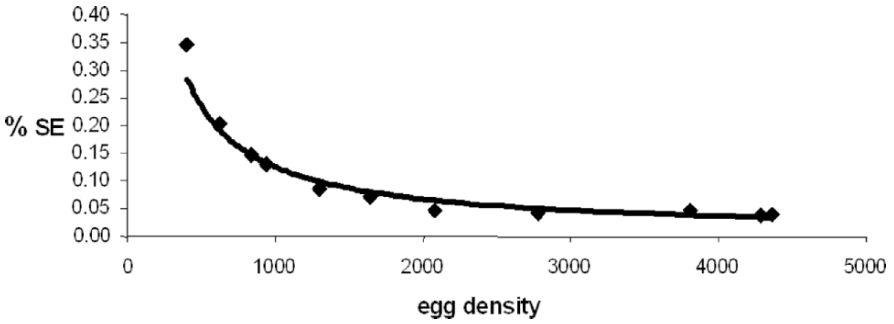

Figure 2. *Digital egg counting standard error versus human direct counting at different egg densities (Y = 66.49 x - 0.909, R^2= 0.948).*

made water containers. This "island" distribution and the mosquito's low active dispersal capability make it a potential candidate for the application of the sterile insect technique (SIT) as a complement to other control tactics already being implemented.

In 1999, a project financed by local funds (see acknowledgements) was started to investigate the feasibility of applying the SIT against *Ae. albopictus* in Italy.

2. Mosquito Rearing

2.1. Adult Maintenance

The colony used for the experiments originated from field-collected eggs from Desenzano del Garda (in the Province of Brescia), in the north of Italy in 1993, and has been routinely mixed with wild specimens collected from other areas in northern Italy. Mosquitoes were kept in an insectary under standard laboratory conditions (27 ± 1°C, 85% relative humidity, 15 hour scotophase). Adults were kept permanently in plexiglas cages (50 x 50 x 60 centimetres) with a supply of 10% sucrose solution to which they had constant access. Females were also provided with fresh mechanically defibrinated bovine blood using a special temperature control apparatus (Bellini et al. 2002). Eggs were laid on filter paper placed inside black plastic containers containing water, removed daily from the adult cages, left to dry in the climate chamber for 24 hours and then placed in a closed plastic box with a saturated solution of K_2SO_4. Using this method, eggs could be kept alive for a few months. When needed, the filter papers with the eggs were put directly in water. Larvae were fed on crushed dry cat food (Friskies® Adults) and kept in plastic larval trays containing dechlorinated aerated water.

2.2. Egg Counting

In order to rear larvae at fixed densities a method for automatic and rapid egg counting must be available. Eggs are black and laid individually on a white paper substrate in variable densities. By using an open source image processing and analysis programme (ImageJ, United States National Institute of Health) it was possible to achieve satisfactory egg counting accuracy by scanning eggs on the filter paper. The correlation between the digital analysis data and conventional direct counting was satisfactory (Fig. 1), as was the standard error distribution of the egg density range in current use (2000-4000 eggs per paper) (Fig. 2).

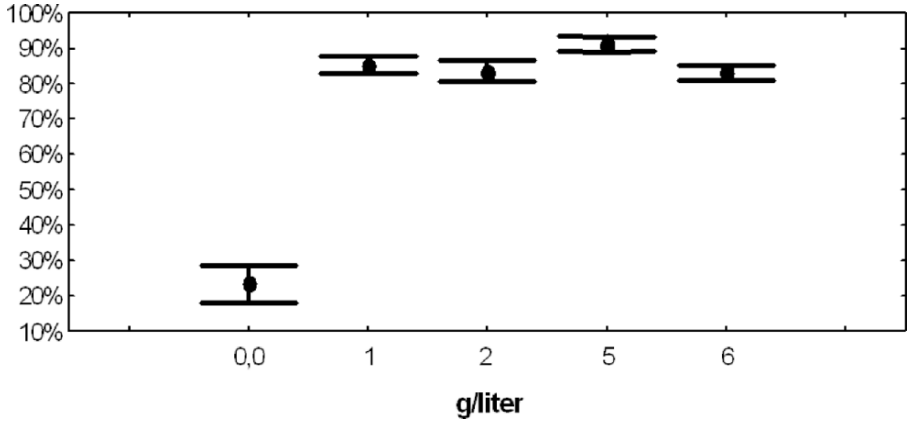

Figure 3. *Percentage of* Aedes albopictus *eggs hatched at different nutrient broth concentrations.*

2.3. Egg Hatching

Ae. albopictus eggs are generally difficult to hatch. Methods were therefore tested to standardize egg hatching in order to obtain a sufficiently precise and synchronized number of L_1 larvae from a known number of eggs. A nutrient broth culture was evaluated in order to deoxygenate the water (Barbosa and Peters 1969, Novak and Shroyer 1978).

Hermetically sealed glass jars (1 litre volume) with 700 millilitres of dechlorinated tap water containing 200-500 *Ae. albopictus* eggs were used to which nutrient broth at different concentrations was added. L_1 larval counts were made 24 hours after egg immersion. Five trials were carried out at the same temperature (27 ± 1°C). The results are illustrated in Fig. 3.

The broth had a strong stimulatory effect on egg hatching at all the concentrations tested with a consistently high percentage of hatching. As there appeared to be no significant difference between the effects obtained with different concentrations, 1 g/litre is currently used. Having observed that the broth had negative effects on larval development, young larvae were removed from the hatching solution. Further investigation is underway to find a way to avoid filtration of L_1 larvae by reducing the broth concentration or changing its composition.

2.4. Larval Density

Studies on larval density were conducted in rectangular white plastic trays (30 x 21 x 8 centimetres) containing 2.5 litres of dechlorinated water. Larvae were provided with standard larval food (Friskies® Adults dry cat food), throughout their development, at a fixed concentration of 4 milligrams per larva, of which 10% was given on day one, 45% on day two and 45% on day five. Evidence obtained from previous studies regarding pupal production (in relation to the initial number of L_1 larvae), and larval development time (calculated from the period between egg immersion and pupation), suggested that better results would be achieved with 1000 larvae per tray than with 100, 500, 750, 1250 and 1500 larvae per tray (Bellini et al. 2002). Following observations made by Teng and Apperson (2000) and Briegel (2003), larger trays (41 x 31 x 11 centimetres) containing three litres of water with a higher larval density and a new larval diet are being evaluated. Some recent results on the effect of larval density on pupal production using the conventional diet are summarized in Fig. 4.

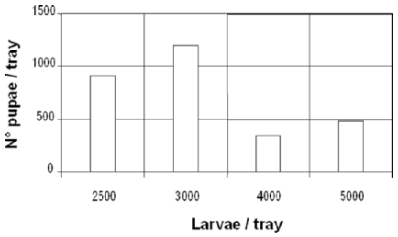

Figure 4. *The production of* Aedes albopictus *pupae at different larval densities.*

Attempts to improve the larval diet involved adding dried brewer's yeast to the standard diet. The dose was fixed at 2.5 milligrams per larva of Friskies® Adults + 1.5 milligrams per larva of yeast. As with the standard method, 10% was given on day one, 45% on day two and 45% on day five. Results are summarized in Fig. 5. A clear increase in

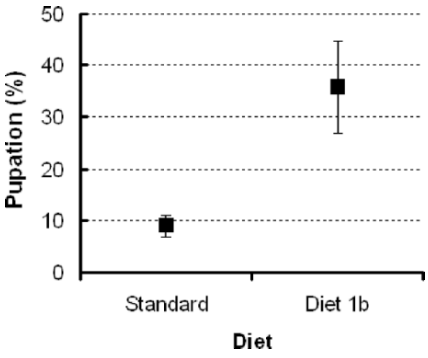

Figure 5. *Mean (± SE) percentage pupation using different larval diets at a density of 1500 larvae per litre (trays with three litres of water).*

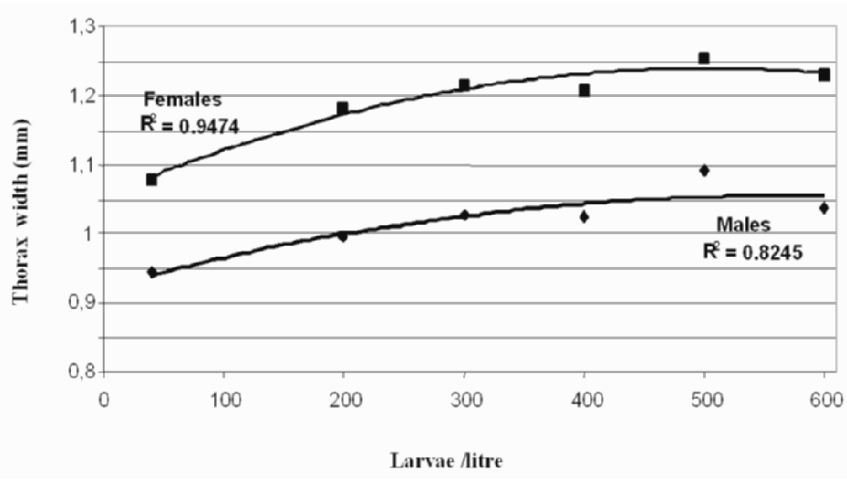

Figure 6. *Size of female and male pupae of* Aedes albopictus *in relation to different larval rearing densities.*

pupal production was achieved when a density of 1500 larvae per litre was used.

2.5. Pupal Sexing

Considering previous findings on several mosquito species and personal observations regarding pupal size and sex in *Ae. albopictus* (Fig. 6), attention was focused on the sieving technique to achieve sex separation (McCray 1961, Sharma et al. 1972, Sharma et al. 1974). The metal sieves used (Giuliani®) have a round stainless steel frame (diameter 20 centimetres and height six centimetres), supporting a square mesh. Trials were conducted with 1250, 1400 and 1500 micron sieves tested independently or in succession. Currently, pupae are collected once per production cycle at a fixed time approximately 24 hours after the beginning of pupation to better exploit proterandry. Pupae are processed together with larvae by being placed in water at 35°C with the sieve for 4-5 minutes. To make it easier to separate residual larvae from male pupae, one part per million of *Bacillus thuringiensis israelensis* (de Barjac 1980) is added to the container to kill the larvae (but not the pupae, as they do not feed) used to transport pupae to the radiation source instead of using the ice technique (Ansari et al. 1975a).

Experiments are planned to check whether exposure to *B. t. israelensis* during the larval/pupal stage has any negative effect on adult males (Zahiri and Mulla 2005). Separated male pupae are collected for radiation while non-separated male and female pupae are reintroduced into the colony. Current average male pupal productivity is ca 15-25% (based on the initial number of L_1 larvae), with the presence of females ranging from 1-3%. Tests are presently being conducted with the aim of increasing male productivity (harvesting pupae at 36 hours instead of 24 hours from the beginning of pupation), and reducing the proportion of females in the released material (shortening the time of sieving, testing sieves with a rectangular mesh, etc.).

2.6. Pupal Irradiation

Irradiation is performed with a ^{60}Co source (Calliope) at the Italian National Agency for New Technologies, Energy and the Environment (ENEA), Rome by exposing pupae in water using a special plexiglas device. External dimensions are 41 x 60 x 3 centimetres (six stacked trays 41 x 10 x 3 centimetres each), allowing the radiation of 20 000

Table 1. *Mean longevity of male* Aedes albopictus *exposed to increasing doses of radiation (100 individuals per cage, 27 ± 1°C, 85% relative humidity, 15 hours scotophase).*

Gy	Longevity of irradiated males (days)			
	No. of replicates	Mean	SD	Test Newman -Keuls
Control	4	35.3	2.3	a
60	3	22.2	1.8	ab
80	4	16.4	6.9	ab
100	4	13.1	3.6	b
110	1	16.2	---	ab

pupae per session. The height of the trays has now been reduced from ten to five centimetres in order to increase the number of pupae.

Dose response studies were conducted in the range of 60-110 Gy at dose rates of 186, 462, and 1190 Gy/hour. Male longevity, male sterility, possible male fertility recovery and sterile male versus fertile male competitiveness were assessed in 40 x 40 x 40 centimetre cages under laboratory conditions. Egg fertility was assessed by standard conditioning of the eggs and application of the hatching method as described above.

The current dose employed for the field pilot studies is 80 Gy, given at a dose rate of 186 Gy/hour, which results in complete male sterility and good competitiveness in cage studies. Further studies are in progress to evaluate the possibility of reducing the dose of radiation and to obtain greater insight into the effect of colonization on the field fitness of sterile males.

Male longevity was affected by radiation at all the doses tested, but not to such an extent as to preclude the planning of weekly releases (Table 1). Surprisingly, 110 Gy gave higher longevity than 100 Gy, and similar longevity to that obtained at 80 Gy.

Tests to establish the optimal radiation dose were conducted prior to the use of the nutrient broth technique, thus making the results difficult to interpret (see low egg hatching in control, Table 2). However, at present, 80 Gy is considered the minimum

Table 2. *Fertility (% egg hatch) following irradiation of male* Aedes albopictus *pupae at different doses (462 Gy per hour if not specified). Competition cages contained 50 sterile males, 50 fertile males and 50 virgin females. Sterile males cages contained 50 sterile males and 50 virgin females.*

		Eggs hatch (%)				
	Gy	Number of replicates	Mean	SD	Test Newman-Keuls	Competitiveness index
Control	0	4	34	27	a	-
Competition cages	60	2	24	20	ab	0.70
	80	3	23	21	ab	0.74
	100	3	27	27	ab	0.63
	110	1	12	8	bc	1.41
Sterile male cages	60	3	2	3	c	-
	80	4	1	2	c	-
	100	4	0	1	c	-
	110	1	0	0	c	-
	60 (1190 Gy/h)	1	3	3	c	-
	80 (1190 Gy/h)	1	0	0	c	-

Table 3. *ANOVA on estimated number of F_1 progeny (i.e. no. eggs x egg hatch) in the sterile male release area as compared to the untreated control area.*

	Mean	SD	n	Diff.	SD	*t*	df	*P*
Release area	36.21	13.53						
Control area	56.77	26.84	9	-20.55	24.72	-2.49	8	0.037

acceptable sterilizing dose although males showed some residual fertility. The relationship between radiation sensitivity and pupal age is currently being investigated (Table 2).

Competitiveness studies in cages showed that the performance of irradiated males was reduced compared with normal males of the same age (Table 2). No clear relationship was evident between radiation dose and competitiveness in the range of doses tested. Again surprisingly, the competitiveness of males irradiated with 110 Gy seemed better than those irradiated at lower doses. Further investigation is needed in order to clarify the relationships between (1) pupal age and sensitivity to radiation in the context of a large-scale mass rearing, and (2) sterile male competitiveness in greenhouses and different radiation protocols.

Female longevity was also reduced by radiation (data not shown), but females were still able to take several blood meals with complete suppression of fecundity at the tested doses of 70-75 Gy. Therefore the release of females, while having an obvious negative effect on the efficiency of the SIT and therefore needs to be reduced to a minimum, is not an interfering factor in the assessment of field release efficacy.

3. Pilot Field Test 2004

During the summer of 2004, a pilot field test was conducted in the centre of Rimini, northern Italy, where the species is well established. Eight weekly releases were implemented during the period 16 June-4 August in an area of ten hectares, while a similar area was used as a control. Regular control activities (larvicide and source reduction) were conducted on the whole urban area by operators who were not informed about the experimental location. Pupae were placed in plastic jars on the ground at 40-45 fixed sites in shaded environments. It is estimated that 50 000 adults emerged from a total of 65 000 pupae. This corresponds to 100-1000 males per hectare per week. Egg monitoring was conducted using standard ovitraps (Bellini et al. 1996), from two weeks before the beginning of releases to two weeks after the releases ended. In each release and control area, 20 ovitraps were positioned at fixed sites and checked weekly. Field-collected eggs were processed regularly to check fertility levels.

Fig. 7 shows the data collected during the trial. A slight, but continuous reduction in the fertility of eggs collected in the release area was recorded, while egg fertility remained quite steady in the control area throughout the whole season. In the release area, egg fertility had increased to a level similar to that of the control area by two weeks after the end of the releases. The trend in the number of field-collected eggs show fluctuations during the season, tending to increase late in the summer in the control area, as is usual, while remaining quite stable at a lower level in the release area.

Comparing the average number of F_1 progeny (number of eggs x egg hatch) produced in the release and control areas over the whole season, a significant reduction was recorded in the release area (Table 3). Despite the low number of sterile males released and the small size of the release area with a presumable high immigration of mated females, this positive result encourages continuation of

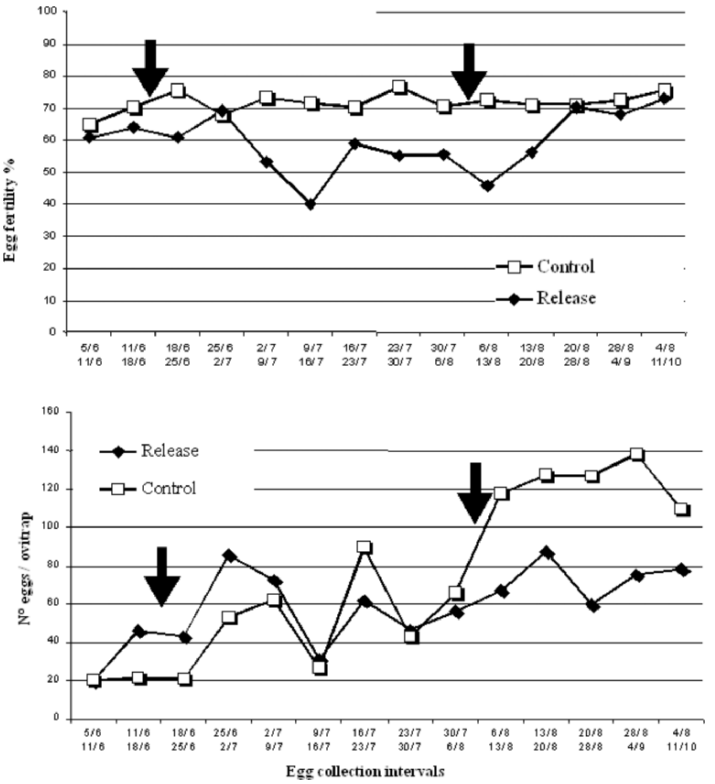

Figure 7. *Number of eggs (lower) and their fertility (upper) collected in sterile male-treated and untreated control areas (Rimini in 2004). Arrows show the beginning and the end of the releases.*

the programme.

4. Considerations for the Future

Integrated application of the SIT for *Ae. albopictus* has not been attempted in the past, although studies of this nature have been made on several other mosquito species including *Aedes aegypti* (L.) which is a closely related species and may be taken as a useful example (McCray 1961, Weidhaas and Schmidt 1963, White 1966, Barbosa and Peters 1969, Jacob and Bevier 1969, Ansari et al. 1975b, Seawright et al. 1975, Curtis et al. 1976, Grover et al. 1976, Lorimer et al. 1976, McDonald et al. 1977, Ogah and Juma 1977, Hagen and Grunewald 1990).

Most of these studies were conducted in the 1970s although at a scale not large enough to be cost-effective and some programmes suffered from political problems. However, new technologies make the SIT approach more feasible and of high potential for future application.

Although positive, the results reported here need to be confirmed through a larger trial, conducted in a more isolated urban area. Field investigations should aim at evaluating: (1) the number of sterile males to be released, (2) the period covered by the releases, (3) the dispersal of sterile males and thus the spatial distribution of the release stations, as well as (4) both the costs and benefits in comparison with conventional control methods.

With respect to mass-rearing techniques, further work is needed on the following aspects: (1) decreasing the nutrient broth concentration or changing the broth type in order to avoid negative effects on larval development and to eliminate L_1 filtration, (2) improving the rearing efficiency by increasing larval density through introducing new larval diets, (3) increasing pupal production by sieving at 30-36 hours after the start of pupation instead of at 24 hours, (4) improving sexing accuracy by testing new types of sieves or alternative methods (e.g. near-infrared spectroscopy), (5) improving sterile male competitiveness by reducing the radiation dose (which may be feasible by exploiting the pupal age-radiation sensitivity relationship), (6) developing larger cages or a greenhouse for colony maintenance, (7) irradiating adult males instead of pupae, and (8) screening new insect growth regulators for their sterilizing effect.

5. Acknowledgements

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Area-Wide Integrated Control of Oriental Fruit Fly *Bactrocera dorsalis* and Guava Fruit Fly *Bactrocera correcta* in Thailand

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ABSTRACT Two tephritid species namely the oriental fruit fly *Bactrocera dorsalis* Hendel and the guava fruit fly *Bactrocera correcta* Bezzi are considered to be the key insect pests of fruit production in Thailand, causing yield loss and quality degradation. This leads to poor commercialization in domestic markets and quarantine restrictions from importing countries. A decade of effective cooperation between Thailand's Department of Agricultural Extension (DOAE), the International Atomic Energy Agency (IAEA), and the Food and Agriculture Organization of the United Nations (FAO) has resulted in the implementation of an area-wide integrated fruit fly management programme which includes a sterile insect technique (SIT) component. The programme consists of two distinctive pilot areas with associations of small-scale mango growers covering 70 square kilometres in the Ratchaburi (western) and Pichit (northern) Provinces. The ongoing programme is aimed at controlling *B. dorsalis* and *B. correcta* through monitoring, orchard sanitation, selective application of bait sprays, and the release of sterile flies. Both species are mass-reared and sterilized at a facility located in Pathumthani Province following standard operational procedures described in this paper. The average weekly production during the mango season is 20 million *B. dorsalis* and 10 million *B. correcta*. After sterilization the pupae are transported weekly to the pilot areas, reared to the adult stage and ground-released at fixed release sites. Quality of the released sterile flies is monitored through the use of a trapping network to measure their distribution and abundance, whilst the success of the control is monitored using periodic fruit sampling to assess the percentage infestation. The integrated approach has been effective in controlling fruit flies by reducing damage from over 80% before programme implementation to an average of less than 3.6% in Ratchaburi Province in the past five years (2000 to 2004). Meanwhile, in Pichit Province where the control programme has been carried out for only two years (2003 and 2004), the infestation has been reduced from 42.9 to 15.5%. This preharvest suppression, combined with postharvest risk mitigation measures, has opened the possibility for exports of mango produced in these selected pilot areas to some of the most stringent and lucrative markets such as Japan. An economic feasibility study conducted in 2002 clearly shows that fruit fly control in Thailand using an integrated area-wide approach with an SIT component could be expanded to other production areas with significant economic returns.

KEY WORDS mango, oriental fruit fly, *Bactrocera dorsalis*, guava fruit fly, *Bactrocera correcta*, suppression, sterile insect technique, male annihilation technique, mass-rearing, ground release, economic feasibility, grower organization, Thailand

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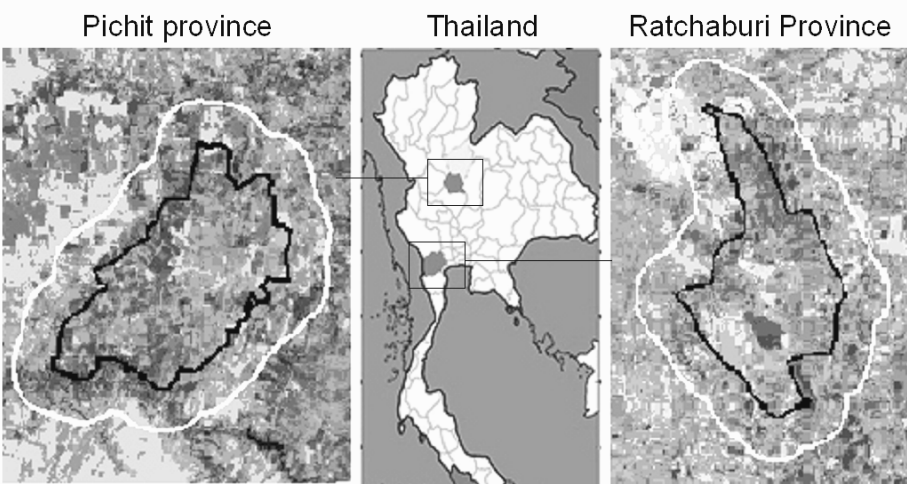

Figure 1. *Maps of the pilot areas at Paktor district, Ratchaburi Province (34 square kilometres) and Sak Lak district, Pichit Province (36 square kilometres) where oriental fruit fly* Bactrocera dorsalis *and guava fruit fly* Bactrocera correcta *are suppressed using an AW-IPM approach with an SIT component.*

1. Introduction

Cooperation between Thailand's Department of Agricultural Extension (DOAE), the International Atomic Energy Agency (IAEA), and the Food and Agriculture Organization of the United Nations (FAO), started in 1991 to control the oriental fruit fly *Bactrocera dorsalis* (Hendel), a major pest of fruits in Thailand. The oriental fruit fly causes losses of several million USD annually to the fruit industry, resulting from significant yield reduction and market restrictions. Widespread insecticide applications to control fruit flies are often carried out on a calendar basis. Concerns about environmental pollution, undesirable chemical residues and the preservation of biodiversity demand new insecticide-minimizing strategies and technologies for combating fruit flies.

Among biologically-based methods, the sterile insect technique (SIT) is the most target-specific, non-disruptive pest control method (Enkerlin et al. 2003). The DOAE implemented the first pilot project in Paktor District, Ratchaburi Province (7.2 square kilometres) which is a lowland mango-producing area involving the association of many small growers, surrounded by an area with cultivation of field crops such as sugarcane, rice and corn. The damage caused by the oriental fruit fly decreased from over 82% in 1987 before the implementation of the area-wide integrated pest management programme (AW-IPM) to 30, 26, 21, 18, 17 and 9% respectively in the following six years (i.e. 1988-1993). From 1994 to 2001, the damage was reduced further to an average of less than 4% (Sutantawong et al. 2004). Since the start of the programme the DOAE set up an oriental fruit fly rearing and sterilization facility in Pathumthani Province, with an average weekly production of 10 million sterile flies.

In 2002, a second AW-IPM pilot project using the SIT was launched in an area of 36 square kilometres in Pichit Province located 450 kilometres north-west of Bangkok. An infestation level of over 40% occurred in this area every year due to the oriental fruit fly and the guava fruit fly *Bactrocera correcta* Bezzi. The small mango growers in this province are well organized and are working in close col-

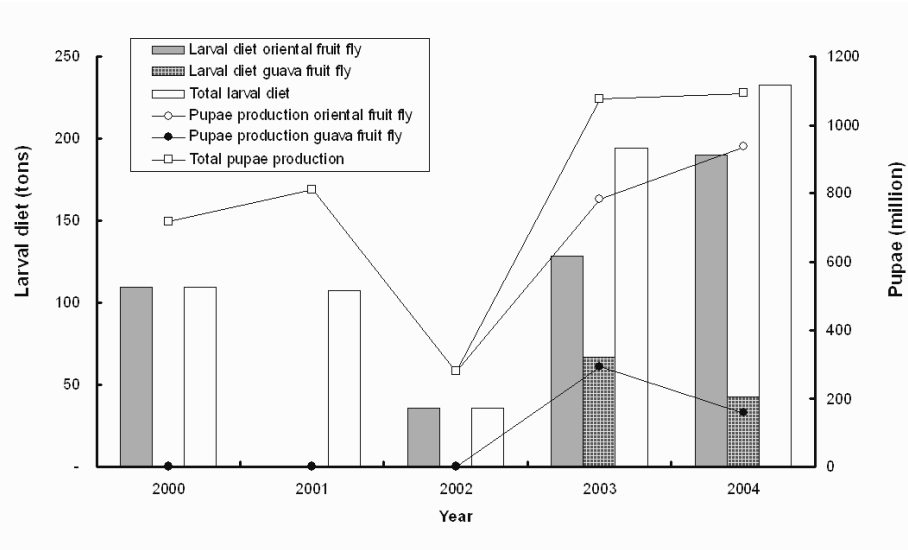

Figure 2. *Oriental and guava fruit fly production from 2000 to 2004 for integration in the pilot control areas in Ratchaburi and Pichit Provinces.*

laboration with the DOAE to implement the AW-IPM pilot project. Simultaneously, the first small operational area in Ratchaburi Province was expanded from 7.2 to 34 square kilometres (Fig. 1). Since 2003, both pests are controlled in these areas by integrating the SIT with monitoring and control methods such as bait sprays and orchard sanitation.

This paper focuses on project activities during the period 2000 to 2004 and discusses the major findings of an economic analysis prepared for the on-going area-wide suppression using the SIT in the selected pilot areas and for other commercial mango production areas in Thailand.

2. Activities and Results

2.1. Mass-Rearing

Oriental and guava fruit flies are being mass-reared in separate rooms and sterilized at the Pathumthani facility, which has currently a maximum rearing capacity of 40 million pupae per week. Two models of rectangular oviposition cages have been used: stainless steel and aluminum sheet cages (40 (width) x 180 (length) x 175 (height) centimetres), and plywood cages (62 x 91 x 120 centimetres) covered with an insect screen (Vargas 1984). Each cage contains 100 000 and 56 000 adults, respectively. The temperature of the adult rearing room is kept at 26 ± 2°C, relative humidity (RH) at 65-70% and light intensity at 2000 Lux with a 12:12 (L:D) light cycle. The adult flies are fed on an artificial diet consisting of a 3:1 volumetric mixture of granulated sugar and yeast hydrolysate (MP Biomedicals, Irvine, CA., USA). Water is supplied to the cages by polypropylene bottles placed over a filter paper. On the 10th day after adult eclosion, perforated bottles are placed into the cage for female oviposition during the day, and removed from the cages for egg collection by rinsing using water (Vargas 1989, Sutantawong et al. 2004). Adults of the breeding colony are kept for 15 days to produce eggs, and then replaced with newly-emerged adults.

The collected eggs are seeded directly into a wheat bran-based diet composed of 26% wheat bran, 12% granulated sugar, 3.6% instant dry yeast, 0.1% sodium benzoate, 0.1% methyl-p-

Figure 3. *Various aspects of the ground release method used for fruit flies in Thailand, (left) loading of boxes at the adult holding and emergence facility in Wang Tob Sai sub-district, Pichit province, (middle, upper) weekly transport of sterile adult flies to release sites using a refrigerated truck, and (middle, lower and right) stationary release devise.*

hydroxybenzonate, 0.2% acetic acid and 58% water by weight, prepared using a 1000 litre capacity industrial mixer (Sutantawong et al. 2004). Approximately 120 000 eggs are seeded on the fibreglass tray (Model Fiber Glass®) containing seven kilograms of diet per tray. The trays are placed on a trolley and covered with a cloth sheet for egg hatch and larval initiation. Six days after the eggs have been seeded, the trays with diet are transferred to a room kept at 22 ± 2°C and 70% RH and remain there until the larvae leave the diet and fall into a metal tray filled with sawdust where they pupate. After 24 hours, the pupae are separated from the sawdust with a mechanical sifting device and placed into pupal trays held on racks and stored in the pupal storage room at 20 ± 1 or 25 ± 1°C.

Quality control tests, conducted for every production batch, include: (1) egg hatch (%), (2) pupal weight (milligram), (3) emergence (%), (4) flight ability (%) and (5) sex ratio, all tests following the international FAO/IAEA/USDA fruit fly quality control manual (FAO/IAEA/ USDA 2003).

During the period 2000 to 2004, the total amount of larval diet prepared for rearing both flies were 109, 107, 36, 194 and 232 tons per year, respectively, while total numbers of oriental fruit fly pupae produced were around 715, 810, 280, 782 and 935 million, respectively. For the guava fruit fly, which has now been produced for two years (2003 and 2004), pupae production was 291 and 157 million, respectively (Fig. 2).

Two days before emergence, pupae are marked with two grams of fluorescent dye powder per litre and then sterilized in a ^{60}Co Gammacell 220 source with doses of 90 and 80 Gy for the oriental fruit fly and guava fruit fly, respectively. They are then close-packed in narrow 400 millilitre polyethylene bags and kept in polystyrene containers with ice packs during shipment to target areas. Between 2002 and 2004, percentage male sterility for the oriental fruit fly was 98.1, 99.4, 99.9% respectively, and for the guava fruit fly it was 99.0% in 2003 and 99.8% in 2004.

The high variability encountered in pupae production was the result of unstable conditions throughout the rearing process. The average production during the mango season was kept at 20 million of *B. dorsalis* and 10 million *B. correcta* pupae per week. The facility has renewed the colony strain once in the past two years in order to assure competitiveness under field conditions.

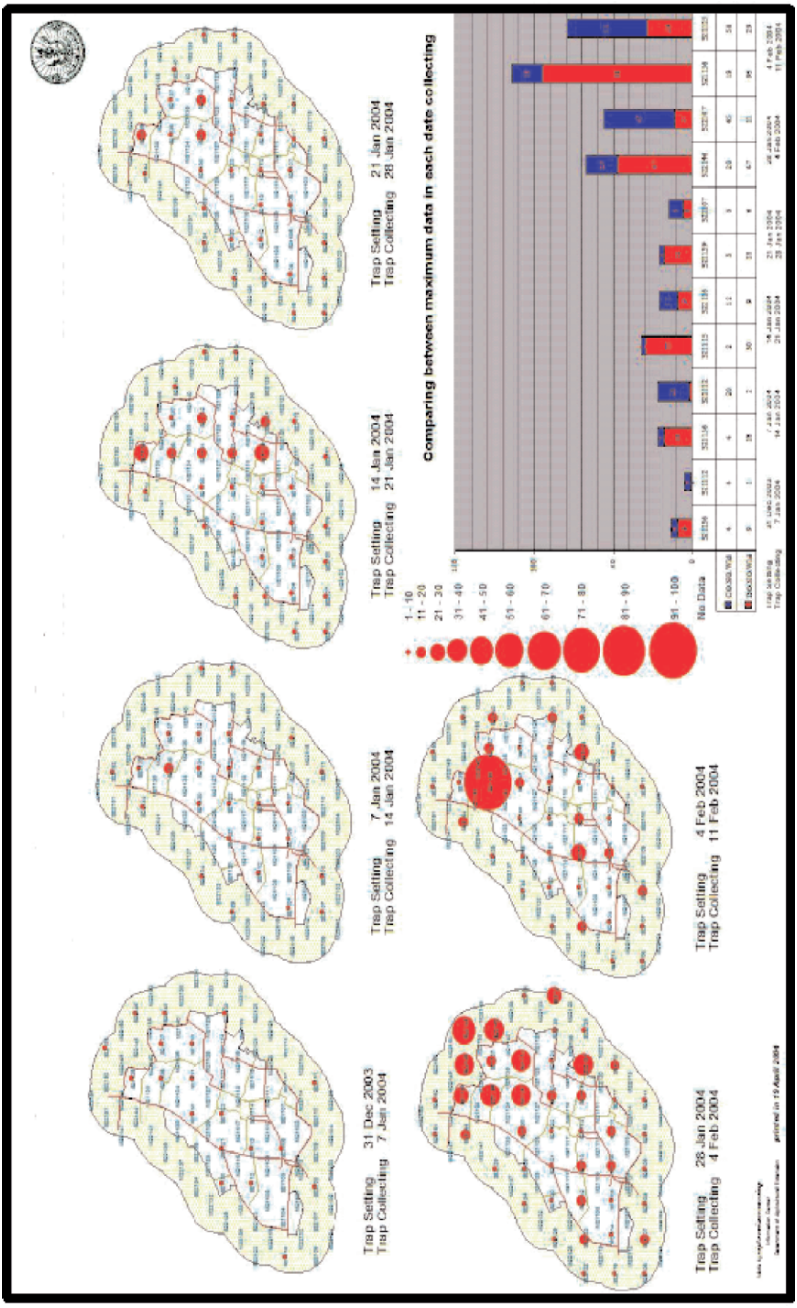

Figure 4. *The temporal and spatial dynamics of the oriental fruit fly population sampled with geo-referenced Steiner traps in Pichit province, Thailand. Number of captured flies in the trapping network were represented weekly as fly per trap per day (the solid points indicate the location and the size of the points is proportional with the size of the trap sample).*

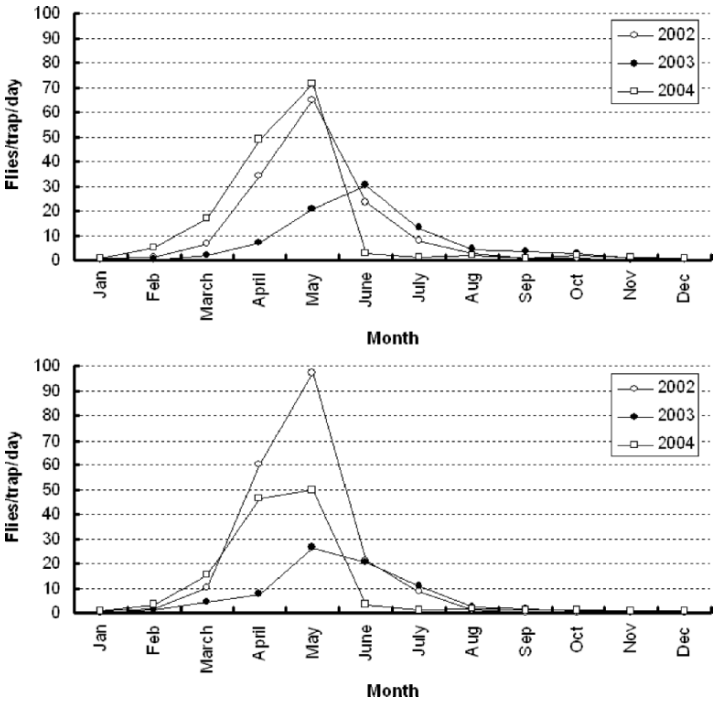

Figure 5. *Number of wild oriental fruit flies sampled per trap per day before (2002) and during integrated area-wide SIT application (2003-2004) in (upper graph) the core area and in (lower graph) the buffer zone in Pichit Province.*

2.2. Sterile Fly Releases

Weekly shipments of sterile pupae are carried out using a refrigerated truck and pupae are delivered directly to the emergence and holding facilities near pilot areas. Upon arrival at these facilities, the bags of pupae are taken out of the container and the pupae placed in plastic 800 cubic centimetre boxes (approximately 36 000 pupae per box) (Fig. 3).

At the release facility, sterile pupae are incubated at room temperature (26-27°C), and after emergence, sterile adults are taken in the boxes to the field for release. Fixed release

Table 1. *Ratio of average captured wild male* Bactrocera correcta *and* Bactrocera dorsalis *in the core area and the buffer zone of the project area in Ratchaburi and Pichit in 2002-2004.*

Province	Ratio *Bactrocera correcta* : *Bactrocera dorsalis*					
	2002		2003		2004	
	Core area	Buffer zone	Core area	Buffer zone	Core area	Buffer zone
Ratchaburi	3.4:1	No data	0.7:1	0.5:1	0.3:1	0.3:1
Pichit	1.5:1	1.4:1	1.1:1	1:1	0.9:1	1:1

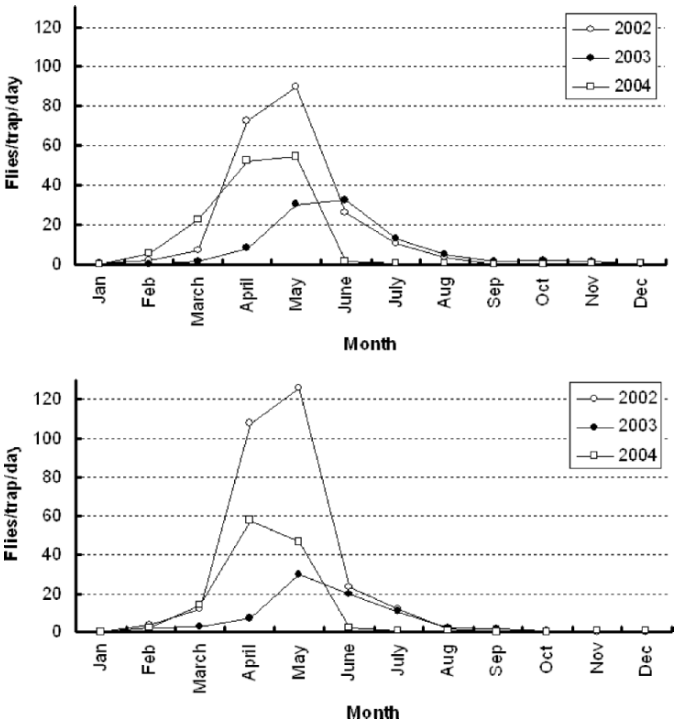

Figure 6. *Number of wild guava fruit flies sampled per trap per day before (2002) and after releasing sterile flies as part of an integrated area-wide pest management programme (2003-2004) (upper graph) in the core area and (lower graph) in the buffer zone in Pichit.*

sites are uniformly distributed in a grid-like pattern throughout the release areas using global positioning system (GPS) and geographic information systems (GIS) technologies. During the critical months (October to March), 2000 sterile oriental fruit flies and 1000 sterile guava fruit flies are released per hectare.

2.3. Monitoring Activities

Steiner traps baited with methyl eugenol and insecticide are placed for monitoring throughout the release areas at a density of one trap per square kilometre (IAEA 2003) in carefully selected sites. Traps are distributed in a grid-like array by using GPS and GIS. All the traps are inspected weekly. The flies are removed from the trap, identified and recorded into a GIS (Figs. 4, 5 and 6).

Based on data collected in 2002 from captured flies, it was found that the ratio of oriental:guava fruit flies in the core area (i.e. mango orchards) of Ratchaburi Province was reduced from 3.4:1 in 2002 to 0.7:1 in 2003 and to 0.3:1 in 2004. In Pichit Province they fell from 1.5:1 in 2002 to 1.1:1 and to 0.9:1 in 2003 and 2004, respectively as shown in Table 1. The target ratio of sterile males to wild males was set taking into account the strategic objective of the project, which is area-wide population suppression rather than eradication.

Fruit evaluation was done throughout the release area. Mangoes with signs of infestation were collected and maintained in plastic

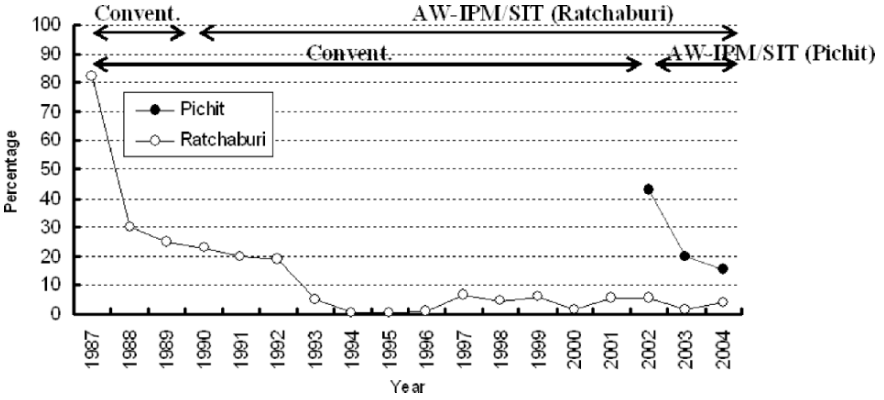

Figure 7. *Percentage of mangoes infested by the oriental and the guava fruit fly in pilot areas under area-wide integrated control in 2000-2004.*

boxes to observe fruit fly emergence and to assess fruit damage. After years of effort, the percentage infestation in Ratchaburi Province has been reduced to an average of less than 3.6% in the past five years (2000-2004). The effort has paid good dividends to a number of small mango growers. After two years under the control programme in Pichit Province, the percentage of fruit infested has been reduced from 43% in 2002 to 15.5% in 2004, as shown in Table 2 and Fig. 7.

2.4. Male Annihilation

The male annihilation technique (MAT), was applied area-wide to reduce populations of wild male oriental and guava fruit flies prior to implementing the SIT in Pichit Province in 2002. The field plan consisted basically of three MAT cycles for male population suppression followed by weekly sterile fly releases. MAT blocks were prepared by using wood fibreboard squares (4.5 x 4.5 x 0.9 centimetres) impregnated with approximately 10 millilitres of a solution containing 75% methyl eugenol, 20% malathion and 5% xylene. Ground distribution was done by hanging four MAT blocks per hectare per month over urban, commercial, non-commercial and wild host areas. The efficacy of the MAT blocks in reducing male *B. dorsalis* and *B. correcta* populations was evaluated by weekly trapping and transforming the trap catch into number of flies per trap per day (Figs. 5 and 6). The third MAT cycle was finalized at the end of January, after which sterile fly releases were routinely carried out since mid February 2002. Other integrated control methods applied along with the SIT included cutting wild hosts, orchard sanitation, applying baits and implementing a monitoring network.

3. Economic Assessment

An economic analysis, done retrospectively and also projected over 14 years (2001 to 2014), predicted, for an AW approach, a benefit/cost ratio of 7.5:1 and a net benefit of USD 7.5 million for the mango growers of Paktor, Ratchaburi Province compared to a benefit/cost ratio of 2:1 and a net benefit of USD 0.17 million for the low-input conventional control method (Enkerlin 2001). Furthermore, an economic assessment for current areas and areas proposed for expansion was conducted by Knight (2002) using a probabilistic approach and Monte Carlo simulation modelling. Results show that economic returns were favourable for all but one of the mango producing provinces in Thailand (Table 3). For the provinces with positive economic returns and given the parameters and

Table 2. *Percentage of mangoes infested by the oriental and guava fruit fly after implementing an integrated control programme that included the release of sterile flies in 2000-2004 in Ratchaburi and 2002-2004 in Pichit.*

Year	Ratchaburi		Pichit	
	No. of sampled fruits	% infested fruits	No. of sampled fruits	% infested fruits
1987	No data	82.0[1]	---	---
2000	1727	1.3[2]	---	---
2001	209	5.6[2]	---	---
2002	403	5.5[2]	119	42.9
2003	2316	1.5[3]	871	19.7
2004	2956	4.2[3]	2856	15.5

[1]Conventional control

[2]Release of sterile oriental fruit flies only

[3]Release of sterile oriental and guava fruit flies, integrated with other related methods

range of values used for the calculations, the most likely benefit/cost ratio values range between 1.26 and 2.47 and the most likely net present value (NPV) between USD 0.98 and 28.02 million. (Table 3) (Knight 2002). Thus expansion to other potential areas appears to be economically viable.

4. Conclusions

Mango growers in the pilot area of Paktor, Ratchaburi Province exported about 240 and 300 tons of mango respectively in 2003 and 2004 to countries where a fly-free certificate is not required (such as Canada, Malaysia, Singapore). However, further expansion of export from other mango production areas in Thailand can only be realized when issues related to pesticide residues and fruit quality are resolved. In addition, more than 2000 tons of mango production from the Pichit area has been exported to countries such as Japan only after attaining compliance using postharvest treatment and a phytosanitary certificate. As results indicate, infestation levels at the Pichit area are substantially higher than in the Ratchaburi area. This is mainly due to the fact that the implementation of an area-wide IPM programme in Pichit started only in 2003. Nevertheless, the Japanese market prefers mangoes from Pichit where growers are better organized, apply good agricultural practices and produce higher quality mangoes. This achievement has encouraged mango producers in other areas to adopt application of the SIT as part of an area-wide integrated fruit fly management programme.

This programme is one of an increasing

Table 3. *Net present value (NPV) and benefit/cost ratio (BCR) of the pilot AW-IPM project areas in Ratchaburi and Pichit provinces and predicted NPV and BCR values derived from a probabilistic and Monte Carlo simulation model of six other areas proposed for expansion.*

Province	NPV (USD million)	BCR
Phichit	0.98	1.26
Ratchaburi	5.72	2.21
Phetchaburi	41.48	3.11
Khon Kaen	-1.99	0.93
Phitsanulok	14.80	2.03
Prachuap Khiri Khon	8.38	2.06
Chacheksao	22.02	1.52
Uthai Thani	13.43	2.47

number of examples of the routinely integrated use of the SIT for the effective suppression of fruit flies. Nevertheless, substantial improvement and economics of scale are needed in order to apply cost-effectively the SIT on a larger scale. The basic requirements for scaling up the programme from a pilot to a nationwide project are available. However, this is a politically and logistically complex procedure that requires government commitment, grower organization, and continous international technical cooperation.

5. Acknowledgements

The authors wish to gratefully acknowledge the Joint FAO/IAEA Division and the Division of Asia and Pacific of the IAEA Department of Technical Cooperation for their commitment and strong support to the project. In addition, the efficient technical support from all recruited experts is much appreciated. Finally, the project could not have been successfully implemented without the cooperation and active involvement of the many growers and programme staff who broadly understand the concept and who have rendered support in many ways, resulting in tangible achievements.

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Establishment of a Mediterranean Fruit Fly *Ceratitis capitata*, Fruit Fly Parasitoids, and Codling Moth *Cydia pomonella* Rearing Facility in North-Eastern Brazil

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ABSTRACT The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is a major pest of fruit crops worldwide and its presence in many countries poses a threat for production and export. The methodology that has been integrated in many countries to contain, to eradicate, or to suppress Mediterranean fruit fly populations is the sterile insect technique (SIT). Besides the Mediterranean fruit fly, *Anastrepha* fruit fly species of quarantine importance are also important pests affecting fruit crops in Brazil. Parasitoids that are natural enemies of fruit flies will be mass-reared, together with Mediterranean fruit fly and *Anastrepha* species in the facility that is being established in Juazeiro, Bahia with the objective of suppressing fruit flies in the expanding commercial fruit production areas in the São Francisco river region. To take advantage of the availability of a large complex of buildings, the facility will eventually also produce sterile codling moth *Cydia pomonella* (L.), a pest recently introduced into southern Brazil, where it threatens the continuously growing apple and pear industries. The production of sterile Mediterranean fruit flies and sterile codling moths, as well as sterile host larvae for natural enemy production, will use gamma radiation as the sterilization method. The area-wide integrated pest management (AW-IPM) approach, including the release of these beneficial insects in the field, is the most effective means to control (suppress or in some situations even eradicate) such pests. The consumption of fresh fruits has increased worldwide and production in Brazil, either for domestic or export markets, has been intensified in the last decade. As food safety is becoming a major concern for consumers, the use of environment-friendly technologies such as the SIT will be increasingly required. The final clients for the sterile flies and moths will be the fruit growers. The foreseen weekly production of 200 million sterile Mediterranean fruit flies and 10 million *Diachasmimorpha longicaudata* (Ashmed) wasps will be released in the tropical fruit-growing areas of northern Brazil: Bahia, Pernambuco (São Francisco Valley), Ceará, north of Minas Gerais and north of Espirito Santo. The sterile codling moths would be sent by air to the apple and stone fruit production areas in the south of Brazil, Rio Grande do Sul and Santa Catarina, where this pest species is still confined to urban areas and hence amenable to eradication.

KEY WORDS *Ceratitis capitata*, *Anastrepha* spp., *Cydia pomonella*, *Diachasmimorpha longicaudata*, area-wide control, sterile insect technique, Brazil, São Francisco river valley

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1. Introduction

The sterile insect technique (SIT) is used in many countries as part of an area-wide integrated pest management (AW-IPM) approach to control (suppress, contain, prevent or eradicate) the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and other fruit fly pests. This expanding use has proven successful in protecting critical production areas from Mediterranean fruit fly infestation and embargos on fresh fruit exports worth thousands of millions of USD. The SIT is incorporated into fruit fly control programmes to minimize the continuous use of insecticides, protect the environment, and meet food safety standards. Fruit fly rearing facilities are in operation in Argentina, Australia, Chile, Guatemala, Israel, Mexico, Peru, the Philippines, Portugal, South Africa, Thailand, Tunisia, and the USA (Hawaii and Texas). Others are being planned or under construction in Costa Rica and Spain, and now in Brazil (Dyck et al. 2005). The Okinawa melon fly *Bactrocera cucurbitae* (Coquillett) mass-rearing facility in Japan is now producing low numbers of insects sufficient to maintain a continuous preventive release programme in the southern-most islands of the Okinawa archipelago that are susceptible to reinfestation since this pest was eradicated from that country (Koyama et al. 2004). In these programmes, the SIT has proven to be successful in the suppression, containment, prevention or eradication of fruit flies (Wong et al. 1992, Hendrichs 1996, Barry et al. 2003, Hendrichs et al. 2005). Other sterile insect factories are in operation to combat lepidopteran pests, screwworms, and tsetse flies (Dyck et al. 2005).

2. Fruit Production in Brazil

Brazil is one of the largest fruit producers in the world (38 million tons in 2004), being first in oranges (18.3 million tons in 2004), second in papayas (1.7 millions tons in 2003) and seventh in mangos (925 000 tons in 2003; also second in exports) (Brazilian Fruits Yearbook 2005). In the last 20-30 years, the federal and some state governments have developed many irrigation programmes in the semi-arid north-eastern part of Brazil focusing on the production of tropical, subtropical, and temperate fruit crops. As a result of such actions, the north-eastern region is the largest producer in the country of mangos, table grapes, melons, bananas, Antilles cherry, and guavas. The cultivated area is continuously increasing and at the end of 2004 there were, for example, 68 455 hectares of mangos in the whole of Brazil, of which about 20 000 hectares are in the São Francisco Valley (Brazilian Fruits Yearbook 2005). Most of the citrus production (800 000 hectares, as well all 35 000 hectares of apples) is concentrated in the southern states of Brazil.

Brazil exports annually around USD 400 million in fresh fruits (850 000 tons, which is only 2% of total production), and USD 1100 million in concentrated juices to Canada, Japan, and the USA, and to countries in the European Union (EU) and in the South Cone Free Trade Agreement (Mercosur) (Brazilian Fruits Yearbook 2005).

To a large extent, the fresh products export market requires some type of quarantine treatment. Fortunately, however, large fruit producing areas in north-eastern Brazil are deemed “low prevalence” in terms of pests and diseases, especially fruit flies. The very low humidity, high air temperature, and lack of host availability year-round keep fruit fly populations low. However, in areas where the harvest time for mangos was extended from the original 2-3 months to 6-8 months due to improvements in technology, wild fly populations can now increase dramatically in a few months. It is exactly in such areas of the North East that the integration of the SIT for suppression is both required and highly feasible.

The new agricultural frontier represented in the semi-arid North East is a consequence of four main factors: (1) improved infrastructure developed through governmental support, (2) high technology such as computer-controlled irrigation, hormone and physiological control of blossoming, IPM techniques, and

advanced postharvest processes, (3) positive environmental factors such as good soil, clean water, low relative humidity, high ambient temperature, and long periods of sunlight, and (4) a new generation of young entrepreneurs.

3. History of the Project

At the end of 2000, a group of researchers proposed the implementation of a Mediterranean fruit fly rearing facility in north-eastern Brazil to the Federal Government of Brazil in response to demands from the private sector for better (i.e. economically feasible and environment-friendly) technologies to control key fruit fly pests in this region. During 2001, the Plant Protection Department of the Ministry of Agriculture, Livestock and Food Supply called a series of meetings to bring together different stakeholders with an interest in the rearing facility. A Mediterranean fruit fly rearing facility working group was created by the Ministry of Agriculture, Livestock and Food Supply to carry out studies to assess the feasibility of a facility. In January 2002, the Ministry of Agriculture, Livestock and Food Supply called for an international mission to visit potential sites for the facility, make a first evaluation of its feasibility, and recommendations concerning the size and numbers of sterile flies to be produced by the facility. The mission from the International Atomic Energy Agency (IAEA), the United States Department of Agriculture (USDA) and Instituto de Sanidad y Calidad Agropecuaria in Mendoza, Argentina (ISCAMEN) recommended that the facility should be sited in the São Francisco Valley (Juazeiro-Petrolina) in the centre of the largest fruit production area of the country and also equidistant from most other fruit producing areas in north-eastern Brazil. This site was also selected because of the availability of an empty cotton processing plant that ironically was shut down due to the collapse of cotton production in the region because of heavy infestation by the boll weevil. The mission concluded that construction of a mass-rearing facility to produce sterile flies (SIT) for suppressing or eradicating medfly is feasible in the north-eastern fruit production areas of Brazil (Enkerlin et al. 2002). In addition the report states that:

> *...this environment-friendly technology, complemented by minimum use of organic bait to lower wild fly populations, is an important addition to the technologies supporting Brazilian agriculture. The introduction of this IPM component opens the door to other pest control tools such as fruit fly parasitoids and expanded use of sterile insects to control other fruit fly species of economic importance.*

Following these recommendations, the Mediterranean fruit fly rearing facility working group carried out studies to define the best legal framework for creating the new entity. Special attention was given to its format in order to avoid inflexibility and excessive bureaucracy, and to enable the establishment of alliances with the federal and state governments, international organizations and the private sector. In November 2002, the Biofábrica Moscamed Brasil (Mediterranean Fruit Fly Facility Brazil) was formally created as a social organization attached to the Ministry of Agriculture, Livestock and Food Supply, and organized with an administration council with federal, state and private representatives, and an executive board with three executive directors.

In January 2003, a technical group was created by the Biofábrica Moscamed Brasil to establish the technical parameters for the rearing facility on premises obtained from the Government of the State of Bahia. This group from the Food and Agriculture Organization of the United Nations (FAO), IAEA and USDA defined the baselines for the facility including the basic drawings, rearing flow, and technical specifications in terms of power and water requirements, air conditioning, and other utilities. Its general conclusions (Cáceres et al. 2003) were:

> *The building site in the State of Bahia located in the city of Juazeiro is ideal if the entire 5-hectares facility could be devoted to the rearing plant. This dedicated use would simplify management and provide adequate space for all pro-*

posed activities with some space for expansion. As built, the location and buildings would provide an excellent site close to all services, but with a great deal of isolation. Most of the covered buildings are in very good condition and the warehouses would be excellent structures to contain an insulated inner building where the rearing would take place. The covered structures would provide shade from the sun and protection from the rain. Air conditioning and other support equipment would also be protected from the elements and air temperatures would be much reduced, minimizing air conditioning needs and costs. The other numerous buildings could be used as warehouse space, offices, quality control, cafeteria, methods development, and other support activities.

An engineering company was then contracted with resources from the Ministry of Agriculture, Livestock and Food Supply. It prepared the master plan for the facility with inputs from the technical personnel in the programme and two visits to the El Pino Mediterranean fruit fly mass-rearing facility in Guatemala. During the planning phase in 2003, the executive board and administration council raised government funds for the necessary modifications to the existing facilities. In 2004, a Technical Cooperation project was approved by the IAEA to support the Brazilian government in implementing the SIT component. Funds from the Ministry of Agriculture, Livestock and Food Supply and the Ministry of Science and Technology became available in the middle of 2004 and construction was initiated late that year. The first building for the installation of the administration began to operate in April 2005, and it is planned that the rearing unit will start its operations in early 2006.

4. SIT Application in Selected Areas of Brazil

The increasingly expanding fruit crop growing areas in the Brazilian semi-arid north-eastern region need an innovative solution for the fruit fly problem since importing countries have imposed quarantine and sanitary barriers for the fresh fruit market. Although Brazil exports to the EU, Japan, and the USA, in order to retain its current markets, bring new fruit production areas into export markets, and reduce the use of insecticides, it is mandatory that there be low or no fruit fly populations present. To achieve these aims, it will be necessary to apply the SIT against the Mediterranean fruit fly and expand its use to other fruit flies and other agricultural pests.

The objective of applying the SIT in Brazil will admittedly be suppression of the Mediterranean fruit fly. Eradication might be reached in some ecologically isolated islands (called irrigation districts), that are, in general, tens of kilometres apart. However, all fruit production areas in the country contain multiple fruit fly species. Therefore, even though the suppression or selected eradication of the Mediterranean fruit fly is the most important first step, the need to control the other pest species will also be important. That is why it is planned to have, in a second phase, a facility for mass-rearing the parasitoid *Diachasmimorpha longicaudata* (Ashmed) for augmentative releases, in combination with sterile flies.

An outbreak of codling moth *Cydia pomonella* (L.) in southern Brazil is a threat to the apple industry in the region. Although a control programme is underway using mainly a host removal strategy, which has to a large extent been a success in confining the outbreak to four urban areas (Kovaleski et al., this volume), the use of the SIT for final eradication is highly recommended. For this reason, and because of wider biosecurity considerations (codling moth cannot become established where the facility is located due to high temperatures and lack of hosts), a small to medium-sized colony of *C. pomonella* is planned in the same complex, as a second phase to be initiated in the middle of 2006.

The SIT is not an easy technology to apply and needs careful planning and continuous supervision. Hence, in addition to providing sterile males for the private sector, the programme could also provide a "total package" that would include monitoring fruit fly densities in the field, processing, retrieving, and storing information, chemical control when

necessary to reduce populations prior to releases of sterile males, and public relation and extension activities.

Regarding the SIT *per se*, a key element to the success of the programme is the design, construction, and operation of a sterile fly mass-rearing facility capable of producing sufficient sterile insects of good quality and at reasonable cost, in combination with efficient SIT programme implementation and support to growers, the public and government. Since a high degree of responsiveness to clients' needs promotes sustainability, the initial focus of the programme is on effectiveness and cost efficiency unless other mitigating issues take precedence. Factors such as the environment, public health, public and political opinion, and the future direction of plant health regulations could influence the use of the SIT, even if the initial cost efficiency is less than optimal.

5. Plans and Concept

The initial kick-off for the programme was the positive political decision by the Federal Government of Brazil, especially the Ministry of Agriculture, Livestock and Food Supply, with strong support from the Government of the State of Bahia. Providing the plant site was the first step, followed by the building process that required a large investment. The federal and state governments are responsible for all funds earmarked for the building phase (around USD 4.5 million), with the fruit industry providing the essential political support to the government agencies. After the operation is initiated, industry – through the growers associations and cooperative farms – will gradually compensate the Biofábrica Moscamed Brasil for the services being delivered to the farms, which will encompass monitoring and releases of sterile males.

Presently, in most fruit production areas, the trapping system in place is grower-operated with different arrangements in place according to the region and always supervised directly by the Ministry of Agriculture, Livestock and Food Supply or by the state plant protection agencies. Hence the growers are already paying for the trapping services. The proposal now – which is supported by the industry – is to have Biofábrica Moscamed Brasil manage the trapping operations since these provide essential information for use of the SIT and for the other control measures that are needed.

The integrated area-wide approach, fundamental for a successful programme, is the concept to be applied and a public dissemination campaign to explain the concept and its operation will be essential to have large, middle and small growers join the programme. The broad acceptance of, and participation in, the fruit fly management programme is largely expected because a low prevalence of fruit fly populations is one of the requirements to export mangos into the USA and Japan, and the EU also now requires new processes for fruit production.

After the government allocates the seed money to build the facility, the operation is expected to be sustainable within a few years. An additional source of funding could be the export of sterile Mediterranean fruit flies. Although a large number of sterile Mediterranean fruit flies are being produced in other mass-rearing facilities around the world, demand is still larger than this production capacity. In addition, Biofábrica Moscamed Brasil production can be destined for local use in future area-wide IPM projects involving the SIT in other regions of Brazil. For example, the El Pino mass-rearing facility in Guatemala exports millions of flies per week to California and Florida in the USA and also to Israel in the past. Argentina, Mexico, Portugal, and USA mass-rearing facilities in Texas and Hawaii have also supplied other users. Modest amounts of profit obtained from such transactions reduce plant production costs. The ability to ship pupae long distances and the technology under development for shipping Mediterranean fruit fly eggs to international clients, adds greatly to plant utility, financial viability, and overall cost effectiveness.

6. Strategic Alliances

A key aspect for a fruit fly management pro-

gramme that includes the SIT is to have alliances in the national and international arenas. The federal and state governments are the natural national alliances. These are key partners since, amongst others, the importation of biological material from foreign sources requires permits and federal government approval, the transshipment of flies from one state to another requires state government collaboration, and the development of technical alliances with other international entities is dependent on federal and state government participation. Within these, the Ministry of Agriculture, Livestock and Food Supply, the Ministries of Science and Technology and of National Integration, and the state governments through the agencies for plant protection in Bahia, Pernambuco, and Ceará are particularly critical for the success of the programme.

Important support for research and development is provided by two research centres, the Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)), i.e. Embrapa Cassava and Fruits, and Embrapa Tropical Semi-Arid. Other important partners are the Centre for Nuclear Energy in Agriculture (CENA) and the Institute of Biosciences of the University of São Paulo (USP). These have helped the Biofábrica Moscamed Brasil implementation since the beginning, including in planning, training, carrying out applied research, and permanent overseeing.

In the industry, the Biofábrica Moscamed Brasil has the support from three major grower associations, the Fruit and Vegetables Export Association of São Francisco Valley (Valexport), the Brazilian Fruit Institute (IBRAF) and the Brazilian Papaya Export Association (BRAPEX). The private sector has also contributed financial and political support.

The IAEA/FAO, as international organizations have also given support. This included supporting the first mission to define the site of the plant in 2001, and a current Technical Cooperation Project that covers the endowment of a ^{60}Co irradiator, and the costs of short-term expert visits and training for the newly-hired personnel. Two professionals from EMBRAPA and CENA were trained for three months in the rearing, irradiation and quality control aspects of the Mediterranean fruit fly *temperature sensitive lethal* (*tsl*) genetic sexing strains at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf in Austria. Additionally, through the El Pino Mediterranean Fruit Fly Facility in Guatemala, the United States Department of Agriculture's Animal and Plant Protection Service (USDA-APHIS) has systematically provided technical assistance since the beginning of the project, with directors' visits to Brazil, and Brazilian professionals visiting Guatemala for training and obtaining information on the facility in order to design and prepare the operational aspects of the Biofábrica Moscamed Brasil. Finally, ISCAMEN, Argentina, has given technical advice and sent sterile Mediterranean fruit flies for preliminary tests in Brazil.

7. Research and Development Component

The Biofábrica Moscamed Brasil is the first facility in Brazil to produce sterile insects on a large scale and, as a result, there is a small critical mass of trained personnel in the country. There is however, no information regarding the effectiveness of the SIT under semi-arid conditions. A set of experiments and large-scale field tests were planned and are being carried out to answer basic questions concerning the mating competitiveness of *tsl* strain VIENNA 8 (Franz 2005), sterile males competing with wild males for wild females, dispersion and longevity of sterile males under semi-arid conditions and related topics. In collaboration with the Agricultural Research Service (ARS) of USDA, the University of Tessaly in Greece, and the Joint FAO/IAEA Programme, one staff member is leading a group in EMBRAPA to answer these questions with financial support from the Bank of Nordeste.

Large pilot tests are also planned for two

Table 1. *Production and basic biological data of the VIENNA 8 genetic sexing strain colony at USP-CENA, Piracicaba, SP, Brazil.*

Generation[1] (month/year)	Eggs[2] (ml)	Egg viability (%)	Number of pupae (x 1000)	Male viability (%)	Female viability (%)	Sex error in brown pupae (%)	Sex error in white pupae (%)
F_{10} (Dec/04)	12.5	24.7	48	63.1	85.1	1.18	0.00
F_{11} (Jan/05)	19.1	77.2	1257	84.9	80.3	0.30	0.00
F_{12} (Feb/05)	64.3	75.8	1680	76.9	86.6	0.85	0.18
F_{13} (Mar/05)	52.2	63.8	1430	83.5	90.7	0.50	0.00
F_{14} (Apr/05)	98.2	71.6	1390	83.1	89.6	0.26	0.08
F_{15} (May/05)	243.5	68.3	3240	n.a.	n.a	n.a	n.a

[1]The VIENNA 8 pupae received from the FAO/ IAEA Agriculture and Biotechnology Laboratory were from generation F_9 (Carlos Cáceres, personal communication)
[2]Not all collected eggs were used for pupal production

mango areas – 3000 and 5000 hectares – where the Mediterranean fruit fly is historically reported as being of economic importance. Sterile males will be brought from ISCAMEN and released in the experimental areas after a detailed survey and following suppression measures taken when the feral population is high. For these pilot tests, partnerships were established with EMBRAPA, the Bahia Animal and Plant Protection Agency (ADAB), and the University of south-western Bahia (Universidade Estadual do Sudoeste da Bahia (UESB)) to have good entomological and logistical support to carry them out.

At the USP-CENA laboratories, the VIENNA 8 strain received from the FAO/IAEA Agriculture and Biotechnology Laboratory in December 2004 was successfully adapted to a larval diet, which was developed in 2002 with local ingredients (Walder 2002). This diet contains sugarcane bagasse as a bulking agent, wheat germ and brewer yeast as protein sources, sugar and wheat flour as phagostimulants and carbohydrate sources, an antibiotic as bacterial growth inhibitor, sodium benzoate to avoid fungal growth and hydrochloric acid as pH regulator. The rearing protocol is very similar to that recommended by Cáceres (2002). Studies using several quality tests including gamma sterilization are being applied under laboratory conditions (FAO/IAEA/USDA 2003). After six generations the strain shows good quality and stability (Table 1), enabling colony expansions to be planned for subsequent generations.

USP-CENA will be responsible for maintaining the original strain imported from the FAO/IAEA Agriculture and Biotechnology Laboratory and transferring it to the Biofábrica Moscamed Brasil when the mass-rearing process starts. Also the tests for improving the local diet and the quality control of the rearing will be supervised by USP-CENA.

8. Conclusions

The location of the Biofábrica Moscamed Brasil in the São Francisco Valley is right in the centre of mango and many other fruit production farms. This close proximity to production areas should promote more private sector interest and commitment. Private sector investment would be enhanced if the Mediterranean fruit fly rearing facility was an integral part of the community where the stakeholders live and work, since local producers and public sector officials are in a better position to support the plant and resolve any

problems that might arise locally or regionally.

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Pilot Mediterranean Fruit Fly *Ceratitis capitata* Rearing Facility in Tunisia: Constraints and Prospects

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ABSTRACT The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) mass-rearing facility, located at the Centre National des Sciences et Technologies Nucléaires, Sidi Thabet, northern part of Tunisia, was specifically designed for rearing genetic sexing strains of this species. Rearing operations were initiated in 2004 to supply sterile males for a pilot project to control the Mediterranean fruit fly in 5800 hectares of citrus plantations in the Cap Bon peninsula. The peninsula contains the largest growing area in Tunisia for citrus destined for export. The project is supported by the Tunisian Government, the International Atomic Energy Agency (IAEA), and the Food and Agriculture Organization of the United Nations (FAO). During the first year of operations, fly production was unstable due to fluctuations in the environmental conditions, the lack of regular supply of essential larval diet ingredients, and the frequent breakdown of some essential equipment. As a result, the numbers of flies produced was reduced and/or the quality of the sterile males impaired. These problems were solved through the fine-tuning of all rearing procedures, adjustments to the environmental control system, and the introduction of quality control procedures consistent with those described in the FAO/IAEA/USDA manual for each procedure and phase involved in the production of sterile male adults. By the end of 2004, an average of six million sterile pupae were produced per week. Further improvements and adjustments in all the rearing steps and protocols are necessary to obtain the maximal sterile pupae capacity of 12 million per week.

KEY WORDS *Ceratitis capitata*, Mediterranean fruit fly, mass-rearing, quality control, genetic sexing, sterile males, Tunisia

1. Introduction

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is a major problem for fruit production in Tunisia although data on exact losses are not readily available. Infestation rates in fruit in orchards treated with conventional broad-spectrum insecticides are around 10%, but this can increase to 55% for areas without chemical treatment (Knight 2001). The Cap

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 535–543.

Bon, with a total surface area of 300 000 hectares, has 186 000 hectares of cultiviable land of which 14 000 hectares are planted with citrus. This makes the Cap Bon the main citrus production area in Tunisia, contributing to 80% of the total national production. Ninety eight percent of the citrus area is cultivated with the cultivar "Maltaise", that is mostly destined for export. New local regulations and the increasing demand of foreign export markets for fresh commodities without pesticide residues, makes the use of the sterile insect technique (SIT), as a component of an area-wide integrated pest management (AW-IPM) approach of this pest, an attractive option.

The SIT for the control or suppression of the Mediterranean fruit fly was tested for the first time in Tunisia in the 1970s through a joint project between the Tunisian Government and the United States Department of Agriculture (USDA) (Cheikh and Ben Salah 1975). This pilot project used releases of a conventional bisexual strain that was reared in a small rearing facility at the Institut National de Recherche Agronomique de Tunis (INRAT). Sterile insect releases were carried out in a 600 hectare pilot area in northern Tunisia (Ghar el Melh), but due to inefficient distribution of the sterile flies, insufficient geographic isolation of the target area, and the absence of an effective buffer zone between the target area and the surrounding areas with non-commercial hosts, this project did not lead to the successful control of the Mediterranean fruit fly population.

During the early 1990s, Tunisia participated in a regional feasibility study, co-funded by the International Atomic Energy Agency (IAEA), and the Food and Agriculture Organization of the United Nations (FAO), that assessed the potential of controlling the Mediterranean fruit fly in the Maghreb region through an integrated pest management approach with an SIT component (Buyckx 1994). During ten consecutive months, an experimental project was conducted in the mountain oases in southern Tunisia to assess the field effectiveness of a genetic sexing strain that carried a *temperature sensitive lethal* (*tsl*) gene. The pupae were produced at the FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf, Austria, and then shipped by air to the fly emergence and release centre at Tozeur (Cayol and Zarai 1999). The results from this trial confirmed that systematic releases of only sterile males induced sterility in the wild population and were able to reduce the density of the wild population. The project likewise demonstrated that considerable savings could be made using the male-only strain as compared to the release of conventional bisexual strains.

In 2001-2002, the Tunisian Government together with citrus growers initiated a project supported by the IAEA and the FAO, with the main objective of suppressing the Mediterranean fruit fly through an integrated area-wide approach in commercial citrus production and export areas and thereby reducing insecticide applications against this pest. In the first phase of this project, a small-scale rearing facility was established at the Centre National des Sciences et Technologies Nucléaires (CNSTN) in the Sidi Thabet technopole to provide a reliable local source of sterile flies for release in a commercial citrus orchard. The 300 square metre facility, specifically designed to rear the *tsl* genetic sexing strain of the Mediterranean fruit fly (Franz 2005), has a production capacity of 10-12 million of sterile male pupae per week. The facility was constructed in 2001-2002 and rearing operations were initiated in February 2004. The flies produced in the facility were used for integrated fly suppression operations in a 5300 hectare pilot area near Hammamet located in the Cap Bon peninsula. The main control area consisted of 2500 hectares of citrus and 2800 hectares as a buffer zone to prevent the immigration of fertile female insects into the core area. The scheduled release density of 1000 sterile male flies per hectare per week demands a stable production of at least six million sterile male pupae per week.

2. Materials and Methods

2.1. Fly Strain

The VIENNA 8 genetic sexing strain of the

Mediterranean fruit fly that carries a *temperature sensitive lethal* (*tsl*) gene is the strain produced in the rearing facility at Sidi Thabet. In addition to the *tsl* mutation that makes the females sensitive to temperatures above 32°C (Franz and Kerremans 1994, Willhoeft et al. 1996), the females carry a second mutation resulting in white pupae (*wp*) – this in contrast to the male pupae that are brown. The rearing of such a genetic sexing strain requires the maintenance of two parallel lines of production: (1) the first one is dedicated to the maintenance of the colony, and (2) the second one for the production of males (Cáceres et al. 2000). In the male production process, the *tsl* selectable marker is used to separate the sexes at the embryo stage by killing the females through exposure to high temperature. The *wp* selectable marker is used to monitor the genetic stability of the strain: emergence of males from white pupae or females from brown pupae is indicative of the degree of breakdown of the genetic sexing strain (Franz et al. 1996).

2.2. Production System

To prevent the accumulation of undesirable genotypes that would result in the genetic breakdown of the strain, a filter rearing system was introduced that maintains a small mother colony under relaxed conditions and low insect densities in the cages (Cáceres et al. 2004). A certain proportion of pupae from this mother colony are subjected to a selection process so that only males and females emerging from pupae with the correct pupal colour are returned to the mother colony. Eggs from the mother colony are used to initiate an amplification process for three to four generations to build up a large colony, from which the resulting male pupae are sterilized for subsequent releases. In such a filter rearing system, no insects that have been through this amplification process are returned to the mother colony, and therefore there is no accumulation of undesirable genotypes in the colony (Cáceres et al. 2004). The mother colony provides sufficient offspring to sustain itself and also to provide surplus eggs for the system of unidirectional amplification. At the end of amplification phase sufficient eggs are produced for thermal treatment and elimination of the female embryos to produce only male flies for sterilization and field release.

2.3. Facility Description

The rearing facility at Sidi Thabet has two egging rooms that contain the cages for the amplifying colonies and separate areas for the different larval rearing stages, separation of the pupation medium, pupal synchronization, quality control, washing, and diet disposal. The adult rearing cages were locally designed based upon the specifications for rearing the

Table 1. *Climatic conditions of the different Mediterranean fruit fly rearing rooms of the rearing facility in Sidi Thabet, Tunisia.*

Room type	Surface area (square metres)	Temperature (°C)	Relative humidity (%)
Egging	20	23	60-70
Larval initiation, male-only colony	10	30	80
Larval initiation, injection and release colony	10	25	80
Larval maturation	15	22	80-85
Larval collection	20	18	85
Pupae maturation	15	20	65-70

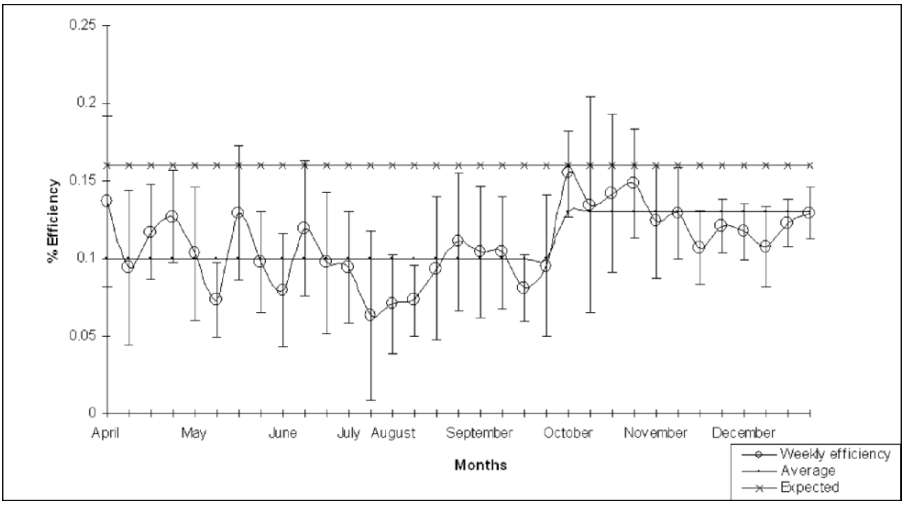

Figure 1. *The weekly percentage rearing efficiency (± SD) of the genetic sexing strain VIENNA 8 of the Mediterranean fruit fly maintained in the rearing facility at Sidi Thabet, Tunisia in 2004, expressed as pupal recovery per eggs seeded. Weekly percentage efficiency was calculated by averaging the daily pupal recovery for each week. The top line (pupal recovery of 0.16) indicates the expected value for the VIENNA 8 strain.*

VIENNA 8 strain (Cáceres et al. 2004). The colony is maintained in 24 cages that produce 400 millilitres of eggs daily for the three amplification streams. The eggs are seeded on

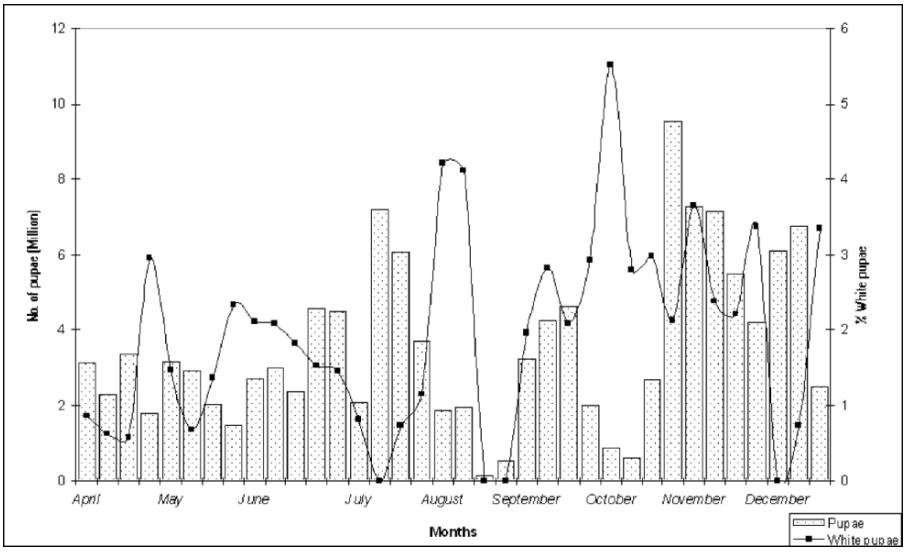

Figure 2. *Total numbers of male-only pupae of Mediterranean fruit fly shipped to the release and emergence centre in Cap Bon during 2004 (bars) and the percentage of white pupae (line) within these shipments.*

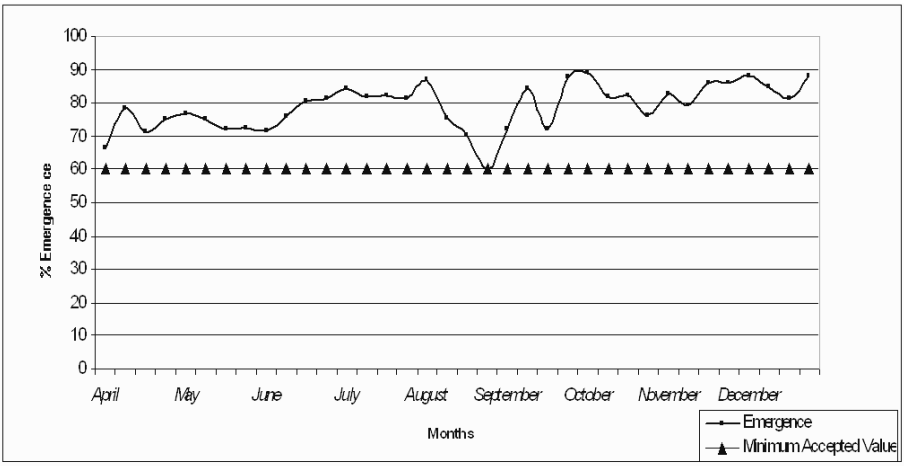

Figure 3. *Emergence of sterile male Mediterranean fruit flies in 2004 after irradiation of pupae with 100 Gy. The minimum value accepted for emergence is 60% (line with solid triangles).*

the larval diet that is based on wheat bran as described by Tanaka et al. (1969). The rearing area for the larval stage, either for male-only or colony production, is divided into different rooms with specific environments for each development stage as specified in Table 1. There are common rooms for the larval maturation and collection stages. Mature larvae are collected every 24 hours in specific pans that contain sawdust as the pupation medium. Pupae are separated from the pupation medium 24 hours after pupation and then each two litres of pupae are put into a pupae maturation tray. Forty eight to 24 hours before adult emergence, pupae are dyed with a fluorescent powder (DayGlo®) and bagged after two hours of hypoxia. The mature male pupae are then irradiated at a minimum dose of 100 Gy before being shipped to the packing and release centre.

3. Results

3.1. Efficiency of Male-Only Pupal Production

Since sterile male pupae are the final product delivered to the field operations, the production of male pupae was selected to measure the stability of the production process. Rearing efficiency was quantified by measuring the egg to pupae survival (i.e. recovery of pupae from a given number of eggs that were transferred to the larval diet). During the first seven months of mass production (April-October 2004), the average weekly rearing efficiency (i.e. the daily recovery averaged over a week) fluctuated from a maximum of 0.15 to a minimum of 0.06 (Fig. 1). These rearing efficiency values deviated considerably from the recovery value of 0.16 established as the expected efficiency for the genetic sexing strain VIENNA 8 (Cáceres et al. 2000). The suboptimal recovery values could mainly be attributed to: (1) the substitution of hydrochloric acid by citric acid, which is less efficient in preventing microbial development in the larval diet, (2) the use of locally-purchased wheat bran or barley bagasse of irregular quality, and (3) suboptimal climatic conditions due to frequent breakdowns in the air conditioning system.

Significant improvements in production were observed from October 2004 onwards when appropriate larval ingredients were used and the environmental control problems were

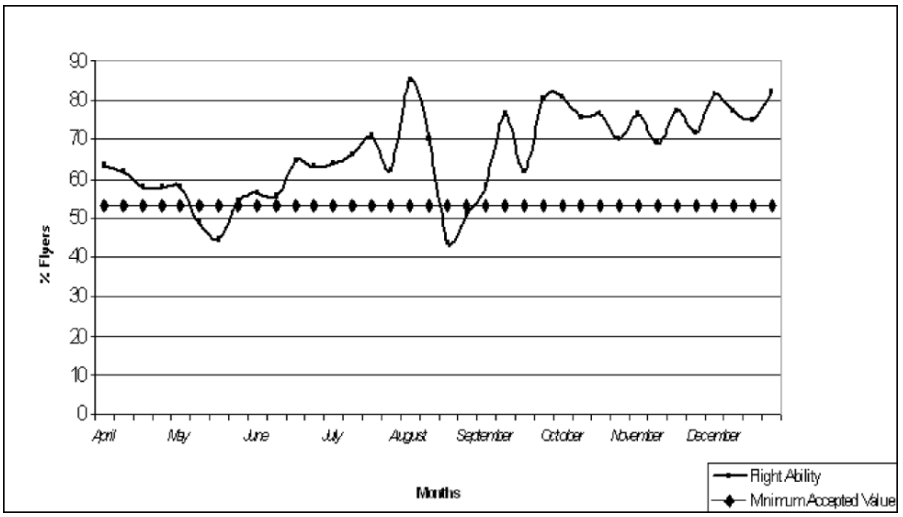

Figure 4. *Flight ability of sterile male Mediterranean fruit flies in 2004 after irradiation of pupae with 100 Gy. Line with solid diamonds indicate the minimum accepted value.*

fixed. Under more stable production conditions it was possible to reach acceptable values of recovery (86% of the expected efficiency for this strain) (Fig. 1).

In February 2004, shipments of sterile Mediterranean fruit fly pupae to the emergence and release centre near the pilot field site in Cap Bon were initiated. Due to the frequent interruptions in fly production, the number of sterile male pupae delivered per shipment between week 1 and 32 fluctuated between 0.5 and 4.5 million.

Improvements of the rearing efficiency resulted in a more stable fly production as of week 34 in 2004 and an average of 6 million sterile male pupae were shipped per week to the project site. Of these, an average of 98% were males (Fig. 2).

3.2. Quality Control

The procedures used to monitor the quality of all development stages were those described in FAO/IAEA/USDA (2003). Summaries of the results of the quality control test for the first year of production (Table 2) show these to be satisfactory. Pupal weight for the male-only production system was on average 7.94 ± 0.96 milligram, and emergence and flight ability for male-only production after irradiation increased during the year, becoming more stable thereafter at levels above acceptable means (FAO/IAEA/USDA 2003) (Fig. 2, 3 and 4).

4. Discussion

Production at the Tunisian Mediterranean fruit fly pilot rearing facility has the potential to meet the sterile fly needs for the selected pilot area. In the last two months of 2004, suitable rearing conditions were established with acceptable quality control values and the facility produced on average 6 million sterile male pupae per week (Fig. 2), which is only half of the facility production potential. During the period reported, 98% of the delivered pupae were male flies with acceptable values of quality as shown in Table 2 and Fig. 3 and Fig. 4. Unstable production during the early stages was due to several constraints, e.g. lack of or low quality of the local larval diet ingredients, deficient environmental control and rearing equipment breakdown. This

Table 2. *Average values for the main quality control parameters at the Mediterranean fruit fly rearing facility for sterile male-only production located in Sidi Thabet, Tunisia.*

Parameter	Average	SD	Acceptable mean[1]	Minimum[1]
Pupal weight (milligrams)	7.94	0.96	7.2	6.7
Proportion of males (%)	98.14	2.75	99.0	95.0
Emergence rate (%)	78.49	10.76	70.0	60.0
Proportion of male flyers (%)	69.09	14.82	65.0	53.0

[1]As defined in the "FAO/IAEA/USDA manual for product quality control and shipping procedures for sterile mass-reared tephritid fruit flies" (FAO/IAEA/USDA 2003)

confirms that mass-rearing of insects is an industrial operation that requires stable supplies of good quality dietary ingredients for larvae and adults, as well as appropriate and stable environmental rearing conditions (Vargas et al. 1983, Schwarz et al. 1985).

Diet quality had a direct impact on the production profile. The most important factors that determine the quality of the diet are the physical characteristics, which are mainly defined by the type and characteristics of the bulking agent. Bulking compounds must provide a suitable structure for the larval medium to allow uniform distribution of the other nutritive ingredients and to serve as larval feeding substrate. Lack of these characteristics affects the normal development of larvae or can induce larval mortality, resulting in poor quality and low numbers of insects (Singh 1977, Vargas et al. 1983, Vargas and Mitchell 1987, Vargas 1989). This happened when the imported wheat bran was replaced by barley bagasse, although it was reported that the latter was an efficient bulking ingredient for rearing Mediterranean fruit flies on a large laboratory scale (Cheikh and Ben Salah 1977). Also, the local wheat bran tested did not have the desirable characteristics and gave unsatisfactory results. Thus, until a suitable local bulking agent is identified, it is essential to continue using imported wheat bran.

As females are sensitive to high temperatures during their entire life cycle (Cáceres et al. 2000), stable environmental conditions are required particularly for the colony maintenance. Some of the production breakdowns, especially at the beginning, were due to the failure of the environmental conditioning system, which caused female mortality in the adult and larval stages. In the filter rearing system the number of females available is almost the exact number required for each amplification colony. Therefore, any breakdown during the amplification process can lead to lack of eggs for male-only production until the size of each amplification stream is re-established (Cáceres et al. 2004).

The main conclusion is that the integrated use of the SIT for suppression of the Mediterranean fruit fly, in the Cap Bon region of Tunisia compares favourably with the current control methods employed when considering the economics (Knight 2001). This encouraged the search for potential markets that might justify the development of a large-scale production facility. The Tunisian authorities and the private fruit industry (Groupement Interprofessionel des Fruits) are willing to expand the SIT project to the commercial fruit production area of Cap Bon and to enlarge its application.

5. Conclusions

Results indicated that the establishment of a control programme with an SIT component in Tunisia for suppression of Mediterranean fruit fly based on the regular release of good quality sterile insects, locally produced at competitive cost, could be achievable. Preliminary

results obtained at the Tunisian Mediterranean fruit fly pilot rearing facility have shown that industrial production of sterile insects in Tunisia is possible at a reasonable cost. At present, the Tunisian Government through the Centre National des Sciences et Technologies Nucléaires is investing around USD 200 000 per year to maintain the operation of the facility. This means that due to the low rearing efficiency, the cost per million of sterile pupae is on average USD 750, which is much higher than the cost of USD 480 per million under stable production of at least eight million per week and in relation to the cost reported for other facilities (Hendrichs et al. 2002). Further efforts are therefore needed to stabilize production and reduce operational costs. The success of the SIT pilot project in the Cap Bon peninsula is of high importance since it could be used as reference to facilitate the implementation of large scale integrated SIT programmes in Tunisia for the suppression of Mediterranean fruit fly.

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Section 7

Operational AW-IPM Programmes

Progress of Boll Weevil *Anthonomus grandis* Eradication in the United States of America, 2005

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ABSTRACT The boll weevil *Anthonomus grandis* Boheman eradication programme in the USA began in 1983 to rid the Cotton Belt of this pest. To date, the boll weevil has been eradicated from nearly 5.3 million hectares of cotton in Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Kansas, California, Arizona, and portions of Tennessee, Mississippi, Missouri, Arkansas, Louisiana, Oklahoma, Texas, and New Mexico; as well as from the neighbouring regions of Caborca, the Mexicali Valley and Sonoita in Mexico. The programme is currently operating in the remaining 1.42 million hectares of cotton in Arkansas, Louisiana, Mississippi, Missouri, New Mexico, Oklahoma, Tennessee, and Texas. As of this writing, 100% of the US Cotton Belt is involved in boll weevil eradication, with nearly 80% having completed eradication and the remaining 20% nearing eradication. Nationwide eradication in the USA is expected by 2008. The remarkable environmental and economic benefits realized within the eradicated regions make boll weevil eradication one of the most important agricultural programmes in the history of the USA.

KEY WORDS *Anthonomus grandis*, cotton, area-wide eradication, USA, environmental and economic benefits

1. Introduction

The boll weevil *Anthonomus grandis* Boheman, a native of Mexico and Central America, was first introduced into the USA near Brownsville, Texas, about 1892 (Hunter and Hinds 1905). By 1922, the pest had spread into cotton-growing areas of the USA from the eastern two thirds of Texas and Oklahoma to the Atlantic Ocean. Northern and western portions of Texas were colonized by the boll weevil between 1953 and 1966 (Newsom and Brazzel 1968).

In view of the economic and environmental problems posed by the boll weevil and its control, and in recognition of the technical advances developed during more than 80 years of research, a cooperative boll weevil eradication experiment was initiated in 1971 in southern Mississippi and parts of Alabama and Louisiana (Parencia 1978, Perkins 1980). This experiment used an area-wide integrated control approach including chemical treatment, release of sterile male weevils, mass-trapping, and cultural control. Based on this experiment, a special study committee of the National Cotton Council of America concluded that it was technically and operationally feasible to eliminate the boll weevil from the USA.

Subsequent discussions among federal and state research agencies, extension and regulatory officials, and grower organizations led to a decision by the United States Department of Agriculture (USDA) in 1977 to conduct two additional area-wide boll weevil eradication trials in Mississippi, and in North Carolina and Virginia. The success of these three-year boll weevil eradication trials, initiated in 1978 on 14 528 hectares in North Carolina and

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 547–558.

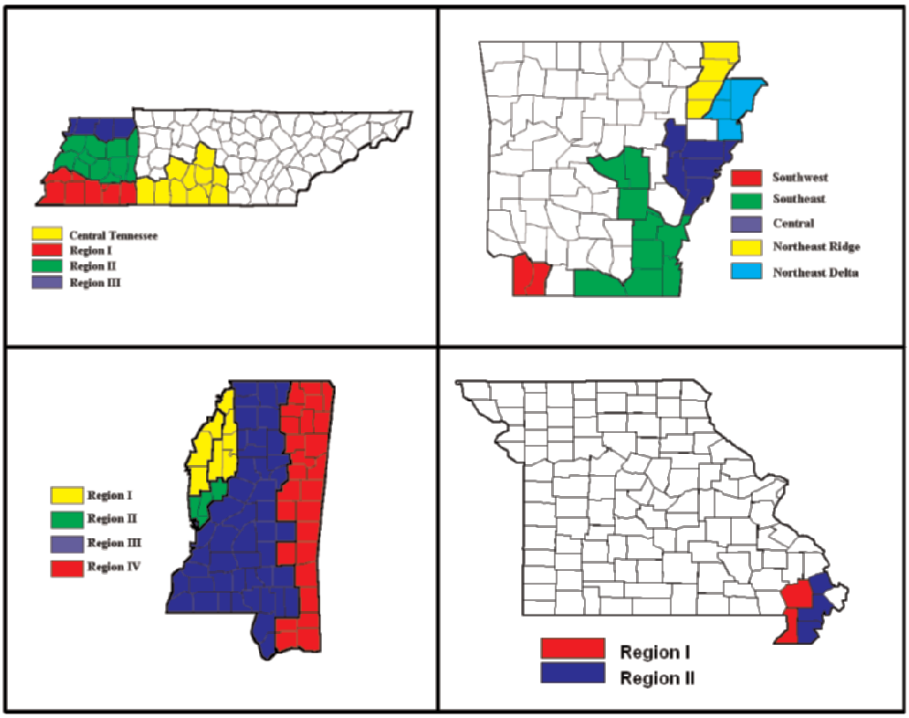

Figure 1. *The boll weevil eradication zones in (upper, left) Tennessee, (upper, right) Arkansas, (lower, left) Mississippi, and (lower, right) Missouri.*

Virginia, and on 12 950 hectares in Mississippi, led to the cooperative boll weevil eradication programme in the USA (USDA 1991).

The use of pheromone-baited traps for detection, along with sound cultural practices and timely insecticide treatments, represented the overall strategy for boll weevil eradication. Although, extensive work had been devoted to make the sterile male technique a part of boll weevil eradication, it was evident in the 1980s that pheromone traps and the cultural and chemical control methods were simpler, more reliable, more effective, and cheaper than sterile males (McKibben et al. 2001). Irradiation was one of the first methods used to sterilize boll weevils, and it was the last method to be tried. Doses of irradiation large enough to produce sterility caused unacceptably high levels of mortality (Davich and Lindquist 1962). Feeding, dipping, or fumigation with various sterilizing chemicals was tried along with irradiation (Haynes 1963, Lindquist et al. 1964, Davich et al. 1969, Gassner et al. 1974, Earle and Leopold 1975, Haynes et al. 1975, McHaffey and Borkovec 1976, Borkovec et al. 1978), with similar results and drawbacks.

The cooperative boll weevil eradication programme began in southern North Carolina (6000 hectares) and South Carolina (28 000 hectares) in 1983, after the successful three-year boll weevil eradication trial in North Carolina and Virginia. The programme expanded into Georgia (116 500 hectares), Florida (43 000 hectares) and south-eastern Alabama (25 000 hectares) in 1987, and into middle Tennessee (4500 hectares) in 1994 (Brazzel 1989, Cousins 1991, Grefenstette 1996). Between 1987 and 2000, boll weevil

eradication was completed in North Carolina, South Carolina, Georgia, Florida, and Alabama (excluding the north-western portion), and the middle part of Tennessee.

The programme also began in the Imperial Valley of California (24 300 hectares) in 1983, western Arizona in 1985 (28 300 hectares), the central part of Arizona in 1988 (170 000 hectares), the Mexicali Valley of Mexico in 1988 (65 000 hectares), and the Sonoita cotton region of Mexico in 1988 (2600 hectares). In 1991, boll weevil eradication was successfully completed in southern California, Arizona, and north-western Mexico.

Environmental and economic benefits realized as a result of the success of the boll weevil eradication programme in the South East and the South West (Carlson et al. 1989, USDA 1991, Haney et al. 1996), led to programme expansion into the rest of the US Cotton Belt:

Tennessee: the programme began in Region I (70 000 hectares) of West Tennessee in 1998 (Brumley 1999), and expanded into Regions II and III (140 000 hectares) in 2000 (Fig. 1, upper, left).

Mississippi: after a brief pause, the programme restarted in Region IV (28 000 hectares) and began in Region III (162 000 hectares) in 1997. It then expanded into Region II (91 000 hectares) in 1998 (Brumley 1999), and finally into Region I (243 000 hectares) in 1999 (Fig. 1, lower, left).

Missouri: the programme began in 2001 and included the entire cotton-growing area (164 000 hectares) of the State of Missouri (Fig. 1, lower, right).

Arkansas: the programme started in the South West Zone (2500 hectares) in 1997 (Fig. 1, upper, right) in conjunction with the Louisiana Red River programme, expanded into the South East Zone (120 000 hectares) in 1999, the Central Zone (86 000 hectares) in 2000, the North East Ridge Zone (55 000 hectares) in 2001, and into Poinsett County (10 000 hectares) in 2002 (Kiser et al. 2002). In 2003, the programme expanded again into the North East Delta (120 000 hectares) (Kiser and Catanach 2005).

Louisiana: the programme started in the Red River Zone (27 000 hectares) in 1997 (Fig. 2, upper, left), and expanded into the remainder of the State of Louisiana, referred to as the North East Zone (220 000 hectares) in 1999.

Oklahoma: the programme began in 1998 and included the entire cotton-growing area (100 000 hectares) of the State of Oklahoma (Fig. 2, upper, right).

Texas: the programme began in the Southern Rolling Plains (89 000 hectares) in 1994 (Fig. 2, lower, left). The programme was expanded in 1996 into the Rolling Plains Central (243 000 hectares) and South Texas/Winter Garden (263 000 hectares) Zones. In 1999, it expanded again into the El Paso/Trans Pecos (20 000 hectares), Western High Plains (324 000 hectares), Permian Basin (283 000 hectares), North West Plains (223 000 hectares), and Northern Rolling Plains (142 000 hectares) Zones (El-Lissy et al. 1996, 2000). In 2001, the programme expanded into the Southern High Plains/Caprock (460 000 hectares), Northern High Plains (223 000 hectares), and Southern Blacklands (40 000 hectares) Zones (Smith et al. 2002). In 2002, it expanded again into the Upper Coastal Bend Zone (76 000 hectares) (Allen et al. 2003). In 2004, the programme expanded into the St. Lawrence Zone (61 000 hectares) and the Panhandle Zone (15 000 hectares) (Allen et al. 2005). In 2005, the programme started in he Northern Blacklands (40 000 hectares) and the Lower Rio Grande Valley (100 000 hectares) Zones, which are the final two cotton-growing areas in the USA to join the eradication effort.

New Mexico: the programme started in the South Central New Mexico and Luna County (13 000 hectares) Zones in 1998 (Fig. 2, lower, right) and expanded into the Pecos Valley Zone (6000 hectares) in 2000. The Lea County (7000 hectares) programme began in 2001 as part of the Western High Plains of Texas, along with the Roosevelt/Curry programme in conjunction with the North West Plains Zone of Texas.

This report provides a summary of boll

weevil eradication in 2004/2005, as well as an expected time frame for nationwide eradication in the USA.

2. Materials and Methods

The operational success of the boll weevil eradication programme hinges on three separate, yet interdependent components including: mapping, detection, and control.

2.1. Mapping

Mapping is one of the first phases of operation in any eradication zone. Mapping identifies the exact location of each cotton field and defines the surrounding environment. The methodology of mapping used in boll weevil eradication evolved from hand-drawn cotton fields on topographic county maps in the mid 1980s, to aerial photos in the late 1980s, to the Global Positioning System (GPS) in the early to mid 1990s. Currently, all active eradication zones are using differentially-corrected GPS units in the same or similar manner as described previously (El-Lissy et al. 1996, El-Lissy and Moschos 1999). Additionally, each field is identified with a unique number to provide for accurate data management.

2.2. Detection

All eradication zones use the boll weevil pheromone trap as the primary tool of detection (El-Lissy and Grefenstette 2002). Barcode systems are utilized in the same or similar manner as described previously (El-Lissy et al. 1996, El-Lissy and Moschos 1999) to assist programme managers in collecting and managing all trapping information. Field personnel are equipped with hand-held scanners programmed to read the barcodes and

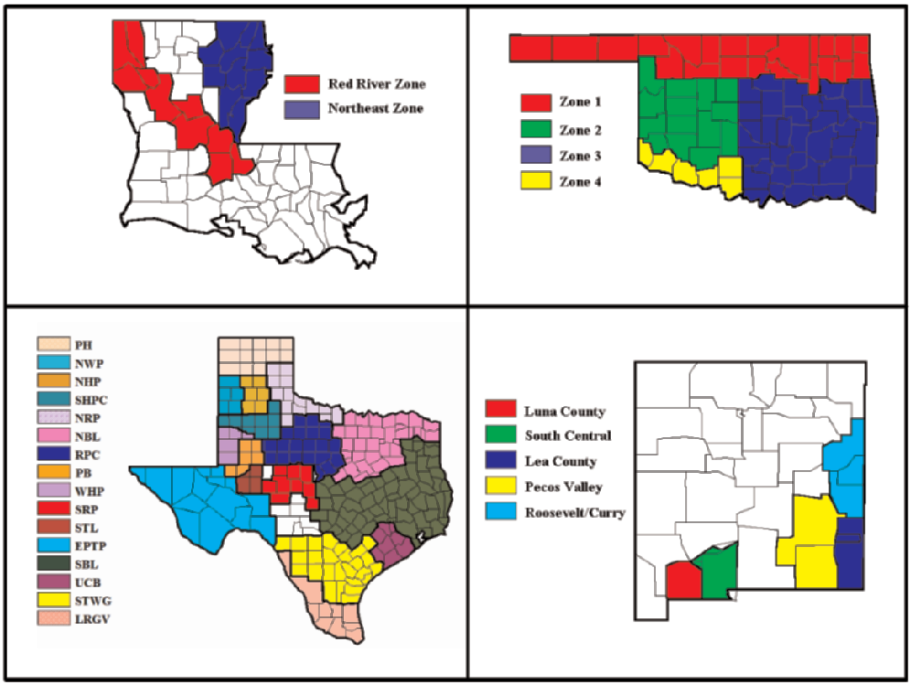

Figure 2. *The boll weevil eradication zones in (upper, left) Louisiana, (upper, right) Oklahoma, (lower, left) Texas, and (lower, right) New Mexico.*

store relevant information regarding each trap and corresponding field. Trapping information is transmitted daily to the database for data analysis, quality assurance, and reporting. Unique regional, ecological and environmental differences across the US Cotton Belt have resulted in slight variations in trapping density and placement.

2.2.1. Post-Eradication Zones

Post-eradication zones include the cotton-producing areas that have already completed boll weevil eradication. Trapping in post-eradication zones is designed to provide early warning of any reintroduction of boll weevils through natural migration or artificial movement. Early detection allows an immediate response in containing and eradicating the reintroduced population before it becomes established. The plan is to continue post-eradication trapping until nationwide eradication is complete, at which time a reduced but still effective trapping density will be employed for long-term protection.

South East: in Florida, Georgia, North Carolina, South Carolina, Virginia and most of Alabama, traps were placed at approximately one trap per eight hectares in mid June (pre-bloom growth stage) and inspected bi-weekly through November (defoliation and harvesting). In Arkansas, Louisiana, Mississippi, and Tennessee traps were also placed at one trap per 4-8 hectares, and inspected weekly and season-long (planting though harvesting).

South West: in southern California (Imperial Valley), traps were strategically placed along major highways and interstates (All American Canal, I-8, and Highway 98) at one trap every eight kilometres and inspected monthly. In Arizona, boll weevil traps were placed around all cotton fields in southern Arizona (within 80 kilometres of Mexico) and the south-eastern counties at one trap per 16 hectares. In central and western Arizona, traps were placed at a density of one trap per 65 hectares. All traps in Arizona were deployed at planting and inspected bi-weekly until defoliation. In north-western Mexico, traps were also placed at a density of one trap per 65 hectares and inspected bi-weekly beginning at planting and until defoliation.

Kansas: boll weevil traps were placed at a rate of one trap per field shortly after planting and inspected bi-weekly until harvest or a killing freeze.

Arkansas, New Mexico, Oklahoma, and Texas: traps were placed at approximately one trap per eight hectares and inspected weekly throughout the growing season.

2.2.2. Active Eradication Zones

Tennessee: traps were placed around the perimeter of all cotton fields, approximately 60 metres apart (averaging one trap per 0.4-0.8 hectares), at or shortly after planting and inspected weekly through harvest or a killing freeze.

Mississippi: traps were placed at planting, approximately 105 metres apart, around the perimeter of each field (averaging one trap per 0.8-2 hectares), in all regions, baited and inspected weekly through harvest or a killing freeze.

Missouri: traps were placed around the perimeter of all cotton fields, approximately 105 metres apart (averaging one trap per 1.6 hectares), at or shortly after planting (2nd week of April) and inspected weekly (beginning May 6) through harvest or a killing freeze.

Arkansas: traps were placed around the perimeter of all cotton fields shortly after planting at approximately 90 metres apart (averaging one trap per 1.2 hectares), and inspected weekly through harvest or a killing freeze.

Louisiana: traps were placed at planting, approximately 45 metres apart, around the perimeter of each field (averaging one trap per 0.8 hectares), and inspected weekly through harvest or a killing freeze.

Oklahoma: traps were placed at one trap per two hectares at planting and inspected weekly through harvest or a killing freeze.

Texas: traps were placed approximately

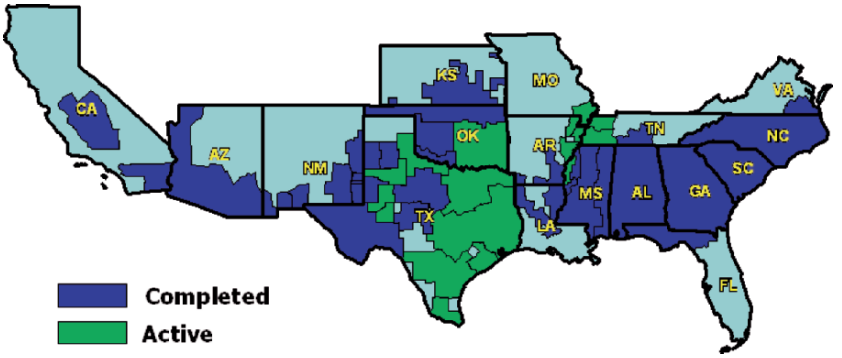

Figure 3. *The boll weevil eradication programme in the USA, 2005.*

150 metres apart around the perimeter of each field (averaging one trap per 2-3 hectares) in all eradication zones and inspected weekly until harvest or a killing freeze.

New Mexico: traps were placed at a rate of one trap per two hectares and inspected season-long.

2.3. Control

The control part of the eradication programme consists of cultural, mechanical, and chemical control.

2.3.1. Cultural Control

Time frames for uniform cotton planting and harvesting, as organized by growers, local agricultural extension services, and in some cases state regulatory agencies, are key components of cultural control in providing the necessary host-free period. In some states such as Arkansas and Texas, growers were offered a rebate to destroy crop residues as soon as possible after harvest in an effort to reduce overwintering populations and insecticide treatments.

2.3.2. Mechanical Control

Although the primary function of the trap is detection, an indirect benefit of trapping, especially in low weevil populations, is that it removes a percentage of the population (Lloyd et al. 1972).

2.3.3. Chemical Control

Season-long phase: a single aerial application of malathion (ultra-low-volume) is made, beginning at the pinhead-square growth stage, to fields that had reached the treatment criteria (action threshold). The 2004 season-long action threshold for treatment was a trap catch of 1-2 adult boll weevils per field (16 hectares or less) in all active zones.

Diapause phase (2005): in the Rio Grande Valley and the Northern Blacklands of Texas, weekly aerial applications with malathion ultra low volume (ULV) begins on June 15 and July 15, respectively, and continue until cotton fields are defoliated and harvested.

In 2004, malathion (Fyfanon® ULV) was used at a rate of 731 ml/ha in Arkansas, Mississippi, Missouri, and Tennessee at 1168 ml/ha in Louisiana, and 877 ml/ha in Oklahoma, Texas, and New Mexico. All aircraft were equipped with differentially-corrected GPS instruments for documentation and quality control purposes in the same manner as described previously (El-Lissy et al. 1996).

Fields located within close proximity of sites designated as environmentally sensitive, or near permanent obstacles, were treated with high-clearance ground equipment. Mist-blowers mounted on pick-up trucks were also used

to provide accurate placement of insecticide on corners and edges of fields, and under power lines or other obstacles, where airplanes had less accessibility.

3. Results and Discussion

To date, the boll weevil has been eradicated from nearly 5.3 million hectares of cotton in Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Kansas, California, Arizona, and portions of Tennessee, Mississippi, Missouri, Arkansas, Louisiana, Oklahoma, Texas, and New Mexico (Fig. 3), as well as from the neighbouring regions of the Mexicali Valley, Sonoita, and Caborca in Mexico.

3.1. Post-Eradication Zones

3.1.1. South East

All post-eradication programme activities in Alabama, Georgia, Florida, and South Carolina are carried out by the Southeastern Boll Weevil Eradication Foundation (SEBWEF), headquartered in Montgomery, Alabama. In Virginia and North Carolina, the state agriculture departments carry out the post-eradication activities with support from SEBWEF. In 2004, there were no weevils detected or plants treated by the programme or producers in the entire States of Alabama, Florida, Georgia, North Carolina, South Carolina, and Virginia. Further, there were no weevils captured in zones that have not yet been declared eradicated, including eradication zones totalling over 1.2 million hectares in, Louisiana, Mississippi, Missouri, and Tennessee.

3.1.2. South West

Post-eradication programme activities in southern California are carried out by the Imperial County Commissioner of Agriculture in El Centro; in Arizona by the Arizona Cotton Research and Protection Council in Phoenix, Arizona; and, in Mexico by Sanidad Vegetal in cooperation with the USDA's Animal and Plant Health Inspection Service (APHIS). In 2004, there were no weevils detected or acres treated by the programme or producers in the entire States of Arizona (123 000 hectares), California (372 000 hectares), or in North West Mexico (24 000 hectares).

3.1.3. Kansas

With the support of cotton producers in Kansas, programme activities are cooperatively carried out by the Kansas Department of Agriculture and USDA-APHIS. There were no weevils captured or fields treated in the entire State of Kansas (36 000 hectares) in 2004.

3.1.4. Arkansas, Texas, Oklahoma, and New Mexico

Post-eradication programme activities in Arkansas are carried out by the Arkansas Boll Weevil Eradication Foundation headquartered in Little Rock, Arkansas. In Texas and eastern New Mexico, the programme is implemented by the Texas Boll Weevil Eradication Foundation headquartered in Abilene, Texas. In Oklahoma, the programme is implemented by the Oklahoma Boll Weevil Eradication Organization, headquartered in Hobart, Oklahoma. In South Central New Mexico, the programme is implemented by the New Mexico Boll Weevil Eradication Control Committee, headquartered in Las Cruces, New Mexico. In 2004, no weevils were captured or fields treated by the programme or producers in the cotton-growing areas of eradicated zones in Arkansas (2500 hectares), New Mexico (35 000 hectares), Oklahoma (102 000 hectares) or Texas (1 844 000 hectares). A seasonal total of 346 weevils were captured in the Southern Rolling Plains of Texas, presumably from migration and artificial movement from neighbouring zones that had just started eradication in 2004.

3.2. Active Eradication Zones

In 2004, the programme was implemented on approximately 1.4 million hectares of cotton in Arkansas, Louisiana, Mississippi, Missouri,

New Mexico, Oklahoma, Tennessee, and Texas. Nationwide, the overall boll weevil populations in all active zones remained significantly lower than in the years when the programme began in each zone.

3.2.1. Tennessee

The 2004 season-long mean number of weevils captured per trap per week in all active zones (88 000 hectares) was significantly less than in the years when the programme started in each zone. The 2004 season-long mean in Region I was reduced by 68.1% as compared to 1998; in Region II, it was reduced by 48.8% as compared to 2000; and in Region III, it was reduced by 46.7% as compared to 2000 (Ron Seward, personal communication) (Table 1).

3.2.2. Mississippi

The 2004 season-long mean number of weevils captured per trap per week in all active zones (49 000 hectares) was significantly less than in the years when the programme started in each zone. The 2004 season-long mean in Region I was reduced by 99.9% compared to 1999; in Region II, it was reduced by 99.9% compared to 1998; and in Region III, it was reduced by 99.6% as compared to 1997 (Farrell Boyd, personal communication) (Table 1).

3.2.3. Missouri

The 2004 season-long mean number of weevils captured per trap per week in all active zones (123 000 hectares) was significantly less than the first year of the programme. The 2004 season-long mean in Region I was reduced by 82.4% compared to 2001; and in Region II, it was reduced by 79.4% compared to 2001 (Dewey Wayne King, personal communication) (Table 1).

3.2.4. Arkansas

The 2004 season-long mean number of weevils captured per trap per week in all active zones (240 000 hectares) was significantly less than in the years when the programme started in each zone. The 2004 season-long mean in the South East Zone was reduced by 99.9% compared to 1999; in the Central Zone, it was reduced by 99.9% as compared to 2000; in the North East Ridge Zone, it was reduced by 99.3% as compared to 2001; and in the North Delta Zone, it was reduced by 83.4% as compared to 2003 (Kiser and Catanach 2005) (Table 1).

3.2.5. Louisiana

The 2004 season-long mean number of weevils captured per hectare per month in the Red River Zone was reduced by 99.9% as compared to 1997. In the North East Zone, the mean was reduced by 99.7% as compared to 1999 (Table 1). The combined active area in these two zones was 141 000 hectares.

3.2.6. Oklahoma

The 2004 season-long mean number of weevils captured per trap per week in the statewide active zone (11 000 hectares) was significantly less than in the first year of the programme. The 2004 season-long mean was reduced by 99.7% compared to 1998 (Bill Massey, personal communication) (Table 1).

3.2.7. Texas

The 2004 season-long mean number of weevils captured per trap per week in all active zones (610 000 hectares) was significantly less than in the years when the programme started in each zone. The 2004 season-long mean in El Paso/Trans Pecos (EP/TP) was reduced by 99.96% as compared to the mean in 1999; in the Northern High Plains (NHP), it was reduced by 99.99% as compared to 2001; in the Northern Rolling Plains (NRP), it was reduced by 99.99% as compared to 1999; in the Permian Basin (PB), it was reduced by 99.7% as compared to 1999; in the Rolling Plains Central (RPC), it was reduced by 99.9% as compared to 1996; in the Southern Blacklands (SBL), it was reduced by 96.1% as compared to 2001; in the Southern High Plains/Caprock (SHPC), it was reduced by 99.99% compared to 2001; in the South Texas/Winter Garden (ST/WG), it was reduced by 94.8% compared to 1996; in the Upper Coastal Bend (UCB), it was reduced by

Table 1. *Percentage reduction in the mean number of weevils captured per trap in the 2004 season as compared with the first year of the programme in each active eradication zone.*

State	Eradication Zone	Year Programme Start	First Year Mean	2004 Mean	Percentage Reduction
Tennessee	Region I	1998	1.50	0.077	94.86
	Region II	2000	0.16	0.001	99.19
	Region III	2000	0.06	0.001	97.84
Mississippi	Region I	1999	2.50	0.001	99.96
	Region II	1998	2.00	0.000	99.99
	Region III	1997	13.80	0.005	99.96
Missouri	Region I	2001	19.50	3.430	82.41
	Region II	2001	6.30	1.300	79.37
Arkansas	Southeast Zone	1999	5.54	0.001	99.99
	Central Zone	2000	15.66	0.029	99.81
	Northeast Ridge Zone	2001	8.70	0.081	99.10
	North Delta Zone	2003	9.58	0.260	97.27
Louisiana	Red River Zone	1997	0.05	0.000	99.92
	Northeast Zone	1999	0.85	0.000	99.99
Oklahoma	State-Wide	1998	1.70	0.023	98.65
Texas	El Paso/Trans Pecos (EP/TP)	1999	0.21	0.000	99.86
	Northern High Plains (NHP)	2001	0.89	0.000	99.99
	Northern Rolling Plains (NRP)	1999	18.54	0.000	99.99
	Permian Basin (PB)	1999	9.99	0.018	99.82
	Rolling Plains Central (RPC)	1996	16.99	0.003	99.98
	Southern Blacklands (SBL)	2001	13.68	0.188	98.62
	Southern High Plains (SHP)	2001	1.16	0.000	99.98
	St Lawrence	2004	3.56	0.361	89.86
	South Texas/Winter Garden	1996	12.82	0.210	98.36
	Upper Coastal Bend (UCB)	2002	18.22	0.301	98.35
	Western High Plains (WHP)	1999	18.20	0.000	99.99
New Mexico	Pecos Valley	2000	10.00	0.000	99.99
	Lea County	2001	10.30	0.000	99.99

90.9% compared to 2002; and in the Western High Plains (WHP), it was reduced by 99.99% compared to 1999 (Table 1).

3.2.8. New Mexico

The 2004 season-long mean number of adult weevils captured per trap per week in the Pecos Valley (1400 hectares) and Lea County (607 hectares) was significantly less than previous years. The 2004 mean in the Pecos Valley was reduced by 99.97% as compared to 2000; and in Lea County, it was reduced by

99.99% as compared to 2001 (Table 1).

3.3. Programme Expansion in 2005

In 2004, cotton producers in the Upper Blacklands (40 000 hectares) and Lower Rio Grande Valley (101 000 hectares) Zones in Texas approved referenda to join the eradication programme. As a result, all cotton-growing regions of the USA are either involved in active eradication, or have already completed the programme.

4. Conclusions

The boll weevil eradication programme has eliminated this plant pest in eradicated areas, resulting in higher yields and a dramatic reduction in the overall use of insecticides. A number of studies conducted in several cotton-producing regions have all concluded a positive economic return and environmental benefits resulting from boll weevil eradication. Carlson et al. (1989) found that the programme led to a reduction in pesticide costs of about USD 74/ha/year in its early years of operation in the South East. More recently, Tribble et al. (1999) estimated the annual net benefit of the programme in the State of Georgia to be at least USD 220/ha. Bryant et al. (1997) estimated increased yields of at least 2.5 kg/ha and a reduction in the cost of insecticides used of at least USD 111/ha in Arkansas. Larson et al. (2000) estimated increased revenues from cotton lint and seeds of USD 143/ha/yr and a decline in insecticide costs of USD 42/ha/year in Tennessee. These remarkable and sustainable environmental and economic benefits, realized within the eradicated regions, make boll weevil eradication one of the most important agricultural programmes in US history.

5. Acknowledgements

The nationwide boll weevil eradication programme exemplifies an unsurpassed effort by federal, state, and industry cooperators in ridding the US cotton industry of its most devastating pest. The operational success of the programme is entirely due to the tireless efforts of grower organizations, including the Southeastern Boll Weevil Eradication Foundation, Mississippi Boll Weevil Management Corporation, Arkansas Boll Weevil Eradication Foundation, Louisiana Department of Agriculture and Forestry, Texas Boll Weevil Eradication Foundation, Oklahoma Boll Weevil Eradication Organization, South Central New Mexico Boll Weevil Control Committee, Pecos Valley Boll Weevil Control Committee, Arizona Cotton Research and Protection Council, Imperial County Commissioner of Agriculture, and Kansas Department of Agriculture. The leadership of the National Cotton Council and technical and operational support of the extension service, state agriculture departments, and USDA continues to play an instrumental role in the success of boll weevil eradication in the USA.

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Regional Management Strategy for Cotton Bollworm *Helicoverpa armigera* in China

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ABSTRACT Cotton bollworm *Helicoverpa armigera* (Hübner) is one of the most important insect pests in cotton, corn and vegetables in China. Transgenic cotton that expresses a gene derived from the bacterium *Bacillus thuringiensis* (Berliner) (*Bt*) has been deployed for combating cotton bollworm since 1997, and in 2005 its use had expanded to 3.3 million of the total 5.1 million hectares used for cotton production in China. The area-wide integrated pest management (AW-IPM) tactics associated with the planting of *Bt*-cotton have resulted in dramatic reductions in insecticide use and the occurrence of the pest in the country has been controlled effectively. Susceptibility of *H. armigera* field populations to the *Bt*-insecticidal protein *Cry1Ac* was monitored from 1997 to 2005. The results indicate that field populations are still susceptible to *Cry1Ac* protein and that a shift toward resistance among *H. armigera* populations is not apparent. The natural refuges derived from the mixed planting system of *Bt*-cotton, with non-*Bt*-corn, soybean and peanut, on single small-scale family holdings, possibly in combination with migration of the pest, play an important role in delaying the evolution of cotton bollworm resistance

KEY WORDS *Bt*-cotton, China, *Helicoverpa armigera*, area-wide management, resistance management

1. Introduction

Since 1984, China has become one of the largest producers of cotton in the world. An area of 4-6 million hectares under cotton cultivation in China meets 20% of the annual worldwide demand for cotton. On an agro-ecological basis, these cotton-growing areas can be grouped into three major regions: the Changjiang River Region, the Yellow River Region, and the Northwestern Region (Fig. 1). The climate in these regions varies greatly in terms of rainfall, temperature, and length of the growing season, affecting significantly pest populations (Wu and Guo 2005).

In the middle of the 20th century, protection of cotton from insect pests relied solely on chemical insecticides. These chemicals were used intensively and often on fixed schedules. Insecticide input increased from zero or a few applications per season in the early 1950s to 10-15 applications by the end of the 1970s. Insecticide input increased even more rapidly in the 1980s, and in the early 1990s many fields received in excess of 30 applications of chemical insecticides per year. Some cotton insect pest populations in China increased rapidly after insecticide treatments. Insect pest populations gradually became resistant to many insecticides and thus control became difficult or impossible in some areas. By the mid 1990s, the combination of insecticide resistance and resurgence of cotton bollworm *Helicoverpa armigera* (Hübner) and other pests had become a major threat to cotton production in China (Xia 1994).

Facing many serious problems caused by cotton bollworm, in recent years China has conducted a substantial body of research and development on the regional management of cotton bollworm associated with *Bt*-cotton deployment, and this is summarized in this review.

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 559–565.

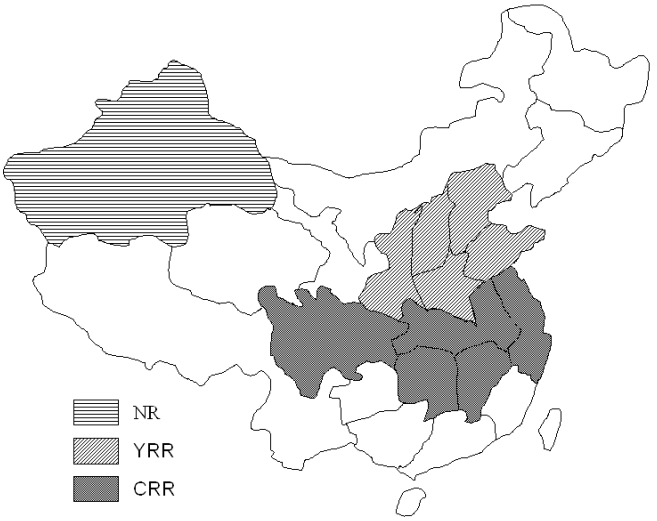

Figure 1. *Geographic regions of cotton production in China. Changjiang River Region (CRR), Yellow River Region (YRR), and Northwestern Region (NR).*

2. Regional Population Dynamics of *Helicoverpa armigera*

H. armigera completes three to four generations in the Yellow River Region and the Northwestern Region and four to six generations in the Changjiang River Region annually. Rainfall during cotton growth is an important climatic factor influencing the regional population dynamics of the pest during a season. Especially in the Changjiang River Region, high levels of rainfall early in the season drastically inhibit *H. armigera* population development, which usually reaches outbreak status in later generations that occur during the drought season. The pest status of cotton bollworm comprises a suite of four physiological, behavioural, and ecological characteristics that enable the insect to survive in unstable habitats and in turn to colonize and exploit agricultural systems successfully: polyphagy, high mobility, high fecundity, and facultative diapause (Fitt 1989).

The populations of *H. armigera* in all of China can be divided into four regional groups: the tropical, subtropical, temperate, and Xinjiang geotypes (Wu and Guo 2005). Their adaptive zones are respectively in southern China, the middle and lower Changjiang River Region, which includes the Provinces of Sichun, Hunan, Hubei and Zhejiang, Yellow River Region, which includes Henan, Hebei and Shandong Provinces, and the Xinjiang Uygur Autonomous Region and Gansu Province (Wu and Guo 2005). Damage to cotton by larvae of the temperate zone geotype can extend into areas of north-eastern China such as Liaoning and Jilin Provinces by long-distance, facultative migration during the East Asia summer monsoon (Feng et al. 2004, 2005).

In consideration of the regional population dynamics associated with its migratory behaviour, a national monitoring system was established in the late 1990s. The visual examination of cotton plants every three to five days during the cotton growing season was conducted in most planting locations to obtain information on the population dynamics of eggs and larvae, and adult densities of cotton bollworm are monitored daily by means of

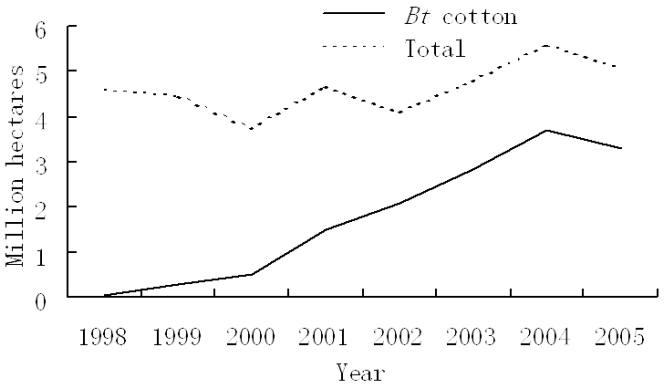

Figure 2. *The increase in the total cotton and* Bt*-cotton planting area in China from 1998 to 2005.*

light and pheromone traps. Forecasting of pest occurrences in crops has been standardized across all growing regions and is used to provide timely information to farmers. The area-wide control strategy against the pest in overwintering areas has been carried out to reduce its damage in the immigratory zone (Wu and Guo 2005).

3. *Bt*-Cotton Deployment and its Ecological Consequences

Field trials of *H. armigera*-resistant transgenic cotton were conducted in the early 1990s in China. *Cry1A* cotton and *Cry1A+CpTI* cotton were approved formally for planting by the Chinese government in 1997 and 1999, respectively. Planting of insect-resistant transgenic cotton was limited to the Yellow River Region before 1999, and then extended to the Northwestern Region and the Changjiang River Region subsequently. By 2003, *Bt*-cotton had been planted in (1) Hebei, Shandong and Henan Provinces, (2) Shanxi Province in the Yellow River Region, (3), Anhui, Jiangsu and Hubei Provinces in the Changjiang River Region, and (4) Xinjiang in the Northwestern Region. Plantings of *Bt*-cotton totalled less than 0.1 million hectares in 1997, but increased rapidly to 3.3 million hectares by 2005 (Fig. 2). This represents 65% of the total cotton area of 5.1 million hectares in that year. *Bt*-cotton has been planted exclusively in most cotton-growing areas of the Yellow River Region since 2000.

On the basis of field evaluations, *Bt*-cotton is highly efficient in resisting the insect (Wan et al. 2005), and in recent years, the regional occurrence of cotton bollworm in northern China has decreased drastically due to the large-scale deployment of *Bt*-cotton (Fig. 3). A continuous resistance-monitoring programme of *H. armigera* to commonly used insecticides was undertaken for assessing the impact of *Bt*-cotton planting on evolution of the pest resistance in *Bt*-cotton planting regions. The bioassay results showed that the average resistance levels in field populations of *H. armigera* to lambda-cyhalothrin, phoxim and endosulfan decreased drastically after cultivation of *Bt*-cotton. This significant increase in insect susceptibility to insecticides is expected to result in reductions in insecticide application for *H. armigera* control in *Bt*-cotton. It can therefore be concluded that *Bt*-cotton is playing an important role in the long-term management of *H. armigera* by increasing the potential for natural and chemical control of the pest (Wu et al. 2005).

A number of studies on the influence of *Bt*-cotton on the structure and diversity of arthropod communities have been carried out in

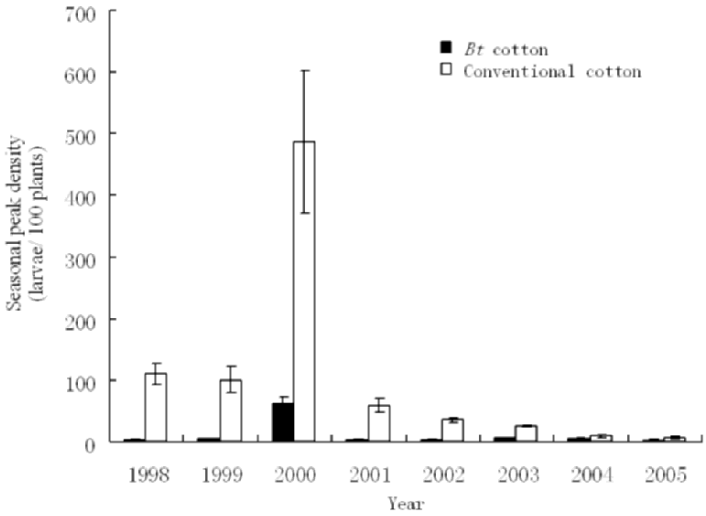

Figure 3. *Seasonal peak density (number of larvae per 100 plants) of* Helicoverpa armigera *larvae during 1998-2005 in* Bt-*cotton and conventional cotton fields (Langfang, Hebei Province). Values shown are means ± one standard error.*

recent years to assess the ecological consequences in cotton ecosystems (Liu et al. 2002, Men et al. 2003). The results showed that planting *Bt*-cotton can increase the stability of arthropod diversity in cotton ecosystems and benefit cotton pest management.

4. Resistance Management of *Helicoverpa armigera* to *Bt*-Cotton

The greatest threat to the continued efficacy of *Bt*-cotton against *H. armigera* is evolution of resistance in this pest. Continuous monoculture of varieties that express *Bt*-toxins is likely to select the insect intensively for resistance, particularly when *Bt*-toxin levels decrease as the plants age (Gould 1998). Field studies in China demonstrate that about 5-20% of naturally occurring *H. armigera* larvae can survive on *Bt*-cotton toward the end of the growing season (Wan et al. 2005). This indicates that *Bt*-cotton does not maintain a high dose relative to the tolerance of *H. armigera*. Simulation studies and some experimental data (Gould 1998) have shown that, without refuge populations, pest resistance to *Bt* could evolve rapidly with widespread use of *Bt*-crops. The need to implement resistance management strategies to delay the development of pest resistance to *Bt*-toxins in *Bt*-crops is therefore widely recognized.

The most promising strategy entails the use of plants with a high dose of toxin in combination with the maintenance of refuge crops that produce *Bt*-susceptible insects within the pest population (Burd et al. 2003). In the USA, the Environmental Protection Agency requires that each farm sets aside some land for non-*Bt*-producing cotton (Gould et al. 2002). In Australia, the government and farmer groups have decided to restrict *Bt*-cotton to 30% of the land, leaving a large refuge for susceptible bollworms (Gould et al. 2002). Although this strategy seems reasonable, it is difficult to implement in the Yellow River Region and the Changjiang River Region because of the challenges associated with educating and monitoring more than ten million farmers in China.

In the Yellow River Region and the Changjiang River Region the cropping system

is quite different from the large-scale farming in the USA and Australia. Mixed plantings of cotton, corn, soybean, and peanut are common. In general, wheat is the main host of first-generation *H. armigera* larvae, and cotton, corn, peanut, and soybean are the major host plants of subsequent generations. In the Yellow River Region, it has been reported (Wu et al. 2002) that the host crops of *H. armigera* are planted annually on approximately 14 million hectares in the proportions of 5, 55, 15, 15 and 10% for cotton, corn, peanut, soybean and other crops, respectively. Field trials indicate that both soybean and peanut can supply refuges for the second and third generations of cotton bollworm (Wu et al. 2002). As the most abundant crop, corn is planted widely, but it has a long sowing period from April to June in the Yellow River Region. The varied planting time for corn in this region increases the overlap between the moth oviposition period and the occurrence of corn in the silk stage, which could serve as the refuge for the third and fourth generations of *H. armigera* (Wu and Guo 2004). A planting system consisting of wheat, soybean (peanut), corn, and *Bt*-cotton can supply refuges for cotton bollworm throughout the year. Adequate provision of refuges on an area-wide basis, and successful production of susceptible insects will increase the probability that a rare resistant homozygote will mate with a susceptible individual to produce heterozygous progeny susceptible to *Cry1Ac*. This strategy has been recommended for areas where farmers exclusively grow cotton without natural refugia from other crops (Wu and Guo 2004).

Resistance/susceptibility monitoring is an integral tool for managing resistance. The susceptibility of *H. armigera* field populations to the *Bt*-insecticidal protein *Cry1Ac* was monitored from 1997 to 2004 in China (Li et al. 2004). The results indicate that the field populations sampled are still susceptible to *Cry1Ac* protein and that a shift toward resistance among *H. armigera* populations is not apparent. This suggests that *H. armigera* resistance genes are still rare and the existing refugia in corn, soybean, peanut, and other crops may be a major factor that contributes to the maintenance of *H. armigera* susceptibility to *Bt*-cotton after several years of large-scale commercialization. In addition, gene flow arising from migration of cotton bollworms over a large area may also be an important factor in delaying the evolution of *Bt*-resistance (Li et al. 2004).

5. Strategies for Integrated Control of *Helicoverpa armigera* in *Bt*-Cotton

With high egg densities, potentially damaging bollworm larval densities may develop in transgenic cotton. Because of the extremely high bollworm egg densities that may occur in northern China, the control of cotton bollworm in *Bt*-cotton fields in some years is still essential. Control thresholds for *H. armigera* in conventional cotton were investigated in different planting areas of China using egg density as an indicator (Zhu et al. 2000). Since the *Bt*-protein only acts on larvae, egg density is not a reliable standard for *H. armigera* control in *Bt*-cotton fields. Through field evaluation in Hebei Province, a yield loss model on larval damage in late season in *Bt*-cotton was established (Wu and Guo 2005). Under the condition of a 3% yield loss caused by the bollworm, the economic threshold is defined as 13 larvae per hundred plants in *Bt*-cotton in northern China.

Biological control is a good option to complement the integrated control of the pest. There are two ways of utilizing natural enemies of insect pests in agricultural systems. One is to preserve existing predators and parasitoids, and another is to mass-rear natural enemies for augmentative releases to regulate the population density of the target insect pest. The egg parasitoid *Trichogramma* spp., and the larval parasitoid *Campoletis chloridae* Uchida, play a major role in the natural control of *H. armigera*. In addition, lacewings (*Chrysopa* spp.), minute flower bugs (*Orius* spp.), lady beetles, paper-net wasps (such as *Polistes antennalis* Perez), and various

species of spiders are all important predators of the bollworm.

The practice for insect pest control in cotton has demonstrated that preservation and augmentation of existing predators and parasitoids by means of rational application of insecticides and planting type can effectively decrease population densities of cotton aphid and cotton bollworm. Another approach to increase the impact of natural enemies is to use selective pesticides instead of broad-spectrum insecticides. In general, pyrethroids are highly toxic to natural enemies, and their use is therefore restricted to the late period of cotton growth. *H. armigera* overwinters as diapausing pupae in the soil of cotton and corn fields. Irrigation in winter and early spring can kill most pupae in the field, which is thought to be an effective method to decrease population density in the next season.

6. Challenges and Future Developments

Great success has been obtained in recent years with the planting of *Bt*-cotton for control of cotton bollworm in China and the AW-IPM tactics associated with the planting of *Bt*-cotton have resulted in dramatic reductions in insecticide use. The main challenge in the near future is the evolution of resistance in this pest to *Bt*-cotton. However, more than a hundred *Bt*-cotton varieties have been bred and commercialized by many companies since 1998, with different expression levels of *Cry1Ac* protein. To decrease the numbers of pest larvae surviving in *Bt*-cotton fields, the Chinese government issued a special regulation in 2004 for stopping poor quality *Bt*-cotton entering the market.

Bt-corn commercialization in China is another important factor in relation to the resistance of *Bt*-cotton to insect pests. Although *Bt*-corn is not grown in China, its commercialization is currently under consideration by the Chinese government. If the government decides to commercialize *Bt*-corn, a key refuge for cotton bollworm will be lost and resistance to *Bt*-cotton may evolve more rapidly.

Second-generation *Bt*-cotton varieties, which express different *Bt*-endotoxins are now available in the USA and Australia (Jackson et al. 2003). The increased level of control gained by two *Bt*-gene cotton varieties can be valuable in delaying the evolution of *Bt*-cotton resistance to cotton bollworm. Undoubtedly, the major change in future management of the insect will rely heavily on the introduction of such second transgenic cotton varieties.

As one of the largest cotton producing countries in the world, China will face the challenges from the cotton bollworm pest in the long term. A national management strategy will have to take into account regional migration, overwintering areas, and the existence of alternative hosts of the pest, for any control to be sustainable. Bollworm population control in the main overwintering areas in the southern Yellow River Region will drastically decrease its occurrence in both the northern Yellow River Region and in north-eastern China, and will be for the benefit of the management of this pest in the entire country.

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Integrated Systems for Control of the Pink Bollworm *Pectinophora gossypiella* in Cotton

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ABSTRACT The pink bollworm *Pectinophora gossypiella* (Saunders) is a major pest of cotton *Gossypium* spp. in the growing areas of the south-western USA and in many other cotton-producing areas of the world. High chemical control costs, excessive economic losses, secondary pest problems, and environmental and social considerations have suggested the need for ecologically oriented pink bollworm management strategies. Extensive research over the years has produced monitoring, biological control, cultural, behavioural, genetic and host plant resistance methods that can be integrated into effective pink bollworm management systems. Pink bollworm moth mobility necessitates integrated pest management (IPM) implementation over large geographical areas. Local uncoordinated efforts have not reduced the economic status of the pink bollworm in any area where it is an established pest. The cotton-growing areas involved in the south-western USA present a wide range of pink bollworm population densities, in cotton production methods, and social and environmental considerations. Tailor-made systems for targeted management areas with the selection of IPM components based on pink bollworm population density, crop production methods, and economic feasibility are the most likely to succeed as long-term population suppression programmes. The breakthrough in host-plant resistance through transformation of the gene from *Bacillus thuringiensis* (Berliner) subsp. *kurstaki* (*Bt*) into cotton varieties for the production of insect toxic crystalline protein provided the framework for efficient pink bollworm population management. The success of area-wide pink bollworm management is highly dependent on participation by all segments of the agricultural community in the planning, site selection, implementation, and assessment phases of the programme. A highly effective extension-education communication programme is an essential component. The outstanding performance of *Bt*-cotton and pheromone behavioural control for pink bollworm, and the availability of historically-proven effective pink bollworm population suppression technologies (cultural controls, crop residue destruction, water management, planting dates, and sterile moth release), encouraged formulation of a multi-agency and transboundary pink bollworm eradication plan. The eradication programme was initiated in 2001-2002 in the El Paso/Trans Pecos area of Texas, in South Central New Mexico and in Chihuahua, Mexico. The results of area-wide suppression have been exceptionally encouraging and provide promising expectations for the other infested areas of the south-western USA and north-western Mexico. The pink bollworm population has been reduced to levels that can be targeted for sterile pink bollworm releases to pursue the goal of eradication.

KEY WORDS *Pectinophora gossypiella*, area-wide IPM, *Bt*-cotton, transboundary eradication, south-western USA, north-western Mexico

1. Introduction

The pink bollworm *Pectinophora gossypiella* (Saunders) was described by W. W. Saunders in 1842 from specimens damaging cotton in India. Its exact origin remains unknown (Ingram 1994). The pink bollworm apparently reached Egypt in infested cottonseed from India about 1906-1907. It was introduced into the Western Hemisphere between 1911 and 1913 in cottonseed shipped from Egypt to Brazil, Mexico, the West Indies, and the Philippine Islands (USDA-APHIS 1977).

Infestations in the USA first occurred in Texas cotton in 1917 and the source was traced to cottonseed shipped from Mexico to

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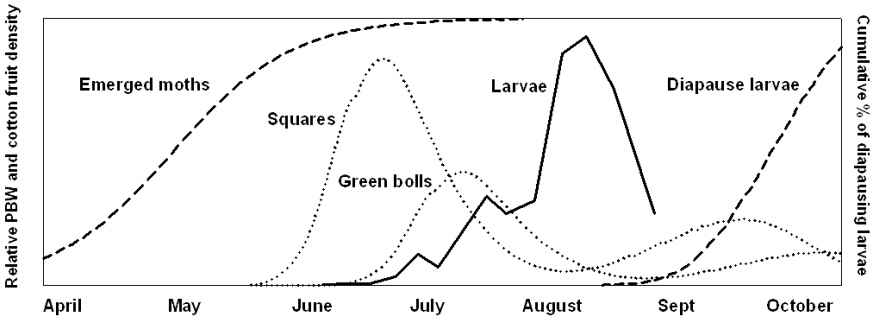

Control tactic	Early season	Mid season	Late season	Post season
Natural mortality	+	-	-	+
Natural enemies	+	+	+	-
Planting date	+	-	-	-
Heat units	+	+	+	-
Plant phenology	+	+	+	-
Host plant resistance (transgenic cotton)	+	+	+	-
Mating disruption (pheromone)	+	+	-	-
Sampling (pheromone traps)	+	+	+	-
Models	+	+	+	-
Augmentative biological control	+	+	+	-
Chemical control (pin-head)	+	+	-	-
Resistance management	+	+	-	-
Sterile insect release	+	+	+	-
Crop rotation	+	-	-	-
Irrigation timing (stimulate emergence)	+	-	-	-
Plant growth regulators	-	-	+	-
Irrigation cutoff	-	-	+	-
Defoliation	-	-	+	-
Stalk cutting/shredding	-	-	-	+
Plough down date	-	-	-	+
Winter irrigation	-	-	-	+

Figure 1. *Seasonal pink bollworm (PBW) population dynamics, cotton plant phenology and some available control tactics for the different phases of the cotton season.*

Texas oil mills (Spears 1968) in 1916. The Texas infestation and one in Louisiana in 1919 were eradicated (USDA-APHIS 1977). Reinvasions in 1936 in the lower Rio Grande Valley of Texas, eventually spread by the mid 1950s to other areas in Texas, New Mexico, Oklahoma, Arizona, Arkansas, and Louisiana. Infestations were reported in eastern Arizona in 1926 and at intervals thereafter in other parts of the state. The Arizona infestations were suppressed through cooperative federal, state, and industry programmes. Termination of the efforts in 1963 resulted in spread to the Imperial and Palo Verde Valleys of California in 1965. Pink bollworm moths were detected in the high desert areas as early as 1967 and in the San Joaquin Valley most years thereafter, but established populations have not occurred (Henneberry and Naranjo 1998). The partial explanation appears related to extensive cul-

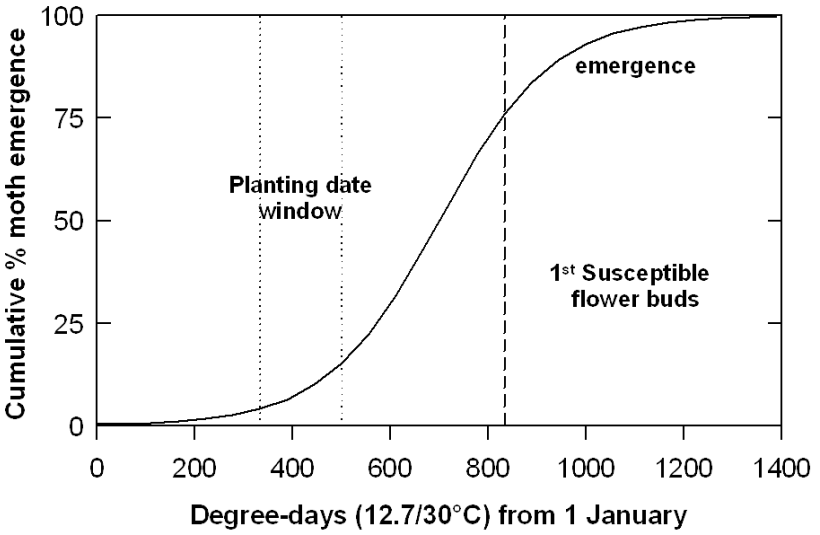

Figure 2. *Cotton planting date management to maximize pink bollworm emergence in the spring before host material is available.*

tural control, pink bollworm pheromone monitoring and treatment, and a sterile moth release system initiated in 1968.

Cotton growers in Arizona and southern California have experienced economic losses from the pink bollworm due to reduced yields, low lint quality, and increased costs of insecticides (Burrows et al. 1982). Chemical control has been heavily relied on, but has not provided a long-term solution for the pink bollworm problem. Focus on attacking localized insect populations on a farm-by-farm basis has given way to area-wide suppression and management with increasing awareness of the limitations of attacking local infestations that represent only a small part of the total pest population (Knipling 1979).

2. Integrated Pest Management (IPM) Components for Area-Wide Pink Bollworm Population Suppression or Eradication

Research by numerous scientists and summarized by Henneberry and Naranjo (1998), has identified pink bollworm control tactics applicable to early-, mid-, late- and post-season periods of the cotton growing season (Fig. 1). The tactics focus on weak links in pink bollworm biology and/or optimum periods of cotton phenology for the most efficient control action. Although all the tactics are important, those currently being implemented with pink bollworm eradication as a goal (Anonymous 2001) are summarized below.

2.1. Biological Control, Natural Control, Cultural Control and Sampling

Natural enemies abound in cotton fields and their impact and role in management systems with minimal insecticide use is only beginning to be quantified and understood. Also, pink bollworm moth emergence before host material is available (suicidal emergence), low reproductive capability and adverse high soil temperatures' impact on the first pink bollworm generation, are all contributing factors to environmentally acceptable management systems for pink bollworm population suppression (Henneberry and Naranjo 1998). Thus, pink bollworm sex pheromone-baited moth traps, sampling, monitoring of natural

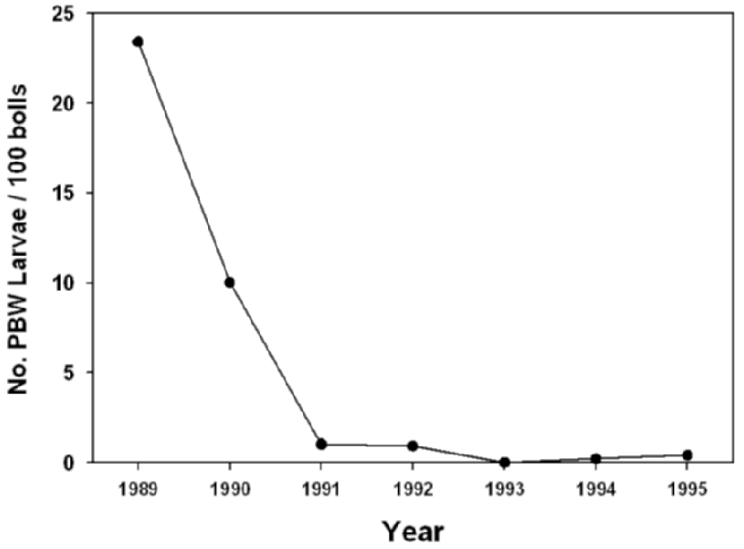

Figure 3. *Pink bollworm (PBW) larvae per cotton boll in 1989 prior to the integrated pest management programme using gossyplure mating disruption and chemical control and for six years following initiation of the programme (data from Antilla et al. 1996).*

enemies, and accumulated heat-unit mediated planting dates to exploit suicidal pink bollworm emergence are essential pink bollworm-IPM components (Fig. 2).

2.2. Host Plant Resistance

Transgenic cotton, the most recent breakthrough in host-plant resistance, has become the key pink bollworm-IPM component that supports and melds other components of the system into a functioning, highly effective management system. The successful development of cotton plants carrying the gene controlling the production of a *Bacillus thuringiensis* (Berliner) subsp. *kurstaki* (*Bt*-cotton) toxic protein was reported in 1989 (Gasser and Fraley 1989). Cotton growers in Arizona enthusiastically accepted *Bt*-cotton costs and more than 60% of the cotton area in Arizona since 1997 has been *Bt*-cotton types (Ellsworth and Jones 2001). *Bt*-cotton production has resulted in reductions in overall area-wide pink bollworm populations in Arizona (Carrière et al. 2003); a phenomenon that has not occurred over the last 40 years using conventional control.

2.2.1. Bt-*Cotton Trials in Arizona*

The first field trials using *Bt*-cotton for pink bollworm control were conducted at Maricopa, Arizona (Wilson et al. 1992, Wilson et al. 1994). Live larvae recovered from bolls, and percentage seed damage were reduced 97-99% compared with non-*Bt*-cottons. The *Bt*-lines tested were also highly resistant to other lepidopteran cotton insects, but not plant bugs or thrips. Beneficial insect populations were not affected. Evaluation of improved transgenic cotton lines and a commercial cultivar NuCOTN 33B® (Flint et al. 1995, Flint et al. 1996, Flint and Parks 1999), verified the high efficacy pink bollworm control results. The registration of *Bt*-cotton by the United States Environmental Protection Agency (EPA, Washington, D.C.) in 1996 signalled the beginning of a remarkable change in cotton pest management for western cotton growers, particularly with regard to pink bollworm. Resistance to *Bt*-cotton and the ramifications of the effect on *Bt* performance in all

its formulations used in agriculture were of concern (Mellon and Rissler 1998). Resistance management plans have been required as part of the registrations. The refugia/high-dose resistance management plan mandates that non-*Bt*-cotton plant refugia are grown in close proximity to *Bt*-cotton varieties. In theory, susceptible pink bollworm developed in the refugia mate with toxin-resistant individuals that survive in the *Bt*-cotton. Progeny produced by the matings are expected to have low or moderate *Bt*-toxin resistance and should not be able to survive on the high-toxin-level *Bt*-plants (Gould 1986).

2.2.2. Commercial Bt-*Cotton Production in Arizona*

Transgenic cotton with the Bollgard® gene (Monsanto Co., St. Louis, MO) was first planted commercially for lint production in Arizona in 1996 (Flint and Parks 1999). A summary of the eight-year history of the *Bt* performance for pink bollworm control and *Bt*-resistance management (Dennehy et al. 2004) showed that pink bollworm infestations have been smaller than 0.1% compared to pink bollworm infestations that develop annually to more than economic levels of 5-10% in non-*Bt*-cottons. However, laboratory strains have been selected for high levels of *Bt*-resistance (Bartlett 1995, Patin et al. 1999, Sims et al. 2001, 2002; also see Dennehy et al. 2004 for references).

2.3. Mating Disruption

Behavioural control with gossyplure is based on the concept that permeation of the pheromone material into the atmosphere of cotton fields results in the disruption of moth communication, the inhibition of male moth orientation and the prevention or reduction of mating (Shorey 1976). Cardé and Minks (1995) reviewed the pink bollworm sex pheromone chemistry, and early mating disruption research with dispensers ranging from steel planchets, polyethylene hollow fiber plastic laminate flakes, rope, encapsulated and other formulations for pink bollworm mating disruption. Readers are encouraged to read that publication for early research and results. A more recently developed pink bollworm-ROPE® slow release pheromone formulation (Shin-Etsu Chemical Industry Co., Ltd, Tokyo, Japan) (Flint et al. 1985, Flint et al. 1995) was used in pheromone behavioural control efforts in large-scale demonstration trials under low pink bollworm population densities in the Imperial and Coachella Valleys in California and the Mexicali Valley, Mexico (Staten et al. 1987a,b), and in Arizona from 1990 to 1995 following establishment of a pink bollworm population dynamics database in 1989 (Antilla et al. 1996). Over the six years of the latter study, pink bollworm larval infestations in cotton bolls were progressively reduced from around 23% in 1989 to less than 1% in 1995. Infestations were below the 5-10% economic threshold for the entire treatment programme (Fig. 3). Conventional insecticide use was reduced from over 89 000 thousand cumulative hectares treated per season before behavioural control was implemented compared to less than 1000 cumulative hectares treatment on year six of the programme. Costs of pink bollworm control were reduced from historic amounts of USD 173 or more/ha to USD 70/ha.

2.4. Bt *and Mating Disruption Integration in Pink Bollworm Management*

The outstanding performance of *Bt*-cotton and pheromone behavioural control for pink bollworm and the availability of historically-proven effective population suppression using other technologies (cultural controls, crop residue destruction and plough-down, water management, planting dates to provoke suicidal emergence, and sterile release) encouraged formulation of a multi-agency pink bollworm eradication plan (Anonymous 2001). The long-term objective is to eradicate the pink bollworm in stepwise programmes in western Texas, southern New Mexico and Chihuahua, Mexico, western New Mexico and Arizona, California and northern Mexico, respectively, while continuing to prevent pink

Table 1. *Mean numbers of pink bollworm male moths caught in gossyplure-baited traps at the Irrigated Desert Research Station, Brawley, California and in commercial cotton fields in Imperial Valley, California, from 1989 to 1994.*

Month		One year before mandatory[1] short-season	No. male moths per trap per night[2] Years following short-season programme				
			1	2	3	4	5
Irrigated Desert Research Station							
March to August	Range	0.01 - 255.1	0.0 - 26.7	0.0 - 29.0	0.0 - 7.4	0.0 - 0.01	0.0 - <0.01
	Mean ± SE	9.3 ± 41.6	4.6 ± 4.4	5.8 ± 4.7	1.5 ± 1.2	<0.01 ± 0.0[3]	<0.01 ± 0.0[4]
Commercial Cotton Fields							
April to September	Range	2.6 - 7.9	0.1 - 9.6	0.1 - 5.9	0.1 - 1.8	0.0 - 1.4	0.0 - 0.2
	Mean ± SE	2.7 ± 1.1	2.7 ± 1.5	1.5 ± 0.9	0.7 ± 0.3	0.3 ± 0.2	0.04 ± 0.02

[1]Chemical crop termination mandated on 1 September 1989

[2]Lingren live traps at the Irrigated Desert Research Station, Delta traps in commercial fields

[3]Fifteen male moths caught with 20 traps during the cotton season

[4]Three male moths caught with 12 traps during the cotton season

bollworm establishment in California's San Joaquin Valley. The major IPM programme components are area-wide short-season cultural control strategies and *Bt*-cotton to reduce overall populations, and integration with pheromone behavioural control and judicious use of chemical control as needed to complete the management system. The final phase of the programme focuses on the potential of eradication with sterile pink bollworm moth releases that are highly effective under low population densities. The *Bt*-cotton IPM component is considered essential, since in-season or follow-up pheromone behavioural control and sterile pink bollworm release methods are most effective at low population levels.

2.5. Cultural Control and Short-Season Management

Overwintering pink bollworm diapause populations are particularly vulnerable to suppression strategies, providing a weak link that can be manipulated in support of the implementation of an area-wide suppression strategy (Henneberry and Naranjo 1998), and a vital component of the eradication strategy with potential benefit to *Bt*-resistance management (Carrière et al. 2001). Typically, 90% of the total upland yield-producing cotton bolls produced are set by 15 September and over 95% of the diapause larvae mature in immature bolls on plants after 15 September (Fig. 1). Removal of late-season immature cotton bolls reduces host material for development of the diapause population (Kittock et al. 1973). In the Imperial Valley of California establishing the earliest cotton planting date as 1 March and 1 September as the dates for defoliant or plant growth regulator application, with 1 November established for cotton stalk destruction and plant residue plough-down (Chu et al. 1996), resulted in a pink bollworm moth trap catch decline each year for four years following initiation of the programme (Table 1). Fewer pink bollworm larvae per boll occurred during each season (Table 2). Numbers of diapause larvae were reduced over 90% (Table 3). Cotton yields and quality increased, and the need for insecticidal control of pink bollworm decreased. Average lint yield of 5.7 bales per hectare in 1989 prior to

Table 2. *Mean number of pink bollworm larvae per 100 incubated immature cotton bolls from the Irrigated Desert Research Station and in commercial cotton fields in Imperial Valley, California, from 1989 to 1992.*

Month	One year before mandatory[1] short-season	No. Larvae/100 incubated bolls in year		
		1	2	3
Irrigated Desert Research Station				
June	1.3	---	---	---
July	22.4	1.2	4.3	0.0
Aug	19.4	73.4	12.1	0.0
Mean ± SE	14.4 ± 6.6	37.3 ± 36.1	8.2 ± 3.9	0.0
Commercial Cotton Fields				
June	---	2.0	0.0	---
July	22.9	1.1	0.3	2.5
August	89.5	14.9	2.7	12.5
September	91.6	27.2	25.3	0.0
Mean ± SE	68.0 ± 22.6	11.3 ± 6.2	7.1 ± 6.1	5.0 ± 1.5

[1]Chemical crop termination mandated on 1 September 1989
--- No samples taken

programme initiation increased to 6.7 bales per hectare in 1993 and to 6.9 bales per hectare in the third year (1994) following programme initiation. The mean percentage of cotton classified as top lint grade was 54% during 1984 to 1988 and increased from 90 to 99% between 1990 and 1994.

2.6. Sterile Pink Bollworm Moth Release

Sterile pink bollworm release as a component of pink bollworm IPM has a long history beginning with the concept as conceived and implemented by Knipling (1979). The development of an artificial diet (Vanderzant and Reiser 1956) and mass-rearing technology (Richmond and Ignoffo 1964) were essential accomplishments before sterile pink bollworm release could be considered feasible. Early work by Graham (1972) demonstrated radiation-induced pink bollworm sterility and Richmond and Graham (1970, 1971) demonstrated reproductive suppression with sterile releases in field cage tests. Early field studies with sterile moth releases for suppressing

Table 3. *Estimated[1] mean numbers of diapause pink bollworm larvae in commercial cotton fields before and after short-season cotton production in Imperial Valley, California, from 1989 to 1992.*

	No. larvae per hectare	% Reduction
Pre-programme	95 000	---
Year of programme		
1	42 000	56.0
2	300	93.0
3	200	83.0

[1]All immature green cotton bolls collected from four metres of cotton row in ten areas of each field. All bolls dissected and larvae held to determine diapause.

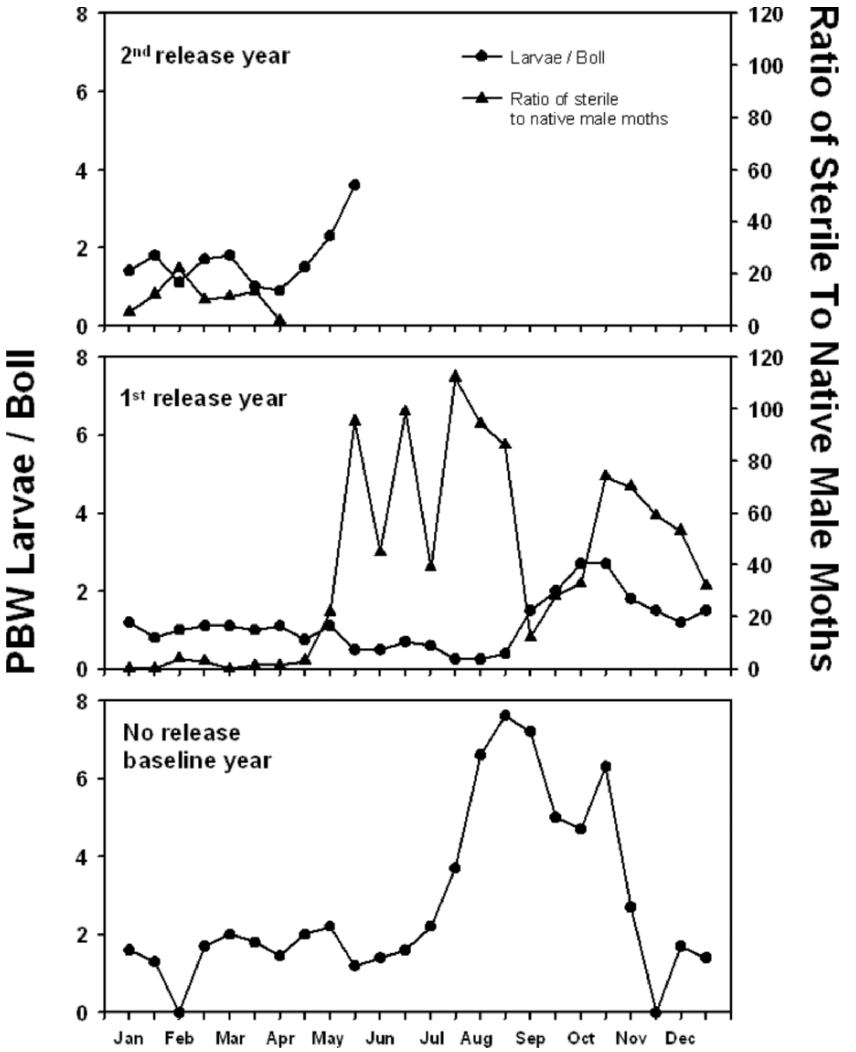

Figure 4. *Mean number of pink bollworm (PBW) larvae per cotton boll (lower graph) before sterile moth releases, (middle and upper graph) ratios of sterile to native moths and larvae per boll during releases and for three months following the last release.*

established populations were only partially successful or failed (Henneberry and Keaveny 1985). However, sterile pink bollworm moth releases in cotton plots on an isolated island (St. Croix) in the Caribbean, verified the effectiveness of the technique for reducing established infestations (Henneberry and Keaveny 1985). Sterile pink bollworm moth releases were made by hand in cotton plots from January 1981 to April 1982. Reduced boll infestations occurred when ratios of released sterile to St. Croix males averaged 72:1. Infestations increased when the sterile to St. Croix male ratios averaged 20:1. However, the 20:1 released sterile to St. Croix male ratio apparently had some population suppression

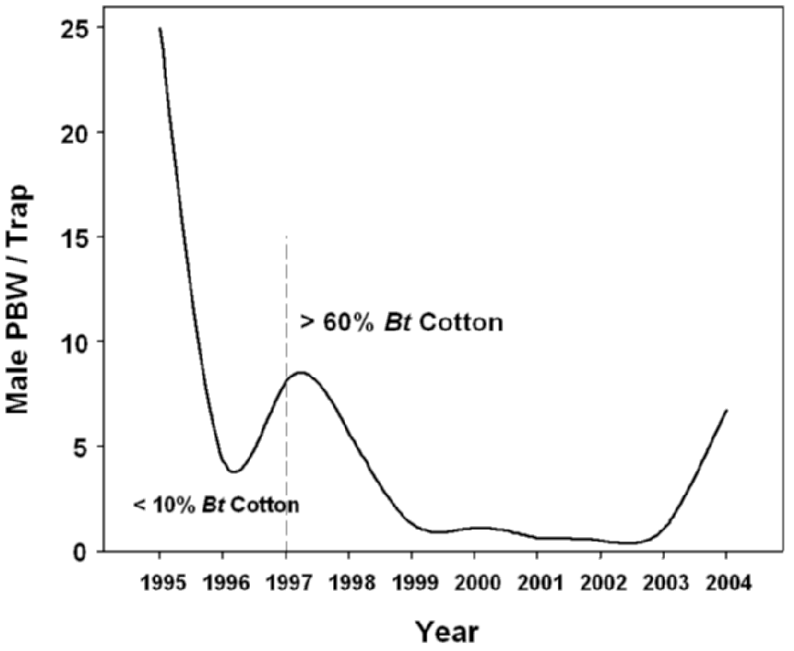

Figure 5. *Yearly male pink bollworm (PBW) trap catches in 1995 and 1996 in Arizona prior to extensive* Bt-*cotton production and from 1997 to 2004 when greater than 60% of the cotton grown were* Bt-*cultivars (Data from the Arizona Cotton Protection Research Council, 600 to 1000 traps per year in 24 cotton growing areas of the state).*

effect, since larval infestations increased from about one larva per boll to 3.7 larvae per boll during the two months immediately after the last release of sterile insects (Fig. 4).

The sterile-moth release component of the eradication trial has not yet been implemented as part of the programme. The high ratios of sterile release to native moths required to start a downward trend in population clearly underlines the necessity for continuing efficiency of the other cultural, biological, and *Bt*-host-plant resistance components of the programme (Fig. 5).

2.7. Pink Bollworm Eradication Progress

The eradication programme was initiated in 2001-2002 in approximately 22 300 hectares of cotton in the El Paso/Trans Pecos area of Texas, 10 500 hectares in South Central New Mexico and 32 400 hectares in Chihuahua, Mexico (El-Lissy et al. 2003). Pink bollworm pheromone was applied only to non-*Bt*-cotton, or *Bt*-cotton with embedded non-*Bt*-refuges (95:5 ratio). *Bt*-cotton was 44, 37, and 58% of the cotton production in the El Paso/Trans Pecos, South Central New Mexico, and Chihuahua, Mexico areas, respectively. Male moth catches were reduced 84, 91 and 95% per trap and pink bollworm larval infestations were 0.81, 0.45, and 0.30 per boll, respectively, in the areas described. The results were exceptionally encouraging and provide promising expectations for the remainder of the programme in other infested areas of the south-western USA and Mexico. After three years of the eradication programme, the pink bollworm is no longer an economic pest in the El Paso/Trans Pecos cotton zone of the targeted eradication area. The population has been reduced to levels that can be targeted for sterile moth releases to pursue the goal of eradica-

tion (Smith et al. 2004).

3. Discussion

The pink bollworm eradication effort is a formidable undertaking. The necessary technologies to achieve eradication are in place, and the *Bt*-component must continue to be effective to move the programme forward on schedule. The *Bt*-refugia/high dose strategy is heavily relied upon to prevent or delay resistance development. Experience and performance of the *Bt*-refugia system suggest that the concept is working, but validation and quantification of the refugia impact remain undocumented. The "high dose" for pink bollworm remains undefined (This high dose strategy suggests that the amount of toxic protein is sufficient to kill the few pink bollworm larvae low resistance level progeny from parents surviving exposure to the *Bt* crop). Additional information on size, placement (distance from *Bt*-cotton), or configuration of refugia for optimum effects will need to be developed for use in area-wide pink bollworm suppression with the goal of eradication.

Pink bollworm *Bt*-resistance development has not been detected in the field at levels affecting efficacy. The lack of reduced susceptibility has not been explained, but there is no reason for complacency or justification for diminished research effort to improve knowledge of the system. The success of the area-wide programme will be heavily reliant on manipulation of refugia to maintain *Bt*-efficacy over the duration of the programme and longer without resistance development. Resistance monitoring and rapid response team coordination to investigate suspected pink bollworm infestations are invaluable segments of the eradication effort. If *Bt*-resistance is identified and verified, a remedial action plan has been clearly described (Carrière et al. 2001), and expected to effectively combat resistance development, but implementation of this plan is voluntary.

The programme aspects, which include behavioural control with pheromone applied to non-*Bt*-cotton varieties and the optional use of selective chemicals only when infestations threaten economic losses, appear as programme-compatible actions. The natural enemy IPM component is facilitated through conservation by judicious chemical approaches, economic threshold decision-making, pheromone behavioural control and heat unit-mediated planting to obtain maximum benefit from suicidal emergence. These approaches melded with the short-season cultural techniques to reduce overwintering survival provide year round area-wide, ecologically-oriented pink bollworm population suppression that has been demonstrated effective, economical, and grower acceptable.

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Pulling Out the Evil by the Root: the Codling Moth *Cydia pomonella* Eradication Programme in Brazil

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ABSTRACT Since the codling moth *Cydia pomonella* (L.) was detected in parts of the Brazilian apple growing area a series of surveillance and control actions have been carried out. Firstly, a trapping survey determined the extent to which the pest had spread in Brazil. Pheromone traps were set up and serviced during one season throughout the temperate fruit growing region, at ports of entry and in the main southern commercial centres (São Paulo, Florianópolis, Santos, Curitiba). This trapping demonstrated that only urban areas within four municipalities were affected: Vacaria, Bom Jesus, Caxias do Sul and Lages. Except for the last, the affected municipalities are important apple growing regions, with more than 3000 hectares of apples each. In 1998/1999, a pilot project of population suppression was undertaken in these affected areas, applying lure-and-kill (male annihilation). This technique proved to be highly efficient in reducing codling moth populations. Results encouraged the elaboration of a series of scientific projects and the development of a plan to eradicate the codling moth from Brazil. The programme has several components: (1) commercial apple orchards adopted detection trapping to identify any new invasion event, (2) removal of host and potential host trees in urban areas and replacement by non-host trees in many cases adopted as an alternative to direct population suppression in the urban areas, and (3) the potential use of the sterile insect technique (SIT) in certain areas. The host tree removal campaign led to a significant decrease in codling moth populations as evidenced by the decrease in the number of catches in pheromone-baited traps used for monitoring. The main constraints to the development of the eradication programme were bureaucratic and legislative. Lack of funds and of national legislation on semiochemicals jeopardized effective actions. In this report, updated data are presented on codling moth population dynamics in four infested areas as well as an overall evaluation of the survey and control activities carried out thus far.

KEY WORDS *Cydia pomonella*, codling moth, SIT, host availability, lure-and-kill, eradication, Brazil, apple, host removal, biological invasion

1. Historical Background

The codling moth *Cydia pomonella* (L.) is a quarantine pest of apples in Brazil. Given its importance in other apple growing countries, efforts to monitor for possible incursions started in the 1980s, using a few traps which invariably returned zero catches. In 1991, a single pheromone-baited trap was deployed in a domestic orchard in the urban area of Vacaria, Rio Grande do Sul. One male codling moth was caught and again in 1992, two males were trapped at the same location.

In January 1993, traps were set up in other municipalities near Vacaria, but no moths were trapped. At that time, pheromone traps were not available in Brazil and had to be imported from Chile. Only in 1997-1998 did traps become available and could be deployed at the appropriate time of the season, in early September.

The programme faced several early obstacles, such as lack of understanding about the importance of preventing the colonization and

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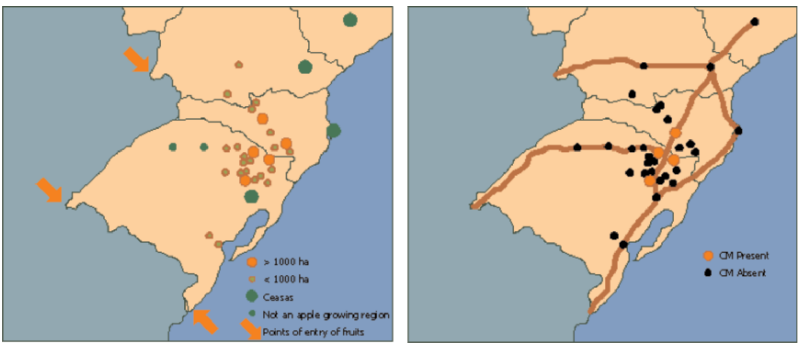

Figure 1. *Delimiting survey of* Cydia pomonella *in the three southern states of Brazil and São Paulo. (left) Municipalities where traps were deployed indicating major apple growing regions (red circles), minor apple growing areas (green circles) and points of entry of fresh fruits from Uruguay, Argentina and Chile (arrows), and (right) uninfested (black circles) and infested municipalities (red circles).*

spread of a new apple pest in Brazil. This was turned around by the dynamism of apple growers who, aware of the importance of the codling moth in other countries, provided financial support to official actions to prevent its establishment. The plant protection actions of the Ministry of Agriculture were improved, and the inspection of imported temperate fruits became routine at all ports of entry.

Now, 15 years after the first detection of *C. pomonella* in Brazil, the programme is quoted throughout the country as an example to be followed with researchers, control officials and growers joining efforts to prevent the establishment and spread of a new pest. This paper presents an overview of the codling moth control programme in Brazil since detection of the pest in the urban area of Bom Jesus, as well as forthcoming activities.

2. Detection Trapping

Detection trapping was initiated during the 1980s, with a few traps baited with codling

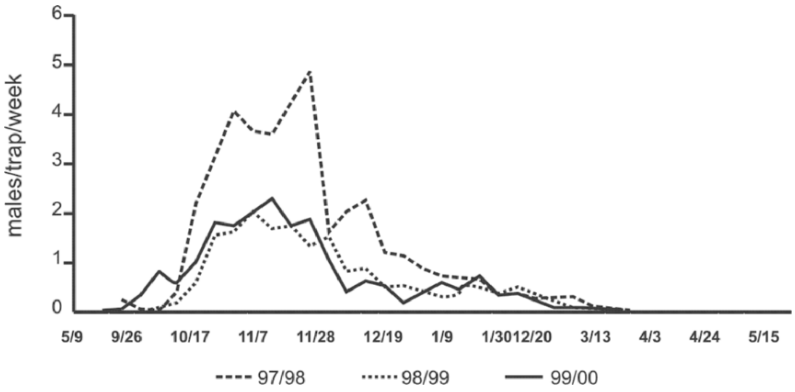

Figure 2. *Population dynamics of the codling moth in Lages, Santa Caterina, Brazil, as indicated by weekly male catches in pheromone-baited traps (1997-2000).*

Table 1. *Number of traps deployed in the urban areas of the four municipalities affected by the codling moth in Brazil.*

Period	Vacaria	Lages	Bom Jesus	Caxias do Sul
1998/1999 to 2003/2004	400[1]	400	100[1]	180[1]
2004/2005	400	1700	200	400
2005/2006	1100	1700	400	550

[1]Incomplete data due to lack of traps in some years

moth sex pheromone. The first male was caught in October 1991. Another two males were caught in the same domestic orchard in October 1992. In January 1993, the number of traps was increased to 32 but no males were caught. In 1994-1995, a network of detection traps was deployed in the field, ranging from the southernmost region of the country to São Paulo (Fig. 1).

Four municipalities were defined as affected: three in the state of Rio Grande do Sul (Vacaria, Bom Jesus and Caxias do Sul) and one in the state of Santa Catarina (Lages). Monitoring in urban areas of affected municipalities was carried out with synthetic pheromone-baited traps (Table 1).

With the establishment of integrated fruit production, detection trapping was adopted as an obligatory action throughout the 30 000 hectares of commercial apple production. Growers are in charge of trap deployment and service and they receive training on codling moth identification. They are also instructed to bring to Embrapa Uva e Vinho, in Vacaria, any trapped individuals that may resemble the codling moth.

In commercial areas located less than 30 kilometres from urban areas where the codling moth was detected, a density of one trap per five hectares is adopted. In other areas the recommendation is one trap per ten hectares. To date, there are no records of codling moth males being trapped in Brazilian commercial apple orchards. Inspection procedures are submitted to external audits by the Plant Protection Service in the states of Santa Catarina and Rio Grande do Sul.

3. Population Dynamics

In Brazil, adult codling moths are first trapped in September and the population peaks from late October to mid December. Laboratory observations corroborate these data on the onset and peak of adult emergence and also indicate that 95% of field-collected larvae enter diapause which may last for up to two years (Fig. 2).

This univoltine pattern differs from that described in other South American countries, where the pest is bivoltine. A possible explanation is the shorter photoperiod during summer, due to the lower latitude of the Brazilian apple growing area compared with more southerly regions in South America. Thus, regardless of the adopted methodology an eradication programme should concentrate actions from October to January.

4. Lure-and-Kill Pilot Project

The delimiting survey demonstrated that the codling moth was restricted to four urban areas. The use of chemical control was deemed unacceptable due to the potential hazard it could present. There were few alternatives: the sterile insect technique was not available in Brazil, nor were pheromone-based techniques due to lack of registered materials.

Brazil did not have a specific legislation for pheromones and their registration had to follow the same procedures adopted for pesticides. However, in 1998, the Ministry of Agriculture of Brazil responded to the prob-

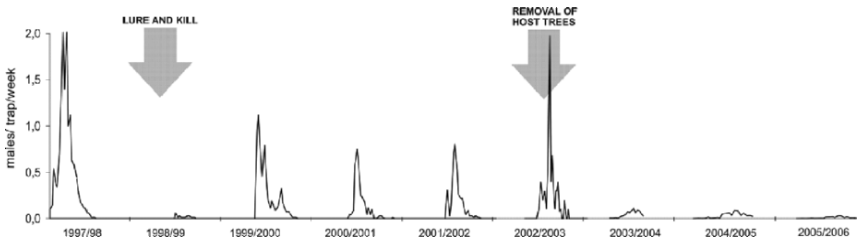

Figure 3. Cydia pomonella *population density in the urban area of Vacaria, Rio Grande do Sul, Brazil, 1997-2006 as revealed through trap catches. In 1998-1999, lure-and-kill was applied throughout the urban area and partially in 2000-2001. In 2002-2003 host plant replacement was initiated and in 2005-2006, only 0.6% of the original host plants remained in the urban area.*

lem and allowed an urgent importation of pheromone dispensers which were used to attract males to paper panels impregnated with naled, an organophosphate with long residual effect.

The technique was applied throughout the urban areas of Vacaria and Bom Jesus and in part of the urban area of Lages. Results were immediately positive and promising. In 1998-1999, the population density of *C. pomonella* in Vacaria and Bom Jesus was reduced to less than 5% of that observed in 1997-1998. Nevertheless, in 1999-2000, there were neither funds nor pheromone dispensers available and the control operations could not be continued. The codling moth population density increased in that season, although not to the levels observed in 1997-1998, but it was clear that lure-and-kill itself would not be sufficient to prevent further population build up. Lure-and-kill was applied a second time in Vacaria during 2000-2001 and in Bom Jesus in 2003-2004 in those locations identified as having high infestation levels. Difficulties in registration of pheromone-based techniques and high costs led the working group involved in the programme to focus on a second technique, the removal and replacement of host plants from urban areas.

5. Host Plant Replacement

It was hypothesized that the elimination of host plants from urban areas would reduce the codling moth population. Activities were started in Lages in 2001 and in Vacaria in

Table 2. *Number of host plants and potential host plants removed and the estimated number remaining in the urban areas of the four affected municipalities.*

Municipality	Urban area (hectares)[1]	Trees replaced (no.)	Trees remaining (no.)	Trees removed (%)
Lages	12 000	34 500	200	99.4
Vacaria	6000	16 020	600	96.4
Bom Jesus	1000	1700	300	85.0
Caxias do Sul	60 000	37 609	22 391	62.7
Total		89 829	23 491	86.9

[1]Approximate values

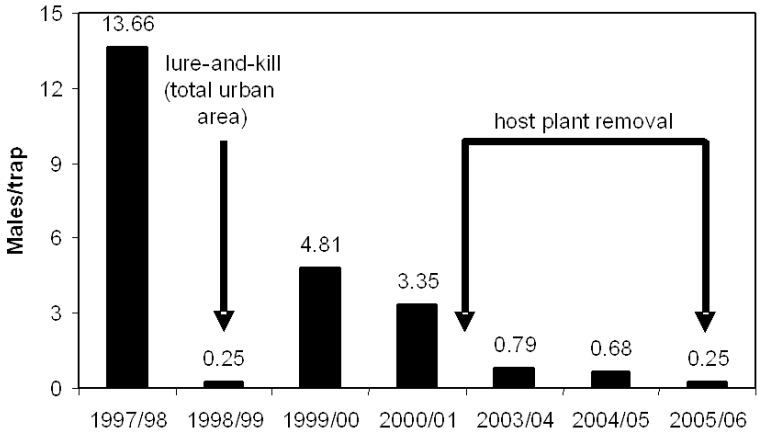

Figure 4. *Number of codling moth males per trap from 1997-1998 to 2005-2006 in Vacaria. Few traps were used for monitoring in 2001-2002 and 2002-2003. For this reason, data are not included in this graph.*

2002. In Vacaria, the replacement of host plants occurred centripetally to avoid the dispersal of codling moth from the urban area to orchards located nearby. In both areas, the work was supported by apple growers and the Ministry of Agriculture of Brazil, with both groups allocating personnel, funds, and equipment to the campaign mainly during the apple off-season. The number of trees removed and replaced and the estimated number of remaining trees is summarized in Table 2.

At this point the cooperation of mass-communication media such as local radio stations and newspapers was crucial for the success of the campaign. Residents became aware of the importance of the apple crop in nearby areas and of the negative impact that codling moth establishment would have on the economy of the region.

The impact of host removal on trap catches was immediate and long-lasting. Taking Vacaria as an example, after the onset of actions to eradicate host plants in 2002, the highest codling moth population density

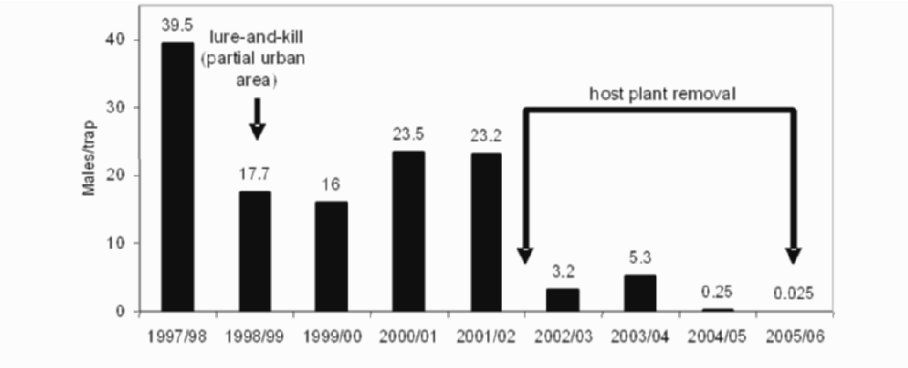

Figure 5. *Number of codling moth males per trap from 1997-1998 to 2005-2006 in Lages.*

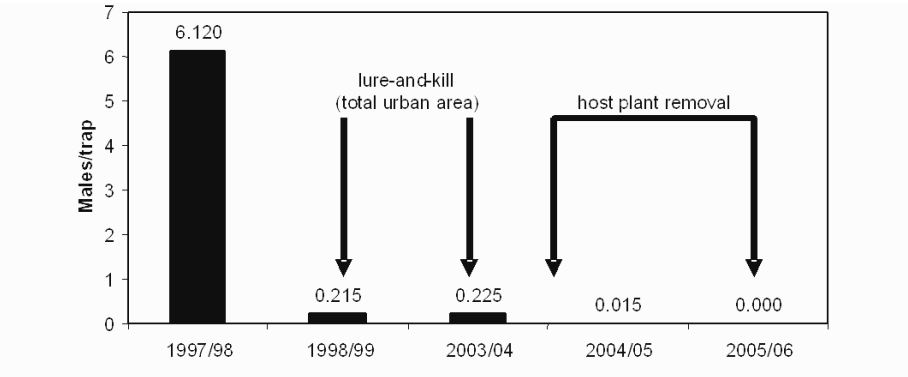

Figure 6. *Number of codling moth males per trap from 1997-1998 to 1998-1999 and from 2003-2004 to 2005-2006 in Bom Jesus.*

decreased from approximately two males per trap per week in 1997-1998 to 0.02 males per trap per week in 2005-2006 (Fig. 3). Similar results were observed in Bom Jesus, Lages and Caxias do Sul (see section 6).

6. Monitoring

Monitoring in commercial and urban areas of the temperate fruit growing region of Brazil had been conducted since the early 1990s, but 1997-1998 can be considered as the starting point of systematic monitoring.

The four areas showed different population densities at the beginning of the systematic monitoring in 1997 (Figs. 4-7). In 1998-1999, lure-and-kill was applied in the urban areas of Vacaria, Bom Jesus and Lages. In Vacaria, 20 000 killing panels were set up in the urban area. These inverted V-shaped panels had an internal surface covered with stick. The paper panel and adhesive surface were dipped in an organophosphate insecticide. After insecticide impregnation, a pheromone dispenser was attached to the system with a pin. The impact of lure-and-kill during the 1998-1999 season was clear: the codling moth population was reduced to almost undetectable levels. It is reasonable to suppose also that monitoring traps may have been affected by the presence of a large number of pheromone dispensers in killing panels. In the three subsequent seasons (September 1999 to March 2002), the population density increased following the suspension of lure-and-kill actions. During the spring of 2002, host trees were replaced by non-host trees in the urban area of Vacaria. The impact of this action was promptly realized in greatly reduced trap catches in subsequent years (Fig. 4).

Lages had the highest initial codling moth density, with almost 40 males per trap during the 1997-1998 season (Fig. 5). A partial application of lure-and-kill was accomplished in 1998-1999 but the population density was high during the next five seasons of monitoring, with mean densities close to or higher than 20 males per trap. In 2003, almost 30 000 host plants were replaced by non-host trees in Lages. As was observed in Vacaria, this action had a profound impact on the population, with a marked reduction in abundance.

In Bom Jesus, Rio Grande do Sul, lure-and-kill was applied during the spring of 1998-1999. Trapping data show that although the population density at the beginning of the programme was very high it was reduced to almost undetectable levels in 2004-2005 and to zero in 2005-2006 (Fig. 6).

During the same period, the codling moth was monitored in Caxias do Sul, Rio Grande do Sul. In this municipality, suppression actions were not undertaken until 2004-2005 when host tree removal was initiated. Actions

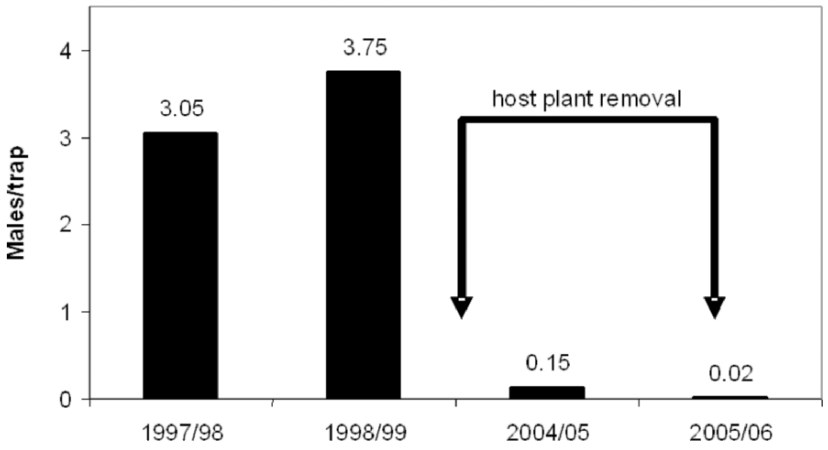

Figure 7. *Number of male codling moths per trap from 1997-1998 to 1998-1999 and from 2004-2005 to 2005-2006 in Caxias do Sul.*

in Caxias do Sul were delayed because it is a much larger urban area and the fact that agriculture plays a much less important role in the economy of the municipality. In 2005-2006, the population density was markedly reduced (Fig. 7). Here, since a significant proportion of city residents did not agree with the replacement of host plants, other techniques may have to be employed to ensure eradication, such as the SIT.

7. External Quarantine Actions

In 2004, after several meetings involving the Brazilian and Argentinean plant protection agencies, an inspection protocol was implemented in the packinghouses in Argentina that market fresh apples and pears to Brazil. They had to be able to demonstrate that fruits originated from areas of low codling moth prevalence or, in cases in which they did not, that growers had adopted effective control methods. This decreases the risk of new invasion events as Argentina is the main supplier of fresh pears to Brazil. Also, an inspection routine of pome and stone fruit shipments from all other countries was established at borders, airports and ports.

8. Benefit/Cost Analysis

The first assessment of benefits and costs associated with *C. pomonella* eradication in Brazil indicated that if the no action scenario was chosen, the codling moth would become established in commercial orchards and lead to control costs much higher than those estimated for an eradication programme based on host plant replacement and lure-and-kill (Kovaleski et al. 2001).

A spreadsheet model was produced by Mumford (2005) to provide a basis for a benefit/cost analysis for codling moth eradication in Brazil based on data from Kovaleski et al. (2005) and interviews with growers and officials involved in the monitoring and eradication. The model is a stochastic simulation model based on best estimates of parameters likely to affect the values of production and control of codling moth host crops (apple, pear and quince) in the three southern states of Brazil, and the options and costs of eradication in the four affected urban areas in Brazil. Fig. 8 illustrates an output. The potential losses to the fruit industry in Brazil if eradication or containment is not achieved in the urban areas are very significant, with an estimated

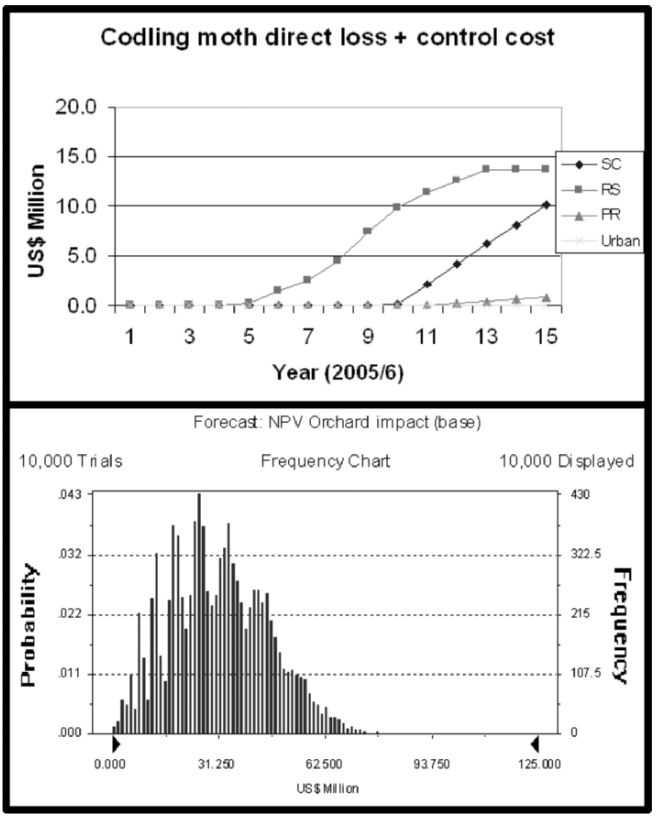

Figure 8. *(upper) An example simulation iteration for spread beginning around five years from present (SC: Santa Catarina, RS: Rio Grande do Sul, PR: Paraná), and (lower) the frequency distribution of net present values (NPV) of commercial losses over 15 years at 10% discount rate (adapted from Mumford (2005)).*

net present value (over 15 years) for losses and costs of up to USD 113 million.

The pathways for reinvasion from Argentina have been eliminated to the degree practical through border quarantine and packing house inspections for Argentine apple exports. Intensive monitoring has shown no spread to commercial orchards in 15 years of infestation in four urban areas of Brazil. Substantial progress has been made in two of the urban areas to remove host trees. Eradication appears to be technically feasible, with further host tree removal and replacement, lure-and-kill in areas with small numbers of remaining host trees (less than 5%) and SIT in areas with over 5% of remaining host trees. It was estimated that the cost of eradication, assuming spread into orchard areas did not occur for 3-7 years, would range from USD 0.286 million to USD 0.644 million, with a mean of USD 0.452 million (Mumford 2005). If SIT were used for the control of codling moth in the four infested areas of Brazil, it is estimated that the numbers of sterile moths required would range from 1-1.5 million per week considering that releases would be directed only against relic moths. This assumes two years of further host

tree removal and concentration of the infestations within the smallest areas of highest host density. Under these scenarios, no infestations or costs occur in commercial orchards. However, without further host tree removal the likelihood of spread into commercial orchards is significant. Under this scenario, costs range from a minimum over USD 0.8 million (in simulations with no spread to commercial orchards), to a mean of around USD 1.5 million (with some impact on commercial orchards), and a maximum cost of over USD 100 million in simulations with extensive infestations occurring in commercial orchards (Mumford 2005).

9. Conclusions

The codling moth is both an interesting issue for scientific investigation in the field of invasion biology and a unique opportunity for plant protection agencies to take action to prevent establishment of a significant new pest. Results obtained thus far are promising and apple growers have agreed to continue investing in codling moth eradication, so as to declare Brazil a codling moth-free country like Japan. Much practical work was necessary, with many setbacks, to secure the credibility of an eradication programme in the eyes of growers and technicians. This is particularly difficult in a country like Brazil with a limited tradition in plant protection. Fortunately, growers are organized in associations and are aware of the direct and indirect costs that the establishment of a new pest would cause them. Currently, the codling moth eradication programme is a major concern for growers. It has been demonstrated that eradication is technically feasible and cost-effective. The increase in the trapping effort in urban areas aims to define hot spots clearly and rapidly. The next step will be the application of the SIT, mainly in Caxias do Sul, an industrial municipality where the eradication programme has been facing difficulties. An analysis of the technical and economic feasibility of importing sterile males from the Okanagan-Kootenay Sterile Insect Release mass-rearing facility in Osoyoos, Canada will be undertaken. Costs and benefits associated with the importation of sterile insects will be compared with those associated with establishing a mass-rearing facility in Juazeiro, Brazil (Malavasi et al., this volume).

10. Acknowledgements

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Suppression of the Codling Moth *Cydia pomonella* in British Columbia, Canada Using an Area-Wide Integrated Approach with an SIT Component

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ABSTRACT Since 1994, the codling moth *Cydia pomonella* (L.) has been the subject of an area-wide suppression programme in the pome fruit producing areas of the south-central part of British Columbia based primarily on the sterile insect technique (SIT). The programme was initially implemented with eradication as its ultimate goal, but since no quarantine measures were implemented, at the end of the 1998 growing season, the objective of the programme became area-wide suppression. The present article summarizes the progress of the codling moth programme since the shift from an eradication to a suppression objective was made and the benefits achieved in terms of reductions in insecticide applications and crop losses. It also discusses new research that could benefit this and future area-wide programmes for codling moth, and presents some thoughts to consider for furthering the long-term sustainability of the programme.

KEY WORDS Lepidoptera, codling moth, *Cydia pomonella*, AW-IPM, suppression, pome fruit, SIT, gamma radiation

1. Introduction

The codling moth *Cydia pomonella* (L.) is considered the key pest of apple and pear in the fruit growing valleys of southern British Columbia (Fig. 1, upper), and in most regions of the world where pome fruit is grown (FAO/IAEA 2000). Codling moth originated in Asia Minor and has been an important pest of apple and pear in North America for more than 200 years (Beers et al. 1993). In British Columbia, infestations of codling moth were first reported from Victoria in 1900, and by 1916 codling moth was a serious pest in the Okanagan and Similkameen Valleys some 600 kilometres away (Marshall 1951). Codling moth typically completes two generations per year in British Columbia (Madsen and Vakenti 1973). After eggs are laid singly on the fruit or leaves of host trees, the emerging larvae burrow into the developing fruit rendering it unmarketable. Traditionally, well-timed appli-

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 591–601.

Figure 1. *Map indicating (upper) the project area in British Columbia and (lower) the different treatment zones.*

cations of pesticides have been used to kill the larvae before they penetrate the fruit.

To delay the development of insecticide resistance as well as to deal with concerns about excessive pesticides in the environment, several attempts have been made to develop and use the sterile insect technique (SIT) as part of an integrated approach against codling moth (Bloem et al. 2005a and references therein). However, only in British Columbia did the research lead to an actual implementation programme – the Okanagan-Kootenay Sterile Insect Release (OKSIR) programme. Research on the SIT for codling moth published by the Canadian entomologist M. D. Proverbs and his colleagues included data on sterilization with gamma radiation (Proverbs 1962, Proverbs and Newton 1962a,b), development of an inexpensive agar-free meridic diet (Brinton et al. 1969), design of a waxed paper rotating oviposition cage (Proverbs and Logan 1970), and design and testing of ground-release devices to distribute irradiated and chilled adults in the orchard (McMechan and Proverbs 1972). The OKSIR programme currently uses all of these components with only very slight modifications (Dyck et al. 1993, Bloem and Bloem 2000). The research conducted by Proverbs and his group over a 15-year period culminated in a three-year (1976-1978) pilot project. This project demonstrated that eradication of codling moth was possible by integrating the SIT with insecticides (Proverbs et al. 1982).

Currently, the fruit growing regions in British Columbia include about 4664 hectares of commercial fruit tree production, as well as several urban centres containing abundant backyard fruit and ornamental trees that are hosts of codling moth. The tree fruit industry in the Okanagan Valley consists of 1800 farms that generate 5000 on-farm jobs and 2500 jobs in packinghouses and supporting industries. The industry generates USD 173 million in revenue and USD 777 million in economic activity per year (BCFGA 2006).

The current OKSIR programme was launched in 1992, the mass-rearing facility was built in 1993, and the first releases of irradiated adults took place during the 1994-growing season. The area-wide programme has continued yearly operations in orchards, urban centres and other potential host areas for ten years. Proverbs et al. (1982) summarized the research conducted during development of the SIT, Dyck et al. (1993) discussed the history and politics of implementing the OKSIR programme, and Bloem and Bloem (2000) reviewed the period between its inception (1992) and the end of 1997. Bloem and Bloem (2000) include data on the construction and operation of the mass-rearing facility, as well as information on yearly activity schedules, target overflooding ratios, issues of insect quality, by-law enforcement in orchard and urban areas, and address the programme costs from construction to public outreach activities. The OKSIR programme has evolved from an eradication programme to a sustainable area-wide suppression programme during the last seven years (1998-2005). In this paper, the progress made since 1997 is reviewed and the changes in philosophy and approach that have taken place during these years are described. In addition, the results obtained by the programme are summarized and a review provided of how well the results have met stakeholder expectations. Finally, a brief summary of recent research that could improve the field performance of irradiated codling moth is included, and conclusions drawn with respect to the future outlook envisaged for the OKSIR programme beyond 2006.

2. Change in Programme Objectives

Following several benefit/cost studies (see Dyck et al. 1993 for references), DeBiasio (1988) developed the initial implementation plan for eradication. The DeBiasio plan had three phases that would be completed over eight years. Phase one (sanitation) would reduce wild populations over two years using conventional controls with the objective of maximizing laboratory-to-wild overflooding ratios once releases of irradiated codling moth were initiated. Phase two (release) concentrated on releases of irradiated male and female codling moths for three years. Two zones, each of about 4000 hectares, would be treated sequentially and urban host trees would be included in the programme. DeBiasio (1988) assumed that the isolation of the valleys would prevent reinfestation following eradication, and suggested that releases of irradiated insects would need to continue indefinitely along the USA-Canada border, considered to be the only plausible route for reinfestation (phase three: prevention).

The OKSIR programme roughly followed the DeBiasio plan from 1994 to 1997 (see review by Bloem and Bloem 2000 for departures from original plan); however, an internal review at the end of 1996 recommended that the treatment area be divided into three (rather than two) treatment zones. Zone 1 in the south (2600 hectares), zone 2 in the central portion (1800 hectares) and zone 3 in the north (1000 hectares) of the Okanagan, Similkameen and Shushwap Valleys (OKSIR 1996) (Fig. 1, lower).

A change in management structure and personnel occurred at the end of 1997. An assessment after the first year under new management concluded that ongoing area-wide suppression rather than eradication was a more feasible and achievable long-term objective for the OKSIR programme. This decision was made by the programme's Board of Directors following a number of considerations: (1) a technical advisory committee concluded that despite the success to date in zone

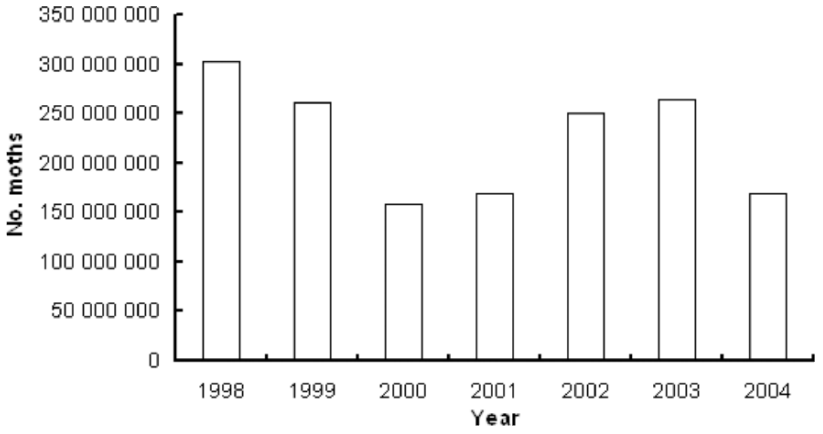

Figure 2. *Yearly number of codling moths produced at the mass-rearing facility in Osoyoos, BC., Canada (1998-2004).*

1, eradication would not be achieved throughout the entire area because of the scale involved and the limited human and and financial resources available, (2) this committee also believed that a quarantine programme would be necessary to ensure no codling moth reinfestation should the SIR programme achieve eradication, and (3) the Federal Government of Canada, under whose jurisdiction a quarantine area would operate indicated that no plans were in place to operate a quarantine programme.

Since the start of the 1999 season, orchard and urban programme operations were directed toward this revised goal. In general terms, full-scale (i.e. 1800-2500 irradiated moths per hectare per release, and releases twice weekly for 20 weeks per season) moth releases in zone 1 continued every year until 2002, when releases were expanded into zones 2 and 3. Even as the size of the treatment area increased from 2600 hectares in zone 1 to include the additional 2800 hectares in zones 2 and 3, orchard and urban sanitation, codling moth control using complementary tactics, and outreach and education efforts continued throughout the entire treatment area.

The sanitation phase for commercial apple and pear orchards in zones 2 and 3 began in 1998 using a combination of well-timed insecticide sprays, codling moth mating disruption, and tree banding (e.g. trees banded with corrugated cardboard strips that trap mature larvae seeking a cocooning site; later the strips are removed and destroyed along with the larvae). As the size of the treatment area increased, releases of irradiated codling moths were reduced in zone 1. These reductions were made in several ways including: (1) fewer moths per hectare, (2) releases once rather than twice per week, (3) releases for only one half of the season, (4) no releases in orchards that had reported no codling moth damage or trap captures in four or more years. Zone 1 orchards continued to be extensively monitored for the presence of codling moth hot spots. When found, hot spots were treated with complementary control measures including fruit stripping, pesticide sprays, and tree banding to reduce overwintering populations.

3. Programme Results 1998-2004

3.1. Codling Moth Rearing

The OKSIR mass-rearing facility located in the town of Osoyoos proved to be extremely well suited for rearing codling moth. The estimated weekly output for the facility was 5.3

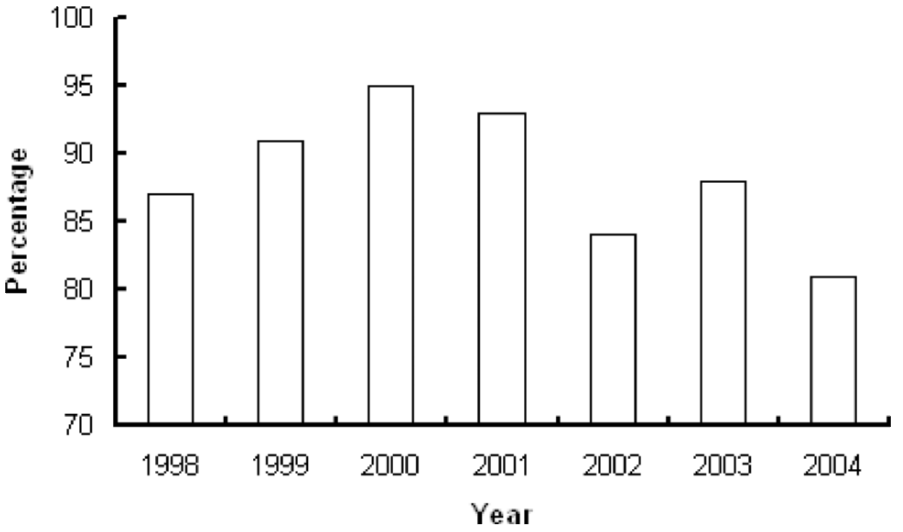

Figure 3. *Percentage of orchards in zone 1 with no codling moth damage at harvest (1998-2004).*

million moths per week (Dyck et al. 1993). However, actual production numbers increased from 8.3 to 14.2 million adults per week between 1994-1997 (Bloem and Bloem 2000). By 2004, the facility was routinely producing 16.3 million moths per week, which represents a doubling of the rearing efficiency in ten years and a tripling of the estimated weekly production output. Strict adherence to process control, unrelenting attention to sanitation as well as staff experience, facility improvements and higher egg densities on the diet were the main reasons for this increased output.

In addition, through gradual refinements in process control, the programme realized significant reductions in the cost of rearing. For example, full-scale production in 1994 required the preparation of 31 584 litres of diet per week. This amount of diet yielded an average of 8.3 million codling moths per week at a cost of USD 5.62 per 1000 insects. In comparison, in 2004 the OKSIR programme was at full production capacity for ten weeks during which time they prepared an average of 37 224 litres of diet per week. Adult moth production per week averaged 16.4 million codling moths at a cost of USD 2.94 per 1000 insects. This difference in the cost of producing (but not releasing) 1000 insects translates into a 48% reduction in operating costs for the facility in ten years.

Yearly production totals (in millions of codling moths) achieved by the facility between 1998 and 2004 are shown in Fig. 2. Due to budgetary constraints that included funding the purchase and implementation of mating disruption in zones 2 and 3, the rearing facility was only operated at 50% capacity in 2000 and 2001 and in 2004 the release season was shortened from 20 to 10 weeks and, as such, the period of full-scale production also was shortened.

3.2. Field Operations

From 1995-1997, the decline in the wild codling moth population in zone 1 was dramatic. The percentage of orchards that showed no detectable level of codling moth damage at harvest increased steadily from 42% in 1995 to 91% at the end of 1997 (Bloem and Bloem 2000). Data on the percentage of orchards in zone 1 with no

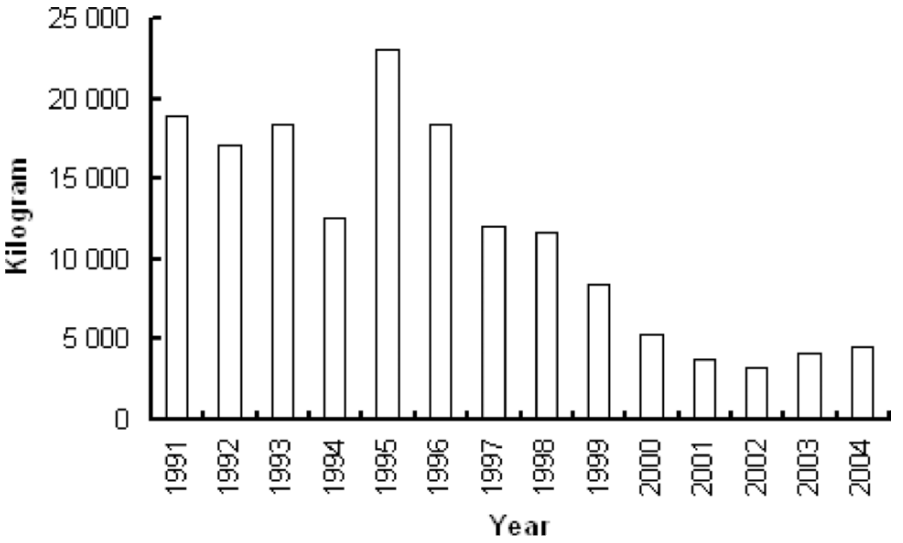

Figure 4. *Sales of organophosphate insecticides (in kilogram) in zone 1 from 1991 to 2004 (data provided by BC. Ministry of Water, Land and Air Protection).*

detectable damage at harvest for the period covering 1998-2004 are presented in Fig. 3. Despite the many changes in the OKSIR programme after 1997, this percentage has been maintained between 81-95%. While overall damage levels at the end of 2004 were economically acceptable to orchardists in the zone 1 treatment area, the proportion of orchards with no detectable damage at harvest dropped slightly (from 88% to 81%), requiring that corrective chemical control measures be used in hot spots. This reality has been a disappointment to many zone 1 growers who have been spray-free for many years. The reasons for the decline can likely be ascribed to a combination of lower release numbers and covering only half the season, the shift to a non-area-wide release approach, and suboptimal monitoring by growers and programme staff alike arising, in the latter case, from reduction in staffing levels.

3.3. Insecticide Use

The significant decline in the total amount of organophosphate insecticides (azinphos-methyl, phosalone and phosmet) purchased by growers located in the zone 1 treatment area from 1991-2004 is shown in Fig. 4 where the majority of organophosphates are purchased for use against codling moth. During 2003-2004 sales appeared to be slightly higher than for 2001-2002, suggesting that codling moth reinfestation was gradually manifesting itself in zone 1.

3.4. Host Tree Removal

An integral part of the OKSIR programme is the removal of infested host trees from urban areas and public lands as well as those in derelict and abandoned orchards. From 1994 to 2004, the numbers of trees removed under the direction of the programme totalled 24 627, 67 890 and 26 418 for zones 1, 2 and 3, respectively (Table 1). While tree removal undoubtedly reduced the codling moth wild population, an accurate quantification of this benefit is difficult to make.

3.5. Urban Compliance

From 1995-1999 the OKSIR programme adopted a policy of “zero tolerance” for

Table 1. *Number of codling moth host trees removed by the OKSIR programme in the three treatment zones by year.*

OKSIR programme host tree removal data				
Year(s)	Treatment areas			Total
	Zone 1	Zone 2	Zone 3	
1994-1998	17 555	37 983	94	55 632
1999	1715	11 664	2230	15 609
2000	1169	5273	10 124	16 566
2001	989	4002	2561	7552
2002	1127	3821	3024	7972
2003	1243	4055	4129	9427
2004	849	1092	4256	6197
Total	24 627	67 890	26 418	118 955

codling moth-infested fruit in all urban and non-commercial orchard properties. Property owners were encouraged to remove all fruit from their trees or to remove the trees entirely. Incentive programmes for homeowners included discounts on replacement fruit purchased at packinghouses or on the purchase of replacement non-host trees. Removal of infested fruit and application of pesticide sprays also were acceptable forms of compliance (Bloem and Bloem 2000).

The zero tolerance policy was abandoned when the programme's overall objective switched from eradication to area-wide suppression. However, backyard growers are still urged to keep their properties free of codling moth. When low codling moth infestation levels are detected, backyard growers are allowed to remove only the infested fruit. Non-commercial orchards that are found harbouring infestation levels above 2% require stripping of all fruit.

3.6. Communications

From 1995-1997, the OKSIR programme maintained an aggressive communication campaign that was coordinated by a privately contracted public relations firm. Commercial growers received information by mail four times per year providing programme updates. News releases and radio clips reminded growers of compliance and spray requirements, as did the Ministry of Agriculture's information network. Packinghouses and local field-men provided weekly updates to interested growers. Urban and non-commercial stakeholders also received information by mail outlining the zero tolerance policy and its compliance dates. Radio and newspaper advertising, presentations to school and community groups, and information booths at shopping malls, garden centres and agricultural fairs also were part of the outreach campaign (Bloem and Bloem 2000). Although the programme still proactively communicates with its various stakeholder groups, the public relations budget has been reduced from USD 86 400 in 1995 to USD 30 250 per year and all outreach and communications activities are now the responsibility of the public information officer hired by the programme since 1999.

4. Supporting Research

Bloem et al. (1997) investigated the possibility of mass-rearing codling moth through diapause to increase the use and overall efficiency of the OKSIR mass-rearing facility. The objective was to find methods to rear large numbers of additional codling moths during the winter months and store them at low tem-

perature until needed. Bloem et al. (1997, 1998) reported that adults reared through diapause were comparable to standard-reared moths when adult longevity, adult weight and mating ability were measured in the laboratory. Furthermore, Bloem et al. (1998) examined the field quality of diapaused and standard-reared codling moth by conducting large-scale release-recapture studies that followed OKSIR programme standard operating procedures. These authors found that the proportion of recaptured diapaused codling moths was significantly higher than that of standard-reared moths treated with the same high dose of gamma radiation (330 Gy) irrespective of the time of year when field trails were conducted. The authors concluded that diapaused codling moths had higher "field quality" than standard-reared moths. However, Bloem et al. (1998) and Bloem and Bloem (2000) cautioned that mass-rearing through diapause was more expensive and less efficient than rearing through standard protocols and suggested that more "methods development" for large-scale diapause rearing was needed before it could be fully implemented.

Bloem et al. (1999a,b, 2001) investigated the effect of substerilizing doses of gamma radiation on laboratory-reared codling moths and documented their positive impact on codling moth performance and competitiveness. This body of work included studies in large field cages to assess direct damage to fruit, pheromone-mediated field dispersal using pheromone-baited as well as female-baited traps, and male competitiveness for virgin females under field situations. These encouraging results obtained prompted additional research that compared the field quality of diapaused and standard-reared codling moths treated with substerilizing doses of gamma radiation (150 and 250 Gy) (Bloem et al. 2004). Here, again, field performance of released males was significantly improved by rearing through diapause and by lowering the dose of radiation used to treat the insects. These effects were observed in spring when temperatures were cool and in summer when evening temperatures were high, and were independent of the sampling method used for evaluation. Bloem et al. (2004) also showed that the reduction in field performance due to radiation was greater when males had been reared through standard rearing, suggesting that diapause might attenuate some of the negative effects of treatment with gamma radiation.

Finally, because of the demonstrated potential of codling moth SIT, the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) are sponsoring a five-year Coordinated Research Project (CRP) entitled "Improvement of Codling Moth SIT to Facilitate Expansion of Field Application". Scientists from ten countries including Canada and the OKSIR programme, as well as Armenia, Argentina, Brazil, Chile, Czech Republic, South Africa, Switzerland, Syria, and the USA are conducting basic and applied research in an effort to expand the knowledge base for the application of this environmentally friendly technology. The research group meets every 18 months to share results and discuss additional ways to cooperate and continue to expand knowledge and refine technologies to improve the delivery of area-wide programmes against this key pest. Some of the topics being investigated include the development of a codling moth male-only strain (Marec et al. 2005), the mobility of different codling moth strains (Bloem et al. 2006a,b), the effect of combining codling moth SIT with other complementary pest control tactics, and the compatibility of codling moth strains from different geographic regions. As part of this CRP, codling moth compatibility studies were conducted between laboratory-reared codling moth from Canada and wild codling moth in South Africa (Bloem et al. 2005b).

Codling moth is the key pest in pome fruit in South Africa and South African growers are interested in using the SIT against this pest. Because the fruit growing seasons in these countries occur opposite one another, the OKSIR programme could rear and ship codling moth to South Africa during the win-

ter when the mass-rearing facility is underutilized. Small cage and release-recapture studies were conducted in 2003 to examine the mating compatibility of these two strains. Data from the small cage and the field studies showed that Canadian codling moth males were equally attracted to calling Canadian and South African codling moth females despite the fact that the Canadian codling moths had a transport time (from Canada to South Africa) of 48 hours and were between one to two days old at the time of transport. Field data also showed that Canadian codling moth females and males were more active than South African codling moths at low field temperatures (Bloem et al. 2005b). These results indicate that laboratory-reared codling moths from Canada are fully compatible with wild codling moths in South Africa and, therefore, could be used for SIT studies in South Africa. This work is continuing and will be further tested in 2006 in a season-long field trial in South Africa using moths purchased from the OKSIR programme under IAEA project SAF/5/007.

5. Future Outlook

After ten years of operation, the OKSIR programme continues to struggle with its long-term financial, political, and operational demands including how to deal with and respond to the needs of its broad constituent group. The stakeholders include the Governments of Canada, British Columbia, and five regional districts that represent 14 municipalities, organic and conventional growers and their organizations, nurseries, packinghouses, farm suppliers, scientists, and homeowners. Additional challenges to the assessment and comparison of programme results from one year to the next have arisen because modifications to programme delivery are constantly being made. Examples of such modifications include non-standardized wild codling moth field monitoring and damage assessments at harvest, varied rates and schedules for field delivery of irradiated codling moth, non-systematic use of complementary pest management tactics, and inconsistent short- and long-term technical directives.

Timely detection and management of very low codling moth wild populations remains an ongoing challenge, particularly in zone 1 where releases have been scaled back. In addition, regulatory and enforcement obstacles, such as the unregulated movement of codling moth infested fruit, wood, and packing bins, continue to seriously impact OKSIR programme operations in all treatment zones.

Nevertheless, key stakeholders continue to support the suppression programme. For example, zone 1 growers, who have been enjoying the benefits of effective and environment-friendly codling moth control for many years, are a positive influence on growers in newer treatment areas. As conventional pest control methods continue to become more expensive, less effective and less tolerated, there is an ever-increasing need to improve and refine the SIT technology for the OKSIR programme, and to implement this improved technology where appropriate in other pome fruit growing areas around the world. The OKSIR programme is poised to play a crucial role in this endeavour as it refines its long-term strategy for programme delivery in the years to come.

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Eradication of the Australian Painted Apple Moth *Teia anartoides* in New Zealand: Trapping, Inherited Sterility, and Male Competitiveness

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ABSTRACT The incursion of the native Australian painted apple moth *Teia anartoides* Walker into Glendene, West Auckland in May 1999, prompted an area-wide eradication programme by the New Zealand Ministry of Agriculture and Forestry Biosecurity Authority. The Australian painted apple moth is a polyphagous pest of horticulture and plantation forestry and threatened New Zealand's native vegetation. The economic and ecological impact of the moth's incursion was estimated at NZD 50-350 million (approximately USD 30.5-212.9 million) over 20 years if no action was taken to eradicate the insect. The eradication programme (1999-2006) used a combination of tactics, including the first use of the sterile insect technique (SIT) in New Zealand. The SIT component was added to the eradication programme in 2002 but releases started in 2003 as an end game tactic once the pest population was brought down to ca 1% of the population level in 2001-2002, as indicated by trap catches. The aerial spray programme using *Bacillus thuringiensis* (Berliner), subsp. *kurstaki* (*Btk*) accompanied by release of sterile males drove the wild population to extinction, with overflooding ratios up to 100:1 based on trapping data. Sterility was assessed from the egg hatch of the F_1-F_3 generations and competitiveness examined using emergence rates and wind tunnel flight performance. When males exposed to 100 or 160 Gy mated with non-irradiated females, there was no significant effect on female egg production, but a lower egg hatch was observed for both doses. When F_1 and F_2 offspring were outcrossed to fertile moths, 100 Gy irradiation gave relatively similar inherited sterility levels to 160 Gy, with full mortality achieved at the F_3 generation. The lowest effective dose of radiation needed to induce inherited sterility is likely to offer the best competitiveness and mating success of the released males, representing a potential trade-off between sterility and competitiveness. Subsequently, the induced dominant lethal mutations carried by the released males (when mated to wild females), will be inherited through the surviving F_1 proportion of the progeny. Moth emergence rate was not affected at 100 Gy, but the response to seek and mate with wild calling females in the wind tunnel was reduced by 33%. The use of wind tunnel for quality assurance in integrated pest management programmes is discussed.

KEY WORDS Australian painted apple moth, *Teia anartoides*, eradication, aerial spray, trapping, *Btk*, sterile insect technique, fitness, wind tunnel

1. Introduction

The Australian painted apple moth *Teia anartoides* Walker has been the target of a large-scale (NZD 90 million equivalent to USD 54.7 million) eradication programme in Auckland, New Zealand since 1999 (Suckling et al. 2002, 2005). Female painted apple moths are flightless, and ballooning larvae are the main means of dispersal in this species. It

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 603–615.

has reached its widest host range in Auckland (Stephens et al. 2007). The insect was considered to have potential for significant economic and ecological damage in New Zealand because its host range includes plants of importance to horticulture and forestry, as well as to natural ecosystems. Estimates of NZD 50-350 million (approximately USD 30.5-212.9 million) of costs over 20 years were developed (Self 2003), and a response was mounted in mitigation.

1. 1. Operational Aspects of the Eradication Programme

The eradication programme operated by the Ministry of Agriculture and Forestry Biosecurity Authority ("Biosecurity New Zealand"), has included a pheromone trapping programme based on caged female moths, in order to map the distribution of the pest, from June 2001 onwards. The use of geographic information systems (GIS) proved invaluable for tracking the population spatially (ground finds and trap catches), as well as for managing the response (aerial spray flight lines and releases of sterile insects). Although the pheromone has been identified (El-Sayed et al. 2005), it is highly unstable and virgin females have been used to bait traps throughout this programme. The female moths were mass-reared for this purpose, and trap arrays of up to 2000 geo-referenced traps were serviced weekly (Suckling et al. 2005). There was concern at one point about bioterrorism through deliberate spread of the organism, and a solution was demonstrated which involved sterilizing females by irradiation, since this had no effect on male catch (Suckling et al. 2006).

The geographic area of the infestation was relatively small, ca 12 000 hectares (Fig. 1). Trap spacing varied from 1500 metres around the periphery, to 500 and 250 metres spacing in the central zone. By 2002, a male catch in a trap was followed immediately by the deployment of a higher density network of traps in order to locate the breeding population.

A targeted programme of ground searches was also used to define the area occupied by the pest, and host removal was used where possible. Insecticides applied from the ground to host trees (e.g. *Acacia mearnsii*) included chlorpyrifos, deltamethrin and the insecticidal pathogen *Bacillus thuringiensis* (Berliner), subsp. *kurstaki* (*Btk*), which was also aerially applied on a regular basis from January 2003 onwards (Charles et al. 2005, Richardson et al. 2005). The sterile insect technique (SIT) was proposed as part of an end game strategy, once densities had been lowered with other tactics (Suckling 2003).

In New Zealand, the SIT has the advantage of meeting the stringent requirements of the Hazardous Substances and New Organisms Act (1996), which seriously limits the application of many technologies with potential value for eradication (Suckling 2003), such as exotic organisms (e.g. insect pathogens), or unregistered pesticides. The SIT had the advantage that the mass-rearing was already underway to provide female moths for the surveillance trapping programme.

Male moth trap catches well outside the known area of breeding populations raised questions about moth dispersal, since it was possible that the infested area was much wider than had been thought. A dispersal study was therefore undertaken, using marked irradiated males, after it was shown that the progeny of males irradiated at 160 Gy were essentially sterile by the F_2 generation (Suckling et al. 2002, Wee et al. 2005). Released moths dispersed up to five kilometres, and 17% of irradiated males released were recaptured (Suckling et al. 2005). This indicated that irradiated insects were sufficiently fit for field recapture, and therefore a full programme of sterile insect release was proposed. Subsequently, releases of a total of around 350 000 male moths irradiated as pupae at 100 Gy were made from January 2003 until April 2004 (Suckling et al. 2005). The lower dose was chosen in an attempt to increase the field competitiveness, while work was being done to quantify the difference in competitiveness from the two doses.

Importantly, the information on recaptures

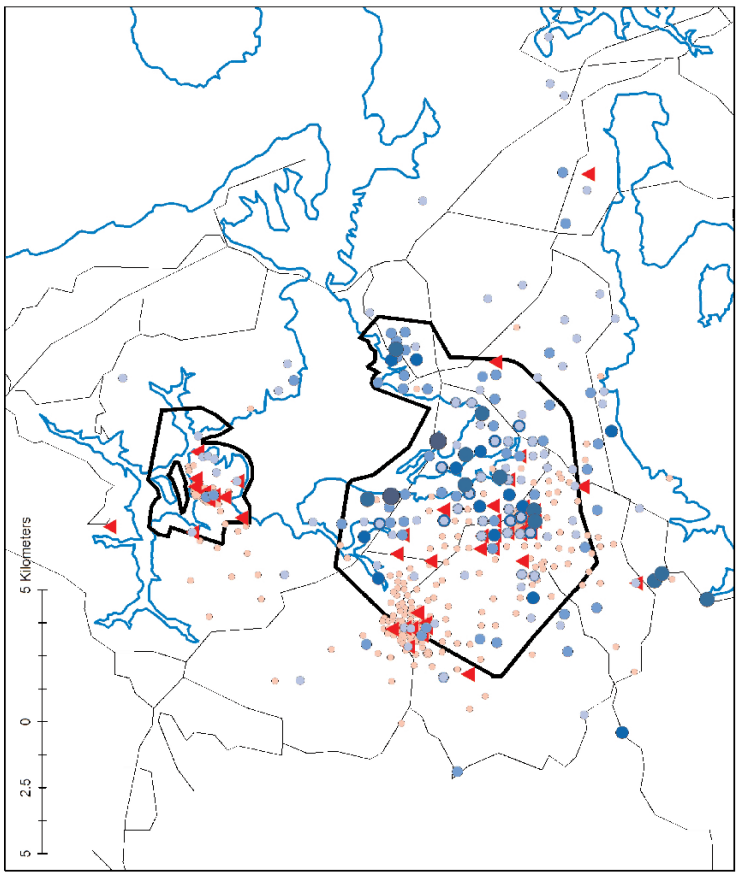

Figure 1. *Distribution of Australian painted apple moth trap catches, in relation to the spray zone at the 15th aerial application of* Btk *in west Auckland New Zealand (solid black lines). Wild moth catches (light to dark blue circles) expressed as general linear model residuals scaled to the observed catch rate corrected for trap location, distance from larval finds, and time of catch over the 2001-2002 period (courtesy D. Baird, AgResearch) with the locations of the final 19 catches of male painted apple moth in 2003 (red circles). SIT recaptures (pink circles) tended to cluster within one kilometre at the three release sites shown.*

was used to assist with the development and use of a decision-support tool for managers of the eradication programme, to estimate changing confidence levels in the success of the eradication programme over time (Kean and Suckling 2005). This model relies on sterile moth recaptures for the best quality estimate of trap efficiency, and takes into account weather, the relative competitiveness of irradiated insects, insect development rate, spatial deployment of trapping effort, duration of trapping effort in the absence of any recent wild catches and other factors. Further work has examined the cost effectiveness of release strategies (Wee et al. 2006) and ways of improving sterile insect quality (Stephens et al. 2006).

The work reported here outlines the trapping programme used for catching both wild and released males since 2001, and looks at the occurrence of catches over time and space, as part of the operational programme from 2001. The releases were continued until May 2004, they were terminated, fifteen months after the last catch. Subsequent trap catches are still under forensic biosecurity examinations of various kinds.

1.2. Competitiveness

Competitiveness of the irradiated insects is a key requirement for the success of the SIT (Lance and McInnes 2005, Itô and Yamamura 2005). The application of irradiation should not diminish the longevity or significantly impair the ability of treated insects to fly, mate and transfer sperm. The lowest effective irradiation dose to achieve sterility is more likely to optimize the mating competitiveness of the released insects (i.e. maximizing mating with the target population), representing a potential trade-off between sterility and competitiveness. With the codling moth *Cydia pomonella* (L.), as with Lepidoptera in general, a high radiation dose resulted in complete sterility but considerably reduced competitiveness, but the application of a lower dose yielded partially sterile insects of superior competitiveness, which were more effective in population suppression in the F_1 generation (Bloem et al. 2001).

A range of radiation doses produced different levels of inherited sterility in *T. anartoides* (Suckling et al. 2002). A 100 Gy treatment was projected to give 80% sterility of the F_1 generation and more than 99.5% sterility in the F_2 generation. The few viable individuals from the F_1 or F_2 generation would carry the inherited sterility into the population which, when crossed with wild-type insects, would later lead to eradication of the target population. Suckling et al. (2004a) showed that exposure of 6-day old pupae to 100 Gy did not increase wing deformities (which at even a low level would correlate with an effect on the flying ability of adult males).

The work reported here was instigated to determine the impact of irradiation on male competitiveness; female fecundity and fertility were assessed as well as the inherited genetic damage, expressed as mortality in the F_1 and F_2 generations (inherited sterility).

2. Materials and Methods

2.1. Trapping

Pheromone traps were deployed at 500 metres spacing, baited with virgin females (Suckling et al. 2005), and operated weekly from 2001 until 2006, although only the period immediately before the *Btk* spraying operations and then the SIT component is presented here. The phenology of *T. anartoides* was determined from a subsample of 102 traps in 2001/02, but in the 2002/03 year all traps were included due to the scarcity of wild moths, after densities fell dramatically. A generalized linear model with Poisson errors was fitted to the weekly counts of moths caught in the traps (D. Baird, personal communication). The components to the model were a smoothing spline on the distance from the trap to the closest larval find, which modelled the dispersion of the male moths, a factor for the week of the catch, allowing for a changing population of male moths. The fitted values from this model were subtracted from the observed catches to give a residual. This was fitted with Genstat® ver-

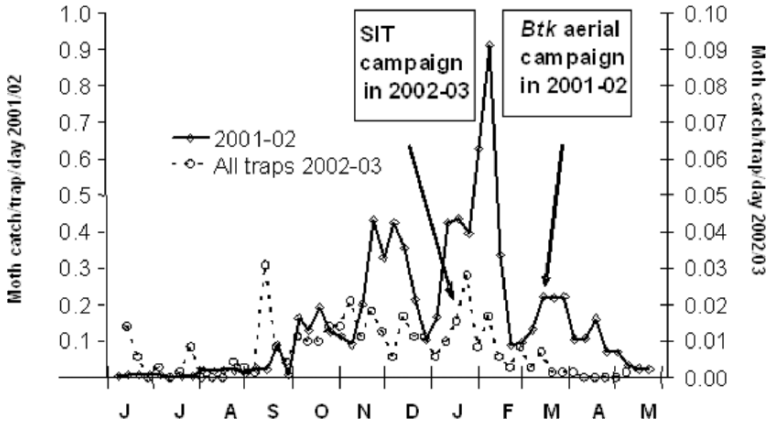

Figure 2. *Pheromone trap catch of the Australian painted apple moth from June to May in 2001/02 (*Btk *aerial campaign commenced in January 2002) and 2002/03 (SIT commenced 17 January 2003). There was a minimum of 100 traps for each data point.*

sion 8 (Payne 2005). High residual values from the model indicated spatial and temporal hot spots of trap catch.

2.2. Release Programme

Male moths were irradiated as pupae (below), dyed with fluorescent powder, emerged and released weekly from paper bags from 17 January 2003 until April 2004 (Suckling et al. 2005). Males were released at four main locations in Auckland, using a single release point but after 2005 multiple release points were used a few hundred metres apart from single nearby trap catches.

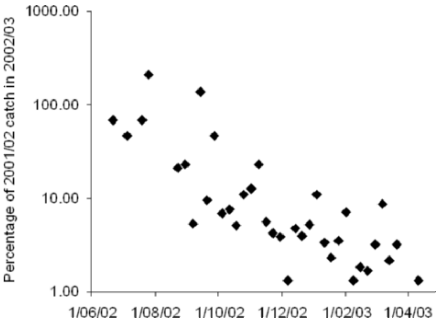

Figure 3. *Log-linear change of Australian painted apple moth trap catches over time as a function of the previous year (2002/03 compared to 2001/02) due to the aerial* Btk *spraying programme, prior to the commencement of the releases of sterile moths in Auckland, New Zealand.*

2.3. Irradiation of Insects

Male and female Australian painted apple moth pupae were obtained from the quarantine facility in Mount Albert Research Centre, HortResearch, Auckland. The colony was established from field-collected insects, and reared on an artificial diet (Charles et al. 2006). Six-day old male pupae were irradiated using 1.25 MeV gamma rays from a ^{60}Co source. Larvae were separated by sex during rearing. The pupae were irradiated for 5015 seconds, giving a dose of 100 Gy, or 8024 seconds, resulting in 160 Gy (Wee et al. 2005).

2.4. Female Fecundity and Fertility

Newly emerged males were mated singly with untreated females for each treatment dose. Total eggs laid (fecundity) and hatch rate (fertility) per female per treatment were assessed

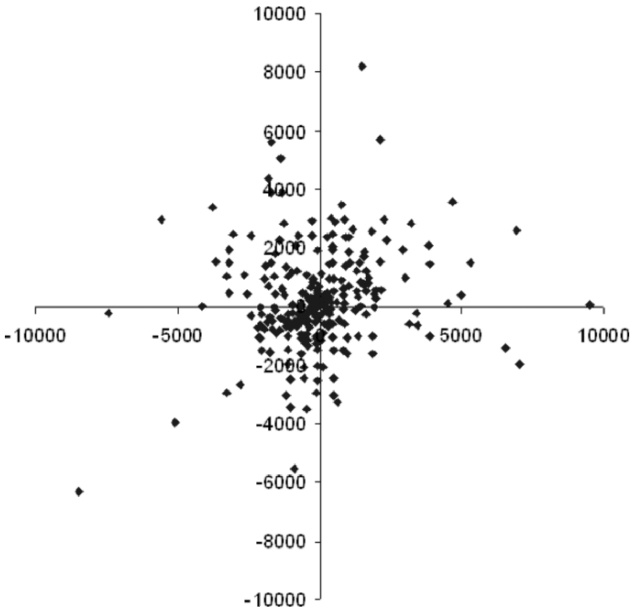

Figure 4. *Dispersal distances (metres) and directions of sterile male Australian painted apple moth, released and recaptured (n = 2145) in Auckland from 17 January to 9 May 2003.*

with 12 to 54 replicates for each cross, depending on survival rates. Egg counts were transformed using log10(n+1), and the proportion of eggs that hatched was angular-transformed (Anscombe 1948) and analysed using one-way ANOVA, with mean comparisons using Tukey's test ($P < 0.05$).

2.5. Post-Embryonic Mortality

All possible crosses of emerging F_1 adults were made according to the number of surviving insects in each line, and were conducted as described above (between nine and 33 emerged F_1 adults of each gender from each treatment were outcrossed to wild-type). The total number of eggs oviposited per female (fecundity) and the number of eggs that hatched (fertility) were counted for each treatment.

All larvae that hatched for each female from each cross were counted to obtain fertility data. Neonates from each P and F_1 cross were reared at 25 ± 2°C, a photoperiod of 16:8 (L:D), and 60% relative humidity. Daily observations were made to ascertain insect development and mortality. The total number of larvae that pupated and the number of pupae that successfully emerged as adults were recorded daily. Data from each developmental stage (egg-larval-pupal mortality for F_1 to F_2, and egg mortality at F_3 generations) were corrected using Abbott's formula to account for control mortality (Abbott 1925). Subsequently, the cumulative mortality at different life stages was calculated as the product of the proportional survivorship of each stage. Results were pooled for reciprocal crosses with no significant difference.

2.6. Male Flight Ability

A perspex flight tunnel (50 x 50 x 50 centimetres) operating at a wind speed of 25-30 cm/sec under fluorescent light was used to determine the flight ability of male moths (Suckling et al. 2004a). Comparisons of treated and untreated males were done weekly as

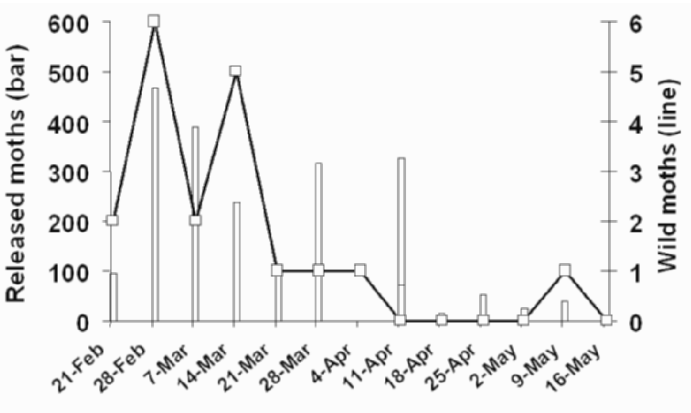

Figure 5. *Comparison of the last catches of wild and dyed released male Australian painted apple moth in Auckland from 17 January to 16 May 2003.*

part of the quality assurance programme. A single calling female (1-2-day old) was placed in a small wire-mesh cage (4 centimetres diameter x 5 centimetres height) with a sticky lid, which was then placed on a sticky base (18 x 19 centimetres) in the upwind area. At a distance of 130 centimetres from the upwind source, ten newly emerged (1-2-day old) irradiated males were released from a plastic tube placed at the downwind area, and their responses to the calling female were observed for six minutes. Similar procedures were repeated for control males, with 128 replicates of groups of males for each treatment. Flight ability expressed as arrival success was defined as when a male moth responded to the female pheromone plume by flying in "zig-zag" anemotaxis and successfully landed on

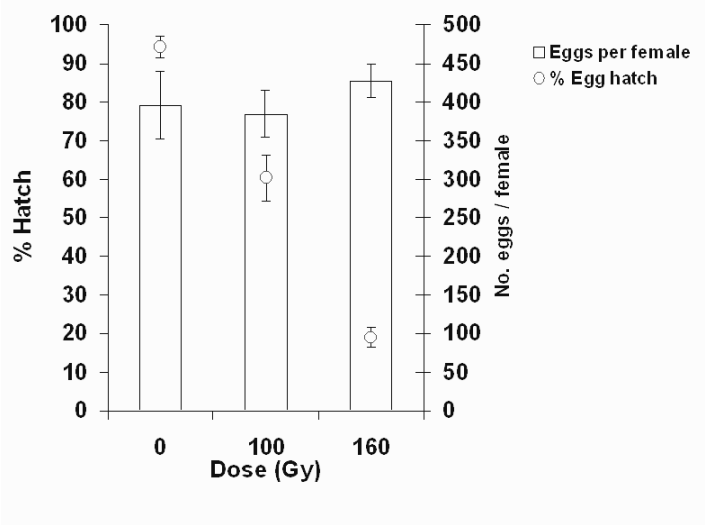

Figure 6. *Effect of 100 and 160 Gy gamma radiation of male pupae on female egg production and F_1 egg hatch of the Australian painted apple moth* Teia anartoides*. Bars show SE.*

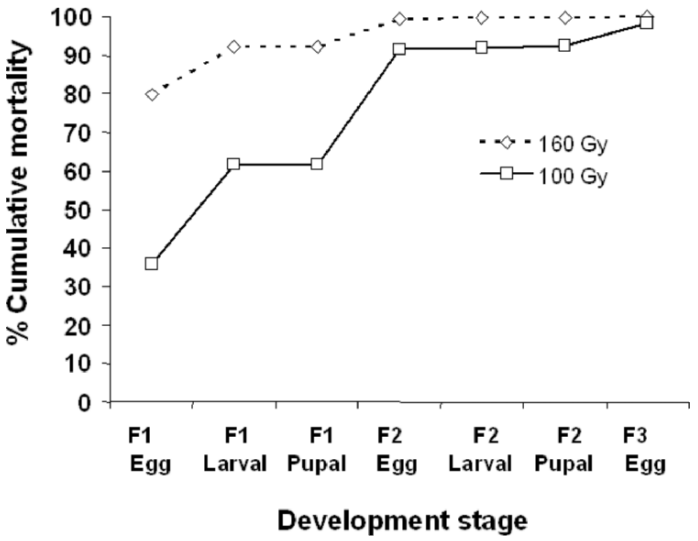

Figure 7. *Cumulative Abbott's corrected mortality at different life stages of Australian painted apple moth* Teia anartoides*, irradiated as male pupae at 100 and 160 Gy.*

the sticky base (female source) within the stipulated time. Numbers of successful male arrivals were noted for each replicate. A simple flight ability ratio was derived by dividing the mean arrival success of irradiated males by that of untreated males.

3. Results

3.1. Population Assessment from Trap Catches

The map (Fig. 1) shows a solid core where there was a breeding population that generated moth catches, followed by circles indicating significance of the catch outwards indicated as residuals from the generalized linear model. The area within the aerial spray zone (the solid black line, approximately 12 000 hectares) can be compared with the spatial distribution of the final 19 catches of male moths (red circles), that occurred between 17 January 2003 and 9 May 2003, and also the recaptures of sterile males.

The population in 2002/03 was much lower, and showed a 100-fold decline in catch to ca 1% of that in the previous year, over several months (301 days) that led up to the releases of irradiated moths in January 2003 (Fig. 3). This was likely to be predominantly due to the applications of *Btk* (Charles et al. 2005, Richardson et al. 2005), although some areas such as Waikumete Cemetery had heavy vegetation cover with less efficacy, evident as the region with the last catches of wild males (Fig. 1). The relative catch compared to the same week in the previous year shows how quickly the population dropped (Fig. 2).

3.2. Phenology

The phenology of painted apple moth was assessed in a geo-referenced grid of 102 traps from June 2001 to May 2002 and assessed from all traps in the second year (Fig. 2). In the first year of trapping, the population showed evidence of several apparently doubling generations of increase, before the

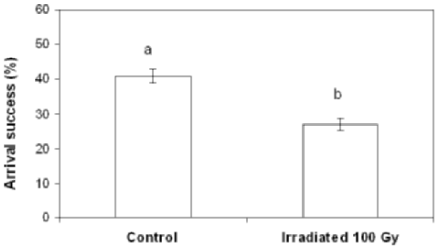

Figure 8. *Arrival success of control and irradiated (100 Gy) males, flown separately to a calling virgin female in a flight tunnel. Bars (SE) topped with different letters were significantly different at* P = *0.01, Student's* t*-test (128 replicates of each treatment; each replicate consisted of 10 males).*

effect of the regular aerial *Btk* spray programme, started in January 2002, began to measurably reduce the moth population after one generation by May 2002 (Fig. 2). A total of more than 9300 male moths were caught in the first 35 weeks of trapping from June 2001 onwards (Suckling et al. 2005).

Recaptures have been reported elsewhere in detail (Suckling et al. 2005), and this information has been used in a model of trap efficiency and therefore the success of the eradication effort (Kean and Suckling 2005). There was no overwhelming evidence of a directional component to overall released male dispersal (Fig. 4), although individual release points could expect some effect of topographic features, and local wind conditions would likely play a large part in a given catch. Geographic bias is obvious as apparently reduced catches in the south-eastern quadrant which includes the harbour at Hobsonville, so cannot be compared with catch in traps present at other quadrants. This leaves room for further analysis at greater resolution.

The estimated ratio of substerile to wild males caught varied with weekly catch (Fig. 5), but was frequently over 100:1, as can be seen by comparison of the axes. The temperature flight threshold of 17ºC (Suckling et al. 2005) was not met later in the season.

3.3. Effect of Gamma Radiation on Fecundity and Fertility

There was no significant difference between the fecundity of females mated with males from the two radiation treatments or the controls ($F = 0.650$, d.f. = 2, 88, $P > 0.05$) (Fig. 6). However, the percentage of F_1 egg hatch (fertility) decreased significantly with increasing radiation dose ($F = 44.06$, d.f. = 2, 88, $P < 0.001$) (Fig.6).

3.4. Post-Embryonic Mortality

The cumulative mortality for the post-embryonic development stages from the F_1 to the F_3 generations was calculated for the partially sterile P males (irradiated as pupae at 100 and 160 Gy) and their progeny (Fig. 7). For 160 Gy, the cumulative F_1 egg mortality was twice that calculated for insects treated at 100 Gy. By the end of the F_1 pupal stage, more than 90% cumulative mortality was recorded at 160 Gy (Fig. 7). Beginning from the egg stage of the F_2 generation, the cumulative percentage mortality for both male and female lines reached 99% (Abbott's corrected). These results were used to choose 100 Gy for the operational programme.

3.5. Male Flight Ability

Both irradiated and control males exhibited similar behavioural responses to the calling females. Responding males initiated wing activation, followed by take-off and flying upwind via zig-zag anemotaxis towards the females. At the up-wind end, upon arriving at the females, males would be stuck on either the sticky base or the sticky lid of the cage. The irradiated males showed 27.0 ± 1.8% arrival success (334 out of 1236 males tested) compared to 40.9 ± 1.9% in the untreated controls (504 out of 1233 males tested) ($t = 5.34$, $P < 0.001$) (Fig. 8). In terms of irradiated/non-irradiated male competitiveness, the simple competitiveness ratio showed that irradiated

males were approximately 66% as successful as the untreated males. These values were tied to the fixed duration of the bioassay, but would be expected to remain constant over time.

4. Discussion

The population of the Australian painted apple moth was dramatically reduced by various measures, including delimitation using virgin female-baited traps, ground searches, habitat removal, and aerial application of *Btk*. The population reduction was an essential precursor to the SIT component of the programme, and it is clear that the combination of these measures was effective in reducing the wild moth population as shown by trap catches in the treated areas, although in some areas heavy vegetation cover affected deposition and impeded the spray programme (Richardson et al. 2005). Sterile releases began with declining catches of wild moths, and only very few were subsequently caught, enabling overflooding ratios in excess of 100:1 to be achieved. Whether the contribution of the releases to eradication was significant compared to the Allee effect acting at such a low insect density (Allee 1938) is unclear, although overflooding was readily achieved at an insect density numbering less than six catches in 12 months to May 2006. As an ongoing case study in mid 2006, it faces operational and scientific challenges similar to other programmes.

Mortality from irradiation treatment of male pupae accumulated across several life stages up to the F_3 generation, but the biggest effect was on eggs at the F_1 and F_2 generations. The level of inherited sterility at the dose chosen for the field programme (100 Gy) was capable of providing nearly complete cumulative sterility of all progeny by the F_3 generation.

In general, sterilizing doses do not alter the lifespan of adult moths (Snow et al. 1972, Lu et al. 2002), and a related study on the Australian painted apple moth showed no effect on the longevity of irradiated males or their progeny under laboratory conditions (Wee et al. 2005), suggesting that this parameter was not a useful measure of competitiveness. Flight and mating competitiveness were therefore used in routine quality assurance (Stephens et al. 2006).

The flight ability of groups of sterile males treated at 100 Gy was only 66% of that of groups of untreated males (with over 1200 males tested per treatment). The estimate of the flight ability of irradiated males tested as a 1:1 pair with untreated males was even lower (32%), although the sample size was also lower (83 males per treatment) (Suckling et al. 2004b). Hence it is not clear whether the inferior performance of irradiated males in a competitive situation was really half of that of irradiated males alone, although reduced flight ability of irradiated males was statistically significant in both experiments. The more representative estimate of flight ability is likely to be from the larger sample size, given that simultaneous arrival of sterilized and wild males at a female would be rare. Some degree of reduced competitiveness of males is inevitable in return for the associated inherited sterility described above. However, it remains unclear whether the increased competitiveness associated with lower irradiation doses is "sufficient" as a trade-off to lower inherited sterility. Population suppression depends on both mating competitiveness (of treated and F_1 males), and the survival of potential non-sterile progeny. Modelling the impact of lower levels of inherited sterility may offer the best approach to optimizing this trade-off.

While the irradiated males showed a lower arrival success, presumably indicating a reduced ability to seek a female in the field, their ability to copulate was not affected (Sucking et al. 2004b). Generally, it is thought that total sperm transfer is positively correlated with copulation duration in insects. In the Mediterranean flour moth *Ephestia kuehniella* Zeller, total time *in copula* was dose (irradiation) and sperm volume-dependent (i.e. the higher the treatment dose and the lower the sperm number, the longer the total time *in*

copula) (Koudelová and Cook 2001). Irradiated males tended to copulate for longer than did untreated controls in gamma irradiated cabbage looper *Trichoplusia ni* (Hübner) (Holt and North 1970).

This study used wind tunnel flight success as a surrogate for competitiveness estimates in the field. Irradiated males showed significantly reduced ability to arrive at calling females compared to untreated males. However, other experiments showed that upon arrival, they did not show a lower probability of mating compared to untreated males. This, together with the lack of other differences in mating behaviour, suggest that flight success may be an adequate surrogate for the probability of mating of irradiated males, and thereby introgression of inherited sterility into the population. It can therefore be concluded that wind tunnel assessment is a valuable tool for quality assurance, and can provide useful insights into the competitiveness of irradiated males in the implementation of the SIT.

The recapture rate from field releases of fluorescent dye-marked 100 Gy-treated males in a trapping grid was also used to assess the programme (Kean and Suckling 2005). Field recaptures of irradiated males indicated dispersal up to ten kilometres from the release point (Suckling et al. 2005), indicating a surprising level of flight capacity.

5. Conclusions

The eradication of Australian painted apple moths has been considered to be successful in West Auckland, with no breeding populations located since 2003, or males in this area trapped since January 2004. Formal eradication in this area was declared in 2006 (http://www.maf.govt.nz/mafnet/press/20030 6pam.htm). However, the simultaneous use of multiple eradication tactics prevents quantification of the contribution of the sterile releases. The more recent catches of several males (May, August, October, November and December 2005 and May 2006) in other parts of the city appears to be due to a separate incursion, although isotopic and mtDNA analysis is underway to determine point of origin of these catches (R. Frew, personal communication). This type of new forensic biosecurity tool appears to have potential and work is underway to extend the validation using another species of moth. Two mtDNA haplotypes have been identified (K. F. Armstrong, personal communication), but their frequency in Australia requires more work to assist interpretation. The response to the catches has been to recommence the releases of irradiated moths during 2005 and to increase trap density around each catch, but no other control tactics have been deployed.

The use of this approach against the Australian painted apple moth has highlighted the potential of the SIT in New Zealand to eradicate other exotic insect pests in the future. In addition, sterile recapture information can be used to assess the effectiveness of the surveillance grid (Kean and Suckling 2005). This programme has introduced the concept of "boutique" SIT, referring to a small-scale programme where only a few thousand sterile insects are released each week, targeted at the eradication of well delimited, small populations. There are considerable social advantages of the SIT over aerial spraying and other interventions in an urban setting, including the lack of inconvenience to the public (Dowell et al. 2000). Further work is underway on this and other areas of terrestrial plant biosecurity research in New Zealand under the heading "Better Border Biosecurity".

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Area-Wide Management of the Formosan Subterranean Termite *Coptotermes formosanus* in New Orleans' French Quarter

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ABSTRACT The Formosan subterranean termite *Coptotermes formosanus* Shiraki is an invasive species estimated to cause annually USD 1000 million in damage in the USA including the costs of preventive and remedial treatments and structural repair. The termite is also known to infest living trees that line New Orleans' boulevards. Subsequent loss of these century-old trees is aesthetically unacceptable as their value is inestimable. Populations of the termite are estimated to have increased 35-fold in the French Quarter of New Orleans during the last decade of the 20th century. Traditional termite control practices used highly toxic chemicals that rapidly killed the insects, or were highly repellent to them. In both cases, such liquid chemical treatments surrounding a structure served to protect it but with no appreciable effect on the size of the termite population. Common wall construction and other unique architectural features in the French Quarter complicate traditional termite treatments using liquids. An area-wide treatment programme was initiated in 1998 using population-reducing strategies. The area-wide approach of treating all properties within 15 blocks of the French Quarter was designed to reduce infestations of structures through population suppression. The area-wide approach has since been extended to an additional 47 blocks. Populations in the original treatment area have been significantly reduced compared with surrounding areas not receiving area-wide management. Some impediments to the successful conduct of this area-wide termite management programme include: (1) common walls that make treatment difficult, (2) construction practices that provide ready access to termites, (3) ample moisture, and (4) failure to treat all structures on a property because of long-held industry practices. Improved detection and more thorough treatments by pest management professionals should lead to improved area-wide termite control.

KEY WORDS Formosan subterranean termite, *Coptotermes formosanus*, population management, area-wide termite management

1. Introduction

The Formosan subterranean termite *Coptotermes formosanus* Shiraki is an invasive species believed to have entered the mainland USA in military shipments from the Pacific realm during and after World War II. The initial sites where the termite was discovered during the mid 1960s were Charleston, South Carolina, New Orleans and Lake Charles, Louisiana, and Galveston, Texas (Beal 1987, LaFage 1987). Since these early introductions the termite has been discovered in eleven southern states including Hawaii, where it was introduced much earlier, probably through sandalwood trade with the Far East. Damage caused by this termite is estimated at approximately USD 1000 million per year including both treatment and repair costs. This termite species also readily infests living trees and causes structural failures of the main trunk and large branches, which result in loss of the aesthetic quality of the trees and collateral damage to structures and vehicles. Nests located in the voids of infested trees may also serve as reservoirs for the termites to attack

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nearby structures or other trees.

Until recently, the termite control paradigm was to protect structures from attack by applying a chemical barrier. This either repelled the termites, causing them to search elsewhere for a food source or so rapidly killed individuals penetrating the chemical barrier that a behavioural repellency was established, again forcing the foraging individuals to forage elsewhere. Such approaches provided a measure of protection from termite structural attack but had little impact on termite populations thus allowing the termites to attack nearby structures or continue foraging until a "crack" in the barrier could be discovered.

1.1. Formosan Subterranean Termite Biology

As with other subterranean termite species, the Formosan subterranean termite colony is comprised of multiple castes including workers, soldiers and reproductive individuals. Workers are the largest of these classes and are responsible for foraging, nest building, cellulose consumption and feeding nest mates. During the spring, reproductive adults undertake dispersal flights, drop to the ground and lose their wings, and upon selection of a suitable mate and nesting location may establish a new colony. It is estimated that such colonies require approximately five years before reaching full maturity, i.e. to produce further reproductive individuals. During the first few years, populations of the newly formed colonies are low and foraging is confined to a minimal area. These small colonies are difficult to detect and control with current technologies.

As the colony size increases, the foraging territory greatly expands. Excavations have demonstrated that colonies may extend 100 metres (King and Spink 1969). Population estimates of a mature colony vary, but mature Formosan subterranean termite colonies are estimated to number from several hundred thousand to several million individuals; about ten times the number in a native subterranean termite colony. Individual queens are known to survive longer than 20 years (Zhong 2003). Supplementary reproductive individuals may also contribute to the rapid increase in individuals in a colony. The termite has essentially three requirements for survival: a food source, moisture and appropriate temperatures. Removal of any of the three essential factors results in death of the colony.

While the Formosan subterranean termite is a subterranean termite, when isolated above ground with sufficient moisture, it can survive in aerial carton nests without contact with the soil. It is speculated that repellent soil treatments can isolate a colony within a structure by disrupting the colony's contact with the ground. Treatment of a colony within a structure using liquid termiticides has been suspected to cause the splitting of the colony into two independent groups rather than eliminating the colony depending on the treatment and the availability of moisture. Their large foraging territories and the termites' ability to establish successful above-ground nests, coupled with the rapid reinvasion of vacated territory by a neighbouring colony, requires the elimination of all colonies simultaneously to prevent reinfestation of a treated property (Messenger and Su 2005), particularly in tightly packed urban areas such as the French Quarter.

2. Structural Protection

2.1. Repellent Chemicals

Chemical treatments of the soil surrounding a structure formed the basis for structural protection of homes from termite damage since the beginning of the 20th century (Randall and Doody 1934, Potter 1997). From the early 1950s through the late 1980s, cyclodiene compounds prevailed as the most used insecticides for structural protection against termites. They were effective in protecting structures because of their longevity in the soil and because they could be cheaply applied to the soil. However, concerns about the effects of these chlorinated hydrocarbons on the envi-

ronment and on human health led to their withdrawal from the marketplace in 1988.

Since then organophosphates and pyrethroids formed the basis of termite control products. These products were either "fast-killing" insecticides or were themselves repellent to the termites and thus a continuous soil barrier prevented the termites from entering a structure. However, members of a colony that did not come into contact with the insecticide were simply directed to forage for a source of food that was unprotected from such a barrier. Thus, although structures were protected from termite assault by these chemical barriers, colony populations were largely unaffected. Disruption of the chemical barrier through landscaping or construction activities, degradation of the barrier itself, or bridges of untreated material inadvertently placed over the chemical barrier could then allow the remaining population to enter and attack the structure. On the other hand, when applied to the soil of an infested structure, such repellent barriers could prevent any termites that had created a nest within the walls or attics of the structure from returning to the soil; they could also entrap the colony within the structure if a source of moisture was available. Organophosphate termiticides have recently been removed from the market as a result of the Environmental Protection Agency's (EPA) Food Quality Protection Act re-registration review, leaving pyrethroids as the main class of repellent barrier chemicals.

2.2. Non-Repellent Liquid Chemicals

Several new termiticides have recently been introduced which are marketed as "non-repellent" to the termites. Termites are believed to readily penetrate such chemical barriers and ingest or contaminate their cuticles with the toxins. On returning to the nest, the chemicals are spread to nest mates through mutual grooming or feeding others. The initial concept was that such chemicals could provide population reduction because of their slow spread throughout the colony. Potter and Hillery (2002) have reported success in killing termites at monitoring sites distal to perimeter-applied barrier bands using a non-repellent chemical. Other researchers have reported similar results (Waite et al. 2004), and EPA has approved a perimeter plus limited direct treatment application label for one of the non-repellent liquid chemicals to control subterranean termites. Since the approval for this label is so recent, data concerning the effectiveness of this treatment approach on termite populations surrounding such a structure are not available. Recent research has cast some doubt on the ability of one such non-repellent chemical to affect the entire colony of the Formosan subterranean termite (Osbrink and Lax 2003).

2.3. Baits

Termite baits are cellulose materials laced with a slow acting non-repellent toxin that when ingested can be taken back to the colony and shared with nest mates through feeding and trophallaxis. The earliest bait systems for the control of subterranean termites were arsenic dust or mirex-treated wood blocks (Esenther and Gray 1968, Esenther and Beal 1974). These were shown to reduce termite populations but were never incorporated into commercially viable products. Later bait formulations included slow acting metabolic inhibitors (stomach poisons) or relatively slow acting inhibitors of the insect nervous system. Other active ingredients include insect growth regulators such as the chitin synthesis inhibitor, hexaflumuron (Su and Scheffrahn 1998, Su 2003a). Extensive field trials using these chitin synthesis inhibitor-containing baits have demonstrated population reduction or "functional elimination" (Su and Scheffrahn 1998, Su 2003b) of the treated termite colony.

In several of the bait strategies termite populations are monitored by placing untreated cellulose (usually wood blocks) in a regular pattern surrounding a structure. These monitors are checked on a regular schedule (usually monthly) for the presence of termites. If termites are present, the wood blocks are

replaced or supplemented with a toxin-containing cellulose substrate that the termites consume and distribute through the colony. In addition to the monitoring/baiting stations within the soil, above-ground bait stations may be placed in the vicinity of a termite infestation when it is discovered within a structure. In such instances, active ingredient is added immediately upon installation of the station. When sufficient toxin has been introduced into the colony the population is eliminated. Thus baits protect structures through colony elimination and ultimately through population reduction of the termites in the surrounding areas, rather than merely by protecting a single structure, which would allow the colony to discover nearby unprotected food sources.

2.4. Physical Barriers

There are several physical barriers, such as stainless steel mesh, thin metal termite shields, a pyrethroid-laced vapour barrier, and basaltic particle barriers that can help prevent termite entry into the structure (Yates et al. 2000, Wege et al. 2003). These are installed beneath a structure or incorporated into the structure during construction but have no direct effect on the colony's population and merely cause termites to search elsewhere for a food source.

3. Integrated Area-Wide Management

When only chemical or physical barrier treatments were available, there was little opportunity for area-wide termite management without the application of huge volumes of long-lived toxins over large areas. With vast areas untreated, the termite populations simply found new sources of food including untreated homes or trees. Only with the advent of the newer chemicals that could achieve termite population reduction was an area-wide management approach possible. Reducing termite populations within an area rather than merely repelling colonies makes it possible to accomplish area-wide population management of termites rather than protecting individual structures. In the integrated management scheme, emphasis is given to the use of advanced termite detection technologies and the application of population reducing treatments to remove the termite pressure in the area. Certainly other termite management strategies are incorporated into the scheme including improved construction practices, the use of pressure-treated wood, moisture management and physical devices to exclude termites. However, the centrepiece of the area-wide management concept is large-scale termite population reduction.

3.1. Formosan Subterranean Termite Populations in New Orleans

Climatic factors in the New Orleans area are quite favourable to the Formosan subterranean termite. Once introduced into the ports in and around New Orleans, infestations increased steadily and spread undiscovered until 1967. Researchers with the Louisiana State University Agricultural Center (LSUAC) have been monitoring the relative population of the termite in New Orleans' French Quarter since 1989. During the ensuing decade populations of the termite increased rapidly with a 35-fold increase in light trap captures being noted from 1989-1999 (Henderson 2000). During this time increased swarms and termite damage were noted even in properties having repellent termiticide barriers, although it is estimated that only around 20% of the properties within the French Quarter had been treated.

Because of the increased presence of the termite and the damage that it was causing, and because of the historic nature of the French Quarter, the US Congress established a research programme in 1997 to develop new technologies to control the termite and to demonstrate the effectiveness of products already available on the market. The approach chosen for the latter was an integrated area-wide pest management strategy. The United States Department of Agriculture-Agricultural

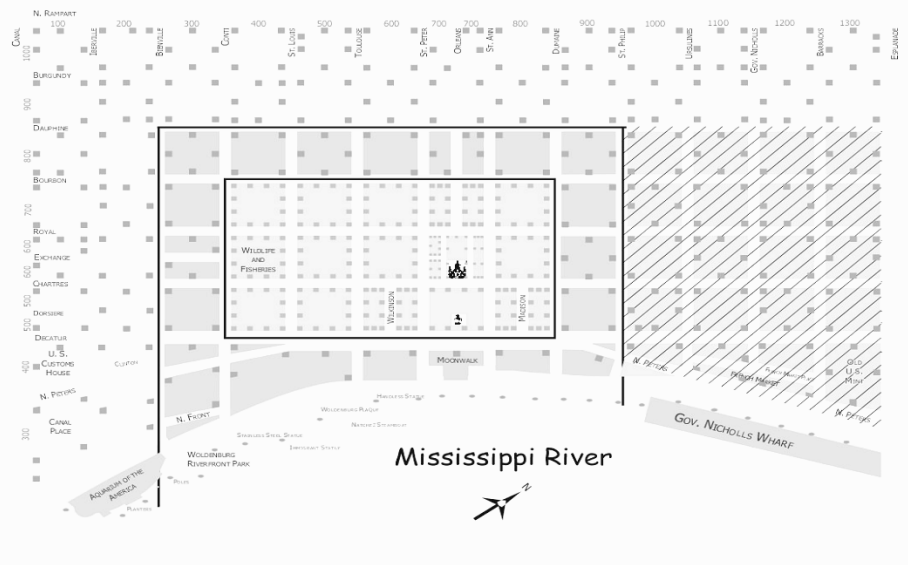

Figure 1. *New Orleans French Quarter test area. The original 15 block treatment area (Area 1) is outlined near the centre of the figure. The test area was expanded in 2002 to include the second bold line and includes all blocks surrounding the original treatment area and extends to the Mississippi River constituting Area 2. The third treatment area was initiated in 2004 and is indicated by hatching (Area 3). Small squares indicate the placement of in-ground monitors while alate traps were located at each intersection within the French Quarter. Area 4 comprises the Mississippi River Levee and Area 5 is comprised of the remaining blocks.*

Research Service (USDA-ARS) in New Orleans led the development of the area-wide strategy within the French Quarter to reduce the termites' density and thereby lessen the threat of further damage to structures through a comprehensive programme of inspection and area-wide treatment.

Cooperation was established among LSUAC, the New Orleans Mosquito and Termite Control Board, and local pest management professionals to provide a non-repellent liquid or monitoring/baiting treatment to every property within the treatment area. To establish area-wide management, only those treatments that were believed to have population-reducing effects were allowed to be applied to properties enrolled into the programme. Independent monitors of two types were established in both treated and untreated areas to determine population densities: in-ground traps and alate sticky traps (Fig. 1).

Commencing in 1997, property owners within a 15 square block area in the heart of the French Quarter were notified through town hall style meetings, the news media and through direct contact letters to begin enrolment in the programme. Cooperators launched the programme through educational efforts to explain the treatment options and to obtain citizen cooperation. Standard commercial pest control contracts were established between the property owners and pest control operators, while payments were made with funds provided by the programme. Enrolment reached 100% of the properties within two years. The greatest impediment to enrolment was making contact with property owners or managers since many of them were located out of town or country.

Since the inception of the programme the treated area has been twice expanded, increasing from 15 centrally located square blocks to

Figure 2. *Typical city block within New Orleans' French Quarter. Note that many of the buildings share walls and there are numerous trees in courtyards.*

encompass 47 square blocks largely spanning the entire French Quarter. All of the properties within these areas have been treated using population reducing treatment technology. In addition to the complete treatment of structures within the French Quarter, bait treatments have been established in the Mississippi River levee and along heavily infested railroad tracks between the French quarter and the levee (Fig. 1).

Regular meetings are held with citizens to report progress and to continue to solicit their assistance in rapid identification of areas of termite outbreaks so that immediate remedial control treatments can be initiated. In addition, programme participants meet regularly with participating pest management professionals to ensure compliance with the programme guidelines and to reinforce the notion of termite control through area-wide population management, a major shift in management paradigm for many of the operators.

3.2. Physical Impediments to Establishing Area-Wide Management

New Orleans' French Quarter is one of the most heavily termite-infested areas in the continental USA, and its architectural styles and construction contribute both to the rapid build-up of termite populations and to making treatment to mitigate the termites more difficult. Throughout the roughly 78 square blocks that comprise the French Quarter, most buildings on each block are connected to one another through shared common walls (Fig. 2).

A large majority of the buildings date to early to mid 19th century constructions of sandstone brick and lime mortar which wick the plentiful moisture available in New Orleans' subtropical climate up from the ground and into the upper storeys of the properties. Frequently, wooden structural beams were directly inserted within the multiple course brick walls, thus providing moisture

directly into the beam. Many properties have flat roofs and parapet walls that retain moisture and allow the attic beams to remain moist. Frequently the structures lack crawl spaces or attic access to allow for thorough traditional termite inspections.

The common wall construction prevents liquid termiticides from being applied in a continuous band into the soil surrounding the structure; it also prevents the typical placement of in-ground baiting stations at the prescribed (approximately 300 centimetres) spacing interval. The multiple course brick construction also makes difficult the application of liquid termiticides to all possible voids within the walls and thereby prevents a complete chemical treatment of the structures. Moreover, the physical connection of many of the structures within a block allows termites infesting one property direct access to other properties within that block without having to travel through the subtending soil. Thus, even complete and proper soil treatments as typically applied would never have the opportunity to contact and thereby control such aerial colonies.

Many properties within the French Quarter also have central courtyards containing planters with large living trees that are themselves susceptible to termite attack and termite population build-up. These planters often have irrigation systems that provide a constant source of moisture for the termites even in times of periodic drought (Fig. 2). Treatment of trees for termites has never been traditional among pest management professionals and until recently no protocols even existed for such treatment. This situation certainly contributed to the rapid build-up of termite populations and until area-wide management was implemented, these termite populations had neither been considered nor targeted for treatment.

Table 1. *Percentage ground monitoring stations visited by termites in each French Quarter area. Declines in termite populations over the four years are highly significant for only Areas 1 and 2.*

Year	Area 1	Area 2	Area 3	Area 4	Area 5
2001	6.45	8.33	6.25	n.a.	10.35
2002	5.17	5.43	5.66	22.9	6.88
2003	2.26	7.04	7.59	36.8	9.04
2004	3.20	4.94	9.01	33.2	8.99

3.3. Effect of Area-Wide Management on Termite Populations within the French Quarter

To assess the effectiveness of the area-wide population management programme in the French Quarter, two methods were used to estimate populations. Sticky traps established at every intersection within the French Quarter were used to monitor alates throughout the lifetime of the programme. Statistically significant reductions in alate population densities were noted in the first two treatment areas when compared with populations of the untreated areas within the French Quarter (Guillot et al. 2005). As shown in Table 1, differences were also detected in the frequencies of in-ground monitoring stations that were infested when comparing treated versus untreated areas. The frequency of visits by termites to in-ground monitoring stations within the areas treated for greater than two years (Areas 1 and 2) was also reduced by approximately 50%, while no reduction was noted in the untreated or newly treated areas (Areas 3, 4 and 5) (Table 1).

A 50% reduction in the alate populations was noted in Areas 1 and 2 after a period of two years (Fig. 3). It should be noted that it takes approximately a full year for all properties to be enrolled within the programme and for treatment to be completed. Furthermore, it must also be noted that control may take longer to achieve particularly with the bait treatments.

Prior to bait placement, termites must be detected within the monitoring stations and only then is the toxin applied. While some bait stations are discovered by termites almost immediately after installation, it may take

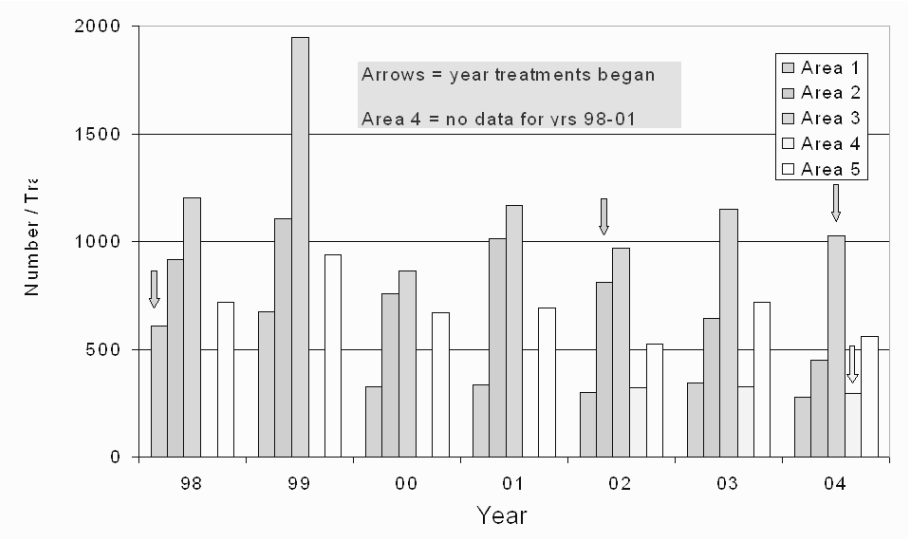

Figure 3. *Alate captures in each of the zones in the French Quarter by year (1998-2004). Downward arrows indicate the initiation of treatments. Note the decline in alate captures in each of the treatment zones after two years. Treatments in Areas 3 and 4 were initiated in 2004 while Area 5 remains untreated.*

many months for termites in the area to manifest themselves in the ground monitors. Moreover, the bait toxins are also designed to be slow acting to enable the toxin to spread throughout the colony; thus the time to achieve control is expected to be longer than with direct application of liquid termiticides. The results to date also indicate that while the termite population has been significantly reduced within the first two years of treatment, it has remained fairly stable at that level even after additional years of continued monitoring.

To understand the residual levels of termites within the treated zones, an extensive inspection programme has begun using infrared cameras, microwave motion detectors and acoustic devices in addition to thorough traditional visual inspections for the presence of termites. Rigorous inspection of the trees within courtyards in the French Quarter has also been included. Furthermore, during the course of these inspections it became readily apparent that the pest management professionals were using less than the ideal number of bait station placements because of the common wall issues and inability to install the stations.

4. Conclusions

During the course of this programme, communication with the industry termite control professionals has improved and the programme is now targeting courtyard planters and outbuildings within the French Quarter that were routinely untreated previously. The industry has also accepted the notion that treatment of detected infestation with above-ground bait stations is imperative for population management of the Formosan subterranean termite. With improved inspections and continued area-wide treatment, further declines in the termite population to less than 10% of its initial level are expected.

Termite population monitoring and treatment will need to be maintained to prevent reinvasion and rapid increases in the termite

population. Pest management professionals are expected to continue to provide this service through commercial contracts with property owners. It is anticipated that monitoring/baiting and non-repellent treatments will form the backbone of the continuing programme, while research continues in an effort to discover new long-term and sustainable treatments such as effective biological control agents which could be incorporated into the programme.

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A Multi-Institutional Approach to Create Fruit Fly-Low Prevalence and Fly-Free Areas in Central America

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ABSTRACT New approaches to facilitate fruit and vegetable exports, through the establishment of fruit fly-low prevalence and fly-free areas, were used in Central America and Panama, by implementing an area-wide integrated pest management approach (AW-IPM), including in some cases a sterile insect technique (SIT) component. These included: (1) the establishment of multi-institutional strategic alliances; instead of the historical isolated efforts scattered throughout the region; involving four international organizations, two donor government institutions and the Ministries of Agriculture of El Salvador, Costa Rica, Guatemala, Honduras, Nicaragua and Panama joining efforts under the umbrella of a regional technical cooperation project coordinated by the International Atomic Energy Agency, (2) the selection of naturally isolated medium-sized pilot areas for intervention where application of fruit fly AW-IPM with a SIT component could be technically and economically feasible. Thereafter, several isolated fruit fly-low prevalence or fly-free areas could be merged to increase the area under fruit fly control, instead of the traditional approach of fruit fly suppression or eradication on a country or region-wide basis, and (3) a focus on exports, with application in the pilot areas of a package of procedures which included the key elements required for exporting fruits and vegetables from fruit fly-low prevalence and fly-free areas, instead of focusing only on applying the technology per se in the field. In this approach, the industry played an important role. Outcomes included: (1) the establishment of a number of fruit fly-low prevalence and fly-free areas in each of the participating countries, (2) involvement of the fruit and vegetable industry which has invested around USD 150 million to export fresh pepper and red tomato from the areas of low fruit fly-prevalence in Guatemala, El Salvador, Nicaragua and Costa Rica that were established through a systems approach, and (3) exports of papaya from the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) fly-

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free area in the Department of Peten, Guatemala, without undergoing quarantine treatment. Major constraints to be overcome for future sustainability include: (1) strengthening alliances among the international organizations and donors since these are still fairly weak, (2) improving coordination between governments and stakeholders, (3) reducing the drastic turnover of civil servants in the national plant protection organizations, which affects continuity, and (4) overcoming insufficient funding from public and private sectors to be able to extend actions to larger or new areas.

KEY WORDS Mediterranean fruit fly, West Indian fruit fly, Mexican fruit fly, area-wide integrated pest management, sterile insect technique, strategic alliances, pilot areas, areas of low pest prevalence, pest free areas, industry

1. Introduction

For the past decade, countries in Central America have been affected by low international prices for coffee, banana and sugarcane and there is no indication that this situation will improve. The governments of these countries and Panama have therefore been seeking new alternatives for international trade through production and export of non-traditional fruits and vegetables. To assist them in this task, support was provided by the International Atomic Energy Agency (IAEA), through a regional project RLA/5/045, to establish fruit fly-low prevalence and fruit fly-free areas using an area-wide integrated pest management (AW-IPM) approach that included in some cases the sterile insect technique (SIT), and by strengthening the countries' phytosanitary frameworks.

After the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) was found in Costa Rica in 1955, many efforts were made to eradicate it from Central America and Panama. The first of these was by the United States Department of Agriculture (USDA) at the request of the Government of Costa Rica, and focused on the transfer of methodologies for monitoring and control using ground protein bait sprays (Salas 1958). In the 1960s, the Mediterranean fruit fly had spread into Nicaragua, and the IAEA and the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) launched a pilot project to eradicate it through the integrated use of the SIT (Rhode 1970). However, this failed due to lack of support and understanding of the area-wide application of SIT by the United Nations Development Programme (UNDP) (UNDP 1970), and the fly invaded the three neighbouring countries of Honduras, El Salvador, and Guatemala, reaching southern Mexico in 1977.

Since then, over 20 projects have been implemented focusing on the Mediterranean fruit fly including: its taxonomy, temporal and spatial distribution, determination of its hosts, use of biological control and implementation of pilot scale mass-rearing. Moreover, over 18 initiatives were funded or presented for funding by national and international institutions for regional eradication of this pest, and in recent years these have been extended to cover native *Anastrepha* fruit flies. These institutions include: the Instituto Interamericano de Cooperación para la Agricultura (IICA), IAEA, the Food and Agriculture Organization of the United Nations (FAO), the Secretaría de Agricultura Ganadería, Desarrollo Rural, Pesca y Alimentación of México (SAGARPA), OIRSA, the UNDP, the United States Agency for International Development (USAID) and USDA (Vo et al. 2003).

In spite of these efforts, the entities involved did not succeed in achieving a single fruit fly-low prevalence or fly-free area in Central America except for the Mediterranean fruit fly-free area of Peten in Guatemala and preventing the establishment of the Mediterranean fruit fly in Belize. This was achieved through a containment programme managed since the late 1970s by the Governments of Guatemala, Mexico and the

USA to prevent the spread of this pest into Mexico and the USA. The main obstacles that prevented success with these efforts included: (1) unaligned phytosanitary policies among the countries, (2) lack of clear regional agricultural goals, (3) isolated and duplicated projects in the region, and (4) lack of a holistic approach to solving the problem.

Consequently, a new approach was proposed to overcome earlier constraints and increase the likelihood of establishing fruit fly-low prevalence or fly-free areas. This involved the integration of three main elements: (1) a project based on developing multi-institutional strategic alliances, (2) the use of pilot areas as a territorial strategy for suppression/eradication of fruit flies, and (3) a focus on promoting export of fruits and vegetables.

2. Multi-Institutional Strategic Alliances

In order to coordinate efforts and overcome constraints arising from working in isolation, the following strategy was implemented. The Ministries of Agriculture of Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama joined resources and efforts with IICA, FAO, OIRSA, USDA and SAGARPA. This initiative was coordinated by an IAEA-employed full-time regional manager, and the establishment of a Regional Coordination Group (RCG) with representatives of the National Plant Protection Organization (NPPO) of each country. In addition to the resources provided by the ministries of agriculture and private sector stakeholders, the RCG effectively coordinated the financial and in-kind contributions provided by (1) the IAEA through a Regional Technical Cooperation Project (RLA/5/045) for establishing fruit fly-low prevalence and fly-free areas in Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama, (2) the USAID through the "Mitch funds" for establishing Mediterranean fruit fly-free areas in El Salvador, Honduras and Nicaragua, (3) the FAO contributions through a Technical Cooperation Project (TCP/072) for establishing Mediterranean fruit fly-free areas in Belize, Costa Rica and Panama, (4) the USDA, the MAGA and the SAGARPA through the "Programa Moscamed" to maintain the Department of Peten, in northern Guatemala, and the border between Guatemala and Mexico free of Mediterranean fruit fly.

3. Strategic Approach for a Succesful and Sustainable Project

The strategy focused on demonstrating the technical and operational feasibility of establishing fruit fly-low pest prevalence areas (FF-ALPP) and fruit fly-pest free areas (FF-PFA) to promote fruit and vegetable exports and the viability of sustaining them through the resulting economic and social benefits to ministries of agriculture, national plant protection organizations and the fruit and vegetable industries.

According to FAO's Glossary of Phytosanitary Terms (FAO 2001) an ALPP is:

> *An area, whether all of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures.*

A PFA is:

> *An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained.*

Implementation was based on an AW-IPM approach. In some cases the SIT was a major component to achieve the desired levels of pest control (Tween 1993). In others, where populations occurred naturally at low levels, only marginally attacked the relevant host, or did not exist, the approach taken was through certification of the phytosanitary status by means of an effective surveillance system. Exports from FF-ALPP would then be done under a systems approach (USDA 1997) to assure negligible level of risk of moving live pests.

This strategic approach is quite different from a country or region-wide fruit fly suppression, containment or eradication effort, which requires a different level of financial commitment, infrastructure and phytosanitary framework. By using pilot areas, the human and financial resources and infrastructure to develop and sustain FF-ALPP and FF-PFA was minimized compared to taking a country or region-wide approach. Additionally, project objectives were aligned with the sanitary and phytosanitary regulations of the US-Central American Free Trade Agreement (CAFTA); with the US-Panama free trade negotiations, and with the goals of the Regional Council for Agricultural Cooperation in Central America and Panama (CORECA) and of the Regional International Committee for Health in Agriculture and Livestock for Central America (CIRSA), which are the bodies that set up regional agricultural and phytosanitary policies.

Thus, it was expected that once it became fully aware of the potential benefits that can be derived from the project, the industry would contribute not only to sustain the project but also to gradually expand the areas from where fruits and vegetables could be exported. It was also expected that governments would act more decisively to implement policies that encouraged trade of agricultural products within and from the region.

4. Pilot Areas

4.1. Target Pests

The list of target fruit fly species considered of economic importance in the region was determined. These are: Mediterranean fruit fly, West Indian fruit fly *Anastrepha obliqua* (Macquart), Mexican fruit fly *Anastrepha ludens* (Loew), guava fruit fly *Anastrepha striata* (Schiner), sapote fruit fly *Anastrepha serpentina* (Wiedemann), and to a minor degree the South American fruit fly

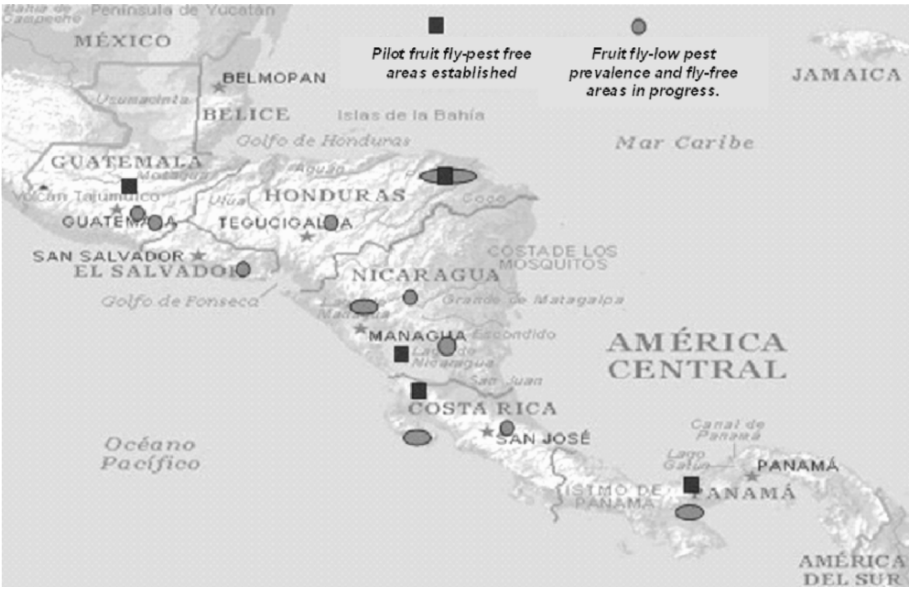

Figure 1. *Pilot Mediterranean fruit fly-pest free areas established from 2001 to 2004 and fruit fly-low pest prevalence and fly-free areas in progress.*

Anastrepha fraterculus (Wiedemann), which is not the same aggressive biotype that occurs in South America (Hernandez-Ortiz 2004).

4.2. Selection of Pilot Areas

From the outset, it was evident that the infrastructure and financial resources available were not adequate to suppress or eradicate even one species of fruit fly if it occurred at high population levels, even when present only during certain periods in the year. In addition, it was necessary to select suitable areas in which all steps required for exporting fruits and vegetables (from detection of the pest to the phytosanitary negotiations for export) could be transferred to the countries during the project lifespan (four years). Therefore, it was decided to select areas in which fruit fly populations were relatively low throughout the year, regardless of whether fruits and vegetables were produced commercially. Accordingly, one naturally isolated medium-sized area (20 000 to 40 000 hectares) was selected in each country.

Fortunately, previous studies had clearly demonstrated that the distribution of fruit flies was not uniform throughout the Central American region (OIRSA 1987), and seasonal fluctuations of each fruit fly species showed a similar year-round pattern. Moreover, although they differed among countries, the major hosts for each species of fruit fly were well known. Hence, sufficient baseline data were available in 2001 to select at least one feasible area in each country to establish either an FF-ALPP or an FF-PFA (Fig. 1).

5. Focusing on Exports

Activities carried out in the pilot areas not only addressed field aspects (e.g. surveillance and control), but also included all phytosanitary actions needed to ensure the export of commodities produced in FF-ALPP or FF-PFA. Therefore, the project focused both on the needed critical actions prior the development of the FF-ALPP or FF-PFA, and those that are essential once a FF-ALPP or FF-PFA is already established.

5.1. Preparatory Activities for the Establishment of FF-ALPP or FF-PFA

Activities were aimed at conducting baseline studies to support the establishment of the specific pilot FF-ALPP or FF-PFA. The first action was to gather and analyse existing data to determine and recommend to governments the specific areas for intervention through the project. Secondly, actions were aimed at preparing technical and economic feasibility studies for each of the pilot areas that were initially selected. Thereafter, the same studies were undertaken for the additional areas selected by the governments. Moreover, a public information programme was prepared for the entire region, which included communication strategies for the pilot areas.

5.2. Establishment of FF-ALPP or FF-PFA

Activities focused on building the technical capacity of national staff who were trained both *in situ* through *ad hoc* training courses, and through hands-on training within operational programmes using AW-IPM with an SIT component in Argentina, Chile, Guatemala, Mexico and Peru. In addition, high-level officials from ministries of agriculture and national plant protection organizations visited countries using FF-PFA and/or FF-ALPP as the core measure in a systems approach for exporting fruit fly hosts to fly-free countries or areas within countries such as Chile, Mexico and the USA. Also, the heads of national fruit fly programmes were trained in leadership and management of AW-IPM projects with an SIT component, as well as in using strategic planning and logical frameworks for planning and evaluating these projects. Special training was given for using geographic information systems (GIS) and global positioning system (GPS) technologies for developing accurate field databases on the occurrence of target fruit flies in each country. Particular attention was given to developing fruit fly identification capabilities and on

training for preparing public information programmes. In the case of Costa Rica, attention was given to transferring the know-how for establishing and managing the mass-rearing and sterilization of the West Indian fruit fly.

5.3. Maintenance of FF-ALPP or FF-PFA

Activities were directed towards preparing a phytosanitary framework for export of horticultural products. This involved provision of assistance to develop national phytosanitary standards and regulations for establishing FF-ALPP and FF-PFA and preparing official quarantine resolutions to recognize and validate them based on the standards of the International Plant Protection Convention (IPPC).

Assistance was also provided to prepare and implement the protocols for emergency actions in case of fruit fly outbreaks, pest risk analyses were prepared for mangosteen and litchi, and support was given for a pest risk analysis on tomato from Honduras, all of them prepared by the Fundación Hondureña de Investigación Agrícola (FHIA 2004a,b). Moreover, under the project, work plans were developed to export papaya without quarantine treatment from the Mediterranean fruit fly-free area in Peten, Guatemala, and to export several species of peppers and red tomatoes from Central America to the USA through a systems approach from a FF-ALPP.

5.4. Role of Industry in the Development of FF-PFA or FF-ALPP

Meetings were held and visits made by experts on trade of horticultural products considered to be fruit fly hosts. These meetings were aimed at advising the governments and industry on the procedures for negotiating exports of fruits and vegetables from FF-ALPP and Mediterranean fruit fly-free areas to fruit fly-free countries. In addition, officials from the USDA who visited the pilot areas supported the goal of exporting fruit and vegetables from FF-PFA and from FF-ALPP through the establishment of a systems approach specific for these areas. Thus, in 2003, the industry actively joined the project, being convinced about the possibility of establishing FF-PFA and FF-ALPP and the likelihood of exporting from these areas.

6. Outcomes and Perspectives

After four years of activities, and with the exception of El Salvador, one pilot Mediterranean fruit fly-free area has been established in each of the participating countries. Based on this experience the governments have selected additional areas to establish FF-ALPP and/or FF-PFA (Fig. 1).

The presence of fruit flies in the pilot and additional areas was determined through a surveillance programme conducted in a manner consistent with the definition contained in the FAO Glossary of Phytosanitary Terms (FAO 2002). The traps used for surveillance were Jackson traps (Scentry Biologicals Inc. Billings, MT 59102, USA) baited with trimedlure (two to four weeks rebaiting interval) to target Mediterranean fruit fly males. This trapping system is the standard trap recommended to demonstrate the presence or absence of this pest. In addition, Multilure® traps (i.e. McPhail type) (Better Trap Inc. Fresno, CA 93727, USA) baited with BioLure® (ammonium acetate, putrescine and trimethylamine) (Suterra LLC Bend, OR 97702, USA) were used to target female flies (four to six weeks rebaiting interval). This female-biased attractant is a powerful tool to detect low numbers of Mediterranean fruit flies (IAEA 2003). The presence of *Anastrepha* fruit fly populations was determined by Multilure® traps baited with torula yeast (one to two weeks rebaiting interval) (Scentry Biologicals Inc. Billings, MT 59102, USA) (IAEA 2003). Traps were serviced at weekly intervals. Trap density is indicated in the sections below dedicated to each country. Fruit fly populations were expressed using the fly per trap per day (FTD) index (IAEA 2003).

Although there is currently no international value for determining the level of fruit fly-low prevalence, for the purpose of this project

the level of low prevalence requested by the USA for export of fruits (mango, papaya, oranges and avocado) from Central America and Mexico through postharvest treatments and/or a systems approach was equal to or less than 1.0 FTD.

Areas were considered as fruit fly-free after one year of zero captures. Once declared as fruit fly-free, a detection was considered an outbreak if it was consistent with the definition contained in FAO's Glossary of Phytosanitary Terms (FAO 2002):

> *An isolated pest population, recently detected and expected to survive for the immediate future.*

6.1. Costa Rica

The selected pilot area of Los Inocentes in the Province of Guanacaste was declared officially free of Mediterranean fruit fly by the Ministry of Agriculture in 2003. The Government of Costa Rica notified the World Trade Organization (WTO) about the establishment of this area. The area encompasses around 40 000 hectares with more than 10 000 hectares of commercial orange and grapefruit orchards grown by a private company that produces fruit juice. The rest of the area includes a national park of forest and dry tropical forest where there are no fruit fly hosts present. In 2001, surveillance was established in fruit fly host areas using a total of 300 traps and a trap density of two traps per square kilometre of hosts for Mediterranean fruit fly detection and one trap per square kilometre of hosts for other fruit flies. Results from 17 000 trap inspections out during 2001-2003 demonstrated that the Mediterranean fruit fly was absent and that the prevalence of other fruit flies of economic importance was low (less than 0.01 FTD). In 2004, several Mediterranean fruit fly outbreaks were detected around the juice facility but the pest was eradicated each time through implementation of an emergency action plan.

In 2003, the Government selected an additional area, the Peninsula of Nicoya, consisting of 500 000 hectares. Although most of the area is used for cattle farming, 2000 hectares are used for producing mango, cantaloupe and watermelon. This area has potential for growing star fruit *Averroha carambola* L., pitahaya *Hylocereus undatus* Britton & Rose, guava, orange and other tropical fruits. In 2003, a surveillance programme was established with a total of 375 traps placed in areas where fruit fly hosts are present using a density of two traps per square kilometre of hosts for Mediterranean fruit fly and one trap per square kilometre of hosts for other fruit flies. Results from the inspections of 40 500 traps carried out during 2003-2005 demonstrated that the West Indian fruit fly was the major pest of concern, with population densities surpassing 2.0 FTD during April, May and June (end of the hot-dry season and beginning of the rainy season). The Mediterranean fruit fly and other species of *Anastrepha* fruit flies of economic importance occurred at low prevalence levels (less than 0.01 FTD) all year round, and Mediterranean fruit fly populations were usually higher in towns and villages.

At present, the Ministry of Agriculture continues trapping against all fruit flies of economic importance, and it is foreseen that the eradication programme will start once the West Indian fruit fly mass-rearing facility is fully operational. Since 2003, the project has supported the Ministry of Agriculture's efforts to upgrade and modify a laboratory for mass-rearing and sterilization of this fruit fly, the main pest of mangoes in the region. The Ministry of Agriculture is acquiring a Gamacell-220® source through a cost-sharing agreement with the IAEA. The sterile flies produced will be used to start the eradication of fruit flies from the peninsula of Nicoya. It is envisioned that this laboratory will have a catalytic effect in the region for controlling *Anastrepha* fruit flies.

In addition, since 2004, the project has supported the development of several FF-ALPP in the Central Valley to export pepper and red tomato through a systems approach. Activities are supported by the National Commission of Agricultural Production in Protected Environments, which is investing over USD 50 million in the initiative. Agreement to export these commodities to the USA requires

approval of the USDA, which is foreseen to occur in 2005-2006.

6.2. El Salvador

The selected area in San Juan Opico, Department of La Libertad, never reached the status of an FF-PFA due to its high Mediterranean fruit fly population densities, which usually reached levels of 3.0 FTD during several months each year. The area encompasses more than 10 000 hectares, including over 500 hectares of oranges and tangerines, which are sold on the local market. Citrus groves are scattered within a zone of 1000 hectares of coffee plantations, and in most of the area there are several species of major fruit fly hosts. Surveillance was set up in 1997 (before the project was launched) using a total of 30 traps placed in areas with fruit fly hosts and using a density of 0.3 traps per square kilometre of hosts for Mediterranean fruit fly and 0.2 traps per square kilometre of hosts for other fruit flies. Results from 8600 trap inspections carried out during 1997-2002 demonstrated that Mediterranean fruit fly populations were always high (more than 1.0 FTD). However, *Anastrepha* fruit fly populations were usually under 1.0 FTD. From the beginning, the NPPO was informed about this risk, but at the request of the local citrus industry it insisted on selecting this area as a pilot for intervention.

When the limitations of the selected area to become an FF-PFA were realized in 2003, the NPPO proposed the Island of Espiritu Santo, Department of Usulutan for developing a FF-PFA. This island has an area of about 3000 hectares, is isolated by swamps and it has coconut, banana and cashew as major crops. All of the target fruit flies are present, and in 2003, surveillance was initiated with a total of 24 traps placed in areas with presence of fruit fly hosts using a density of 0.8 traps per square kilometre of hosts for Mediterranean fruit fly and 0.4 traps per square kilometre of hosts for other fruit flies. The results of 2600 trap inspections carried out during 2003-2004 demonstrated that the West Indian fruit fly was the major pest reaching levels of 2.0 FTD. Occurrence of the other fruit flies, although variable, did not surpass 1.0 FTD. It is foreseen that in early 2006 a programme of ground bait sprays will be launched for population suppression and to prepare the area for an eventual eradication of the fruit flies.

In 2004, the sugarcane industry of San Juan Opico launched a project to produce and export bell peppers and red tomato through a systems approach. Using previous data gathered for this area, the project supported this initiative by carrying out complementary surveillance procedures in 2004 to develop a FF-ALPP. The industry has invested over USD 15 million in building the infrastructure to export these commodities. It is expected that negotiations for exporting these commodities to the USA will be completed successfully in 2005-2006.

6.3. Guatemala

The selected pilot area in the Municipalities of Quetzaltenango and Totonicapan, in the western highlands (over 2500 metres high), is free of Mediterranean fruit fly and *Anastrepha* fruit flies. The area encompasses more than 50 000 hectares, including over 400 hectares producing peaches and apples that are sold in the local market. In 2001, surveillance was set up with a total of 60 traps placed in areas with fruit fly hosts using a density of 0.8 traps per square kilometre of hosts for Mediterranean fruit fly and 0.4 traps per square kilometre of hosts for other fruit flies. Results from 6700 trap inspections carried out during 2001-2004 demonstrated absence of the Mediterranean fruit fly except for three outbreaks that were detected and eradicated in 2004; since then there have been no Mediterranean or *Anastrepha* fruit fly detections. Since these activities are supported by peach and apple producers and because the border with Mexico is nearby, the Ministry of Agriculture of Guatemala is negotiating with its Mexican counterpart for permission to export peaches from these areas into Mexico.

In 2004, at the request of the industry, the

Ministry of Agriculture established four FF-ALPPs in the Departments of Guatemala and Jalapa which qualify for the export of pepper and red tomato to the USA through a systems approach. The project is supporting and working closely with the industry in these areas, and negotiations for exporting these commodities to the USA are foreseen to be completed in 2005-2006.

In 2000, the Department of Peten was established by the Moscamed Programme (Guatemala-Mexico-USA) as a Mediterranean fruit fly-free area. In April 2005, based on a bilateral work plan prepared by the project, the papaya growers of the department began exporting this commodity to the USA.

6.4. Honduras

The selected pilot area in the valley of the river Aguan, Departments of Yoro and Colon, was declared officially free of Mediterranean fruit fly by the Ministry of Agriculture in 2003. The area covers around 400 000 hectares, including more than 25 000 hectares with oranges for fresh fruit domestic and regional markets, and grapefruit for concentrated juice. There are also 40 000 hectares of different commodities such as oil palm, banana, pineapple and cocoa. Also, coffee, avocado and mango are grown to a minor degree. In 2001, surveillance was implemented with a total of 700 traps placed in areas with fruit fly hosts using densities of 1.7 traps per square kilometre of hosts for Mediterranean fruit fly and 0.3 traps per square kilometre of hosts for the other fruit flies. Results of 43 000 trap inspections carried out during 2001-2003 indicated the absence of the Mediterranean fruit fly. At present (2005), results of 58 000 trap inspections carried out from 2001 to early 2005 indicated that this pest is not present. Other fruit flies of economic importance occurred at low prevalence, with total captures of the six economic fruit fly species barely reaching 0.25 FTD, and the Mexican fruit fly being responsible for 0.20 FTD of that value. In late 2004, a Mediterranean fruit fly outbreak was detected in the urban area of the small port of Trujillo (one square kilometre), near the northern edge of the free area. By early 2005, that outbreak had not been eradicated, and thus jeopardizing the Mediterranean fruit fly-free status of the valley.

Once the Mediterranean fruit fly outbreak is eliminated the next step would be to eradicate the Mexican fruit fly, the West Indian fruit fly and the guava fruit fly. The possibility of doing so is based on the low prevalence of all fruit flies of economic importance. Thus, the establishment of a fruit fly-free area is feasible in this vast fertile valley.

6.5. Nicaragua

The selected pilot area, the Island of Ometepe (on Lake Cocibolca), in the Department of Rivas, was declared officially free of Mediterranean fruit fly by the Ministry of Agriculture in 2003. The area covers more than 30 000 hectares, including over 5000 hectares of banana and 500 hectares of coffee. In 2000 (before the project was launched), surveillance was set up with a total of 425 traps placed in areas with fruit fly hosts using a density of 1.5 traps per square kilometre of hosts for Mediterranean fruit fly and 0.3 traps per square kilometre of hosts for the other fruit flies. Results of 71 400 trap inspections carried out during 2000-2003 demonstrated that the Mediterranean fruit fly was not naturally present, that the prevalence of the guava fruit fly and sapote fruit fly was low (0.01 FTD) and that the West Indian fruit fly could reach population levels of 0.5 FTD. In 2003, four outbreaks of Mediterranean fruit fly occurred in Moyogalpa, a small town that surrounds the main harbour of the island, but the pest was eliminated by implementing an emergency action plan.

The experience gained in Ometepe provided the Ministry of Agriculture (with the support of the producers who had been exporting mango to the USA since 1999 from the north of Lake Xolotlan, between the Departments of Leon and Managua, through hot water quarantine treatment) with the confidence to develop

Figure 2. *Fruit fly-low pest prevalence areas (FF-ALPP) planned for production and export of bell pepper and tomato, through a systems approach based on an FF-ALPP and pest free growing structure, Valley of Sebaco, Nicaragua. (upper, left) Location of the FF-ALPP, (upper, right) greenhouse frame, (lower, left) first plantation and (lower, right) bell pepper fruits produced.*

a FF-PFA in that area. The area covers around 40 000 hectares, of which more than 4000 hectares of rice and corn (by irrigation) and around 1000 hectares of mango, papaya, cantaloupe and watermelon. Also there are a few scattered avocado and orange groves. The area has exceptionally favourable conditions for becoming fruit fly-free since it has only two small towns (less than 2000 inhabitants), one road going across the area, a large airstrip (not in use), no more than five species of fruit fly hosts and a very low density of wild host trees (one host tree per 50 square kilometres).

Records of 25 000 trap inspections during the last ten years of trapping (1994-2003) supervised by the Ministry of Agriculture and USDA using a total of 52 traps located in 600 hectares of a mango and a density of 87 traps per square kilometres of hosts for Mediterranean fruit fly and for other fruit flies, indicated that the Mediterranean fruit fly never reached a level of 0.05 FTD, while the West Indian fruit fly, which is the main pest of mango, reaches a maximum level of 1.5 FTD only during June and July. During the rest of the year, this pest occurs at low prevalence levels (between 0.05 and 0.7 FTD). During these ten years, the annual occurrence of the guava fruit fly was negligible (less than 0.0001 FTD).

Based on these data, and under the framework of the regional project, the Ministry of Agriculture and the industry launched an eradication programme in 2004 based on ground insecticide-bait sprays. After 12 weekly bait spray treatments, and an intensive surveillance programme in areas with fruit fly hosts using 14 traps per square kilometre of hosts for the Mediterranean fruit fly and seven traps per square kilometre of hosts for other fruit flies, and with traps inspected weekly for seven months (around 5200 trap inspections), fruit fly populations were eradicated from this area. Based on these results and considering

that the area has enough water resources to irrigate over 10 000 hectares throughout the year, the Ministry of Agriculture, the industry, USAID and USDA developed a three-year plan starting in late 2005 to strengthen current public outreach, quarantine activities and emergency plans to keep the area free of fruit flies in order to further develop agriculture through expansion of the area planted with mango and papaya.

The resulting successes in Ometepe Island (2000-2003) and in the northern Lake Xolotlan area (2004-2005), culminated in early 2005 in another industry/Ministry of Agriculture initiative for establishing a FF-ALPPs in the Sebaco Valley, Department of Matagalpa, and on the south-eastern area of Lake Cocibolca, Department of Rio San Juan. Data gathered by the Ministry of Agriculture during the period 2003-2005 through a surveillance programme using a total of 50 traps placed in areas with fruit fly hosts, using a density of 0.2 traps per square kilometre of hosts for the Mediterranean fruit fly and the same for other fruit flies, indicated that it is feasible to develop a fruit fly-free area in both places.

In Sebaco Valley, the industry has invested USD 50 million to produce bell pepper and red tomato for export through a systems approach including pest-free growing structures (Fig. 2). Negotiations for exporting these commodities to the USA are foreseen to be successfully concluded in 2005-2006.

6.6. Panama

The selected pilot area of Las Churuquitas in the Province of Coclé is in the process of being declared officially free of Mediterranean fruit fly by the Ministry of Agriculture. The area covers about 30 000 hectares, of which more than 2000 hectares are oranges for fresh local consumption. Also, there are 3000 hectares of coffee. In 2001, surveillance was established with a total of 25 traps placed in areas with fruit fly hosts using a density of 0.1 traps per square kilometre of hosts for Mediterranean fruit fly. In 2003, the number of traps was increased to 180 using a density of 2.7 traps per square kilometre of host area. Results from 22 140 trap inspections carried out during 2001-2004 demonstrated the absence of the Mediterranean fruit fly but the presence of the West Indian fruit fly and the guava fruit fly. However, unlike with Mediterranean fruit fly, trapping is not reliable for these species, and so their levels are still unknown. The absence of Mediterranean fruit fly in this area is not surprising, since except for the small (25 square kilometres) Valley of Anton situated 25 kilometres east of Churuquitas, extensive trapping (0.01 traps per square kilometre) by the Ministry of Agriculture in different periods since 1985 had shown that most of the region, from the central part to the eastern border of Panama with Colombia, had failed to result in captures.

In 2003, at the request of the mango industry of the Pensinsula of Azuero, which demanded a fruit fly-free area to boost mango exports, the Ministry of Agriculture decided to select the peninsula for establishing an area free of several species of fruit flies. Azuero is an area of 650 000 hectares mostly dedicated to livestock. However, in the north-eastern part of the peninsula, in a zone called Arco Seco, there is a government irrigation project of 10 000 hectares aimed at producing different fruits and vegetables such as mango, papaya, star fruit, pitahaya and bell pepper.

Since 1995, the Ministry of Agriculture had been operating a trapping network of 200 Jackson and McPhail traps on a bi-weekly basis in the peninsula of Azuero. This activity stopped in 2001 after six years of collecting data that the Ministry of Agriculture had used to determine the absence of the Mediterranean fruit fly. However, these activities were not conclusive for the project since they were neither permanent nor of the required technical quality. Therefore, with the support of the project, the Ministry of Agriculture started fruit fly surveillance in 2003 with a total of 300 traps placed in areas with fruit fly hosts using a density of one trap per square kilometre of hosts for Mediterranean fruit fly and 0.5

traps per square kilometre of hosts for other fruit flies. After a year of consistently improved trapping (i.e. over 95% of traps were serviced using high quality lures), the absence of the Mediterranean fruit fly was confirmed, while the prevalence of the West Indian fruit fly was established at 1.5 FTD.

Since Azuero is characterized by extensive cattle farms with only a few clustered fruit orchards, if the government and industry decide to eradicate the West Indian fruit fly from this area, the establishment of a fruit fly-free area is highly feasible.

6.7. Belize

Under the multi-institutional project alliance, Belize was supported through FAO's Technical Cooperation Project TCP/072. The exotic fruit fly detection network was strengthened as well as the emergency response capabilities. This was done through training of plant protection staff in deployment and operations of trapping networks, population suppression methods, fruit fly identification and the supply of trapping materials and equipment. In 2004, this capacity building allowed Belize to maintain its status of a Mediterranean fruit fly-free country by eradicating an outbreak that occurred in the southern part of the country, in an area near the border with Guatemala.

6.8. Additional Outcomes

As a result of the public relations activities, the ministry of agriculture set up a regional public information group aimed at developing and implementing a uniform framework for public information activities to improve the management of national fruit fly programmes and eventually, a regional fruit fly programme.

The high level of integration and experience gained by members of the RCG on different approaches for dealing with fruit flies in Central America has resulted in OIRSA setting up a fruit fly technical advisory group coordinated by the same members of the RCG. This technical advisory group is presently preparing OIRSA's regional vision for management of fruit flies of economic importance in Central America.

When the regional project was initiated, representatives of the NPPOs of Honduras and Belize participated in the RCG as associated participants because these countries were not Member States of the IAEA. However, once the project generated results, the Governments of Honduras and Belize decided to become a Member State.

7. Constraints

A major external limitation for the sustainability of the project is that with the exception of the IAEA and USDA-APHIS in Guatemala, which have strongly supported the project, the alliances among international organizations have, despite the project, been fairly weak. Various factors are negatively influencing the possibility of a more solid future partnership. One is competition among international organizations for the relatively limited international funds available, which affects the potential commitment that these organizations may have towards the project. Another factor is that even though project partners share common goals, they envision different ways of achieving the goals, thereby opening the prospect that funds and efforts could be duplicated and wasted.

Analysis of the constraints within the national plant protection organizations running the project in the different countries was made possible through the development of a model by IICA with support of the IAEA (IICA 2003). The model evaluates the level of institutional capability that the national plant protection organizations have to implement and effectively sustain pest suppression and eradication projects without active support from international organizations. It enables precise diagnosis of the national plant protection organizations' weaknesses and strengths, thereby making it possible to predict potential setbacks that would affect future project actions. This in turn enables major institution-

al constraints to be addressed before embarking on these projects.

Application of the model in 2004 revealed that coordination between the ministries of agriculture and the industry for operating suppression and eradication activities is still not well developed. Also it was realized that the rapid turnover of civil servants in the national plant protection organizations could lead to loss of the technical capacity that was built up through the project. Finally it was found that in most of the countries there is insufficient funding for operating the national plant protection organizations, much less for implementing emergency action plans or for extending ongoing actions to larger areas.

When taking into account all these constraints, the outcomes of this project are very significant: with a very low budget, each participating country learned how to establish, negotiate and maintain FF-PFAs and FF-ALPPs. In the future the industry will have to play an increasing role in co-financing the expansion of these area-wide phytosanitary projects.

8. Conclusions

Enhancing exports of non-traditional fruits and vegetables as a viable alternative to traditional tropical crops can be achieved by establishing FF-PFA and FF-ALPP as the core phytosanitary measure integrated within a systems approach.

As described for the different pilot areas in the various Central American countries, any attempt to establish these areas through an AW-IPM approach with an SIT component can be successful if: (1) the ministries of agriculture are the driving forces of any initiative, (2) the industry is convinced of the potential benefits that these areas can bring and is an active partner in the activities, and (3) there are alliances between technical and financing organizations present in the region and they commit to work together sharing a common vision.

Pilot projects establishing FF-ALPP and FF-PFA, in which a comprehensive package of procedures is effectively applied (from suppressing or eradicating the pest to negotiating a work plan for exporting fruit and vegetables from the area concerned), are both low-cost and appropriate to demonstrate the benefits of fruit fly AW-IPM with an SIT component. They are also preferable to venturing *a priori* into extensive, costly and unfeasible fruit fly eradication projects.

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The Fruit Fly Exclusion Programme in Chile

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ABSTRACT For Chile, the export of fresh fruit is an important component of the national economy. The annual export volume reaches up to 200 million boxes and represents an annual income of over USD 1900 million. An important comparative advantage for this industry is the absence of the different genera of major tephritid fruit flies. In order to maintain the condition of Chile as a fruit fly-free country, it is necessary to maintain a permanent exclusion programme, which contains active preventive elements that begin internationally through joint work with neighbouring countries, continues at the border level through a strict inspection system and concludes at the national level with a nationwide, multi-target detection system, oriented to the most important fruit fly species. Additionally, the preventive use of releasing Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) sterile males in northern Chile and southern Peru helps to keep the pest free status, even in the area of highest risk in Chile.

KEY WORDS Chile, area-wide, fruit fly-free, exclusion, SIT, preventive releases, Mediterranean fruit fly, *Ceratitis capitata*

1. Introduction

1.1. National Fruit Fly Programme

As a result of the national fruit fly programme conducted by the Servicio Agricola y Ganadero, Chile is a country free of the most economically important fruit flies. In fact, none of the species of the tephritid pest genera *Ceratitis*, *Bactrocera*, *Anastrepha*, *Dacus* and *Toxotrypana* exist in the country. This area-wide programme operates by means of the Fruit Fly National Detection System, which includes detection activities focusing primarily on the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), but also covering the remaining genera. Even though the main component of the detection system is oriented to adult detection by trapping, it also involves fruit sampling. In spite of this pest free status, five species of *Rhagoletis* fruit flies are present in Chile, only two of which might have potential economic importance and all exclusively associated with Solanaceae hosts (Table 1).

Detection activities began in 1963 when it was determined that the Mediterranean fruit fly was present only in the province of Arica, at the northern border of Chile with Peru (Harris and Olalquiaga 1991, Olalquiaga and Lobos 1993). That situation remained until 1995, when the sterile insect technique (SIT) was added to the control programme, resulting in the pest being eradicated from Arica and the whole Chilean territory was ascertained as fruit fly-free (MAG/SAG 1995, Esparza Duque 1999). A severe reinfestation occurred in Arica in 2000, which was finally eradicated in 2004.

1.2. Protecting the Fresh Fruit Export Industry

For the Chilean fresh fruit export industry, this internationally-recognized absence of major fruit fly pests is an important advantage, because there is no need to apply postharvest treatments or any other restriction measure to export products. As a result, Chile has developed a huge fruit industry, whose export revenues in 2004 reached up to USD 1900 million. This fact has encouraged Chile to under-

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Table 1. *The distribution and hosts of* Rhagoletis *species known to occur in Chile (Frias 1992, Gonzalez 1999).*

Species	Host	Distribution
Rhagoletis tomatis Foote	Tomato	Found in La Chimba, II Region, recently detected in III Region[1]
Rhagoletis nova (Schiner)	Chilean pepino	III – XI Regions[1]
Rhagoletis conversa (Brethes)	Weeds (*Solanum nigrum*, *Solanum tomatillo*)	III – XI Regions[1]
Rhagoletis penela Foote	Weeds of the *Solanum* genus	IX Region[1]
Rhagoletis brncici Frias	Unknown	VII Region[1]

[1]See Fig. 1 showing the different regions in Chile

take continuously a major effort to maintain the fly-free phytosanitary condition.

Since Chile is currently (2005) the only fruit fly-free country in Latin America, it continuously faces the potential threat of fruit flies, mainly the Mediterranean fruit fly, invading its territory. However, the country has some important natural advantageous features that hamper the natural spread of these pests. These include the Andes range to the east of the country, thousands of square kilometres of desert in the north, the Pacific Ocean to the west and finally an extremely cold, sub-polar climate in the south (Fig.1). This isolation has led officials of the national plant protection organization (NPPO) to believe that passive spread through smuggling and hidden fruit in passengers' baggage are the most likely source of fruit fly entries. Because of that, Chile has a very strict quarantine system with border control stations at every point of entry.

1.3. Preventive Release Programme

The only exception to this isolated nature of Chile with respect to fruit flies is Arica Province on the border with Peru, which is separated from Peru by only a few kilometres of non-host habitat. In this border area, which is mainly desert but has some "green spots" that allow flies to rest and feed, the Servicio Agricola y Ganadero currently applies a preventive release programme of sterile Mediterranean fruit flies, integrated with bait spraying (Lobos 1995). The use of the SIT began as a containment measure (Hendrichs et al. 2005) and successfully progressed towards achieving eradication of the Mediterranean fruit fly (MAG/SAG 1995). After eradication, the SIT continues to be applied as part of the preventive strategy (Hendrichs et al. 2005).

Additionally, through bi-national agreements, cooperative work is done with Argentina and Peru to create fly-free areas in relevant provinces in these neighbouring countries to serve as buffer zones. These have been beneficial for all parties, and currently, there are advanced discussions to sign a similar agreement with Bolivia.

1.4. Emergency Response Plan

Despite these stringent measures, almost every year some Mediterranean fruit flies are detected somewhere in the country. To address this, Chile follows an emergency response plan (SAG 2001), which is approved by its trading partners and is activated upon detection of any fruit fly stage. Depending on the number of flies trapped, a different response is initiated:

(1) If one fly is captured (male or virgin female) an intense survey is undertaken, including high density trapping, with traps deployed in a grid of 64 square kilometres (8

Figure 1. *Map of Chile.*

x 8 kilometres) as well as systematic fruit sampling at every property. The survey lasts for two insect cycles, calculated according a scientifically validated day-degree-model (Tassan et al. 1982).

(2) If two or more adult specimens or one inseminated female or immature stages are captured, the Servicio Agricola y Ganadero undertakes an eradication campaign, which includes an intensive monitoring programme involving both trapping and sampling in a 196 square kilometre area. Additional control activities are applied such as fruit stripping, soil drenches and toxic bait spraying in an area within a 200 metre radius around each detection site. Also, regulated areas are established for 7.2 kilometres around every detection spot.

Obviously the main goals of the Servicio Agricola y Ganadero are to minimize the risk of passive spread and maximize the chances of early detection. For the latter, about 9000 lure-baited traps are deployed along the country and additionally fruit sampling is performed regularly.

2. The Regular Detection System

The main goal of the regular detection system is to detect early any entry of exotic fruit flies. It extends from the border with Peru (18° south latitude) to the very south of Chile (47° south latitude, XI Region), and includes every town and city in that area, as well as rural areas that have fruit fly hosts. Based on standardized operational procedures, it consists of a trapping network, supplemented by fruit sampling. The traps and attractants used are presented in Table 2.

2.1. Trap Deployment in Urban Areas

The standard trapping grid within urban areas covers 1000 hectares and includes 52 traps, of which 40 are baited with trimedlure, ten with hydrolyzed protein, one with methyl eugenol and one with cuelure. Trap densities are one trap per 25 hectares for trimedlure-; one per

Table 2. *Target fruit fly species and attractants used in the Chilean detection system.*

Target species	Attractant	Trap model
Ceratitis capitata (males)	trimedlure (TML)	Jackson, Steiner
Ceratitis capitata (females)	protein hydrolysate, BioLure	McPhail, Multilure
Anastrepha spp. and others	protein hydrolysate	McPhail
Bactrocera spp. (mostly *dorsalis* complex)	methyl eugenol	Steiner
Bactrocera spp. (mostly	cuelure	Steiner

100 hectares for protein- and one per 1000 hectares for cuelure- and methyl eugenol-baited traps. Traps are deployed in grids, according to the scheme shown in Fig. 2.

The total area is covered by a grid each composed of squares of one square kilometre. Each grid square is divided into four sub-squares each of 25 hectares. Every sub-square must have one trimedlure-baited trap, which will be relocated four times a year, but always inside the assigned area (25 hectares). Every square must have one protein-baited trap, which will be relocated inside the assigned area (100 hectares). In every 10 squares there are one methyl eugenol- and one cuelure-baited trap. Additionally, fixed traps that are not relocated are placed in high-risk areas.

2.2. Trap Deployment in Rural Areas

The trap lines for surveillance in rural areas are located along roads and in commercial

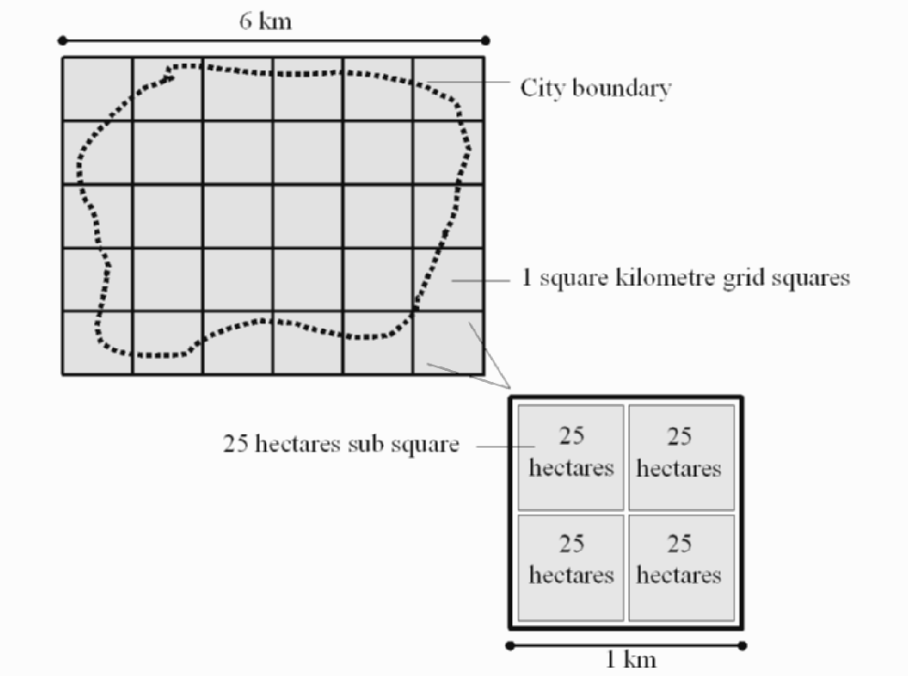

Figure 2. *Scheme of trap deployment in urban areas using one square kilometre grid squares.*

orchards according to the scheme presented in Table 3. The average density of the traps deployed in Chile is given in Table 4.

3. Details of the Response Plan

Upon fruit fly detection, the response plan, which has been approved by most of the Servicio Agricola y Ganadero's commercial counterparts, is triggered (SAG 2001). To date, only Mediterranean fruit flies have been detected repeatedly in Chile and these have been eradicated on every occasion. The following is a description of the response plan for this pest.

3.1. Single Detection

A single detection is defined as one adult specimen caught in a trap (male or unmated female) within an area of 2.25 kilometre radius during one insect generation. Upon detection, a delimitation trapping grid is set up, covering 64 square kilometres (8 x 8 kilometres) around the detection site. Additionally systematic fruit sampling is conducted on every property within 200 metres around that point. No control activities or quarantine regulations are set up. Survey activities last for two continuous insect cycles. Three zones are defined: (1) area A, the core area, is a four square kilometre area surrounding the detection point. Forty trimedlure traps plus 20 protein traps are placed in the square grid where the specimen was caught and ten protein traps in the remaining three squares of the core area, (2) area B, which is immediately outside area A, is a one kilometre wide buffer zone in which 20 trimedlure traps per square kilometre are placed, and (3) area C, a second buffer

Table 3. *Number of hectares for each single trap in relation to the used attractant and risk level in rural areas.*

Trap attractant	High-risk area	Medium-risk area	Low-risk area
trimedlure	50-100	100-150	150-300
methyl-eugenol	1000	1500	2000
cuelure	1000	1500	2000
protein	150	300	500

Table 4. *Number of traps baited with the attractants trimedlure, proteine, cuelure and methyl-eugenol deployed per region.*

Region	Attractant				Total
	trimedlure	protein	cuelure	methyl-eugenol	
I	1041	176	28	17	1262
II	331	98	6	6	441
III	228	101	10	10	349
IV	356	88	9	10	463
V	2111	683	128	129	3051
R.M.	2590	563	52	60	3265
VI	440	125	12	12	589
VII	104	48	6	0	158
VIII	130	44	10	0	184
IX	68	23	0	0	91
X	109	107	0	0	216
XI	10	5	0	0	15
Total	7518	2061	261	244	10 084

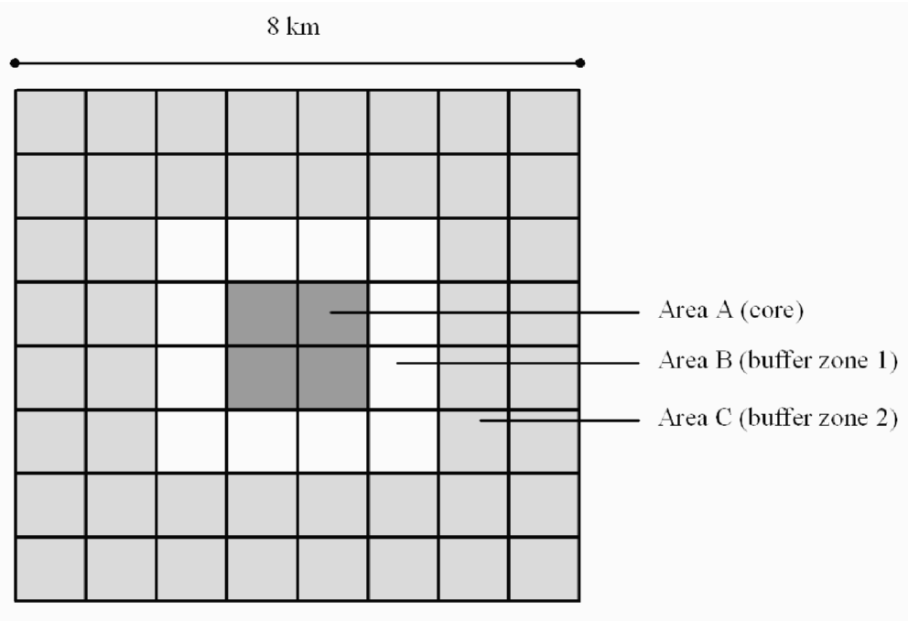

Figure 3. *Delimitation trapping grid after a single Mediterranean fruit fly detection. The grid contains a core area (A) and two buffer zones (B and C).*

zone, extending two kilometres from area B and where ten trimedlure traps per square kilometre are placed. The scheme in Fig. 3 explains the delimitation trapping grid and Table 5 the trap density for single detections.

3.2. Multiple Detection

This is defined when one inseminated female, a multiple detection of males or uninseminated females, or immature stages of the insect are found.

3.2.1. Survey

Following a multiple detection, the eradication phase begins and the delimitation trapping grid is extended up to 196 square kilometres. Detection activities last for three life cycles (Tassan et al. 1982). Besides the trapping network, systematic fruit sampling is conducted in an area with an 800 metre radius around the detection site.

Five different zones are defined from the detection site to seven kilometres away according to the scheme in Fig. 4 and the trap densities in Table 6.

3.2.2. Control Activities

In order to achieve eradication in the shortest time possible, a working area of 200 metres around every detection site is defined and the following activities conducted: (1) toxic bait spraying: a mixture of a protein attractant and a pesticide (malathion) is applied for adult control, (2) fruit stripping: all fruit on every tree within the working area (200 metres around the detection) is destroyed, (3) and soil drench: diazinon to kill immatures is sprayed under the projection of the canopy of every fruit tree in the working area.

3.2.3. Quarantine Regulations

A regulated area is set up 7.2 kilometres around every detection site. Fruit without

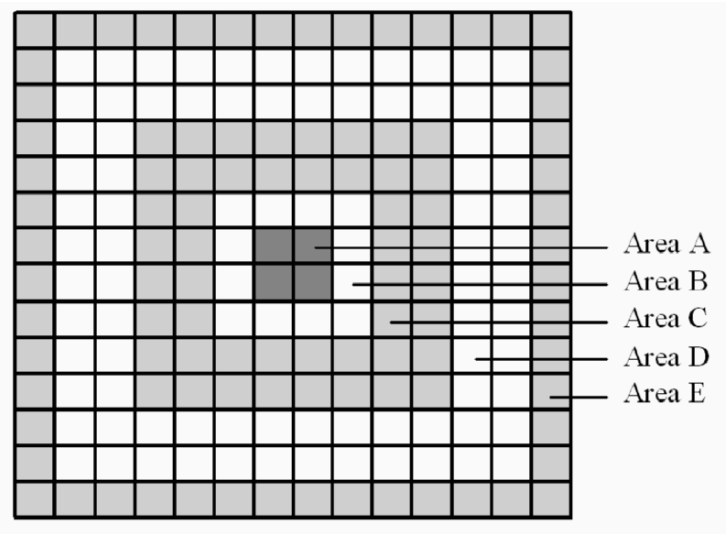

Figure 4. *Delimitation trapping grid after multiple Mediterranean fruit fly detection.*

quarantine treatment is not allowed to leave the area. Safeguard measures are implemented for fruit to be stored within the regulated area. All the quarantine regulations last three life cycles (Tassan et al. 1982).

4. DNA Analysis

The DNA of every specimen found is analysed to determine the most likely origin of a Mediterranean fruit fly introduction. It is based on the polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) technique using four markers developed for the Mediterranean fruit fly by Bruce McPheron, University of Pennsylvannia, which determine the different haplotypes (Mc Pheron et al. 1994, 1995). This technique has been used since 2000, and to date no similar haplotypes in areas under eradication programmes have been encountered. Therefore, it can be concluded that in all cases, eradication of each outbreak was successful. This also means that the outbreaks originate due to continuous introductions from different geographical areas. Also, the technique made it possible to determine the highest risk pathways for entry of the fly (Meixner et al. 2002). The most commonly detected haplotypes are AABB, BBBB and AACB. Peru or countries

Table 5. *Summary of trap deployment in the different areas of the delimitation grid in Chile.*

Area	Size (hectares)	No. of trimedlure-baited traps	Traps per square kilometre	No. of protein-baited traps	Total
A	400	160	40	50	210
B	1200	240	20	0[1]	240
C	4800	480	10	0[1]	480
Total	6400	880		50[1]	930

[1]These trap numbers do not include the traps deployed in the regular programme

Table 6. *Number of traps deployed in the different areas of the delimitation trapping grid after multiple detection.*

Area	Size (hectares)	No. of trimedlure-baited traps	Traps per square kilometre	No. of protein-baited traps	Total
A	400	160	40	50	210
B	1200	240	20	0[1]	240
C	4800	480	10	0[1]	480
D	8000	640	8	0[1]	640
E	5200	208	4	0[1]	208
Total	19 600	1728		50[1]	1778

[1]These trap numbers do not include the traps deployed in the regular programme.

of the South American Pacific basin are the most likely source for AABB, while Argentina is the most likely source for BBBB and AACB. While AAAA and AAAB are sometimes detected, these haplotypes are not known to occur in South America. This means that the source of introduction is more distant than only the neighbouring countries (Sheppard et al. 1992, McPheron et al. 1994, 1995, Steck et al. 1996).

5. Use of the Sterile Insect Technique

5.1. Lluta Mass-Rearing Facility

In Arica Province, the SIT is used as a containment, eradication or preventive control tactic. The insects are mass-reared at the Centro de Producción de Insectos Estériles (CPIE), located in the Lluta valley of Arica Province. The sterile males are released in the Tacna valley in Peru and in Arica Province in Chile (MAG/SAG 2003). Periodic quality control activities are implemented based on standardized procedures described in a quality control manual (FAO/IAEA/USDA 2003) to ensure that the material meets international standards.

As Peru and Argentina also mass-rear and release sterile insects, bi-national agreements for joint work in border areas help to reduce the risk of accidental introductions to Chile and also benefit the partner countries. The strain released is the VIENNA 7/Toliman, which is a genetic sexing strain that carries a *temperature sensitive lethal* (*tsl*) gene (Franz 2005). Both air and ground releases are conducted twice a week.

As Arica is currently free of the

Table 7. *Operational parameters of the SIT component in Arica Province.*

Strain	Release density	Release frequency	Rearing centre	Release method
VIENNA 7 /Toliman	2500 flying sterile males per hectare	Twice a week	CPIE[1] (50 million male pupae per week)	Air

[1]Centro de Producción de Insectos Estériles.

Mediterranean fruit fly, Chile uses the SIT as a containment or preventive strategy (Hendrichs et al. 2005). Details of its use are shown in Table 7.

5.2. Mediterranean Fruit Fly Ceratitis capitata *Outbreaks in 2005*

Up to May 2005, three Mediterranean fruit fly outbreaks have been detected in Chile. In all cases the response plan was triggered and populations eventually dropped to zero. Eradication will be officially declared for each case when three life cycles without detection are completed. The following are the specific details of the current situation relating to outbreaks of Mediterranean fruit fly in Chile:

Estación Central, Metropolitan Region: between 30 December 2004 and 11 March 2005, 41 adults and 14 larval sites were detected. The quarantine trigger was met on 3 January 2005, with control activities including toxic bait spray, fruit stripping and soil drench being applied against all the insect stages. After completion of three life cycles without detection, eradication was declared on 2 December 2005. The mitochondrial DNA haplotype was BBBB which is a fairly common haplotype in many populations worldwide. However, it is not present in Peru and in many populations in Central America. The presence of the BBBB haplotype in Argentina could suggest an origin for this outbreak.

Rancagua, VI Region: between 14 March and 26 April 2005, 100 adult flies and 19 larval sites were detected. The quarantine trigger was met on 15 March 2005 and control activities as above applied against all the insect stages. To date, two months without detection have elapsed and according to historic temperatures, the estimated date to proclaim eradication will occur around mid January 2006. The mitochondrial DNA haplotype was AAAB. This may indicate a different origin than the above as the AAAx haplotype has not been found in Argentina. The AAAx haplotype is found in many parts of Central America.

Los Andes, V Region: on 22 March 2005, three adult flies were detected, triggering the response plan on that date. The control activities described above were applied against all insect stages and after three theoretic cycles without detection, eradication was declared on 15 November 2005. The mitochondrial DNA haplotype was AACB which is fairly common haplotype in South America but not in Central America.

The fact that all three outbreaks belonged to different haplotypes suggests that there was no relation between the incursions. It also demonstrates that the pest was not able to spread from the established regulated areas.

6. Conclusions

Since the fruit industry in Chile plays an important role in the Chilean economy, keeping the fruit fly-free status of the country is a government priority. This is facilitated by the fact that Chile has very good natural barriers, which makes it impossible for the pest to fly directly from neighbouring countries. However, among the difficulties faced by Chile is the fact that the pest is widespread in the remaining countries of the region. For this reason, the risk of entry is permanent. Given the above, the national control strategy operates beyond the borders through binational agreements with neighbouring countries, at frontiers by means of a strict quarantine inspection system, and within the country through a highly sensitive detection system able to detect early any specimen that manages to enter the country. Upon fruit fly detection, the response plan is triggered and area-wide control measures as well as regulated areas are applied. The actions last for three theoretical life cycles after the last detection. In Arica Province, located at the border with Peru, the SIT is included for preventive control. Mitochondrial DNA analyses demonstrated that new pathways are jeopardizing Chile´s Mediterranean fruit fly-free status. Nevertheless, all introductions were of different origin rather than one outbreak that managed to spread and infest other regions.

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Expansion of the National Fruit Fly Control Programme in Argentina

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ABSTRACT In the Patagonia and Cuyo regions of Argentina, which specialize in temperate fruit production, the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) occurs mainly in urban areas. Nevertheless, its presence has represented a phytosanitary barrier for exporting fresh fruits to countries free of the pest or with active control programmes. As a result of more than ten years of successful integrated area-wide application of the sterile insect technique (SIT) against this pest, the phytosanitary status of these two regions was redefined, thereby allowing exports without postharvest treatments to previously closed markets. In the north-eastern and north-western regions of Argentina that are mainly citrus-growing areas, there are two main fruit fly species: the Mediterranean fruit fly and the South American fruit fly *Anastrepha fraterculus* (Wiedemann). In these regions they are present in urban areas, in commercial orchards and in wild host areas. Growers using traditional methods based exclusively on insecticides applied as air or ground sprays actively control these pests. Even so, about 143 000 tons of citrus are lost every year in these regions due to fruit fly pests. The gross value of this lost production is estimated at USD 37 million annually. Against this background and within the framework of the National Plant Health Programme, the National Agri-Food and Animal Health and Quality Service (El Servicio Nacional de Sanidad y Calidad Agroalimentaria - SENASA) has made the expansion of the fight against these pests a priority. The expanded programme plans to become comprehensive, extending suppression of the pest to these other major fruit production regions by applying the area-wide concept, and integrating and adapting different technologies to each particular regional condition. The programme will incorporate the use of the SIT over an additional 90 000 hectares. The main economic impact of the expanded programme is expected to be felt in the citrus-growing areas of the north-eastern region, Monte Caseros (Corrientes) - Colón (Entre Ríos), where 56 200 hectares are used for the production of oranges and tangerines by 2400 growers. In this important production area (932 000 tons of citrus), direct benefits will include increased revenues from the higher volumes and quality of fruit produced due to the absence of damage by fruit flies; these are estimated from the fourth year of the initiation of the expanded programme at USD seven million per year. Reduced surveillance and control costs coupled with reduced environmental impacts arising from lower insecticide use will be additional advantages.

KEY WORDS *Anastrepha fraterculus*, South American fruit fly, *Ceratitis capitata*, Mediterranean fruit fly, Argentina, area-wide, suppression, fly-free areas, areas of low prevalence

1. Current Situation

1.1. Cuyo and Patagonia Region

The presence of fruit flies in Argentina varies in different parts of the country. In the provinces of Neuquen and Rio Negro (Patagonia region), as well as Mendoza and San Juan (Cuyo region), the only species present is the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), while in La Rioja province and other fruit-growing areas, the South American fruit fly *Anastrepha fraterculus* (Wiedemann) can also be detected (Fig. 1). Control strategies also vary with the region concerned. In the citrus-growing areas, suppression actions are the exclusive responsibility of fruit growers, while in Patagonia and

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 653–660.

Figure 1. *Map of Argentina showing the current fruit fly control situation. (1) Regions under official fruit fly programme containing pest free areas (Patagonia, Malargüe and El Sosneado Valley, and Uco Valley (Mendoza province)), low-prevalence areas (rest of Mendoza, Bermejo Valley (La Rioja province)), and areas under control (rest of La Rioja; San Juan province), and (2) regions to be included in the expanded programme, i.e. NOA (north-western region) (Catamarca, Tucumán, Salta and Jujuy provinces) and NEA (north-eastern region) (Entre Ríos, Corrientes and Misiones provinces, and northern part of the province of Buenos Aires).*

Cuyo (Mendoza, San Juan and La Rioja) these are implemented by the National Control and Eradication Programme (PROCEM). This programme is jointly funded by growers and the government and aims to establish fruit fly pest free and/or low prevalence areas (FAO 1996, 2001, 2005) based on the concept of area-wide integrated pest management (AW-IPM). Since the early 1990's, the control strategy implemented through PROCEM has been based on integrated use of the sterile insect technique (SIT), cultural and chemical controls, and a strict quarantine system (Aruani et al. 1996, De Longo et al. 2000).

The regions of Patagonia and Cuyo specialize in producing temperate fruits, mainly pome fruits (apple, pear, grape, and quince) and stone fruits (peach, plum, apricot, nectarines, cherry). Due to the climatic conditions and structure of the industry in these regions, and because of the biological characteristics of *C. capitata*, it is the only fruit fly pest species present. Although occurring mainly in some urban areas, the presence of this fly has represented a phytosanitary barrier for exports to countries free of the pest or with active control programmes. However, successful area-wide control actions in Patagonia and Cuyo carried out over the last ten years, have resulted in pest free and low prevalence areas (De Longo et al. 2000, Enkerlin 2001). They have paved the way to overcome the need for postharvest treatments to export fresh fruits from these regions to *C. capitata*-free countries (Acevedo 2004, USDA 2005, FAO 2006, IAEA 2006).

1.2. North-Eastern and North-Western Regions

In the north-eastern and north-western regions of Argentina (Fig. 1), which are mainly citrus areas, *C. capitata* and *A. fraterculus* cause significant losses in orchards unless actively controlled by growers who use traditional methods based exclusively on insecticides applied as air or ground sprays. The high levels of fruit damage registered every year in the north-eastern and north-western regions, in spite of the use of these environment-unfriendly methods, demonstrate the importance of addressing the problem at a regional level.

In the case of the main sweet citrus-producing areas (oranges and tangerines) that are

located in the Corrientes and Entre Ríos provinces of the north-eastern region, the average damage caused by the pest is 13%. In addition to the direct economic impacts in the areas concerned, such levels imply high risks of reinfestation of protected regions under pest free or low prevalence status like Patagonia and Cuyo through trade of infested citrus into these regions.

In the north-eastern region, the fruit-growing area that is most affected by fruit flies stretches from Monte Caseros (in Corrientes province) to Colón (in Entre Ríos province). It includes more than 56 200 hectares and 2400 growers who produce 954 000 tons of oranges, tangerines and grapefruits for commercial markets. Due to fruit fly pests, about 143 000 tons of produce are lost every year with a gross value estimated at USD 37 million. This direct damage of the pests (without considering the indirect damage they cause) justifies expanding the integrated area-wide programme to control the problem.

2. National Fruit Fly Control and Eradication Programme (PROCEM)

Against the above background and within the framework of the National Plant Health Programme, SENASA has declared the fight against fruit flies as a priority.

The aims of the expanded national programme are: (1) to reduce fruit fly populations in the citrus-growing areas in the north-eastern region of Argentina, to levels where fruit damage is reduced to 0.5% and maintained at this level over the long term, (2) to improve control of the pests by establishing a surveillance and phytosanitary emergency response system, and by providing validation methods and technological training in the areas under control in the north-western region of Argentina, north-eastern Corrientes-Misiones provinces, and northern part of Buenos Aires province, and (3) to ensure maintenance of the pest free and low prevalence areas of Patagonia and Cuyo regions.

The programme takes an integrated approach to pest control throughout the national territory, involving implementation of the area-wide concept and adapting different technologies to each particular condition. It will enforce actions that have been implemented for years in Patagonia and Cuyo regions, and will expand control into new areas like the north-eastern and north-western regions, where fruit flies have a direct impact on citrus production.

For the 56 000 hectares of citrus in the Monte Caseros area, the programme represents a change of strategy, moving from chemical control at an orchard level to biological control at a regional level by integrating the SIT. This technological change has the objective of reducing the damage from 13 to 0.5% and ensuring the environmental and commercial sustainability of suppression through reduced use of insecticide sprays.

The SIT will also be incorporated into control activities undertaken in new areas of San Juan and La Rioja provinces, the aim being to bring all growing areas in those provinces under area-wide control.

In the remaining areas under production with fruit fly hosts to be included in the expanded national programme (e.g. Salta, Jujuy, Tucumán and Catamarca provinces, Misiones, north-eastern Corrientes and northern Buenos Aires), a phytosanitary and ecological survey will be carried out as an initial step to obtain detailed knowledge of the fruit fly pests in each area and their relationship with the environment. The programme will also improve current control measures carried out by growers by incorporating surveillance and phytosanitary emergency response systems, training and technology transfer.

The expanded programme will also supply the regions of Patagonia and Cuyo with more tools to maintain their pest free and low prevalence area status by strengthening the quarantine protection system and implementing phytosanitary emergency plans. As it expands into new areas and the prevalence of fruit flies decreases, the level of pressure of the pest on the free or low prevalence areas is expected to decrease and an improved phytosanitary sta-

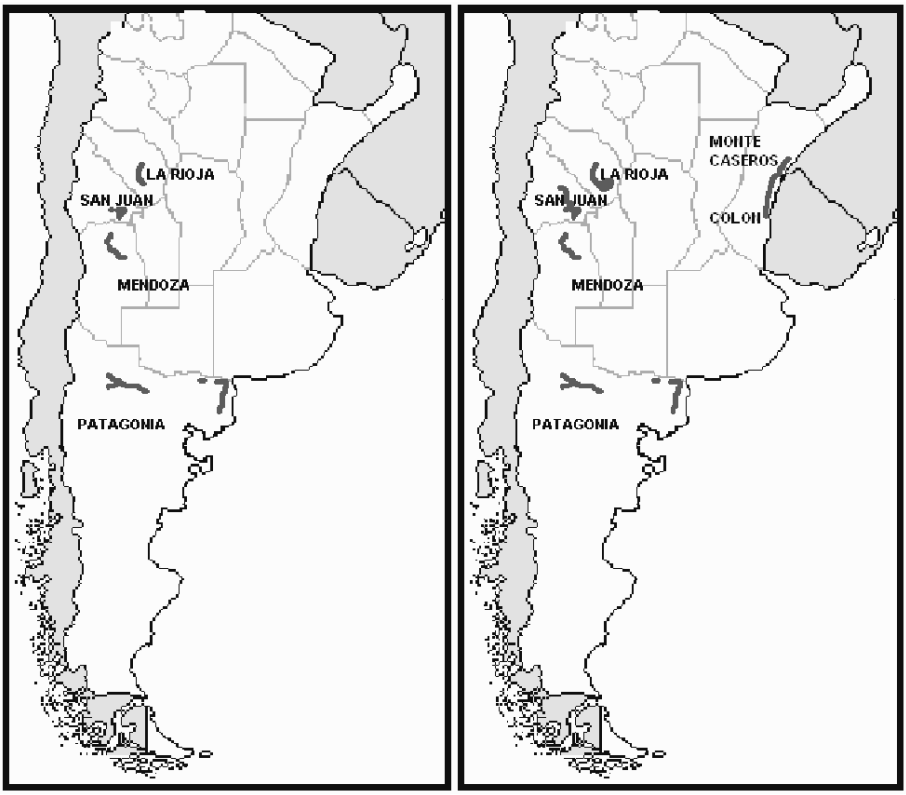

Figure 2. *Current (left) and future (right) areas under integrated SIT application.*

tus obtained.

2.1. Proposal to Expand the SIT Area

At present, about 160 000 hectares are subjected to the releases of sterile Mediterranean fruit flies males in the regions of Patagonia, and the provinces of Mendoza, San Juan and La Rioja. The expanded programme will incorporate an additional 90 000 hectares bringing a total of 250 000 hectares under integrated management with an SIT component in Argentina. In Monte Caseros (Corrientes) and Colón (Entre Ríos), sterile males will be released over 70 000 hectares out of which 56 200 hectares are citrus areas and the rest urban and suburban areas. In San Juan province, the SIT will be integrated over additional 10 000 hectares in the Tulum Valley, and on another 10 000 hectares in La Rioja province (departments of Capital, Sanagasta, Castro Barros and Arauco) (Fig. 2). Expanding the area covered by the SIT in Argentina will increase the number of sterile Mediterranean fruit flies required from 140 million at present to 300 million sterile male pupae per week.

In addition, in preparation of the implementation of the SIT against *A. fraterculus* (Ortíz 1999), studies have also been underway to develop an understanding of the genetics of this pest (Lifschitz et al. 1999, Basso et al. 2003), its ecology (Segura et al. 2006), and to confirm mating compatibility among populations from the relevant regions in Argentina (Alberti et al. 2002, Petit-Marty et al. 2004a,b). Unlike studies among *A. fraterculus* populations from other regions of South

Table 1. *Projected increase (tons) in commercialized fruit production and estimated reduction in fruit volume affected by fruit flies as a result of the project.*

Fruit Production	Present situation	Year 1	Year 2	Year 3	Year 4 and onwards
Commercialized production	811 311	811 311	896 414	922 918	927 709
Production affected by fruit flies	121 018	121 018	35 914	9410	4619
Total production	932 329	932 329	932 329	932 329	932 329

America (Vera et al. 2006), all these studies indicate that Argentinean *A. fraterculus* populations belong to a single biological species. As a result, mass-rearing systems have been developed and established (Jaldo et al. 2001) for future *A. fraterculus* SIT integration into these area-wide programmes.

To ensure more effective pest control, it will first be necessary to improve the chemical control to suppress pest populations. Prior to initiating the SIT, 12 aerial bait sprays with NuLure (500 cc/ha) and malathion 100E (100 cc/ha) will be carried out in the north-eastern region during the first three months. In La Rioja and San Juan, such suppression will be carried out over three months using ground sprays in urban and suburban areas. The programme will supply sprayers (200 litres capacity) and chemicals such as malathion 110E, which will be applied in doses of 0.75 litres/15 ha, and hydrolyzed protein in doses of 3 litres/15 ha.

The necessary sterile Mediterranean fruit fly pupae for application of the SIT will be provided by the insectary in Mendoza province. This mass-rearing facility, which currently meets the total demand of the country, will be replaced by a new facility in Mendoza, increasing the weekly production of sterile male pupae to a minimum of 300 million. The programme will have a new fly emergence and release centre in Concordia (Entre Ríos) and another one in Monte Caseros. In La Rioja and San Juan, the available release infrastructure will be used.

Aerial distribution of sterile Mediterranean fruit flies at a density of 1200 flying males per hectare per week (equivalent to 1700 pupae per hectare per week) will be carried out during 52 weeks per year over the 70 000 hectares of the Monte Caseros-Colón area. In the 10 000 hectares of the Tulum Valley (San Juan) and the 10 000 hectares of Capital, Sanagasta, Castro Barros and Arauco (La Rioja), the density was established at 1000 flying males per hectare per week, equivalent to 1400 male pupae per hectare per week, to be released also aerially during 36 weeks per year. During the release of sterile males, additional chemical control will be conducted at hot spots to control outbreaks of the pest.

In the rest of the north-eastern region (Bella Vista and Saladas, Misiones and north-eastern Corrientes), in the fruit growing area of San Pedro (Buenos Aires province), and in the north-western region, chemical control by the growers will be accompanied by surveillance and phytosanitary emergency response systems and strongly supported by the national programme through training and technology transfer.

2.2. Economic Analysis of the Expanded Programme

The total cost of the expanded programme is estimated at USD 57 million for the five years of execution. Half of this will be funded by

Table 2. *Projected increase in income (USD) generated by the expanded programme.*

	Year 2	Year 3	Year 4 and onwards
Income	5 000 000	6 600 000	7 000 000

the Agricultural Services Programme (PROSAP) of the Secretariat of Agriculture, Livestock, Fisheries and Food (SAGPyA), with the other 50% coming from national and provincial governments and the private sector. The main economic impact of the expanded programme is expected to be in the citrus growing areas of the north-eastern region, Monte Caseros (Corrientes)-Colón (Entre Ríos), where 56 200 hectares are used for growing oranges and tangerines by 2400 growers. Fruit flies currently reduce total production by about 13% and the expanded programme is expected to reduce these losses to 5, 1 and 0.5% in the second, third and fourth year, respectively (Table 1).

In this important productive area (932 000 tons of citrus), direct benefits will be expressed through (1) increased revenues, (2) reduced control costs, and (3) reduced use of chemicals.

Increased revenues will be obtained from higher volumes of commercialized good quality fruit (absence of damage by fruit flies). By the fourth year of the project, an increase of around 116 000 tons of commercialized fruit is expected from the region (Table 1).

Considering present sales values, this increment would generate an additional regional income from the 4th year of the expanded programme of USD 7 million per year (Table 2).

Table 3. *Projected reduction in surveillance and control costs as a result of the project.*

Area (hectares)	Number of sprays	Cost (USD/hectare)
34 270	15	392
15 400	6	218
3900	0	60
2630	0	0

Current annual cost: USD 17 025 040 (USD 303/hectare)

Annual cost after the 4th year: USD 8 900 000 (USD 158/hectare)

Annual Benefit: USD 8 200 000 (USD 146/hectare)

In addition, reduced surveillance and control costs (Table 3), and environmental impacts due to lower chemicals applied (Table 4) are expected.

Table 4. *Projected reduction in costs due to decreased use of malathion (litres per year) as a result of the project.*

Current annual malathion use	83 900
Annual use from the 4th year	16 000
Reduction in malathion use	67 900

3. Conclusions

The national fruit fly programme in Argentina has created the human, regulatory and physical infrastructure to effectively manage fruit flies on an area-wide basis. Over ten years of integrated application of the SIT against *C. capitata* have demonstrated the effectiveness of the approach. They culminated in the official recognition by trading partners of pest free or low prevalence phytosanitary status of important fruit growing areas of Patagonia and Cuyo. Stakeholders in other fruit producing regions are demanding the expansion of the national programme to their areas to also benefit from an area-wide approach to fruit fly control. Feasibility assessments indicate that expanding the approach to other fruit production areas has considerable potential and would result in important economic benefits. However, the complexities in these new areas are significantly larger, including an additional pest species (*A. fraterculus*), a warmer climate with more pest generations per year, and

pest presence not only in orchards and urban areas, but also in surrounding non-commercial areas.

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The Augmentative Biological Control Component in the Mexican National Campaign Against *Anastrepha* spp. Fruit Flies

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ABSTRACT The Mexican Government launched the National Campaign Against Fruit Flies in 1992, in order to create free and low prevalence areas of native fruit flies of economical importance belonging to the genus *Anastrepha* Schiner. The campaign uses an area-wide integrated pest management approach that includes the use of environment-friendly strategies to suppress or eradicate fruit flies, such as the application of selective toxic baits, the use of the sterile insect technique (SIT), and the release of the endoparasitoid *Diachasmimorpha longicaudata* (Ashmead). Fifty million parasitized pupae, produced in the Moscafrut facility in the State of Chiapas, are sent weekly via commercial flights to several states of the country (i.e. Michoacán, Sinaloa, Nayarit and Aguascalientes), in accordance with a national technical plan. In these areas the parasitoid releases are made by air or from the ground, and focused on *Anastrepha* spp. host trees located in marginal areas (including backyard orchards), with the objective of preventing fruit fly populations from moving into commercial orchards. Release densities fluctuate between 1500-2500 parasitoids per hectare, depending on the ecological complexity of the area. Apparently, the effect of the releases has been similar in control areas, showing high rates in the percentage of parasitism reached in *Anastrepha* species (e.g. 33.5-64.7% in the State of Nayarit), and notable reductions in the flies per trap per day indices (e.g. 39% in the State of Michoacán and 41% in the State of Sinaloa). These data show the impact that augmentative area-wide releases of parasitoids can have on fruit fly populations. By carrying out these actions in association with other control measures, the presence of fruit flies inside commercial orchards was greatly reduced, and consequently, their control made easier.

KEY WORDS augmentative biological control, parasitoids, fruit flies, *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha striata*, *Anastrepha serpentina*, *Diachasmimorpha longicaudata*, Mexico, area-wide management, grower associations

1. Introduction

Tephritid fruit flies are one of the greatest problems affecting fruit crops worldwide. In Mexico, the presence of the native genus *Anastrepha* Schiner represents one of the most important problems because of the great number of commercial fruits they attack. Fruit flies affect an area of 1.3 million hectares (which represents 9.5% of the national agricultural area), with a fruit production estimate above 10 million tonnes/year. It is estimated that these flies attack more than 30 fruit species growing in commercial orchards (i.e. citrus, mango, guava, peaches, etc.), as well as more than 60 fruit species grown on a minor scale in backyard orchards (Gutiérrez et al. 1995).

The Mexican campaign against fruit flies began in 1992, under a national plan with a 12-year time frame. This plan considers the suppression, containment or eradication from

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Mexico of the four most economically and quarantine important fruit fly species (i.e. *Anastrepha ludens* (Loew), *Anastrepha obliqua* (Macquart), *Anastrepha serpentina* (Wiedemann) and *Anastrepha striata* (Schiner)), in order to develop free or low-prevalence areas of these pests. To reach this goal, the country was divided into three working regions, which were defined by their agro-ecological characteristics, the number of fruit fly species present in each region, and the size of the fruit-growing areas. In addition, a mass-rearing facility was built to produce 300 million sterile *Anastrepha* spp. flies and 50 million parasitoids per week (Reyes et al. 2000). The technical plan is based on the integration of different technologies and strategies that have been applied using an area-wide approach. These are: (1) the use of specific lures and baits to detect and monitor fruit fly populations, (2) the use of cultural practices as mechanical control to destroy host fruits, (3) the application of selective toxic baits through aerial or ground applications, (4) the use of the sterile insect technique (SIT) against *A. ludens* and *A. obliqua*, (5) the establishment of quarantine procedures, and (6) the release of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead) in specific regions and periods.

On a tripartite basis and through various agreements, the organization and operation of the Mexican fruit fly campaign involves the participation and support of (1) the Federal Mexican Government as the supervisor and driving unit, (2) the support of the government of the states where the campaign is carried out, and (3) the participation of the growers, who are the field operators of the campaign through the establishment of associations or plant health committees.

2. Selection of *Diachasmimorpha longicaudata* for Augmentative Biological Control of Fruit Flies

D. longicaudata is a solitary fruit fly endoparasitoid native to the Indo-Australian region, which attacks at least 14 species of the genus *Bactrocera* (=*Dacus*) (Wharton and Gilstrap 1983). This parasitoid was selected for augmentative releases because it has shown a high capacity to adapt to the different environments where it has been introduced (Jirón and Mexsón 1989, Aluja et al. 1990, Eskafi 1990, Baranowski et al. 1993, Ovruski et al. 2000), reaching in most cases the highest percent of natural parasitism in relation to other parasitoid species, including native ones, with which it competes (López et al. 1999). In addition, efficient methods for mass-rearing and augmentative releases have been developed, and nowadays they are available in several parts of the world (Sivinski 1996). Different laboratory studies have shown that *D. longicaudata* attacks successfully the complex of *Anastrepha* species of economic importance in Mexico (Cancino et al., unpublished).

For other potential candidates for augmentative biological control, such as *Fopius arisanus* Sonan or *Diachasmimorpha krausii* (Fullaway), there is no experience with *Anastrepha* species in Mexico, although from the laboratory studies of Zenil et al. (2004) it is known that *Anastrepha* fruit flies are not as good hosts for *F. arisanus* as *Ceratitis capitata* (Wiedemann). Nevertheless, ongoing research is assessing various native parasitoids with the aim of complementing and reinforcing this augmentative biological control programme.

The biology of *D. longicaudata* is well researched (Ashley et al. 1976, Lawrence et al. 1976, 1978, Lawrence 1981, Leyva 1982, Lawrence 1988a, 1988b), its mass-rearing (Wong and Ramadan 1992, Cancino and Yoc 1994, Cancino 2000, Cancino et al. 2002), and its performance as a natural enemy of fruit flies (Ovruski et al. 1996, Montoya 1999, Montoya et al. 2000a, Montoya et al. 2003). This knowledge led to the proposal to use this species as a primary candidate for an augmentative area-wide release programme. Since natural rates of parasitism in fruit flies are extremely low (this is one of the reasons augmentative biological control is needed), and

since parasitoid releases are performed on hosts of *Anastrepha* species of economic importance, the risk to non-target species was considered practically non-existent. From work on mass-rearing (see section 3), it was known that even at very high densities of parasitoids per cage, or a high ratio of parasitoid to larvae (e.g. 4:1) (Montoya et al. 2000a), the percentage parasitism never reaches 100%.

3. Mass-Rearing of *Diachasmimorpha longicaudata*

Since 1995, the Moscafrut facility located in Metapa de Dominguez, Chiapas, Mexico, has produced each week 50 million pupae parasitized by *D. longicaudata* (De la Torre et al. 1995). This parasitoid is mass-reared on third instar (eight day-old) larvae of *A. ludens* produced in the Moscafrut facility. These are irradiated at 45 Gy to avoid the emergence of adult flies from non-parasitized pupae when the releases of parasitoids are being made in the field (Cancino et al. 2002). The irradiated larvae, plus the retained diet of *A. ludens* larvae, are placed in cassette-type containers covered with mesh, which are inserted in mesh-covered cages (30 x 30 x 41 centimetres) with an aluminium frame. In these cages the larvae are exposed to adult parasitoids at a rate of two larvae per parasitoid female. Adult parasitoids are fed with crystallized honey, and are kept in these cages for ten days. After two hours of exposure, the host larvae are collected and placed in containers with vermiculite to allow pupation. Fourteen days later, the pupae are ready to be packed and sent to different destinations for field releases.

A quality control system to evaluate the process of the mass-rearing is performed for each production lot. The key parameters under constant evaluation are: (1) weight and volume of host larvae, (2) host mortality after exposure, (3) weight and volume of pupae, and (4) percentage parasitoid viability and percentage emergence (Planta Moscafrut/ DGSV/SAGARPA 2000). The full rearing process of *D. longicaudata* has been described by Cancino (2000).

4. Augmentative Biological Control of Fruit Flies

Augmentative biological control is defined as the release of large numbers of natural enemies in the field, with the aim of suppressing a pest population in a short period of time (Greathead and Waage 1983). Augmentative releases of parasitoids against fruit flies have been tested by several authors (Wong et al. 1991, 1992, Burns et al. 1996, Sivinski et al. 1996, Montoya et al. 2000b), who concluded that this kind of strategy offers a good alternative to suppress fruit fly populations if it is used in an appropriate way. According to Montoya and Cancino (2004), the augmentative biological control approach for fruit flies must be confined to those specific circumstances and conditions where it is most efficient. The main conditions are: (1) areas with organic fruit production, (2) areas such as canyons and inaccessible areas where there are important quantities of host fruits, (3) marginal areas (i.e. backyard orchards) where producers are not going to implement control actions, and (4) areas and seasons where climatic conditions (e.g. high precipitation) could make bait spray applications inefficient. All these conditions are common in tropical countries where commercial exploitation of fruits and vegetables is appropriately carried out.

In order to obtain the optimal performance of mass-reared parasitoids once released in the field, Cancino and Montoya (2004) pointed out that the key parasitoid attributes that must be taken into consideration are: (1) longevity, (2) flight capacity, (3) search capacity, and (4) a wide range of adaptations to different climatic conditions. These attributes are highly correlated with the quality control parameters from the mass-rearing process (i.e. weight and volume of the pupae, percentage emergence), and for the final product, longevity with and without food.

In addition to the previously cited studies showing that mass-releases of parasitoids can

Table 1. *Weekly mean and total amount (in millions) of* Diachasmimorpha longicaudata*-parasitized pupae of* Anastrepha ludens *sent to different release areas during 2004 and 2005.*

Release zone	2004			2005		
	Weekly mean	No. of weeks	Total	Weekly mean	No. of weeks	Total
Aguascalientes	3.28	16	52.49	3.46	8	27.69
Culiacán	8.81	51	449.11	9.42	8	75.38
Mazatlán	8.92	52	464.02	9.30	8	74.44
Michoacán	5.07	51	258.34	5.66	8	45.28
Morelos	2.01	19	38.27	1.00	8	8.02
Tepic	10.30	51	525.45	10.37	8	82.95
Zacatecas	5.98	32	191.38	7.66	8	61.26

successfully suppress fruit fly populations, Barclay (1987) and Knipling (1992) argued that the integration of this kind of biological control with the SIT could produce synergistic effects because two different stages of the fly population (i.e. immature and adult stages) would be simultaneously attacked. According to these authors, and under the circumstances described by Montoya and Cancino (2004), the benefits of using augmentative biological control against fruit flies could be substantial.

4.1. Field Strategies of Parasitoid Mass-Releases in Mexico

The destinations and amounts of pupae parasitized by *D. longicaudata* are determined according to a yearly national plan and sent via commercial flights to several states of the country. These are shown in Table 1 and Fig. 1. This plan is derived from industry requirements and/or availability of biological material produced in the Moscafrut facility. In the work zones, parasitoids are released in specific areas where the environmental, biological and social conditions are considered to be adequate. In some rural locations there is strong social opposition to aerial bait sprays outside of commercial areas because of potential damage to human health and the local environment. In these places the parasitoid release help to suppress fruit fly populations before flies move into commercial orchards. The releases are focused mainly on *Anastrepha* spp. host trees located in marginal areas that had been previously identified as fruit fly reservoirs (i.e. backyard orchards, wild host trees, canyons).

Packing and release procedures of parasitoids in most places follow those described by Montoya et al. (2000b). Parasitized fly pupae are packed in paper bags with sucrose as food, at a density of approximately 2500 pupae per bag. Bagged pupae are placed in dark rooms at ca 25°C and 65 ± 5% relative humidity for five to six days, until both males and females have emerged. The average emergence is around 60% (equivalent to about 1500 parasitoids per bag), but this depends on the care taken and the environmental conditions during handling and packing. The female:male sex ratio is approximately 2:1. Parasitized pupae can also be packed in plastic aerial release containers (PARC) (plastic boxes of 60 x 49 x 33 centimetres, with mesh on the sides and top to allow ventilation), using two paper bags at a density of 40 000 pupae per box. Parasitoid releases are made mainly by air, using Cessna 206-type aircraft if available, and when the distribution of host trees is considered uniform. Otherwise, if the host trees are distributed mainly in backyard orchards in small urban places, parasitoids are released using ground release-systems. Release densities fluctuate

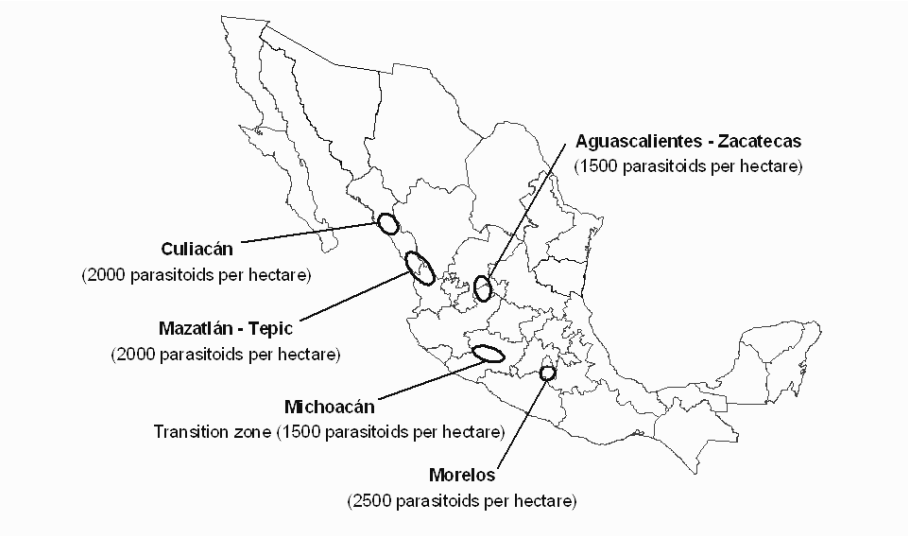

Figure 1. *Release densities of the parasitoid* Diachasmimorpha longicaudata *in the different control areas in Mexico.*

between 1500-2500 parasitoids per hectare.

The impact of parasitoids on fruit fly populations is assessed comparing areas with and without releases, or historical data for the same place, and is determined through (1) the trapping system (i.e. flies per trap per day or FTD indices) using McPhail traps or multilure traps baited with liquid protein (ten millilitres of bait, 235 millilitres of water, and five grammes of borax per trap), and (2) fruit sampling, where fruit infestation levels are determined (number of larvae per fruit and per kilogram) as well as the percentage parasitism. Percentage parasitism is estimated by dividing the total number of parasitoids emerged by the total number of *Anastrepha* spp. flies and parasitoids emerged (Wong et al. 1991). Each fruit sample collected must be around 400-3000 grams in weight, depending on fruit size and availability.

4.2. Results of the Parasitoids Release Programmes

4.2.1. Michoacán State

Ground releases are used to disperse the parasitoids in the State of Michoacán in the "transition zone" (zone between high and low lands) of this state, at a density of 1500 parasitoids per hectare. The transition zone is located near a neo-volcanic mountain range, has an altitude of 600-1200 metres above sea level and average maximum and minimum temperatures of 33°C and 18°C, respectively. The annual average rainfall is 700 millimetres. This zone has a total of 800 producers distributed over 14 counties (i.e. Nuevo Urecho, Parácuaro, Apatzingán, Zirecuarétiro, Peribán, Buenavista, Tancítaro, etc.) and contains 970 hectares of peaches, 625 hectares of mango, 517 hectares of guava and 57 hectares of sweet citrus. Surrounding these areas, there are 2500 hectares of marginal or backyard production, with a high number of fruit fly hosts, such as creole mango, Mexican hog plum, hog plum, citrus, chicozapote, mamey, papaya, caimito, coffee, guava, etc.

Due to its great ecological and host fruit fly diversity, it is possible to find fruit fly populations all year round in this transition zone. According to trapping records, *A. ludens* is the most common and important species captured, with 89% of all individuals sampled. The second one is *A. obliqua* (6%), followed by *A.*

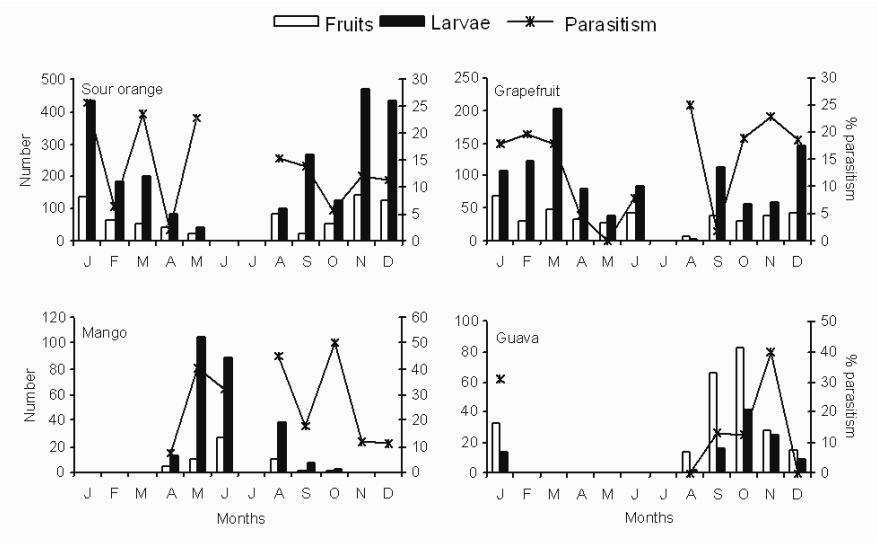

Figure 2. *Number of fruits monitored, number of larvae detected and percentage parasitism by* Diachasmimorpha longicaudata *in (upper left) sour orange, (upper right) grapefruit, (lower left) mango and (lower right) guava in the transition zone of Michoacán, Mexico in 2004.*

striata (3%) and *A. serpentina* (2%). *D. longicaudata* is currently released in this area at a density of 1500 parasitoids per hectare, mainly focused on marginal and backyard orchards, with a total release surface of 1500 hectares. The highest average percentage parasitism was found in creole mango (34.3%), followed by guava (20.5%), sour orange (14.9%) and grapefruit (14.8%) (Fig. 2). The ratio of females to males in emerged parasitoids from fruits is around 3:1. The use of integrated pest management approaches, including mechanical and/or chemical control in specific areas, resulted in a reduction in the FTD index of 39 and 42.7% for 2004 and 2005, respectively.

4.2.2. Other States

In the south of Sinaloa State and in the north of Nayarit State (Mazatlán-Tepic area in Fig. 1), ground releases are used to disperse the parasitoids at a density of 2000 parasitoids per hectare over 10 800 hectares. Here, the host trees are located mainly in backyard orchards in small towns, where it is possible to find high densities of creole mango, guava, hog plumb, chicozapote, and citrus. The percentage parasitism reported for these areas varied between 5 and 21% in 2004, but with the integration of other strategies, the reduction in the FTD index was 41 and 42% for the south of Sinaloa and Nayarit, respectively. During 2003, the percentage parasitism in the State of Nayarit varied between 33.5 and 64.7%, and the reduction in the FTD index was 46%.

In 2002, the average parasitism in the State of Chiapas was higher than 30% on the four economically-important *Anastrepha* species, with maximum levels reaching 72.1, 77.5, 38.1 and 54.5% for *A. serpentina*, *A. ludens*, *A. obliqua* and *A. striata*, respectively, and a reduction of 68.6% of the FTD index was recorded.

In the guava production area (ca 10 000 hectares) located in the States of Aguascalientes and Zacatecas, the FTD indices are now below 0.01, with a very low number (0.00023) of larvae per fruit, after an integrated pest management programme, which included chemical control, releases of

sterile insects and parasitoids.

5. Discussion

Integration of augmentative biological control as a component of a national programme against fruit flies is confronted by different challenges. One of the most important is the assessment of the benefit/cost ratio when using natural enemies (Montoya and Cancino 2004). This is because the environmental and social benefits associated with augmentative biological control are not easy to quantify. The social concerns about intensive and extensive sprays of insecticides (as in fruit fly control), represents in some circumstances a strong barrier to advance the goal of establishing fruit fly-free or low-prevalence areas. Parasitoid releases help in an environment-friendly manner with this task.

The effect of released parasitoids was similar in all areas under control, where an important reduction of the FTD indices was noted. In most cases, the FTD indices correlated well with the percentage of parasitism recorded (e.g. Michoacán and south of Sinaloa). It is evident therefore that parasitoids effectively reduce fruit fly populations. In some release areas, however, the great diversity of host fruits represents a formidable challenge to parasitoids, since some fruits (particularly the largest ones such as grapefruit and other citrus), serves as a refuge for the pest. In such larger fruit, parasitoids cannot reach the larvae with their ovipositors and consequently they only have a minor impact on fruit fly populations. The opposite is observed in small fruits such as creole mango, some kinds of guava and hog plum, where the highest percentages of parasitism are registered.

An important consideration is that fruit sampling removes from the field an important number of first and second instar larvae, which had not reached the necessary development (third instar) to be attacked as a preferential host by parasitoids. According to Wong et al. (1991) and Van Driesche (1983), this results in an underestimation of the percentages of parasitism that could be reached if these larvae had remained in the field. On the other hand, it is known (Montoya et al. 2000a), that parasitoids inflict a higher percentage of mortality on fruit fly larvae than observed from the percentage parasitism. It can be assumed that parasitoids are able to find and parasitize an important proportion of those larvae not removed from the field through mechanical control or fruit sampling, and which also had a high probability to reach the reproductive stage. This has more relevance in those locations where chemical control is either inefficient or is not an adequate option to suppress fruit fly populations.

6. Conclusions

The results presented demonstrate the impact of augmentative releases of parasitoids on fruit fly populations when such releases are focused on hosts of fruit flies in marginal areas. They also reinforce the value of a strategy that uses this kind of integrated approach within an area-wide context, and on a regional level. By carrying out the actions described, the presence of fruit flies of economic importance inside commercial orchards can be greatly reduced, and consequently, fruit fly control and management becomes easier.

7. Acknowledgements

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The Hawaii Area-Wide Fruit Fly Pest Management Programme: Influence of Partnerships and a Good Education Programme

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ABSTRACT In 1999, the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) launched the Hawaii Area-wide Fruit Fly Pest Management (HAW-FLYPM) programme. Its aim was to suppress populations of the melon fly *Bactrocera cucurbitae* (Coquillet), Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), and oriental fruit fly *Bactrocera dorsalis* (Hendel) to levels below economic thresholds while reducing the use of organophosphate insecticides. The programme was carried out on farms in demonstration sites of less than 5000 hectares each on the islands of Hawaii, Maui, and Oahu. The goals involved developing and integrating biologically-based pest control technologies into a comprehensive management package that would be economically viable, environmentally sensitive and sustainable. The technologies included: field sanitation, protein bait sprays and/or traps, male annihilation with male lures and attractants, and if needed, augmentative parasitoid releases, and sterile insect releases. Although many of these technologies were developed in Hawaii by the USDA-ARS, they had never been packaged and transferred to Hawaiian farmers. Aside from the technical issues that underpin a successful area-wide integrated pest management (AW-IPM) programme, attention was given to the development of partnerships to address the large number of non-technical issues that arise. This was accomplished through individuals and institutions that were knowledgeable and willing to assist with key aspects of the programme such as: grower and community-based education on fruit flies (University of Hawaii), regulatory issues related to implementation of the programme and to subsequent registration of any new technologies (Hawaii Department of Agriculture), and industry (Dow AgroSciences, Better World Manufacturing, United Agricultural Products, etc.), who provided products already on the market for use by growers. Finally, in addition to scientists, an advisory group was created to guide the programme. The HAW-FLYPM's educational programme targeted growers and community backyard growers, and utilized the "logic model" approach to organize, plan, execute and evaluate farmer and community educational programmes state-wide. The logic model approach was an outcome-driven rather than activity-based method that employed a linear sequence to develop relationships between programme inputs, outputs and outcomes. This model was used extensively to transfer sustainable, science-based technologies to suppress tephritid fruit fly pests, and served as a "blueprint" for ensuring that programme elements were planned, delivered and executed on a timely basis. The team approach brought enormous success, including six national awards. Systematic, and continuous application of the three tactics (sanitation, GF-120 (spinosad) bait spray, and trapping with lures), resulted in sustained reductions in melon fly and Mediterranean fruit fly populations that in turn led to significant reductions in crop damage and high economic impacts. The programme's success also led to renewed interest in fruit fly programmes in Hawaii, which hopefully will translate into continued opportunities for additional partnerships to reduce the impact of these pests on Hawaiian agriculture.

KEY WORDS fruit fly, area-wide, IPM, melon fly, Mediterranean fruit fly, education, logic model, partnerships

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1. Introduction

The melon fly *Bactrocera cucurbitae* (Coquillett), Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), the oriental fruit fly *Bactrocera dorsalis* (Hendel), and the so-called Malaysian (solanaceous) fruit fly *Bactrocera latifrons* (Hendel), have accidentally become established in Hawaii (Vargas et al. 2003). These flies attack over 400 different host fruits and inhibit the development of a diversified tropical fruit and vegetable industry. They require that commercial fruits undergo quarantine treatment prior to export, and in Hawaii they provide a breeding reservoir for introduction into other parts of the world. Present fruit fly control measures in Hawaii rely heavily on the application of organophosphate insecticides to crops. In 1999, a five-year area-wide integrated pest management (AW-IPM) programme was funded to manage fruit flies in Hawaii (Vargas et al. 2003). This programme integrated two or more control components (field sanitation, protein bait sprays, male annihilation, sterile insects, and parasitoids), into comprehensive packages that are economically viable, environmentally acceptable, and sustainable. It has resulted in area-wide suppression of fruit flies and in reduced use of organophosphate insecticides, as well as providing the impetus for further growth and development of diversified agriculture in Hawaii.

2. Partnerships

Aside from the technical issues that form the basis of any successful area-wide programme, significant attention must be paid to programme organization and in particular, to the development of partnerships to deal with the large numbers of non-technical issues that arise. The recent experience with the Hawaii Area-wide Fruit Fly Pest Management programme (HAW-FLYPM) is a good example of the critical need to develop partnerships when attempting to set up such a programme.

United States Department of Agriculture-Agricultural Research Service (USDA-ARS) researchers from the US Pacific Basin Agriculture Research Center had developed many of the overarching strategies that are used today for the detection, suppression and eradication of the tephritid fruit fly species (especially the Mediterranean fruit fly, oriental fruit fly and melon fly), that had become established in Hawaii over the last 100 years. These researchers were responsible for the development of such seminal technologies as low-cost diets for mass-rearing (Tanaka et al. 1969, Vargas 1989), attractants for several fruit fly species (Beroza et al. 1961, Cunningham 1989), early demonstrations of the effectiveness of the sterile insect technique (SIT) against fruit flies (Gilmore 1989), and more recently, augmentative biological control strategies against fruit flies (Wong et al. 1991, Harris et al. 2000). Although these early discoveries have subsequently been refined and improved by many researchers, the basic technologies have remained the same.

While credit must be given to those pioneers in Hawaii who set the stage for area-wide fruit fly control, the presence of plantation agriculture in the form of sugarcane and pineapple overshadowed any strong movement to apply the Hawaii-based technologies in their backyard. Instead, they were demonstrated outside the State of Hawaii, being utilized by others in other states and countries, resulting among others, in the successful eradication of the melon fly and oriental fruit fly from Okinawa, Japan (Koyama et al. 1984, Kuba et al. 1996), the eradication of the Asian papaya fruit fly *Bactrocera papayae* Drew & Hancock from Queensland, Australia (Cantrell et al. 2002), and the multiple eradications of the Mediterranean fruit fly from California (Penrose 1996). However, the decline of both sugarcane and pineapple production in Hawaii has led to renewed interest in diversifying agriculture on the island, and with it, the resurgence of the fruit fly issue due to the impact of these pests on production and trade, and the fact that they are a source for introduction to fly-free states.

The HAW-FLYPM programme was launched in 1999-2000 to suppress fruit fly

pests using improved control technologies and, if successful, to spur increased awareness of the need for state-wide suppression or eradication programmes. In assessing the opportunities for success (or failure) of a programme of such magnitude, and mindful of previous pilot tests that had failed to move fruit fly control in Hawaii forward, it was recognized by all concerned that for success, this programme would have to develop a broad-based coalition of stakeholders from diverse and interdependent backgrounds on agricultural issues in Hawaii.

The first challenge was to gather a local "team" of stakeholders who could share a common goal and vision for a successful fruit fly programme in Hawaii. This was accomplished through a series of frank discussions with individuals and institutions with knowledge and know-how on key activities and issues. These included: grower training, cooperative extension and community-based education on fruit fly issues (University of Hawaii), technical issues involved with area-wide control (USDA-ARS and USDA-Animal and Plant Health Inspection Service (APHIS)), regulatory issues including registration of any new technology developed (Hawaii Department of Agriculture), as well as private sector entities that could provide products already on the market or in the process of development for use by the growers (Dow AgroSciences, Scentry Biologicals Inc., Better World Manufacturing, United Agricultural Products, etc.).

The importance of creating such alliances is frequently underestimated, and like any large programme of this type, the group had successes as well as failures. Central to the identification of key partners are certain behavioural traits that help to develop the overall partnership team. These include the sharing of common programme goals, willingness to communicate, listen and be flexible, ability to develop consensus and most importantly, mutual trust among team members. In addition to the local partnerships, a "core" management team and secondary technical advisory group were set up to help guide the programme. The local partnership met frequently, and although often not in complete agreement, understood the need for consensus decision-making as a key to programme success. The sharing of common goals was evident in the flexibility that was needed to ensure the continuation of various programme elements, being able to deal with cross-agency administrative rules and regulations, and making the programme work in spite of technical problems.

3. Selection of Fruit Fly Species and Demonstration Sites

Since the four fruit fly pest species affect different crop groups, it was decided to undertake the suppression programmes by pest species. Melon fly caused the greatest overall losses throughout the year to solanaceous, cucurbit and melon crops, and therefore it was the first pest targeted. These crops are commonly grown in adjacent, sequential plots on each farm and are planted throughout the year because of the mild climate on the Hawaiian Islands. Mediterranean fruit fly suppression was undertaken at the same time because of the strong, continuing requests by growers. This is a serious pest of persimmons, which are harvested in the autumn, but it also develops on uncultivated fruits that are found throughout the year. Suppression of Mediterranean fruit fly could only be undertaken at the same time as melon fly suppression because of the voluntary assistance of persimmon growers.

Since it was already known that the programme could not effectively cover the whole state, demonstration sites were chosen for reasons ranging from ease of implementation, farm size, and political realities. Three sites were chosen on three islands and although not in total agreement, the local partners with the concurrence of the core team identified sites on the Waimea region on the Big Island of Hawaii, the Kula area of Maui and at Ewa, Oahu as primary suppression sites to start the programme (Fig. 1). Secondary suppression sites were also chosen, but these are not reported on here. The

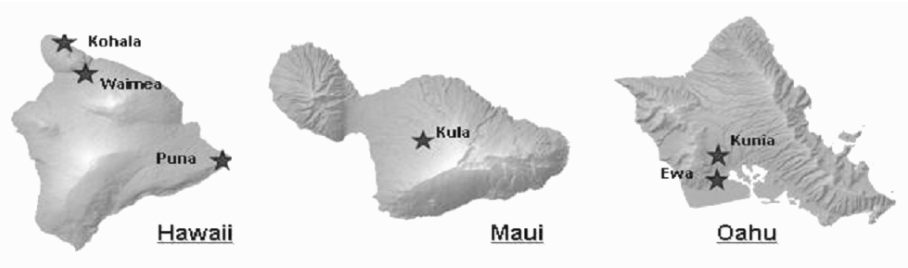

Figure 1. *The islands of Hawaii, Maui and Oahu indicating the location of the suppression zones. The primary demonstration zones were at Waimea, Kula, and Ewa. The additional sites are secondary zones to which programme activities are being transferred.*

decision to select these sites turned out to be fortuitous and added to the credibility of the eventual success of the programme. Decisions regarding the order of flies (melon fly and Mediterranean fruit fly) to control first and the individual technologies to be used also played a large role in the success of the programme. Often the determination of which species to start with in a multiple species control programme as complex as the HAW-FLYPM programme becomes one of the keys to success since early failure could have had a negative psychological impact on those involved and jeopardize the future activities of a multi-year effort. Fortunately, the success of the melon fly control programme allowed the programme to be continued and eventually extended for two additional years.

4. Education Planning and Outreach

The logic model approach was used to organize, plan, execute and evaluate farmer and community educational programmes statewide (Taylor-Powell et al. 1996, 2000, Mayeske and Lambur 2001). This is an outcome-driven rather than activity-based planning method, with a linear sequence being used to establish direct relationships between programme inputs, outputs and outcomes, and implementation schedules to track programme progress. This plan, which targeted commercial and community backyard growers, contributed to the successful suppression of tephritid fruit fly pests and reduced use of organophosphate insecticides.

The logic model is a series of if-then relationships that, when established, can assure achievement of targeted outcomes. For example, if fly population baseline levels are used at the start of the programme and the impact of fruit fly suppression tactics on reducing fly numbers is clearly demonstrated, then farmers will learn the value of the pest management tactic. The plan was initiated by establishing long-term outcomes (impacts), with the following questions being asked to establish each outcome: (1) What does success look like?, (2) What are the ultimate goals?, and (3) Who are the target audiences?

Intermediate and short-term outcomes were established as logical, benchmark steps towards long-term pursuits (Fig. 2), with observable or measurable indicators that represent achievement of each outcome being used for evaluation purposes.

Outputs (activities and participation), were selected to assist targeted audiences and achieve programme outcomes. Much research and time was invested in understanding the target audiences. Assumptions were made about the characteristics of adult learners, these being autonomous, self-directed, relevance-oriented individuals who took responsibility for their learning and enjoyed cooperative learning environments. Based on these assumptions, sequential outputs for achieving programme outcomes were formulated. These were workshops, meetings, presentations,

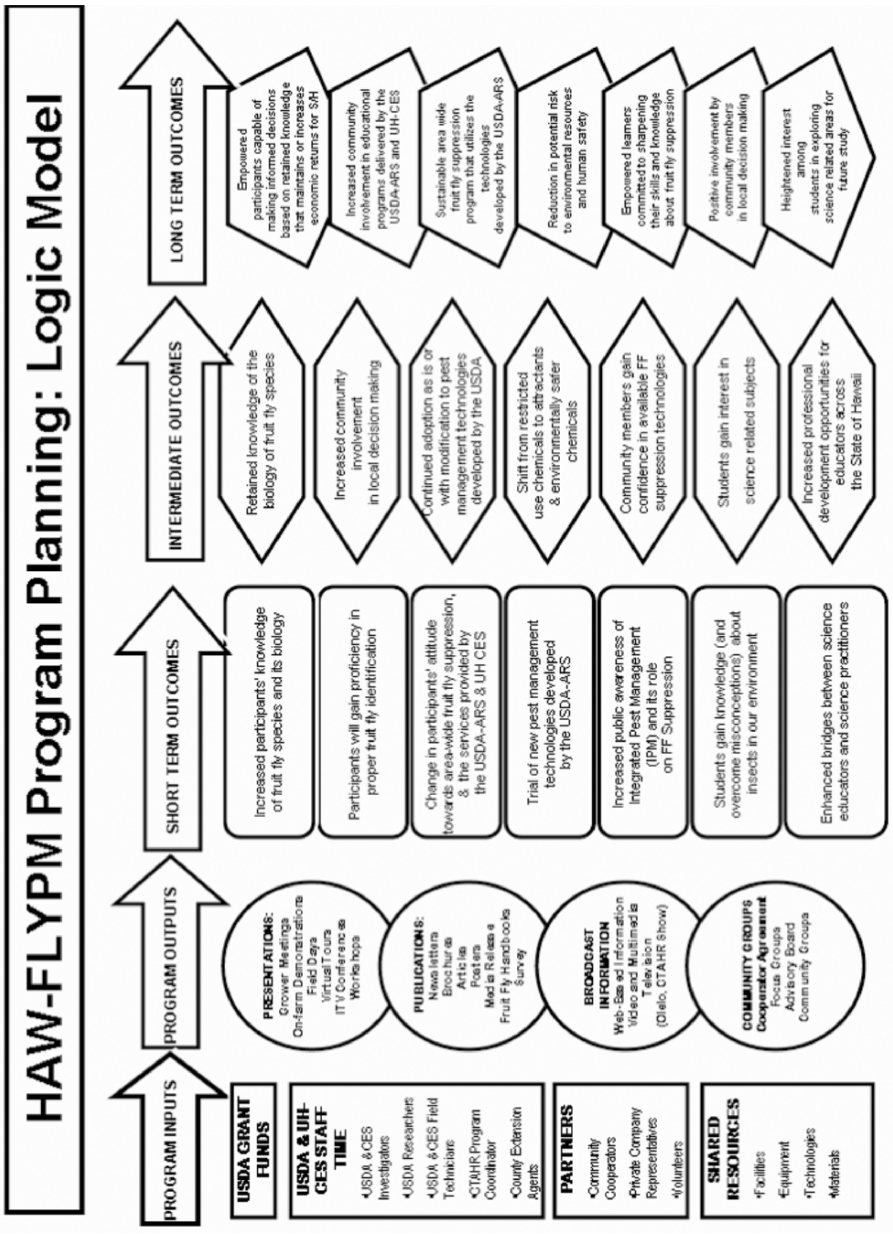

Figure 2. *The Hawaii Area-wide Fruit Fly Pest Management programme (HAW-FLYPM) outcome-based programme logic model that was used for the suppression programme. HAW-FLYPM = Hawaii Area-wide Fruit Fly Pest Management, USDA = United States Department of Agriculture, ARS = Agricultural Research Service, UH = University of Hawaii, CES = Cooperative Extension Service, CTAHR = College of Tropical Agriculture and Human Resources, ITV = Interactive Television Conference.*

demonstrations, publications, broadcast information, and developing and strengthening cooperator and community groups. The final step was to assemble programme inputs (resources); these were technical know-how, equipment, software technology, computers, materials, staff, and collaborative partnerships.

A five-year outreach education plan was devised (Fig. 2) (Mau et al. 2003b). Seven threaded outcomes were chosen, one of the most important being empowered participants who could make informed decisions based on retained knowledge and skills. This effective transfer of knowledge and skills would help to assure sustainability of the HAW-FLYPM suppression programme.

Adult education methods were used. Outreach educational media were developed to ensure that targeted audiences were supported. Four important types of outputs were established early in the educational programme. One was the HAW-FLYPM video, which provided an overview about the suppression programme in lay terms for commercial and community cooperators (Mau et al. 2003a). Another was a series of brochures that described the suppression programme, the identification and life cycle of the four targeted species of fruit flies and suppression elements. These brochures included colour photographs and described in lay terms the importance of species monitoring, male lures, male annihilation, protein baits, and biological control. A third was an internet web site, created to provide ready access to information and updates (http://www.fruitfly.hawaii.edu), and the fourth was a colourful newsletter which was published monthly for cooperators and partners who did not have internet access. Other teaching materials were created and distributed as needed.

Since evaluation began during programme planning, measurement benchmarks were incorporated into every component of HAW-FLYPM outreach to ensure achievement of programme outcomes. The benchmark surveys also helped to produce accountability reports to stakeholders and policy makers.

Although the programme's educational and evaluation benchmarks are not shown in Fig. 2, they were included in the annual schedule. Benchmark evaluation periods were May and December of each year. Significant activities such as benchmark observations, surveys, interviews, testimonials, advisory committee meetings, annual baselines, and a public relations plan were key components of the schedule.

Sustainability was one of the main goals of the programme. An important measure of the success of the outreach programme is whether commercial and community growers can sustain fruit fly suppression after government funding ends. Demonstrations by respected farmers were solicited and basic suppression practices (farm hygiene, male annihilation and food bait treatments), were combined into an easy-to-remember 1-2-3 method. Farmer demonstration results and farmer testimonials convinced their peers to evaluate and adopt the programme. Communication among farmers about programme success was important for the credibility of the programme in each demonstration region.

To assure hands-on learning, participants signed agreements that specified their responsibilities in exchange for project support. They were required to perform suppression work on their farms, and in this way they experienced the impacts of each of the suppression tactics and adopted or adapted these to meet their needs. HAW-FLYPM provided all the materials including traps, lures, and GF-120 fruit fly food bait according to an agreed schedule. Participants were resupplied as needed and project personnel monitored fruit fly populations on farms and provided this information back to them. Farmer successes were monitored through bi-weekly crop infestation surveys, which were also valuable to gain insights on programme sustainability.

5. Outcomes of the Programme

Suppression programmes were implemented on the islands of Hawaii, Maui, and Oahu. The melon fly, Mediterranean fruit fly and

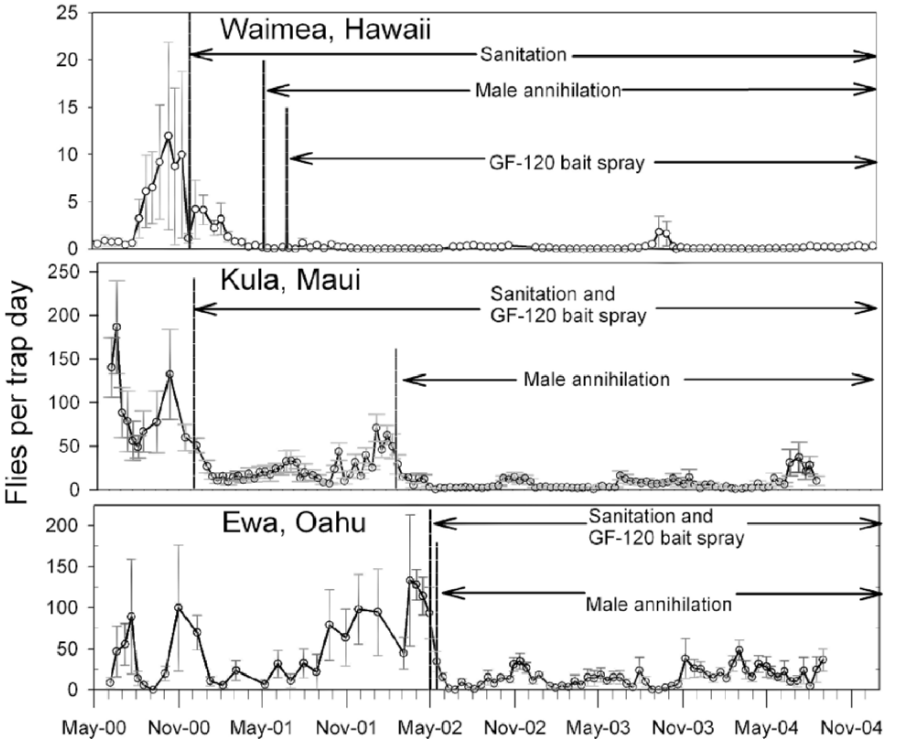

Figure 3. *Population monitoring of melon fly* Bactrocera cucurbitae *with cuelure-baited traps at Kamuela (Hawaii), Kula (Maui), and Ewa (Oahu) from May 2000 to December 2004.*

oriental fruit fly were the predominant species on each, and *B. latifrons* occurred at low population densities on all three. The first demonstration project was initiated on Hawaii Island in the Waimea region. The 3800 hectare-demonstration zone (cucurbits and melons) was surrounded by pastures and was characterized by homes and a small town that separated two farming zones. The second implementation zone (4400 hectares) (cucurbits, melons, tomatoes, and persimmons) was at Kula on Maui Island. This zone was characterized by clustered, small farms (ca 7-10 hectares) that were surrounded by wild fruit fly hosts. Central Oahu was the third implementation zone. This zone encompassed more than 1600 hectares of farmland that were adjacent to large suburban residential, industrial and city areas. Intensively grown fruiting vegetables and melon crops included watermelon, honeydew melon, cantaloupe, Korean melon, zucchini, kabocha squash and pumpkin.

5.1. Use of a Geographic Information System (GIS) for Data Management

Seasonal population densities of the four fruit fly species were monitored to establish pre- and post-implementation baselines. Monitoring grids were established using global positioning system (GPS) handsets and geographical information systems (GIS) software technology (ArcInfo® 8.1; ESRI, 380 New York St., Redlands, CA 92373-8100). A GIS was used for each of the three areas to assist with the complex, multi-function tasks of an IPM programme aimed at multiple fly species (Mau et al. 2003c), enabling programme personnel to: (1) visually follow the impact of the IPM tactics on fruit fly densities

throughout the implementation zones, (2) define and/or delimit suppression areas using a structured grid that could be overlaid on various geographical maps and/or land use areas, (3) monitor fruit fly populations based on trap captures within the grid areas, and (4) identify the location of host crops and alternative host "refugia" breeding areas and the complex of flies and parasitoids emerging from these hosts.

5.2. Implementation of Monitoring Systems

Relative numbers of each fruit fly species were determined bi-weekly throughout the grid using strategically placed traps baited with specific male lures that included trimedlure, cuelure, methyl eugenol and a mixture of latilure plus cade oil. Protein-(Solulys) baited traps were also used to monitor female populations. Population densities were expressed as flies per trap per day. Fly numbers at each trap's geographical coordinates were logged, transferred into the GIS, and superimposed over other maps (land use, grids, etc). Maps for each fruit fly species were constructed. This gave the team detailed, sequential information about how well the fly suppression programme was progressing.

As an example, the implementation zone at Kula on Maui Island encompasses several types of host crops located at different elevations. Melon fly is the predominant species at lower elevations, while the Mediterranean fruit fly is a pest of tree fruits that are grown at higher elevations. The GIS maps were helpful in visualizing portions of the zone where growers may not be successful in implementing fruit fly suppression tactics. Also, the technology allowed comparisons between time periods.

5.3. Suppression Results

The impacts of step-wise implementation of fruit fly suppression tactics are obvious from examining Fig. 3, Table 1 (melon fly suppression) and Fig. 4, Table 2 (Mediterranean fruit fly suppression). Although not specifically shown on Fig. 3, growers plow and rotavate cucurbit and melon fields within one week after final harvest, and this combination of sanitation with GF-120 (spinosad) bait sprays and male annihilation trapping with cuelure was quite effective in reducing pest populations.

Persimmon at Kula, Maui was the only Mediterranean fruit fly-impacted crop used to demonstrate the effectiveness of the suppression programme. Here, the impact of combin-

Table 1. *Summary of impact of melon fly area-wide control at infestation data collection farms on Hawaii, Maui, and Oahu Islands.*

Impact of area-wide programme on melon fly population in targeted area				
	Peak melon fly population		Reductions due to programme	
Suppression area is total contiguous programme zone	Before programme in 2000 flies/trap/day	Population in 2004 flies/trap/day	Melon fly infestation. Pre-programme to mean for 2004	Organophosphate reduction in usage for melon fly control
Waimea, Hawaii 46 hectares	12 (baseline)	0.52 (grid)	> 20 to < 2%	100% (none used now)
Kula, Maui 100 hectares	100 (baseline)	> 5 (baseline)	> 40 to < 5 %	90%
Ewa, Oahu 405 hectares	135 (baseline)	7 (baseline)	>30 to < 6 %	90%

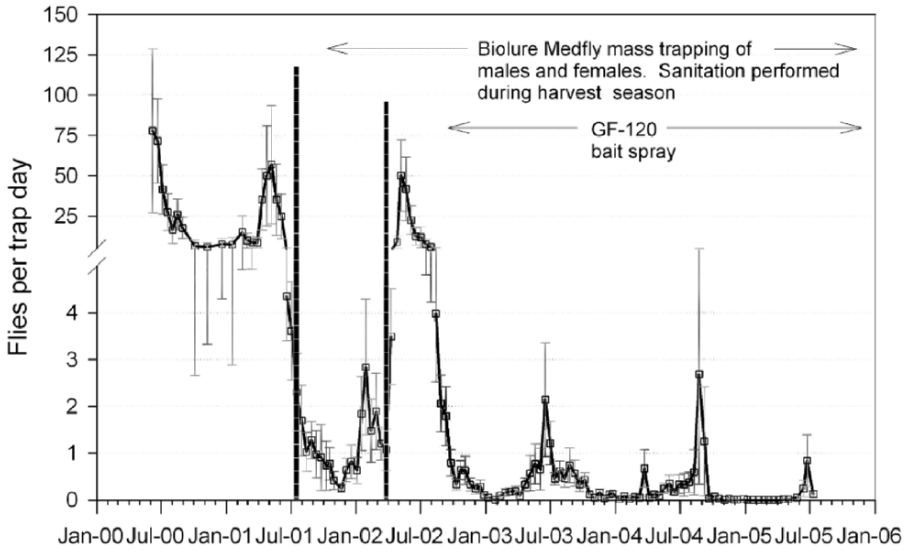

Figure 4. *Population monitoring of Mediterranean fruit fly* Ceratitis capitata *with trimedlure-baited traps at Kula, Maui and Hawaii from January 2000 to July 2005.*

ing field sanitation with weekly spot applications of GF-120 (spinosad) bait sprays to the crop and nearby hosts and adult trap annihilation tactics on fly populations, is clearly depicted. Sanitation was limited only to disposal of infested and fallen fruit during the week after final harvest.

At this point, it can be stated with confidence that the HAW-FLYPM collaborative partnership has been very successful. At the three demonstration sites on the three separate islands, the development and use of the spinosad bait spray GF-120 was an important development (Prokopy et al. 2003) for area-wide melon fly control. Impacts occurred with respect to overall suppression of field populations, with decreases in both melon fly infestations on commercial farms and in crop-directed sprays of organophosphate insecticides (Table 1). One large grower reflected on the programme's success by saying that prior to adopting the programme, melon flies

Table 2. *Summary of Mediterranean fruit fly area-wide control on persimmon farms at Kula, Maui.*

Impact of area-wide programme on Mediterranean fruit fly population in targeted area			
Commerical area under suppression	Peak Mediterranean fruit fly population		Reductions due to programme
	Before programme in 2000 flies/trap/day	Current population in 2004 flies/trap/day	Mediterranean fruit fly infestation
Kula, Maui 100 hectares	100 (baseline)	> 5 (baseline)	> 40 to < 5 %

Table 3. *Estimated benefits to a zucchini farmer applying the suppression programme (Adapted from Table 5, McGregor 2004, unpublished report).*

Benefits	USD/hectare
Returns from adopting the suppression programme	
Increase sales - marketable yield increased by 10 125 kg/ha (infestation < 5% compared with 40%) at USD 1/kg	10 125
Improved quality resulting from a reduction in fruit fly damage (adds to USD 0.11/kg price to 13 608 kilogram)	3697
Improved labour productivity in harvesting and sorting (estimated 7.1 hours improvement in labour productivity per 1000 kilogram)	1896
Cost of previous insecticide cover spraying programme (380 litres mixture per week at each USD 62.5/ha/week; labour at 1 hour/week at each USD 15; equipment time at USD 40/hour)	2884
Total benefit from adopting the suppression programme	18 602
Costs	
Costs of applying the suppression programme	
1. Monitoring (and male annihilation)	
20 buckets at each USD 1, replaced every 12 months	49
20 cuelure traps and killing agent at each 80 c, replaced every 9 months	52
Killing agent assumed 50 c	25
Labour assumed two hours per month	474
Total cost monitoring	600
2. Sanitation	
Estimated an additional 0.5 hour per week at harvesting time	158
1.5 hours to mow/rotovate and bury, using USD 40/hour imputed hire rate (50% attributed to sanitation)	74
Total cost sanitation	232
3. Protein bait spray	
0.6175 litre GF-120 concentrate per acre per week around perimeters of fields on roosting plants for total farm area of 1.6 hectare at each USD 22.37/litre	1773
Application one person hour per week, shared amongst four bearing hectares	104
Total cost bait spray	1877
Total estimated cost of adopting the suppression programme	2709
Benefits minus costs to the farmer from adopting the suppression programme	15 893

Table 4. *Estimated net benefit to a persimmon farmer applying the suppression programme (Adapted from Table 9, McGregor 2004, unpublished report).*

Benefits from applying the suppression programme	
Increased revenue	USD/hectare
Before adoption of the programme, marketable production 771 kilogram (50% grade 1; 50% grade 2)	
grade 1 386 kilogram at each USD 4.44/kg	4231
grade 2 386 kilogram at each USD 2/kg	1907
After adoption of the programme, marketable production 998 kilogram (70% grade 1; 30% grade 2)	
grade 1 699 kilogram at each USD 4.44/kg	7667
grade 2 299 kilogram at each USD 2/kg	1477
Total	9144
Total increase in revenue	3006
Reduction in harvesting and sorting cost per kilogramme	
Harvesting with < 5% damage estimated eight hours to harvest 454 kilogram	
Harvesting with 35% damage estimated 11 hours to harvest 454 kilogram	
Savings per 454 kilogram is three hours - for 998 kilogram the gain in labor efficiency is 6.6 hours at each USD 6/hour	131
Total	131
Previous cost of applying cover insecticide spray	
Cover spray application of insecticide during fruiting season (Malathion, Nulure)	
Materials -7.58 litres each)	247
Labour for application 60 hours	1152
Total	1433
Total Benefit	4570
Costs of applying the suppression programme	
Monitoring	
2 Biolure traps at USD 7 per trap plus three chemical release packages and sticky cards that are replaced four times a year at USD 2.50 per charge	84
Labour for recharging and monitoring (20 minutes/trap/month) at USD 8/hour	94
Total	178
Mass trapping	
30 x Biolure traps plus rechargeables	1260
Labor for recharging (0.12 hrs/trap) at USD 8/hr	284
Total	1544
Sanitation	
Estimated an additional 0.5 hour per week for 10 weeks	99
Total	99
Protein bait spray	
7.58 litres GF-120 spot sprayed on trees for 10 weeks at USD 22.43/ litre	420
Application - four hours per crop	80
Total bait spray	500
Total estimated cost of adopting the "1-2-3" programme	2321
Benefits minus costs of applying the "1-2-3" programme	2249

reduced his marketable yields of cantaloupe from 243 cases per hectare to 24 cases per hectare. Now, he and nearly all of the other growers involved in the programme have minimized melon fly losses in fruiting vegetables, usually to less than 5%.

5.4. Economic Benefits

In order to determine the benefit of the fruit fly suppression programme, an independent economist was contracted to perform an interim analysis of the programme. His benefit/cost analysis on the impacts of the melon fly programme on zucchini, and the Mediterranean fruit fly programme on persimmon (McGregor 2004), are shown in Tables 3 and 4. Farmer gains from both programmes were significant. Clearly, the programmes against the melon fly and Mediterranean fruit fly have been highly successful and they are now being completely transferred to participants.

5.5. Current Focus

Currently, the focus is on suppressing the oriental fruit fly on smallholder fruit farms on Hawaii and Oahu. A papaya area-wide demonstration project at Puna, Hawaii is in its first phase of operation with the goal of harvesting more and better quality mature fruit. Pre-suppression population baseline levels are being assessed and wild hosts are being characterized.

6. Conclusions

The success of the HAW-FLYPM programme has been better than expected. Together with a fruit fly project in Central America (Reyes et al., this volume), it can serve as a model for fruit fly control activities in other tropical regions of the world, that have to address the presence of multiple pest species. Effective partnerships and high level teamwork have been critical in the successful area-wide approach. This was acknowledged and recognized by receipt of the Federal Laboratory Consortium Technology Transfer Award for team effort, the USDA Secretary Group Honor Award for creating an effective area-wide suppression programme for fruit flies in Hawaii, and most recently, by the IPM team award from the Entomological Society of America Foundation. The programme's success has also stimulated renewed interest in fruit fly programmes in Hawaii that hopefully will translate into continued opportunities for additional partnerships to be formed and successes to be fulfilled.

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Area-Wide Management of Fruit Flies in Australia

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ABSTRACT Parts of Australia have endemic populations of pest species of fruit flies while in other parts exotic species have become established. There are also some areas that are free from pest fruit flies and are maintained free from fruit flies using area-wide integrated pest management (AW-IPM) principles. In addition to these, Australian horticultural production, as a whole, is at risk from incursions of other pest fruit fly species. AW-IPM of fruit flies has been practised in Australia for many years when protecting areas, which have only marginal opportunities for fruit fly establishment. In recent years research on AW-IPM in other areas has commenced with the desired outcome being to set up systems assuring fruit fly-sensitive trading partners' freedom from fruit fly infestations in imported produce. The success of AW-IPM programmes is highly dependent on monitoring for fruit flies, appropriate and quick response to incursions and an active participation by all growers and the rest of the community in the area under the AW-IPM programme. This paper describes AW-IPM tools currently in use in Australia at national, regional and smaller area levels. Fruit fly mitigation methods such as trapping, trap arrays, border inspections, community awareness programmes as well as the male annihilation technique (MAT), the sterile insect technique (SIT), baiting and postharvest treatments are discussed.

KEY WORDS Australia, Tephritidae, *Ceratitis capitata*, *Bactrocera* spp., area-wide management, quarantine, sterile insect technique, male annihilation technique

1. Introduction

There are about eighty species of tephritid fruit flies that are native to Australia and that infest mainly native fruit and vegetables (Drew 1989) but, of these, six are classed by the Horticultural Policy Council (HPC) as pests of horticultural significance (HPC 1991). These are *Bactrocera tryoni* (Froggatt), *Bactrocera neohumeralis* (Hardy), *Bactrocera cucumis* (French), *Bactrocera musae* (Tryon), *Bactrocera jarvisi* (Tryon) and *Bactrocera aquilonis* (May). The Queensland fruit fly *B. tryoni* is by far the most destructive of these native Australian fruit fly species. Another, non-native species, now established in parts of Western Australia, which arrived in Australia in the 1890s – the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) – is just as damaging and it, too, is a critical quarantine

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 685–697.

pest (Fig. 1).

The distribution of these flies in Australia is such that not all pest species inhabit the same region. Quarantine restrictions are placed among states, and in the case of fruit fly-free areas also within states, to protect them from pest fruit fly species that are not present there. For example, Tasmania, to the southeast of Australia, is classified as free from pest fruit flies, mainly due to the limitations of cold climate. Fruit fly host produce exported to Tasmania from the rest of Australia is either prohibited or allowed entry following various quarantine requirements such as a postharvest desinfestation treatment approved by the Department of Primary Industries, Water and Environment (DPIWE 2005). Western Australia is free from Queensland fruit fly, while the eastern states are free from Mediterranean fruit fly. Hence quarantine restrictions are in place on trade between the eastern and western parts of Australia. South Australia, midway between the east and west coasts, is fruit fly-free but does have detections of both species, and actively eradicates each incursion (HPC 1991).

Mediterranean fruit fly is now well established in parts of Western Australia and nowhere else in Australia. It is a most destructive pest in Western Australia. On the other hand, the Queensland fruit fly is just as destructive but is restricted to the east coast of Australia. Programmes such as advice on cover sprays, correct use of pesticides, public education, the sterile insect technique (SIT), the male annihilation technique (MAT), roadside vehicle inspections and fruit fly baiting

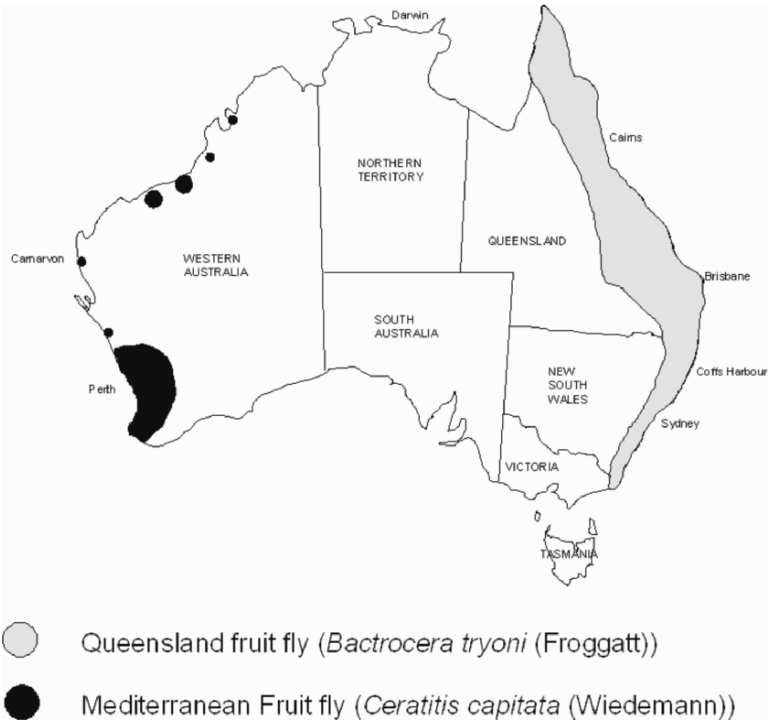

Figure 1. *Distribution of Queensland fruit fly* Bactrocera tryoni *(Froggatt) and Mediterranean fruit fly* Ceratitis capitata *(Wiedemann) in Australia.*

and trapping are underway to reduce the impact of pest fruit flies on commercial and backyard horticulture.

Extensive trade and travel, both commercial and private, from Western Australia to the central areas of Australia (South Australia and the Northern Territory), and the east (Tasmania, Victoria, New South Wales, Queensland, and the Australian Capital Territory), and from east to west, mean that both Mediterranean fruit fly and Queensland fruit fly are serious threats to the states in which they are absent. Protocols for declaration of pest fruit fly outbreaks have been set up so that affected production regions and importing authorities are notified.

2. Fruit Fly Exclusion Zone

There are horticultural production areas situated between the east and west coasts of Australia that have area freedom from fruit flies. The Fruit Fly Exclusion Zone is recognized as free from pest fruit flies by other states of Australia and by some of our trading partners such as New Zealand and the USA. This area comprises most of the horticultural production areas in southern New South Wales, northern Victoria and eastern South Australia (Fig. 2). Being within a Fruit Fly Exclusion Zone is financially and environmentally beneficial to fruit and vegetable growers, packers and exporters in this region in that produce is marketed without needing to apply either long-term cold disinfestation schedules or chemical postharvest treatments (TriState 2003).

A code of practice for actions against Queensland fruit fly describing these conditions and actions, has been compiled by the Standing Committee on Agriculture and Resource Management (SCARM 1996), and agreed upon by most national states and some overseas trading partners. A Mediterranean fruit fly code of practice is in the final stages of approval. These describe rules for the declaration of an outbreak, quarantine distances and

Figure 2. *Map showing the Fruit Fly Exclusion Zone, comprising much of the horticultural production areas in southern New South Wales, northern Victoria and eastern South Australia.*

conditions for reinstatement of area freedom.

3. Protection from External Incursions

Australia also protects its shores from exotic pest fruit fly species. The Australian Government has issued signage, pamphlets and compulsory quarantine declaration forms alerting travellers about the risk of bringing in fresh fruit and vegetables and the penalties for doing so. It also has set up a national trapping grid for fruit flies at all major ports. The grid is administered by personnel from each state and territory government and reports are sent to the Australian Government. Protocols for declaration of pest fruit fly outbreaks have also been set up so that affected production regions and importing authorities are notified. All commercial produce is inspected on arrival and samples taken for analysis of possible infestation. Some produce is prohibited from some countries due to the presence of quarantine pests in the country of origin, while others are allowed to enter following certification of area freedom status or of the correct application of postharvest quarantine treatment or area-wide integrated pest management (AW-IPM) of pest fruit flies.

Principles of AW-IPM of fruit flies are practised in Australia (1) at the national level, where actions are taken to protect the entire country from entry of exotic fruit fly species, (2) at the individual state or territory level to protect their production from non-endemic fruit fly species, (3) at local areas or regions to protect their area freedom from fruit flies status, and (4) even at the single-farm level.

Tools used to implement AW-IPM vary with the size of the area being protected. They vary also with the type of constituent in these areas. For instance, areas populated predominantly with growers and their orchards need applications of different strategies than those with a mixture of townships, growers and other businesses. Often town people do not see or understand the benefits of fruit fly control in their region and AW-IPM of fruit fly is therefore more difficult to implement.

4. Tools for Area-Wide Management of Fruit Flies

4.1. Monitoring

The presence (and absence) of pest fruit fly species is monitored by the national trapping grid. More than 25 000 traps have been deployed throughout Australia (Smith 2000). These traps include a toxicant (such as maldison or dichlorvos) and a male lure (cuelure, methyl eugenol or capilure). Generally traps are placed out on a 400 metre grid in areas of high risk of fruit fly incursion such as cities with airports and shipping terminals, and towns near horticultural production areas. In production areas under incursion risk, traps are placed on a one kilometre grid. Other towns and villages in moderate risk areas have two or more traps of each lure type, depending on the size of the built-up area. These traps are examined on a weekly basis in high fruit fly prevalence seasons (e.g. from November to May in the Fruit Fly Exclusion Zone), and once a fortnight in cooler seasons. Generally, officers of the State Department of Primary Industries carry out the duties of collecting the insects from the traps, replacing damaged traps, recharging with new lure and replacing them on the use-by date. Insects recovered from these traps are packaged and posted to central identification institutions in each state or territory.

The protection of the Fruit Fly Exclusion Zone from incursions of all pest fruit flies can be used as a model for AW-IPM on a large area basis. The Fruit Fly Exclusion Zone, covering an area of about 185 000 square kilometres (Bull 2004) is a region recognized by all Australian states, the Federal Government of Australia and some overseas trading partners as being free from species of tephritid fruit flies of quarantine significance. This region comprises about 70% of Australia's fresh citrus production as well as table grapes, pome fruit, stone fruit, tomatoes and other fruit and vegetables. Due to its being free from fruit flies, it enjoys favourable market access to other Australian states and other desirable

export markets such as the USA. Exports of fresh fruit and vegetables from this area are sought-after by markets due to the lack of fruit flies and the consequent zero need for postharvest treatments such as fumigation with methyl bromide or several weeks' storage at -0.5 to 3°C.

The Fruit Fly Exclusion Zone is closely monitored by state and federal regulatory authorities that use a sophisticated grid system of fruit fly traps, bar code and bar code readers, and an internet-based recording and reporting system. There are about 3000 sites within the Fruit Fly Exclusion Zone being monitored for fruit flies (TriState 2003). Most sites comprise two traps – one with cuelure and the other with capilure – although some have an additional methyl eugenol trap.

There are several triggers that require different responses. If two flies are caught in the Fruit Fly Exclusion Zone within 400 metres of each other within two weeks, then supplementary traps are required to be deployed and a search of fruit for larvae (within the 200 metre outbreak zone). These traps are both cuelure- and protein-based traps, and these techniques are designed to find a potential epicentre. If a threshold number of pest fruit flies – five males for Queensland fruit fly or three male Mediterranean fruit fly (or one gravid female, or one larva in a fruit) – are detected within a specified period (generally two weeks) of each other, then an outbreak is declared and eradication commences. Several action steps are initiated such as (1) deployment of supplementary traps (16 extra male traps within 200 metres of the outbreak site and 16 McPhail traps baited with protein solution to capture female flies, with all supplementary traps emptied twice a week), (2) placement of restriction zones around the detection site (restricting the movement into and out of the restriction zone), (3) release of sterile fruit flies, (4) notification of landowners, Australian states and trading partner governments, and (5) the implementation of postharvest treatments.

In Western Australia the Lynfield trap baited with capilure/trimedlure is the most used for Mediterranean fruit fly monitoring. However, Tephri and McPhail-type traps baited with Biolure® are also effective. Jackson traps have also been used in sterile insect release programmes because of the ease of counting captured flies.

4.2. Community Awareness

There are several community-targetted programmes relating to the dangers of allowing exotic pests and diseases into Australia and the adverse effects on Australian agriculture, the environment and the community. Australian customs and quarantine officials screen over 90% of incoming passenger baggage, 100% of international mail and 100% of incoming sea containers for illegal and accidental products which may be host to exotic pests and diseases (Fullam 2004). This awareness programme is designed to minimize the introduction of pests and infested produce.

Another awareness programme, "Quarantine Domestic" is designed to minimize the movement of pests between states. Community awareness programmes about fruit flies in particular are concentrated on travel in and through regions in Australia with area-freedom status and on travel between islands to the north of Australia close to Papua New Guinea and Indonesia. The latter is through the Federal Government initiative, the Northern Australia Quarantine Strategy.

Again taking the Fruit Fly Exclusion Zone as an example, community awareness is given high priority. Road signage is one of the strategies used to reduce the amount of fruit fly-infested fruit entering the Fruit Fly Exclusion Zone. Large signs are in place in South Australia, Victoria and New South Wales on all primary and secondary roads into the Fruit Fly Exclusion Zone. Signs are highly visible and indicate a fine (Fig. 3) for illegally carrying fruit fly hosts (fruit and tomatoes) into or through the region. On many of the major roads, sites for safe disposal of such goods are supplied. Disposal rates (Fig. 3) increased by 50% when fines were displayed on the signs (Dominiak et al. 1999). An eval-

Figure 3. *Precautionary road signs on highway into the Fruit Fly Exclusion Zone (FFEZ) in New South Wales, a strategy to reduce the amount of fruit fly-infested fruit entering the FFEZ (Photos from B. Dominiak, NSW DPI, reproduced with permission).*

uation of the effects of road signage on limiting the entry of fruit fly host material (Ernst & Young 1999), based on roadblock data, confirmed its effectiveness in informing travellers of their responsibilities to dispose of host produce before entering the Fruit Fly Exclusion Zone. Current estimates are that one vehicle in 2000 carries infested fruit in normal dry years. However, this doubles in wet years; overall, about 6-12% of traffic carries fruit, depending on the site (Dominiak et al. 2000).

The other aspects of community awareness include media campaigns, community service announcements, and the distribution of information to travellers via motels and tourist information centres, and education kits. As with road signage these, too, assist in limiting incursions, but also educate the community of the benefits of area freedom.

An education officer is stationed within the Fruit Fly Exclusion Zone with responsibility to develop and implement a community awareness programme. This targets television advertising, radio announcements, print media advertising, Fruit Fly Exclusion Zone maps, a web site for fruit flies and a school education kit.

Manned roadblocks have also been set up in some situations on roads entering the Fruit Fly Exclusion Zone. South Australia operates four permanent sites on major highways from New South Wales and Victoria (to limit the entry of Queensland fruit fly from the east) and on the Eyre Highway from Western Australia (against incursions of Mediterranean fruit fly from the west). Two of these, one on the west and the other on the east of South Australia's horticultural production area, are manned 24 hours a day, 365 days a year. The other two are manned during peak traffic periods. Victoria and New South Wales used permanent roadblocks up to the 1980s, but found them to be too expensive for the benefits gained. They now deploy random roadblocks. At some roadblock sites, travellers are asked to stop and to answer a questionnaire regarding their carrying fruit and are given a chance to hand over the produce without penalty. The roadblock operators have the power to search vehicles for hidden fruit and spot fines may be issued at some sites. Currently trucks are inspected only at some sites.

In Western Australia there are permanent 24 hour roadblocks where all traffic in Eucla in the south and Kununurra in the north is inspected.

4.3. Climatic Aspects

Some Western Australian growing areas in the south of the state have winter temperatures unfavourable to Mediterranean fruit fly development resulting in lower fruit fly populations with most attacks occurring in towns. In the Kimberley region in the far north, area freedom is maintained at Kununurra. The high temperatures and humidity during the wet season help maintain this area freedom, as Mediterranean fruit fly does not prefer them. This is demonstrated at Broome, on the southern edge of the Kimberley region, where Mediterranean fruit fly populations drop to very low levels during the wet season.

4.4. Cover Sprays

Cover sprays are generally more toxic than bait sprays. In some states it is becoming increasingly difficult to access private property generally, and there is increasing public resistance to the application of cover and bait sprays. Cover sprays of contact insecticides, which target adult fruit flies, must be reapplied after heavy rain. A delay in application may result in fruit infestation. Cover sprays of systemic insecticides are designed to kill eggs and early larvae near the fruit surface. However, if adverse weather delays application, the larvae may crawl further into the fruit and the sprays may be ineffective. The chemicals used in cover sprays are fenthion and dimethoate and these are under increased scrutiny by regulators. In the future it is likely that there may be additional limitations placed on the use of these pesticides.

In Western Australia fruit flies are controlled with insecticide baiting, cover sprays or a combination of both. The organophosphate insecticides fenthion, dimethoate and triclorfon are still widely used.

4.5. Fruit Fly Baits, Traps and Male Annihilation Technique

Bait sprays are based on a protein attractant and a pesticide, usually malathion. The mixture remains attractive as long as the protein degrades and produces ammonia. In dry areas such as the Fruit Fly Exclusion Zone, the bait spray dries out within 24 hours and is no longer attractive to Queensland fruit fly. Most spray schedules are seven days apart and this may need to be reviewed. Baits sprays are likely to be more effective in larger towns with a core humidity not influenced by the dry rural surrounds. Towns with low-cost water supplies also use more water and create favourable environments for bait programmes.

Outbreaks of exotic fruit fly species have occurred in Australia from time to time. Queensland fruit fly invaded Western Australia in the late 1980s and spread through several Perth suburbs (Sproul et al. 1992). The Asian papaya fruit fly *Bactrocera papayae* Drew & Hancock entered the Cairns region of Far North Queensland in the 1990s (Cantrell et al. 2002). Both species have been eradicated. A significant eradication tool used in these programmes was the male annihilation technique (MAT), where caneite blocks impregnated with pesticide (generally malathion), and male parapheromone lure (methyl eugenol for Asian papaya fruit fly and cuelure and SIT for Queensland fruit fly), were distributed throughout outbreak areas. Caneite MAT blocks are also used when outbreaks of Queensland fruit fly, or any other pest fruit fly species, enter the Fruit Fly Exclusion Zone (SCARM 1996).

4.6. Sterile Insect Technique (SIT)

In Eastern Australia the SIT involves the release of sterile Queensland fruit fly to contain, suppress or eradicate Queensland fruit fly outbreaks in fruit production areas. The New South Wales Department of Primary Industries (NSW-DPI) maintains the Fruit Fly Production Facility (which commenced production at the Elizabeth Macarthur Agricultural Institute in 1996), and is part of collaboration between New South Wales, Victoria and South Australia, which together form the Tri-State Fruit Fly Committee. The

facility produces up to 15 million Queensland fruit fly pupae per week, which are sterilized by gamma irradiation at the Australian Nuclear Science and Technology Organization, Lucas Heights. Dyed sterile fly pupae are couriered by plane to Narrandera, which is in the Fruit Fly Exclusion Zone and then taken to the NSW-DPI release facility at Yanco for rearing out to adult stage. Pupae are also sent, at times, to several Victorian towns, Adelaide for eradication of incursions from South Australia, and Alice Springs in the Northern Territory. Adult sterile flies are ground released from refrigerated trucks in towns, villages and orchards, and efficacy is measured using data from the national fruit fly trapping grid.

In Western Australia, the SIT is being investigated as a replacement for area-wide baiting schemes for maintaining Mediterranean fruit fly at low levels. Initial trials have been carried out in country towns with good success. The protocol involves a period of baiting in spring followed by sterile male release for one to three months in summer. VIENNA 7/99mix (Robinson et al. 1999) genetic sexing strain flies are produced in Western Australia to internationally accepted quality control standards (FAO/IAEA/USDA 2003, Smallridge and Hopkins 2003). They are irradiated in nitrogen to maintain competitiveness. Barriers to increased uptake of the SIT include the cost of sterile flies produced in a small facility, the technical and managerial skills required for successful SIT implementation, the need for cooperation between growers, and the unforgiving nature of the technique.

4.7. Interstate Certification Assurance

The Interstate Certification Assurance (ICA) scheme has been set up to govern the movement of fruit in Australia. Plant health certification adopted by the ICA is accepted by all Australian states and territories. Any business that wishes to use ICA treatments must be registered and accredited by the Interstate Plant Health Regulation Working Group (IPHRWG) (ICA 2003). The range of activities covered by ICAs adds up to effectively creating area freedom for that orchard or production area.

The following techniques can be used, or are under consideration for use within the ICA system:

4.7.1. Containment

Fruit fly host crops can be grown in fruit fly endemic areas under exclusion barriers. Such barriers include insect-proof netting applied to the whole crop or to separate trees or even to individual fruit. This technique is currently under consideration in Australia by the IPHRWG for adding to the list of approved ICA treatments.

4.7.2. Preharvest Insecticide Treatment and Inspection

For interstate movement of stone fruit (apricots, cherries, nectarines, peaches and plums) ICA-21 can be used. The procedure is a combination of fenthion sprays followed by 100% destructive examination of one in every 100 packages or cartons of product. Similar, but slightly different treatments exist for strawberries (ICA-11), lychees (ICA-14), custard apples (ICA-18), mangoes (ICA-19), and tomatoes, chillies and capsicums (ICA-26). Area-wide treatments, which reduce fruit fly populations to below detectable limits, have been trialled in areas in the south-west of Western Australia. For example, areas south of Donnybrook/Manjimup in Western Australia have been shown to be free from the Mediterranean fruit fly following the implementation of monitoring, but no proposals have yet been approved.

4.7.3. Climatic Zone Freedom

Some areas in Australia have been designated as having “climatic zone freedom for fruit fly”. This facility is allowed under ICA-23-1. For example ICA-accredited growers and packers can export stone fruit from the Young region in southern New South Wales, to Victoria and the Fruit Fly Exclusion Zone. Fruit can be exported if there are no fruit flies

found in standardized monitor-trap systems set up on accredited orchards.

4.7.4. Preharvest Insecticide Treatment

Preharvest treatments are approved only for capsicum, chilli, kiwi and pome fruit, strawberry and tomato. They involve application of dimethoate or fenthion at a concentration and frequency approved by the Department of Primary Industries/Agriculture in the exporting state for the control of Queensland fruit fly. Monitoring for female Queensland fruit fly using a liquid yeast autolysate in a McPhail trap can be used to identify when the flies are not active and when sprays are not required. Monitoring traps should be spaced on a 400 metre grid and examined weekly by a person accredited to recognize fruit flies. If monitoring is not done then spraying must be undertaken at the rate and frequency recommended by the department.

4.7.5. Non-Host Status

Hard skinned fruit such as mangosteens and pineapples can be naturally unable to host fruit flies or they can be harvested at maturities that are unable to host fruit flies such as papaya (ICA-06), limes (ICA-15) and bananas (ICA-16).

4.7.6. ICA Postharvest Quarantine Treatments

For movement of fruit fly host produce within Australia, a series of Interstate Certification Assurance procedures has been set up when a postharvest treatment is used as the only option, or the last option, available for trade. The following examples illustrate this option:

Postharvest insecticide treatments for Mediterranean fruit fly: (1) fenthion dipping (tomatoes, avocado, tamarillo and mango) – full immersion of the fruit in 412.5 mg/litre fenthion for 1 minute, (2) fenthion flood spraying (capsicum, tomatoes, mango, and tamarillo) – flood spraying the fruit with 412.5 mg/litre fenthion at 16 litres/min/m^2.

Postharvest heat treatments for Mediterranean fruit fly: (1) hot water dipping treatment for mangoes – 46.5°C for 20 minutes; or 47°C for 15 minutes pulp temperature, (2) high temperature forced air for mangoes – 46.5°C for 20 minutes, or 47°C for 15 minutes pulp temperature, (3) vapour heat for mangoes: 46.5°C for 20 minutes, or 47°C for 15 minutes pulp temperature.

Methyl bromide fumigation for Mediterranean fruit fly and Queensland fruit fly: all host-susceptible fruits can be treated at the rates given below, which vary depending on the temperature of the produce to be treated. The rates for two hour fumigations are: (1) 10-14.9°C at 48 g/m^3, or (2) 15-20.9°C at 40 g/m^3, or (3) 21-25.9°C at 32 g/m^3, or (5) 26-31.9°C at 24 g/m^3. This treatment may not be available in the future as the production and use of methyl bromide is in the process of being phased out due to its adverse effects on the ozone layer (UNEP 2000).

Postharvest cold treatment for Queensland fruit fly: the produce is held at one of the temperature/time combinations indicated below. The rates are: (1) 0 ± 0.5°C for at least 14 days, or (2) 1 ± 0.5°C for at least 16 days, or (3) 1.5 ± 0.5°C for at least 18 days, or (5) 2.5 ± 0.5°C for at least 22 days.

Citrus pesticide and waxing treatment for Queensland fruit fly: citrus may be treated with a flood spray of Rogor® (dimethoate 400 g/litre) and Panoctine® (guazatine at 400 g/litre) at the rates of 0.1 litre/100 litres and 1.3 litre/1000 litres, respectively. A non-recovery foam citrus gleam wax may be applied after this treatment but must always be the last treatment applied to the fruit.

5. Australian AW-IPM Research and Development for Fruit Fly Pests

5.1. Research Background

In April 2001, a workshop sponsored by Horticulture Australia Limited identified area-wide management of fruit flies in endemic areas as a priority area for research into fruit fly control options. To scope the opportunities, Horticulture Australia Limited funded a consultant to conduct a feasibility study on AW-IPM in late 2001. He visited nine candi-

date districts throughout Australia and identified the five most appropriate districts for the utilization of AW-IPM as: Corindi, Young, Narromine and Orange in New South Wales, and the Central Burnett in Queensland.

Candidate districts were assessed for their feasibility for successful AW-IPM by several criteria (Jorgensen 2002):

Local climatic conditions adverse to fruit fly infestation for at least part of the year: natural control of fruit fly by low temperature provides an important basis for an AW-IPM. Low temperatures can reduce the size and the spread of fruit fly populations.

Major local crops harvested when fruit fly infestation rate is low: crops harvested in winter, or when fruit fly populations are otherwise low, are least likely to be infested with fruit flies.

Major local crops poor or non-hosts for fruit flies: some crops are unable to host fruit fly infestation due to inherent physical or biological barriers to infestation. Crops such as most pineapple cultivars are resistant to fruit flies due to their impenetrable skin. Cherry tomatoes also resist oviposition because their skins are tough and smooth.

Few uncontrolled sites of fruit fly breeding: the presence in a district of few sites where fruit flies are not controlled reduces the risk of infestation of commercial crops. Uncontrolled sites include untreated orchards, urban areas, feral hosts and native host areas.

Level of community support for area-wide management: support from all sectors of the community, including growers of all host fruit, townspeople, local authorities and state governments, facilitates achieving maximum control of fruit flies.

Level of technical skills and support: Growers with a high level of technical skill in pest management, plus the support of research and extension staff, and one-on-one consultants, are needed to establish and maintain area-wide management of fruit flies.

Benefit to district from area-wide management of fruit flies: the strength of grower and community support for area-wide management will be dependent on the financial benefits expected or achieved, especially the major benefits resulting from market access.

Reliability of AW-IPM system in meeting future market access conditions: the system used to achieve a low rate of infestation needs to be reliable, so that the approved market access conditions can be met year after year. Sudden loss of market access is very disruptive to orderly marketing. Applied treatments such as bait sprays and male annihilation technique are generally quite reliable. Low winter temperatures are moderately reliable. Quarantine measures to prevent the entry of fruit fly into small quarantine districts are likely to have low reliability.

5.2. AW-IPM Research and Development Areas in Australia

5.2.1. Gayndah-Mundubbera, Queensland (A. Lloyd, personal communication)

Orchards: (1) maintain existing protein baiting in citrus and improve control in other crops, (2) introduced male annihilation technology at ten MAT cuelure cups per hectare in all orchards, three times per year, and (3) grower participation entirely voluntary.

Town areas: (1) no previous fruit fly treatments – heavy infestation in backyard fruit, (2) introduced year-round MAT and baiting of backyard fruit trees and encourage disposal of fallen fruit, and (3) year-round trapping and backyard fruit collection.

5.2.2. Corindi, New South Wales (A. Jessup, unpublished)

Within plantation: (1) protein baiting in harvest season (October – March), (2) cuelure traps on 400-metre grid, (3) MAT caneite blocks at four blocks per hectare, (4) strategic cover sprays with dimethoate based on trap captures, (5) removal of fruit fly hosts within plantation (peaches), and (6) larval searches in blueberries and raspberries throughout the year.

Areas surrounding plantation: (1) perimeter protein baiting, (2) perimeter placement of MAT caneite blocks every 20 metres, (3) cuelure traps in bush surrounding planta-

tion (20 traps), (4) sites of feral fruit fly hosts (removal or cover spray), and (5) larval searches of native and feral fruit.

5.2.3. Manjimup, Western Australia (C. P. F. de Lima, personal communication)

Within town of Manjimup: Lynfield traps with capilure and malathion on a 400 metre grid.

Town perimeter (1.5 kilometres from edge of town): Lynfield traps with capilure and malathion 400 metres apart. Orchard trapping (Lynfield traps with capilure and malathion) on one kilometre grid within orchard areas. Additional traps on a 400 metre grid were deployed from November 2003 to May 2004 (harvest period for pome and stone fruit) in all orchards outside two kilometres from the town boundary.

5.3. Results

5.3.1. Gayndah-Mundubbera, Queensland

The following results were obtained: (1) community education and awareness programmes implemented showing town people the impact of fruit flies in their gardens on nearby commercial orchards, (2) for 2004 season, flies trapped in commercial orchards fell by about 90%, and (3) town infestations were 61% prior to AW-IPM and fell to 26% within five months. From May to September 2004 no backyard infestations have been found.

5.3.2. Corindi, New South Wales

The following results were obtained: (1) the combination of perimeter baiting with MAT caneite blocks reduced the ratio of the number of flies trapped outside the plantation perimeter to that inside the plantation from 1:1 at the start of the programme (September 1998) to 3:1 by March 1999, and to 12:1 by November 2002, (2) reduced number of applications of dimethoate from every seven to every 21 days (studies showed extended anti-fruit fly efficacy in blueberries), (3) new, organically-certified toxicant for protein baits (GF120-active ingredient spinosad) tested for replacement of malathion (approved in 2005), and (4) quarantine treatment developed using cold storage as back-up in case of uncontrollable outbreaks.

5.3.3. Manjimup, Western Australia

The following results were obtained:

Zone 1 – Orchards: (1) no Mediterranean fruit flies trapped over harvesting period in all orchards more than two kilometres from the town boundary with a 400 metre trap grid from November 2003 to May 2004, (2) no fly trapped in Manjimup orchards (one kilometre trap grid) for ten years from November 1994 to June 2004, and (3) no hosts and over 20 continuous days below 3°C in winter give environmental control.

Zone 2 – Perimeter: less than 0.002 flies per trap per week in 400 metre grid 1.5 kilometres outside town boundary.

Zone 3 – Town: three flies per trap per week from November 2003 to May 2004.

6. Conclusions

As a result of trial AW-IPM strategies tested in New South Wales and Queensland, there have been significant reductions in the numbers of fruit fly trapped in orchards where the MAT and protein baits were used. In Corindi, New South Wales it was necessary to cover spray when the number of flies trapped caused concern to growers. Cover spray applications were reduced from about 12-15 times a season to 4-5 times. Although there are flies in the township of Manjimup in Western Australia and in very small numbers in a trap line placed 1.5 km from the township, there have been no flies trapped within nearby orchards in the ten years from November 1994.

From discussions with the scientists involved, it appears that there are three major factors, which have significant impact on the success of AW-IPM programmes. Firstly the size of the natural fruit fly population in that area affects the type of fruit fly management strategy that is used. AW-IPM under very high fruit fly populations may be too difficult to achieve without the need for heavy cover

spray use and very strict control of fruit fly host material entering the area. Both techniques are costly to the community or to the environment and would only be used where benefits outweigh those costs. AW-IPM would be more achievable in areas where fruit fly survival is marginal or restricted to short warm periods such as in Manjimup where winter temperatures are lethal to fruit flies. In these cases "softer" fruit fly control options can be used to reduce populations to negligible levels (i.e. to non-detectable levels or complete eradication).

The second aspect with major impact on the success of AW-IPM is farmer and urban householder compliance with fruit fly management strategies employed by the programme. Fruit flies are quite highly mobile betweens orchards and around towns so full regional compliance is ideal.

Thirdly is the cost of AW-IPM and who should pay for it. This question resulted in a review carried out recently (Bull 2004). Among several recommendations, was the desirability for maintenance of area freedom in the Fruit Fly Exclusion Zone to be funded 50:50 between government (national, state and local) and industry. At present, in New South Wales, there is negligible local government and industry investment. Negotiations are currently underway.

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ABSTRACT A total of 8500 hectares were treated with larvicides against several species of mosquitoes in an area of 25 000 hectares of agricultural land with 11 500 hectares of Ramsar Convention-protected wetlands in the plain of Nestos (Prefecture of Kavala, northern Greece). Temephos, diflubenzuron and *Bacillus thuringiensis* subsp. *israelensis* (de Barjac) were used as larvicides. Detailed ecological mapping of vegetation using geographic information systems (GIS) was used to predict potential breeding sites. This allowed a more environmentally-friendly application of insecticides resulting in only 77% of the area being treated using helicopter and ultra-light motorized aircraft. The results, as determined by personal interviews, are satisfactory: 98% considered the mosquito problem large or unbearable before the beginning of the control project while 67% considered it small or non-existent after its implementation.

KEY WORDS mosquito abatement, larviciding, *Ochlerotatus caspius*, mapping, GIS, aerial spraying

1. Introduction

Greece has a long history of mosquito-born diseases (malaria, dengue fever), and there is scientific evidence that some inhabitants have contracted West Nile virus (Hubalek 1999). Rice fields, extended wetlands close to human settlements (e.g. 15 000 hectares of rice fields 15 kilometres from Thessaloniki, the second largest city of Greece with one million inhabitants), in combination with favourable temperatures and misuse of irrigation water and liquid wastes provide ideal mosquito breeding conditions along the 15 000 kilometre coastal zone of Greece.

Especially in northern Greece where the majority of wetlands and rice fields are located, inhabitants and visitors suffer from an unbearable mosquito nuisance for more than five months every year (May-September) and counts of 150-200 mosquito bites per 15 minutes are not unusual (Ecodevelopment 1997). During the 1960s, malaria was eradicated in Greece using DDT (aerial applications) and wetland draining. In Macedonia alone, the richest area nowadays in terms of wetlands in Greece, about 70% of its original wetlands were lost during the previous century (Psilovikos 1990). However, the gradual increase in arable land and the recent emergence of environmental ethics in the form of wetland restoration and protection have exacerbated the mosquito nuisance problem. For a country with an economy largely based on tourism, the mosquito problem has serious implications. Furthermore, every year 40-50 imported malaria cases are recorded in Greece, and the recent emergence of West Nile virus (a mosquito-born disease) around the Mediterranean basin also poses a threat to public health (Gratz 2000).

Ecodevelopment S.A., a pest control company established nine years ago, specializes in mosquito control. Starting with a successful project involving eight municipalities and 15 000 hectares of rice fields situated 15 kilometres west of Thessaloniki, Ecodevelopment has expanded its activities, offering mosquito control services to 11 of the 52 prefectures of Greece ranging from the protected wetlands

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 699–707.

Figure 1. *Mosquito control projects currently implemented in Greece.*

of Rhodopi through the rice fields of Thessaloniki to the arid environments of the islands of Cyclades (Fig. 1).

The project in the Prefecture of Kavala presented in this paper has been implemented since 2000 (with an interruption in 2003), and constitutes a case study in itself in terms of difficulty and complexity since it comprises a wide multitude of mosquito breeding sites in irregularly scattered fragments of urban agricultural and natural environments.

2. The Project Area

The project is implemented in the plain of the Nestos river in a coastal area of 25 000 hectares of agricultural land irrigated by the waters of the river. Within this area, Nestos forms an extensive delta with 11 500 hectares of Ramsar Convention-protected wetlands. About 1500 hectares of these wetlands are dominated by *Arthrocnemum fruticosum* (L.) Moquin-Tandon, *Salicornia europea* L., *Aeluropus littoralis* (Gouan) Parlatore, *Juncus maritimus* Lamarck and *Scirpus maritimus* L., vegetation that creates highly suitable mosquito breeding sites.

During the summer of 2004, 2150 hectares of rice fields scattered in 586 individual parcels of on average 3.7 hectares, constituted the most important mosquito breeding sites. Abandoned agricultural land comprising 232 fields of about 850 hectares received occasional irrigation creating the second most productive breeding site. In addition, 1-2% of the total surface of 6500 hectares of corn fields were poorly drained resulting in about 100 hectares of stagnant water producing at least one mosquito generation per season. Finally surplus water and misuse of water resulted in a wide variety of potential breeding sites in urban and peri-urban environ-

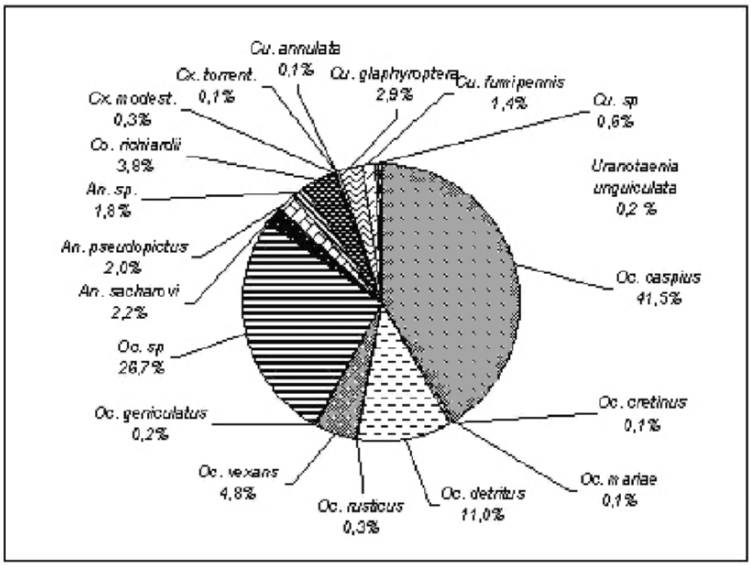

Figure 2. *Species composition in 2002 as determined by the "human bait" method that uses an aspirator to collect mosquitoes that land on a human leg within a certain period of time (usually 15 minutes).*

ments comprising nine villages, surrounded by rice fields and other cultivations, with a total population of about 25 000 inhabitants.

3. Species Composition

Fig. 2 shows the composition of the mosquito species in 2002 as determined by the human bait method. The method uses an aspirator to collect mosquitoes that land on a human leg within a certain period of time (usually 15 minutes). Ten sampling sites were set up mainly around human settlements to record mosquito nuisance and were visited on a weekly basis.

In total, 21 mosquito species could be identified. *Ochlerotatus caspius* Pallas was clearly the dominant species (42%) followed by *Ochlerotatus detritus* Haliday (11%) and *Ochlerotatus vexans* Meigen (5%) while 27% were unidentified *Ochlerotatus* spp. specimens. Anopheline species contributed 6% (*Anopheles sacharovi* Favre, *Anopheles pseudopictus* Grassi, *Cocquillettidia richiardii* Ficalbi 4%, and *Culex* spp. and other species 6%.

4. Equipment and Personnel

The main spraying applications (larviciding) were conducted by one Hiller UE-12E helicopter, an ultra-light motorized aircraft (Air Creation BUGGY 582 SL with FUN 18QC wing) and one low-volume fan sprayer unit mounted on a Unimog 4 x 4 vehicle. Minor applications were conducted by two conventional spraying units mounted on 4 x 4 vehicles. For various spot treatments mainly in urban and peri-urban environments, knapsack sprayers and granule applicators were used.

Adulticiding, only used in cases where emergence of adults was recorded, was performed by one ultra low volume cold fog generator, mounted on a 4 x 4 vehicle.

The personnel, who were employed full-time during the project, consisted of two pilots and two technicians for the aerial team, two ground spraying technicians, six sampling

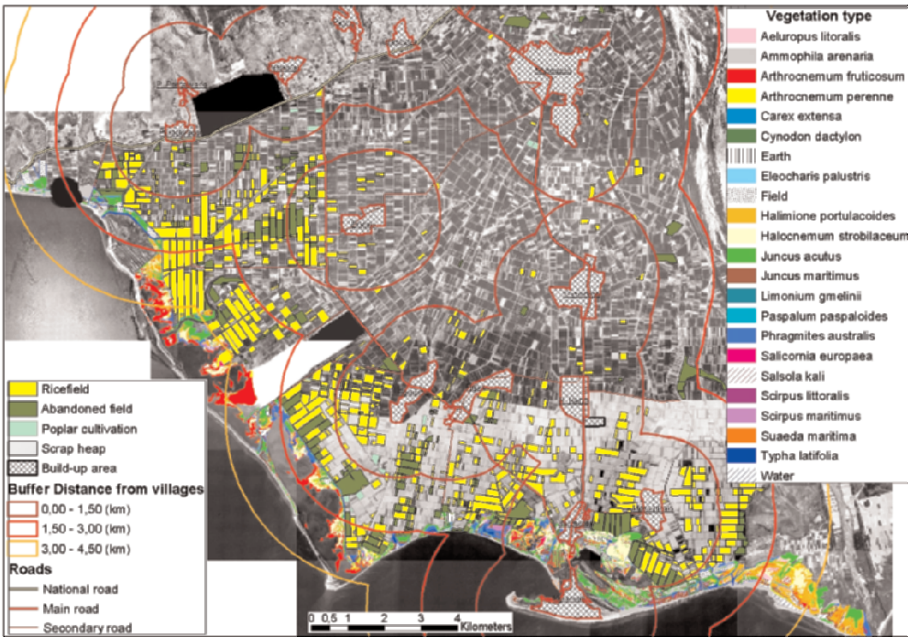

Figure 3. *General map of the Nestos plain presenting the main mosquito breeding sites.*

technicians performing also minor spraying applications and one project manager.

5. Insecticides

Three different larvicides were used in accordance with the type of breeding site: *Bacillus thuringiensis* subsp. *israelensis* (de Barjac) (Vectobac® SL), temephos (Abate® 50 EC, Abate® 1 SG) and diflubenzuron (Dudim® 10 WP). Vectobac® was used exclusively in the natural environment (2-3 litres/ha), Abate® 50 EC in the rice fields (150-300 ml/ha), while Abate® 1 SG (5-10 kg/ha) and Dudim® 10 WP (1 kg/ha) were used in urban and peri-urban breeding sites. Malathion (Fyfanon® 44EW) was used as adulticide (0.25-0.5 litres/ha).

6. Development of a Control Strategy

The Kavala plain was initially divided into four different types of environment, reflecting different abatement approaches: natural, agricultural, peri-urban and urban. In each, preliminary inspections were undertaken to determine all potential breeding sites. Then, permanent larval sampling sites were set up, which were sampled on a weekly basis. A total of 1235 sampling sites were established and checked weekly: 311 in the natural environment, 818 on agricultural land and 106 in the peri-urban system. In the urban environment, and after having checked 2320 private properties, 1096 potential breeding sites were recorded the majority of which (737) were open cesspools. In addition, educational leaflets were distributed to local people with information on how to restrict mosquito breeding on their own properties and complaints of mosquito nuisance were recorded. Buffer zones were drawn on a general map of the area around each village at distances of 1.5, 3 and 4.5 kilometres. These buffer zones helped to set spraying priorities which largely depend on the dispersal abilities of the main mosquito genera in the application area (Fig. 3).

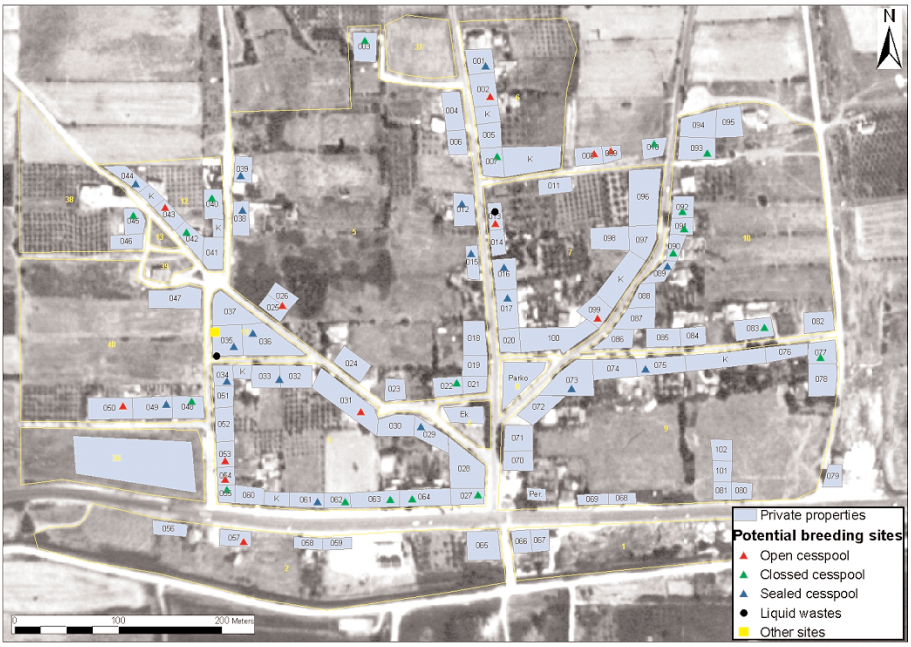

Figure 4. *Example of a digitized orthophoto map that indicates all potential breeding sites in an urban system.*

All potential breeding sites were mapped and digitized on orthophotomaps using ArcGIS® 8.3. Twenty three maps were then developed: six maps at a scale of 1:15 000 for the natural system, four maps at a scale of 1:30 000 for the agricultural system, four maps at a scale of 1:20 000 for the peri-urban system and nine maps at a scale of 1:5000 for the urban system (Fig. 4). These maps were distributed to both air and ground personnel as operational working documents.

7. Sampling Method

Each sampling site was checked for mosquito larvae on a weekly basis. Water samples were collected from flooded sites on a 5-10 metre transect and analysed on-site using a dip net of around 16 centimetres diameter with a mesh size of about 200 micrometres. Data on genus (*Ochlerotatus*, *Anopheles*, *Culex*), developmental stage (1st-4th larval instar, nymphs), abundance using a 5-grade scale (0 = no larvae present, * = 1-7, ** = 8-14, *** = 15-21 and **** = more than 21 larvae per sample) and site characteristics were recorded daily on record sheets. These data were then used to delimit areas for ground and aerial spraying and afterwards entered into Access® databases, which could be integrated with the ArcGIS® data.

8. Ecological Mapping

Ecological mapping in mosquito control is a method to delimit and identify potential breeding sites in coastal wetlands. The method was initially introduced in France (Gabinaud 1975), and adapted to the comparable Greek Mediterranean conditions (Eco-development 2002), and is now successfully applied to the majority of the main coastal areas of Greece. The method uses the vegetation as an index of oviposition site preference of gravid *Ochlerotatus* (*Aedes*) spp. females. Distribution of plant species in a littoral zone

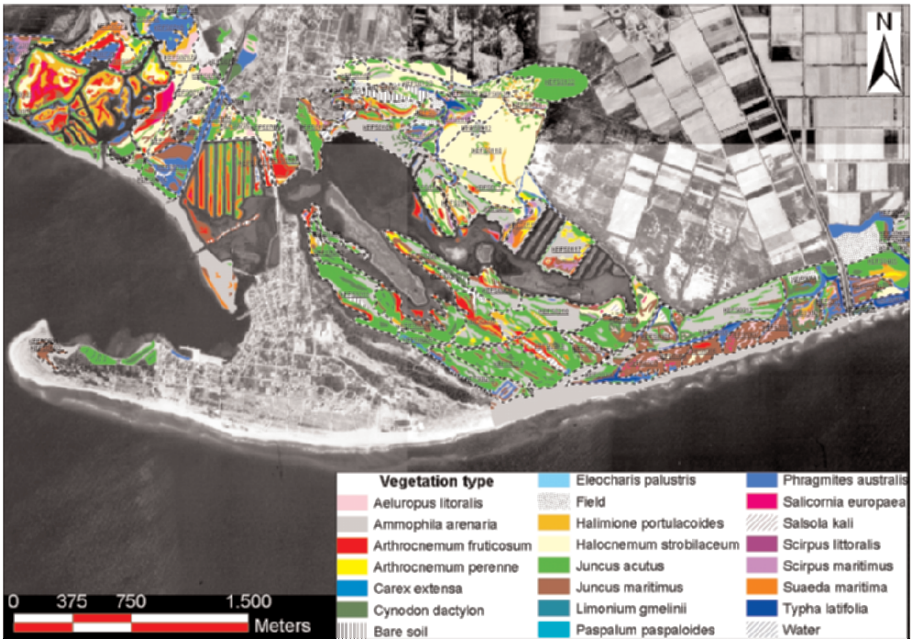

Figure 5. *An example of ecological mapping: one of the six maps displaying the different vegetation types in the project zone.*

mainly depends on their tolerance to salinity and duration of inundation. These ecological factors also determine the preference of *Ochlerotatus* spp. females for oviposition. Thus, vegetation distribution patterns can reflect the egg-laying patterns of *Ochlerotatus* spp. females (Babinot 1982). In total 20 different vegetation types were distinguished according to their dependence on water and salinity tolerance (Fig. 5). Each vegetation type was labelled with its most characteristic plant species using a different colour. The final output is a map of various vegetation types with different colours, each corresponding to a specific *Ochlerotatus* spp. oviposition potential.

The benefit of such a method is that one can more or less predict the mosquito (*Ochlerotatus* spp.) potential of an area before the breeding season and organize promptly the control operation in terms of equipment, insecticides and personnel. Moreover, once the abatement begins, many sampling and spraying efforts can be saved: when larvae are sampled in one polygon, the presence of larvae in the rest of the polygons of the same colour is highly probable. It is therefore not necessary to check each polygon of the same colour and also, excessive spraying of non-productive areas is avoided.

In the present case, ecological mapping was used to characterize 1313 hectares of coastal wetlands into 3200 vegetation polygons and establish 311 sampling sites. Factors that influenced the delimitation of the sampling sites were uniformity of vegetation (dominance of a vegetation type or a series of related types), physical borders and accessibility.

9. Larval Productivity

Record keeping enabled the development of sampling site productivity maps according to the total abundance of *Ochlerotatus* spp. larvae recorded at each sampling site during

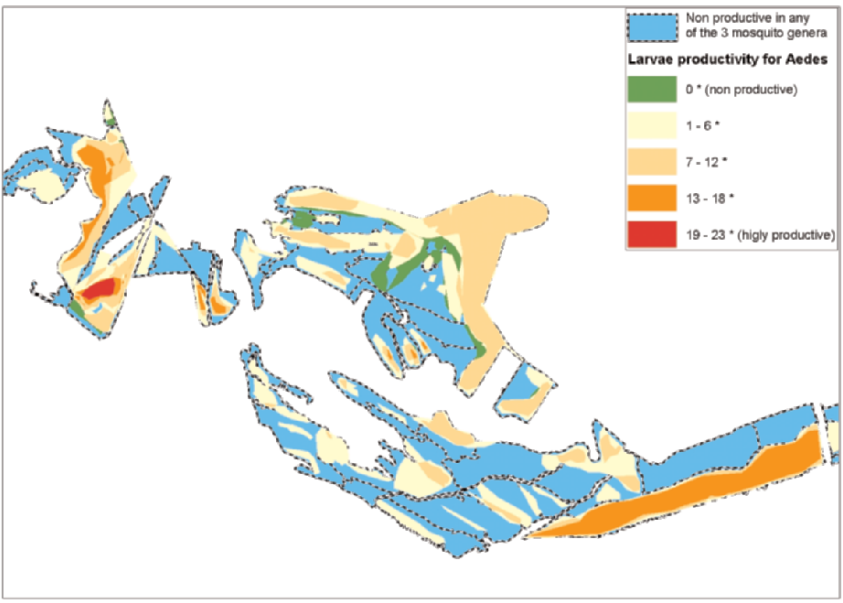

Figure 6. *Example of a larval productivity map. Sampling sites are classified on a five-grade scale, according to the total abundance (sum of asterisks) recorded during a 12-week period in the summer of 2004.*

2004 (Fig. 6).

From 1313 hectares of ecologically-mapped potential breeding sites in 2004 (a very dry year), production of mosquito larvae was recorded in only 657 hectares, limiting the spraying applications to the minimum necessary. In 95% of these sites, *Ochlerotatus* spp. larvae were recorded (629 hectares), while in 23 and 20% of the sites *Anopheles* spp. and *Culex* spp. were observed, respectively. The data on larval productivity in the agricultural system are still under evaluation and will be presented elsewhere.

In general, the productivity of individual rice fields varied considerably, between one to five generations per season, in contrast to the natural ecosystem where both levels and variations in productivity were lower (one to two

Table 1. *Total sprayed surface area (hectares) in relation to the type of breeding site and application equipment.*

	Rice fields	Abandoned fields	Natural ecosystem	Total	Percentage
HILLER helicopter	3950	484	703	5137	60.4
Ultra Light	903	175	316	1394	16.4
UNIMOG 4 x 4	477	1149	348	1974	23.2
Total	5330	1808	1367	8505	
Percentage	62.7	21.3	16.0		

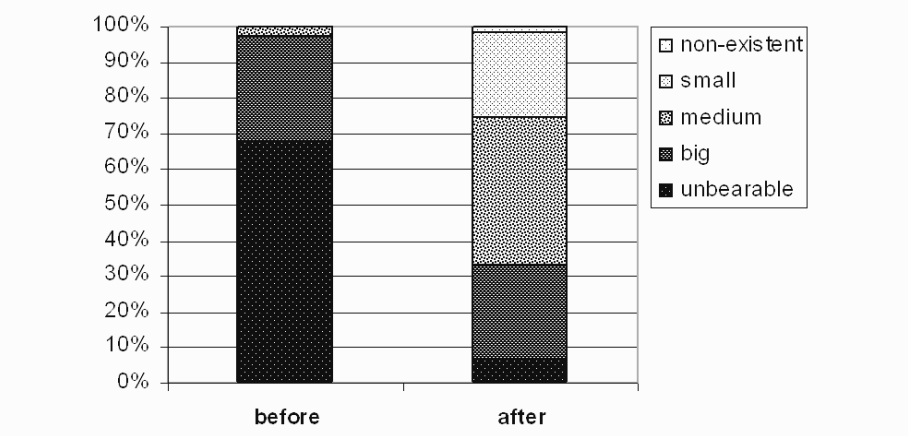

Figure 7. *Results of a survey (comprising 180 questionnaires) conducted in 2004 to assess the severity of the mosquito problem before and after the implementation of the project.*

generations).

10. Spraying Applications

In total 8505 hectares were treated with larvicides using temephos and diflubenzuron in agricultural land and *B. t. israelensis* in the natural environment. 76.8% of the sprayed area was aerially treated (Table 1).

11. Evaluation of Results

In order to evaluate the effectiveness of the project, a survey in the form of questionnaires (personal interviews) was conducted in 2000 (200 interviews) and 2004 (180 interviews). According to the results, 80% of local people considered the problem of mosquitoes the most serious environmental issue in the area among others such as aerial pollution, environmental degradation, misuse of agricultural pesticides, etc. Moreover, the local community fully supported the project, 88% being willing to participate financially to ensure its continuation. Also, 98% considered the mosquito problem a large or unbearable problem before the beginning of the control project while 67% considered it moderate, small or non existent after its implementation (Fig. 7).

12. Conclusions

O. caspius is the most important nuisance species in the area. Detailed mapping of the target area, delimitation of breeding sites, record keeping and continuous communication with local people can ensure the continuing success of the project as well as the public acceptance required for its viability. Ecological mapping is a valuable tool when dealing with sensitive natural environments, saving both labour and insecticides. From a total of 11 500 hectares of wetlands only 1313 hectares were identified as potential breeding sites. From this area, only 50% was found to be productive in 2004, thereby limiting the spraying application to the absolute minimum necessary. The majority of local people questioned considered the mosquito problem crucial for their environment, are satisfied with the results of the project and are willing to participate financially for its continuation.

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The Carribean *Amblyomma variegatum* Eradication Programme: Success or Failure?

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ABSTRACT The tropical bont tick *Amblyomma variegatum* F. was introduced into Guadeloupe in the mid 1700s and into Antigua about 100 years later. It was mainly restricted to these islands until the mid 1970s, when the tick spread rapidly, coincident with the introduction and expansion of the cattle egret (*Bubulcus ibis* L.) population. Thereafter, the tick became established on 18 islands. In late 1994, an eradication programme, the Caribbean Amblyomma Programme (CAP), was initiated using a strategy based on applications every two weeks of the pour-on acaricide, flumethrin (Bayticol®), for a minimum period of 24 to 30 months. Farmers were made responsible for regular treatment of their livestock. The strategy was reinforced through intensive public information programmes. A tropical bont tick surveillance programme was carried out and data entered into a customised database "TickINFO". Progress was slower than originally foreseen, but St. Kitts and St. Lucia (November 2001), Anguilla and Montserrat (February 2002), and Barbados and Dominica (February 2003) were certified provisionally tropical bont tick-free following defined periods of surveillance. Unfortunately, St. Kitts, St. Lucia and Dominica have all had reinfestations or recrudescences of the tick since being certified. In 2004, with the increase in spread of the tick, St Kitts was decertified. St Vincent qualifies for certification, as ticks have not been seen for two years. Three CAP islands remain infested: Antigua, Nevis and St. Maarten/St. Martin. The four islands in the French West Indies programme also remain infested. Three of the four islands under the jurisdiction of the USA remain tropical bont tick-free. St Croix, however, became reinfested again in 2000 and remains so up to 2005. The major conclusions drawn from the programme are discussed in the context of successes and failures, and include administrative and financial management issues, the technical design and methodology and the future of the programme. Notably, if additional funding is not available within a few months to continue and conclude eradication activities, then it is recommended that a tick management strategy is adopted, with full cost recovery.

KEY WORDS tropical bont tick, *Amblyomma variegatum*, eradication, Caribbean, flumethrin, cattle egrets

1. Introduction

The tropical bont tick *Amblyomma variegatum* F. was introduced into Guadeloupe in the mid 1700s and into Antigua about 100 years later. It spread to Marie Galante quickly, but thereafter it was mainly restricted to these islands until the mid 1970s. Thereafter, coincident with the introduction and expansion of the population of cattle egrets, *Bubulcus ibis* L., the tick spread rapidly. During the period 1970-1990, the tick became established on 18 islands (Table 1 and Fig. 1). It was estimated that if it, and its associated diseases, heartwater and dermatophilosis, became widely established throughout their potential range in the Americas, potential losses of USD 762 million annually could occur to the livestock industry.

Pegram and colleagues (2000c) presented

M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-Wide Control of Insect Pests, 709–720.

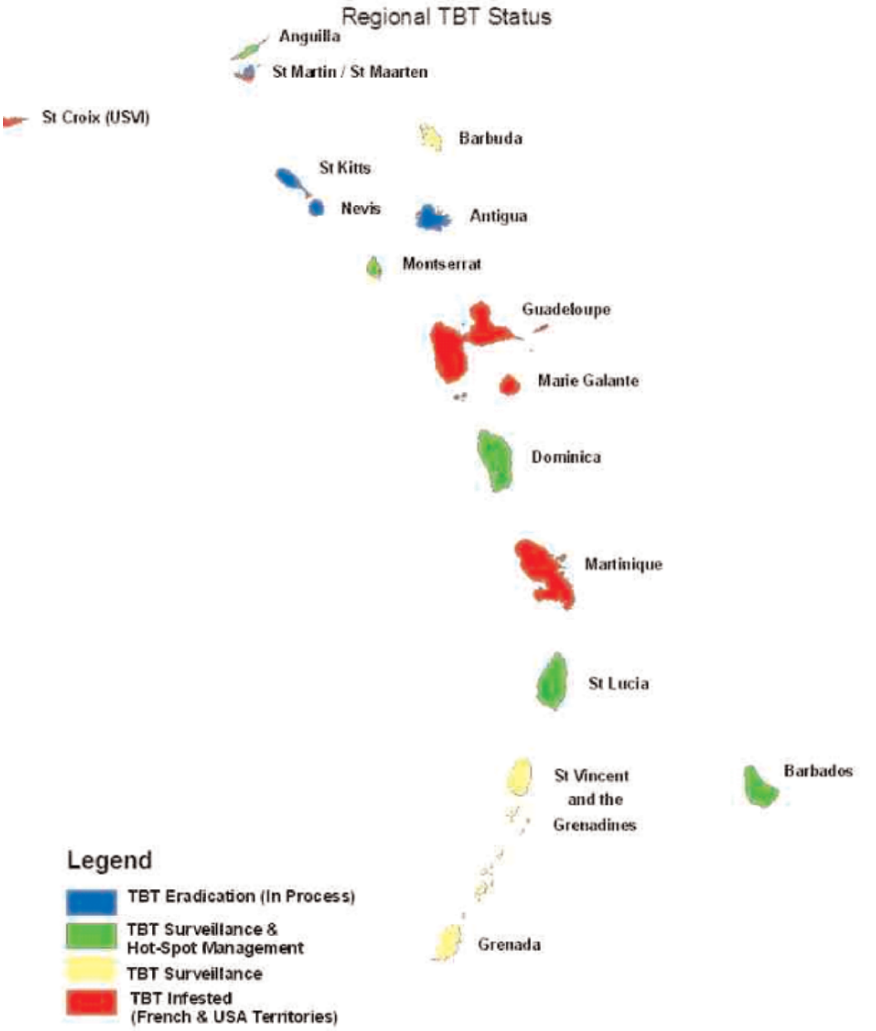

Figure 1. *Map of the Caribbean showing the status of the tropical bont tick (TBT) eradication programme.*

a general introduction to the Caribbean Amblyomma Programme (CAP), its history, and the initial progress towards the eradication of the tropical bont tick. Subsequently, progress was reported in several international symposia and papers (Pegram et al. 2000b, Phillip 2000, Pegram et al. 2002, Pegram and Eddi 2003, Pegram et al. 2003, 2004). This paper reviews further progress and identifies some serious financial, administrative and technical constraints that have impacted negatively on its original progress and successes.

2. Programme Methodology

In late 1994, the *A. variegatum* eradication programme was initiated, with the Food and Agriculture Organization of the United

Table 1. *Islands involved in tropical bont tick eradication programmes in the Caribbean Community and Common Market (CARICOM) (Caribbean Amblyomma Programme), the French West Indies, and USA territories.*

Island or group (Authority)	Year of infestation	Current activity	Tropical Bont Tick	Comments on status
United States of America (USDA)				
Puerto Rico	1974 / 92	S and Q	-	Eradicated / remains free
Vieques	1981	S and Q	-	Eradicated / remains free
Culebra	1986	S and Q	-	Eradicated / remains free
St. Croix (USVI)	1967 / 87	E	+	Reinfested in 2000
CARICOM and Associated States/Islands (CAP)				
Anguilla	1982	E	-	Certified provisionally free - 2002
Antigua	1865	E	+	Process of eradication
and Barbuda		S	-	Not infested / remains free
British Virgin Islands		S	-	Not infested / remains free
Barbados	1990	S and Q	- *	Certified provisionally free - 2003
Dominica	1993	S and Q	- *	Certified provisionally free - 2003
Grenada		S	-	Not infested / remains free
Montserrat	1980	S and Q	-	Certified provisionally free - 2002
St. Kitts	1978	E, S and Q	±	Reinfested and decertified - 2004
and Nevis	1977	E	+	Process of eradication
Montserrat	1980	S and Q	-	Certified provisionally free - 2002
St. Lucia	1973	S and Q	- *	Certified provisionally free - 2001
St. Vincent		S	-	Reinfested in 2000 / Now free
Trinidad & Tobago		S	-	Confirmed tick-free in 2001
Netherlands Antilles (CAP)				
Saba		S	-	Not infested / remains free
St. Eustacius		S	-	Not infested / remains free
St. Maarten	1978	E	+	Process of eradication
French West Indies (FWI)				
Guadeloupe	ca 1700	E	+	All FWI territories remain tick - infested
Marie Galante	ca 1700	E	+	
Les Desirade	1982	E	+	
Martinique	1948	E	+	
St. Martin	1978	E	+	CAP assumed responsibility in 1999/2002

* Tropical bont tick status: periodic reports of "hot spots" that require treatment

E = eradication, S = surveillance, Q = quarantaine

Nations (FAO) providing the lead technical role. The CAP Regional Coordination Unit was responsible for overall operational and technical management, training, and supervi-

Table 2. *Public awareness and training materials used in the tropical bont tick programme.*

Theme	Activities and Items	Comments
General and "Be Aware"	Brochures, clocks, calendars, calculators, diaries, document conference cases, key rings, posters, T-shirts, watches Sponsorship for cricket and football (polo shirts and CAP logo) Animal handling video and field manuals Radio and TV programmes School and TV quizzes / competitions Promotional DVDs, CDs, and videos	General materials and specifics for meetings of the Amblyomma Programme Council
"Pour-it-On"	Training video and supporting print materials (posters, brochures) Coffee mugs	Training package co-funded by Bayer
"Tick Watch" and "Keep Looking"	Training videos and supporting print materials Tick identification field kits in a Fanny Pack Tick identification video, manual and field aids	Training package co-funded by Bayer. Different sub-themes used in tick-infested and tick-free islands

sion of the regional database. The unit also served as the secretariat for the Amblyomma Programme Council that met annually.

2.1. Eradication of Tropical Bont Tick

In the tick-infested countries, the project strategy was based on the application of the acaricide every two weeks, flumethrin (Bayticol®) pour-on, for a minimum period of 24-30 months. Farmers were made responsible for regular treatment of their livestock, and the strategy was reinforced through an intensive public information campaign using various media and services to ensure sensitization of farmers and the general public. Government animal health personnel were responsible for training, monitoring compliance, and surveillance.

2.2. Surveillance for the Tropical Bont Tick

The surveillance protocol was developed in participatory workshops during 1998-1999 that included field staff as well as epidemiologists and national coordinators. In 2000, a revised version was based on the double binomial nested probability function (Beal 1971) to take account of the heterogeneity of livestock management systems in the Caribbean islands. Prevalence rates were determined for both properties and animals. In addition to intensive quantitative surveillance, training was provided in participatory epidemiological methods to enhance the quality and quantity of data generated. This was particularly rewarding in islands with a very low prevalence.

2.3. Public Information Campaigns

A wide variety of techniques, activities and materials was used to increase awareness of the programme among livestock owners and the general public. The programme adopted themes that covered the main field activities, including the initial awareness campaign "Be Aware", the tick eradication activities "Pour-it-On", and for tick surveillance "Tick Watch" and "Keep Looking". These themes, and a summary of activities and items produced, are

Table 3. *Components and functions of Tick*INFO 4 *and GIS.*

Component	Function and Outputs
Village data	Lists all villages with geographic coordinates
Farmer data	Registration of all animal owners and livestock with specific locality coordinates
Visit data	Compilation of tropical bont tick surveillance data, and update of census data
Summary analysis	Facilitates selection criteria for analysis and standardized reporting
	Includes export facility for GIS analysis and mapping
	Provides standardized quarterly reports with zonal and species prevalence rates
	E-mail transmission facility
	Upload facility to CaribVET website
File data	Facilitates safe filing and back-up of all data
Random contacts	Generates a random list of farmers for surveillance purposes

shown in Table 2.

2.4. Database Management

Following tropical bont tick surveillance activities, all data were entered into a customized database, "TickINFO"(Unpublished). The TickINFO database was programmed in Microsoft Access® to process and analyse *A. variegatum* surveillance data. It was developed by CAP and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement-Département d' Elevage et Médecine Vétérinaire des Pays Tropicaux (CIRAD-EMVT) with continuous feedback from national database managers to enhance adaptability and acceptability. The database was used both in the private and public sectors in Guadeloupe and Martinique and in the CARICOM veterinary departments of the Lesser Antilles. Key components and functions of the current version of TickINFO 4 and GIS, which is linked with ArcView® 8.4 to facilitate mapping outputs, are summarized in Table 3.

3. Results and Discussion

It was realized in 2000 that several islands had taken considerably longer than originally projected to attain the goal of tropical bont tick eradication. Several technical, administrative, and management factors contributed to the delay.

The national programme in St. Kitts (Phillip 2000) was considered a model from the onset in 1995. Blanket treatment was terminated in five of the seven parishes within 36 months, and by 2001 it was believed that only one tick-infested property remained (Fig. 2). St. Kitts was granted "tropical bont tick provisional-free" status in November 2001. Immediately thereafter, a series of events had serious detrimental impact on the programme,

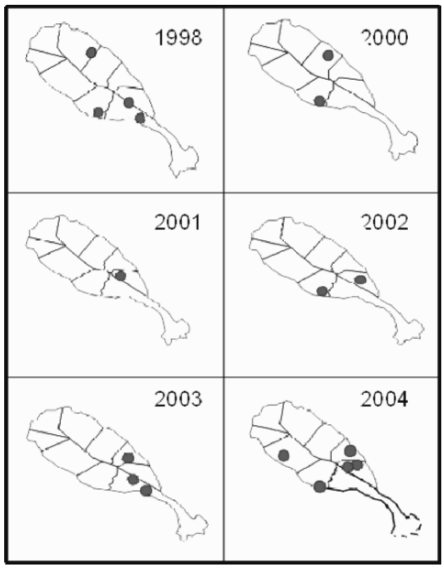

Figure 2. *Progressive decline and reinvasion of tropical bont tick in St. Kitts.*

including major senior staff changes in programme management.

One paradoxical factor was that, due to the initial success of the eradication programme and increased confidence in rearing livestock, cattle numbers increased almost four fold. Unfortunately, little attention was paid to cattle maintenance or confinement and markets for beef had declined distinctly. This, coupled with a parallel decline in the sugar industry, led to increased numbers of free-roaming livestock and inadequate quarantine at a critical time. The subsequent rapid spread of the tropical bont tick through 2003 and 2004 resulted in St. Kitts being decertified from its provisionally-free status in November 2004 (Fig. 2).

In Antigua, a major setback was identified soon after the implementation of the national programme. Livestock numbers had been grossly underestimated at the time of the initial launch of the eradication efforts in 1997: cattle by 25% and small ruminants by some 400%. There were also many animals free-roaming or stray that had not been registered. Consequently, management deficiencies and financial constraints led to a suspension of all field activities in 2000. A new philosophy and strategy was then developed for livestock in general as an essential prerequisite for any possible resumption of eradication activities. These included the compulsory registration of all livestock owners, tagging of all livestock, and the branding of cattle. Data were recorded in the database "Intertrace" (PAN Livestock 2003) and in most respects the current system is on parity with that practised within the European Union (EU).

3.1. Technical and Scientific Factors

The underlying technical issues that emerged during the critical analysis of the problems in 2000-2002 were based on the following observations and analysis: (1) the programme eradication strategy, with only 24 months of treatment, was conceptualized using a simulation model with defective assumptions, (2) the duration of post-treatment surveillance prior to certification of provisional freedom from the tropical bont tick was also deficient. However, both of these technical considerations may have been influenced previously by political and economic factors during project formulation and in the initial stages of implementation.

For the purpose of this discussion, and an understanding of the proposed new pathway for attaining certification for provisional freedom from the tropical bont tick, the original technical model for eradication is briefly reviewed. In the Caribbean, the only hosts for the tick other than domestic livestock, and rarely dogs and donkeys, are the cattle egret and the small Indian mongoose *Herpestes auropunctatus* (Hodgson). Studies in Guadeloupe showed that almost 95% of larvae, 97% of nymphs and of 100% adults feed on domestic hosts.

Two computer simulation scenarios for tick eradication strategies were developed based on the following scientific data: (1) the total accumulated maximum survival period for eggs, larvae, nymphs and adults was 46 months. A treatment programme of 48 months would be required, and (2) the survival period of adult ticks was only 20 months, and theoretically therefore, 24 months of intensive treatment might suffice.

This second scenario, which was considered cost-effective, had been adopted for the eradication strategy. Unfortunately, it made two important, but erroneous, assumptions: (1) that all tick stages would concentrate within the hosts grazing area or range, and (2) that all livestock would graze within that range, and be treated every two weeks.

The latter assumption was based on experiences in Puerto Rico where the intensive treatment duration of two years was deemed successful in eliminating tropical bont tick foci. It had been assumed that it would work throughout the Caribbean, but there are at least two reasons why this may not be so.

Firstly, *A. variegatum* had not become well established in Puerto Rico at the time intensive treatment programmes were implemented. It was most unlikely that *A. variegatum*

immatures had become adapted to feral hosts. In contrast, in most other Caribbean islands the tick had been present for at least 10-15 years and was well adapted and established before intensive treatment programmes were implemented.

Secondly, a further factor influencing the widespread dispersion of the tick was related to livestock management systems. In Puerto Rico, most livestock are kept commercially and maintained in enclosed or fenced commercial properties. Thus, all stages of ticks would be exposed to hosts that have been treated with acaricide. It is also known that immature stages of *A. variegatum* are known to survive longer in bush scrublands than on well-managed pastures.

In contrast, in the other Caribbean islands very few livestock are managed commercially except on government farms. The majority are free ranging. In some islands, for example Antigua and Nevis, there is a high proportion of feral livestock. In these situations, there could well be small pockets of residual infestations of *A. variegatum* that are not exposed to acaricide-treated animals, due to larvae and nymphs feeding on non-domestic small ruminant hosts for periods of up to two years. Drought and other factors influence the grazing areas of domestic animals, especially goats, and as such, untreated or infrequently treated hosts may pick up residual adults some 24-48 months after the original deposition of eggs.

Animals such as mongooses may also play a role in moving ticks among farms and grazing areas. They may support tropical bont tick immature populations that may emerge as adults after the completion of the two year treatment programme (Corn et al. 1996). In 2001, the project carried out a small survey for tropical bont ticks on cattle egrets on St. Kitts, but no ticks were found on 50 birds captured.

It can be concluded that within the CAP islands, the mandatory treatment period should have been a minimum of four years and not two years. Notably, potential donors had raised this critical issue during the project formulation and review before the implementation of the programme in 1994. Moreover, the tropical bont tick-free surveillance period should have been a minimum of 15-18 months after the cessation of island-wide treatments, rather than six months as had also been adopted from the Puerto Rico experiences. These rather complex and varied management factors undoubtedly contributed to the prolonged persistence of infestations of tropical bont tick in the Caribbean islands.

Table 4 summarizes representative tropical bont tick surveillance data for 1999-2004 for Barbados, Nevis, St. Kitts and St. Lucia.

From 2000 onwards, a critical analysis of tick surveillance data led to the need to develop a more stringent pathway to attain certifi-

Table 4. *Animal and farm tick prevalence (%) for selected Carribean islands and years.*

Island		1999	2000	2001	2002	2003	2004
Barbados	Animal	0.05	0.05	0.01	0.01	0.01	0.00
	Farm	n.c.[1]	1.00	0.30	0.09	0.27	0.00
Nevis	Animal	0.11	n.c.	0.10	0.19	0.12	0.04
	Farm	n.c.	n.c.	1.61	1.72	1.31	0.71
St. Kitts	Animal	0.28	0.10	0.05	0.21	0.16	0.76
	Farm	n.c.	0.28	0.17	0.67	0.50	n.c.
St. Lucia	Animal	0.13	0.09	0.02	0.10	0.02	0.04
	Farm	n.c.	1.95	0.24	0.52	0.72	0.12

[1]n.c. = not calculated

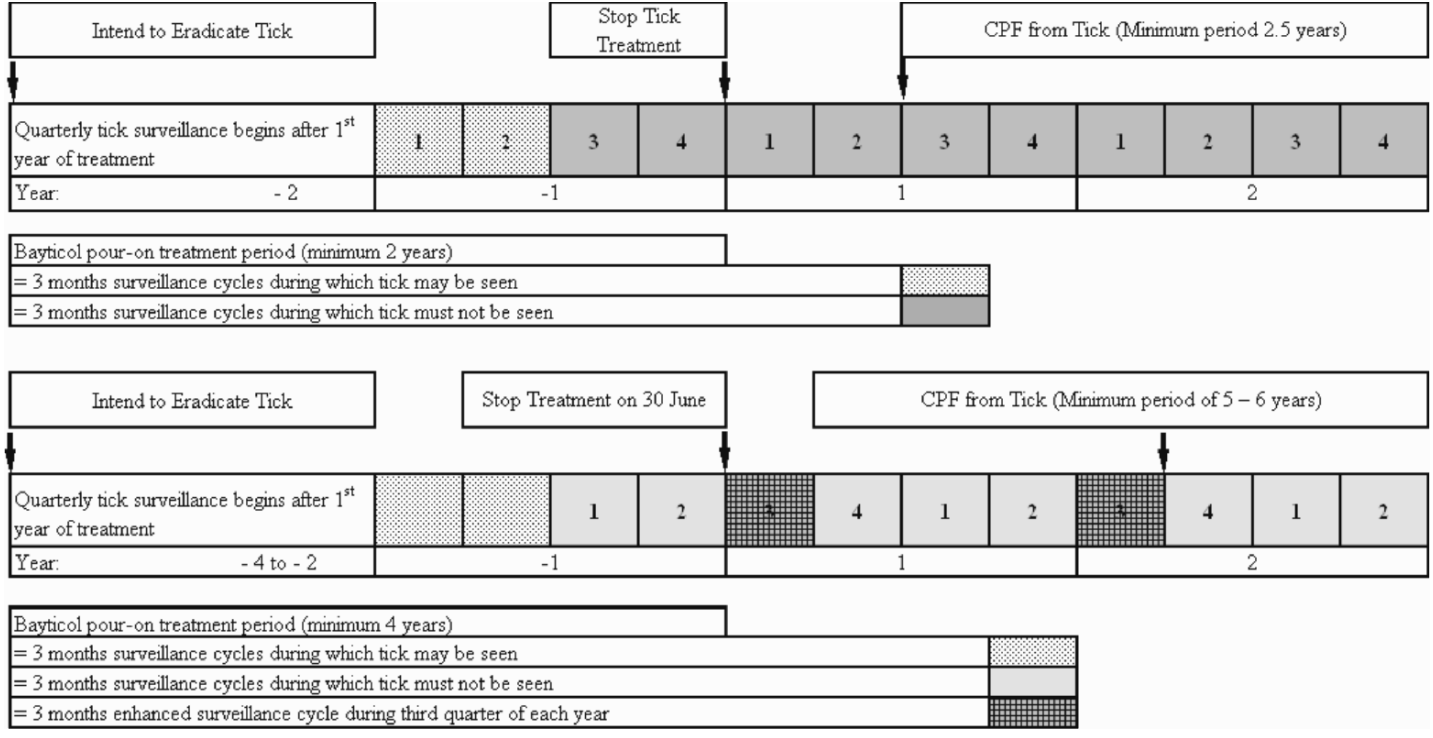

Figure 3. *Original (upper) and revised (lower) pathway to obtain the Certificate of Provisional Freedom (CPF) from tropical bont tick.*

cation of provisional freedom, concurrently with the obvious need for prolonged treatment, and an extended period of surveillance after the cessation of treatment. The original eradication pathway to demonstrate certification of provisional freedom is outlined in Fig. 3 (upper) and the current pathway in Fig. 3 (lower). Both pathways show the shortest route possible under the set conditions. In the new pathway, if the treatment period ends before 30 June, the period of surveillance after treatment must include two third quarters and will be longer than if treatment ends at 30 June (i.e. 15 months).

Surveillance for the tropical bont tick should continue indefinitely after the award of Certificate of Provisional Freedom according to protocols and using a variety of surveillance techniques, including participatory methods. Ultimately, recognition of complete freedom from the tick will depend on demonstration that the region is free, rather than an individual island.

The new quantitative surveillance protocol introduced in 2000 assumed that it was surveying the tropical bont tick population, while in fact it was only sampling that part of the parasitic population feeding on the livestock host at the time of sampling. In cooler, dry seasons, this method is very misleading as the greater part of the tick population at that time is not in a parasitic phase where it can be sampled, but in a terrestrial phase where it will be undetected. A quantitative surveillance protocol requires that the likelihood of tick detection be spread evenly throughout the sampling frame, which in this case is the livestock holding. However, some ticks may survive in small refuges where either livestock are not being treated with acaricide through inaccessibility or owner neglect, or they are using a feral host, which similarly is untreated. Thus, by necessity, this method of surveillance focuses only on the parasitic phases of the tick.

Two other technical factors that were of concern during the formulation and donor review phases had a more positive outcome. Firstly, there was a suggestion that if prolonged, intensive use of a pyrethroid acaricide may lead to resistance problems. During the 10-year period of application, there were only two minor cases, both in government dairies that had been using Bayticol® for several years for control of *Boophilus microplus* (Canestini) before the implementation of the tick eradication programme. In both cases, in St. Kitts and in St. Lucia, after laboratory confirmation of resistance, a switch to amitraz for between six and 12 months resolved the problem. No resistance problems were encountered with *A. variegatum*. The second problem envisaged was the disruption of enzootic stability to anaplasmosis and babesiosis following intensive control of the vectors. Only in St. Kitts following the cessation of intensive treatment in 1998 and 1999, did a few cases of babesiosis occur, and most of these were treated successfully with Imizol®.

3.2. Administrative and Management Factors

The complexity of operating an eradication programme in several countries within a multi-donor and multi-organizational framework, with many conflicting, often incompatible agreements, and the need for maximum operational flexibility was a major challenge throughout the eradication programme.

There are several critical issues to be learnt from this programme, although they are not necessarily new. Many of them were reviewed during the presentations at an international conference convened in 1998 (Lindquist 2000). Other relevant features were considered and adapted from former large-scale eradication and control programmes, including screwworm in North Africa, and tick control and eradication efforts in Africa, Australia, and the USA (Pegram et al. 2000a). Wilson (1996) also reviewed historical tick and tick-borne disease eradication programmes in Zimbabwe and considered the implications for the proposed tropical bont tick eradication programme for the Caribbean.

Critical issues and constraints, include the following: (1) programmes should be managed

independent of political and institutional bodies; that is, managed by an autonomous body. Lindquist (2000) noted that:

> *...some programmes have failed not because of inappropriate technologies, but because of conflicting political and institutional agendas.*

(2) full support of producers and producer associations is essential, (3) appropriate legislation must be in place, agreed upon and implemented, (4) defined goals are essential as well as standard systems to monitor compliance and verify status, and (5) research should not be carried out within the area of eradication or suppression.

However, several key lessons contributed positively to the initial successes. The positive lessons are as follows: (1) the public information and social marketing aspects of the programme, including education and communication, are very important, (2) the important role played by the Amblyomma Programme Council as an independent body and the flexibility of "informal *ad hoc* working groups" as approved by the Amblyomma Programme Council, (3) the continuous informal contact and meetings, particularly between the main technical and donor institutions, for example the USDA and the CAP-Regional Coordination Unit, (4) the commitment and support of the Ministry of Agriculture of the participating governments, (5) the positive response and compliance of most of the target livestock owners, and (6) the use of only proven technical methods.

The main conclusion to be drawn from these experiences is that international collaboration is a valuable tool in the implementation of multi-donor, multi-institutional funded programmes. However, rather than a multitude of individual, potentially incompatible agreements being made, a single multi-organizational agreement is essential to synchronize and harmonize operations under the leadership of a single technical implementing agency. Democracy can be ensured through a body such as the Amblyomma Programme Council, but it should be empowered with some legally acceptable authority. In describing the preparation and implementation of such cooperative agreements, Wyss (2000) makes a very pertinent observation, emphasizing the importance of interpersonal relations during execution:

> *Experience has shown that the best agreement with the wrong mixture of people may not work as well as a bad agreement with the right mixture of people.*

3.3. Funding Issues

The original cost estimates were grossly underestimated. In 1995, CAP recalculated costs for a conventional approach using public service (government) delivery and concluded that it was prohibitively expensive particularly for manpower and transportation. For example, in Antigua alone about USD 5 million were required, whereas in the project document only USD 500 000 had been allocated. Notably, in 1988 the proposed USDA/United States Agency for International Development (USAID) pilot project for Antigua was valued at USD 4.8 million. It was then decided to change the approach with much greater involvement of livestock owners to make them responsible for the mandatory treatment. Serious funding problems occurred periodically throughout the programme.

Two other issues impacted on effective implementation. Some agencies managed their own funds independently and staff inputs were not always complementary or compatible, and continuity was often a problem. Secondly, the question arose: was there a conflict of interests between the major donors/partners? The EU and FAO perceived the eradication of the tropical bont tick as having livestock development potential, that is, to rid the region of a pest that limits livestock production. In contrast, the USDA perceived the tick as a major potential risk to the livestock industry in the USA. The contrasting philosophies complicated the implementation and approach, and the sourcing of complementary funding.

3.4. The Future?

There is inadequate funding for 2005 to con-

tinue the eradication programme on Antigua and to support surveillance and database management on other islands. Both USDA and FAO continue to seek additional funding to complete the eradication by 2009, but if it is not found by the end of the year, then the eradication efforts must be terminated and the programme may need to revert to a tick management programme. It is recommended that if a tropical bont tick management strategy is to be adopted on all islands, then full cost recovery for acaricides and a stronger application of the existing legislation, must be enforced to minimize further spread of the tick.

4. Conclusions

International collaboration is a valuable tool in the implementation of multi-donor, multi-institutional funded programmes. Nevertheless, rather than several individual, conflicting agreements being made, a single multi-organizational agreement is considered essential to synchronize and harmonize operations under the leadership of a single technical implementing agency. In order to achieve the final goal of tropical bont tick eradication in the Caribbean, various aspects of the programme including administrative, management, political and technical, require better harmonization. The time-lapse experienced between decision-making and actual funding has been a major challenge throughout.

The original time frame, activities and budget indicated for the various phases of the project were markedly underestimated and required several readjustments in respect of specific situations in each island. Moreover, revision of the treatment and surveillance protocols was required taking into consideration the life cycle of the tick and the different animal management systems in the Caribbean.

On a positive note, the project highlighted the fact that under an informal umbrella of the Amblyomma Programme Council, a cross-section of islands in the region was able to work together and overcome considerable constraints to attain the various intermediate goals, including farmer registration, animal identification, implementation of livestock database(s) and, in several cases, attain provisional tropical bont tick-free status. Those achievements could still lead to total eradication if adequate funding becomes available in the near future. The importance of public information campaigns and legislation was demonstrated during the project. However, increased government support, particularly to ensure implementation of the legislation, is required to guarantee full compliance by all parties.

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Section 8

Lessons Learned

Lessons from Area-Wide Integrated Pest Management (AW-IPM) Programmes with an SIT Component: an FAO/IAEA Perspective

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ABSTRACT Area-wide integrated pest management (AW-IPM) programmes that integrate the release of sterile insects are complex and very management intensive undertakings. Their success depends upon continuous interactions between essential components of the system of pest suppression. Although there are many AW-IPM programmes that very effectively incorporate the release of sterile insects, success cannot be taken for granted. From an analysis of successful programmes and those beset with difficulties, several essential technical and managerial prerequisites for success were extracted. Technical requirements included: the availability of high-quality baseline data to develop an appropriate strategy, adequate competitiveness and mating compatibility between the strain used for release and that of the target population, persistence of the quality of the release strain, and sound monitoring. On the managerial side, the prerequisites of success were: commitment of all stakeholders, adequate funding, a flexible and independent management structure with dedicated full-time staff, independent peer reviews and consistency in the implementation of critical programme components taking into account differences in local ecological, socio-economic and political conditions.

KEY WORDS area-wide integrated pest management, sterile insect technique, competitiveness, monitoring, management

1. Introduction

Area-wide integrated pest management (AW-IPM) entails the integration of various control tactics against an entire insect pest population within a delimited geographical area (Klassen 2005). This approach is undoubtedly the most rational available against major insect pests of agricultural and medical importance. Although the approach is area-bound, management intensive and requires the coordinated involvement of local stakeholders and national and/or regional authorities, it results in sustainable long-term pest control (Lindquist 2000). The AW-IPM approach is proactive, i.e. action is taken before a pest population reaches damaging levels, and aims at protecting agriculture and/or human health in an entire area. This strategy stands in stark contrast to the more traditional approaches of

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localized pest management, where a pest problem is addressed on a field-by-field or household-by-household basis in a more reactive way, and the pest is not suppressed in surrounding areas that are economically unimportant such as wild hosts, abandoned crops and backyards (Klassen 2005).

The sterile insect technique (SIT), which interferes with the reproduction of a pest, has become a significant component of many AW-IPM campaigns against selected insect pests. The SIT relies on the production of large numbers of the target insect in specialized production centres, the sterilization of one or both of the sexes, and the systematic and sustained release in the natural habitat in numbers large enough to outcompete the wild pest population. Simple theoretical models developed by E. F. Knipling in the 1950s demonstrated that the sustained, sequential release of large numbers of sterile male insects could drive a target insect population to extinction within a few generations (Knipling 1955). The concept was first demonstrated on the island of Curaçao, Netherlands Antilles in 1954, with the elimination of the New World screwworm fly *Cochliomyia hominivorax* (Coquerel) after only six months of sterile male releases (Baumhover et al. 1955, Baumhover 2002). These encouraging results stimulated a bigger programme in Florida, USA, which ultimately culminated in the largest and most successful SIT-based eradication campaign ever undertaken against a major insect pest, i.e. the elimination of the New World screwworm from the southern USA, Mexico and Central America (1957-2002) (Wyss 2000).

The SIT is not a stand-alone technique and is used concomitantly with several complementary methods within an integrated pest management (IPM) system. In addition to eradication, the technique can also be used for suppression, containment and prevention (Hendrichs et al. 2005). Moreover, a series of technological improvements have reduced the overall cost of the SIT component and optimized rearing procedures (Tween 2004), making possible the weekly production and release of 500 million sterile New World screwworm flies (Meyer 1994) and 2500 million sterile Mediterranean fruit flies *Ceratitis capitata* (Wiedemann). Improved diets, development of genetic sexing strains and automation of segments of the rearing process have resulted in more and better quality sterile Mediterranean fruit flies (Enkerlin 2003), and have made the SIT for these flies competitive with more conventional pest control methods (Hendrichs et al. 2002).

Despite being complex and management intensive, most AW-IPM programmes with an SIT component have yielded remarkable achievements against selected insect pests (Table 1). Undoubtedly, the importance and application of the SIT as part of AW-IPM approaches will continue to increase in the future. Unfortunately, complications have surfaced during implementation of some programmes (e.g. the New World screwworm eradication campaign on Jamaica between 1999 and 2004), or even before activities reached the operational phase, e.g. the ill-fated Mediterranean fruit fly programme in Egypt (Fischer 1997) and various tsetse research and development projects in different sub-Sahara African countries. It is not the objective of this paper to review the many successful AW-IPM programmes with an SIT component as these are well documented in the literature (Dyck et al. 2005a), but rather to provide a comparative analysis of several successful and troubled programmes from which some lessons and essential prerequisites for success are extracted.

2. FAO/IAEA Support to AW-IPM Programmes that Integrate the Release of Sterile Insects

As an independent, intergovernmental, science and technology-based organization, the role of the International Atomic Energy Agency (IAEA) in the field of agriculture is to support its Member States in implementing projects that yield tangible socio-economic impact and contribute to sustainable development through peaceful applications of nuclear

Table 1. *Examples of past and ongoing successful AW-IPM programmes that integrate the SIT to suppress, contain, prevent or eradicate important insect pests.*

Pest species	Target area	Date	Control strategy
Diptera			
New World screwworm *Cochliomyia hominivorax* (Coquerel)	North and Central America	1958-2001	Eradication
	Panama - Colombia border	2002-present	Containment
	Libya	1988-1992	Eradication
Tsetse fly *Glossina austeni* Newstead	Unguja Island (Zanzibar)	1994-1997	Eradication
Mediterranean fruit fly *Ceratitis capitata* (Wiedemann)	Israel/Jordan	1997-present	Suppression
	Madeira (Portugal)	1995-present	Suppression
	South Africa	1997-present	Suppression
	Spain	2003-present	Suppression
	Tunisia	2001-present	Suppression
	Patagonia (Argentina)	1992-2006	Eradication
	Mendoza (Argentina)	1992-present	Eradication
	Chile	1992-1995	Eradication
	Chile-Peru border	1996-present	Containment
	Mexico	1978-1982	Eradication
	Mexico-Guatemala	1998-present	Containment
	Los Angeles Basin (USA)[1]	1980-1996	Eradication
	Los Angeles Basin (USA)	1996-present	Prevention
	Florida (USA)[2]	1998-present	Prevention
	South Australia[1]	1946-present	Eradication
Oriental fruit fly *Bactrocera dorsalis* Hendel	Thailand	1987-present	Suppression
Mexican fruit fly *Anastrepha ludens* (Loew)	north-western Mexico	1991-present	Eradication
	Mexico-California border	1960-present	Prevention
	Mexico-Texas border	1984-2002	Prevention
	Mexico-Texas border	2003-present	Eradication
Melon fly *Bactrocera cucurbitae* (Coquillett)	Okinawa (Japan)	1982-1994	Eradication
Queensland fruit fly *Bactrocera tryoni* (Froggatt)	south-eastern Australia	1996-present	Containnent
Lepidoptera			
Codling moth *Cydia pomonella* (L.)	British Columbia (Canada)	1994-present	Suppression
Pink bollworm *Pectinophora gossypiella* (Saunders)	California (USA)	1969-2000	Containment
	south-western USA/northern Mexico	2001-present	Eradication
False codling moth *Thaumatotibia leucotreta* (Meyrick)	South Africa	2004-present	Suppression
Painted apple moth *Teia anartoides* Walker	New Zealand	1999-present	Eradication

[1]Not continuous but short, intermittent outbreaks [2] Previously eradication of several outbreaks

science. The mandate of the Food and Agriculture Organization of the United Nations (FAO) includes the provision of assistance in the area of food, agriculture and nutrition through the collection, analysis, interpretation, dissemination and exchange of information; the delivery of advice on policies, laws and standards; and the provision of technical assistance and the mobilization of support, in partnership with governments, private industry, financial institutions and non-governmental organizations, for agricultural, forestry, fisheries and rural development. Both IAEA and FAO respond directly to the priorities, needs and interests of their Member States, with support being provided through the following two major and complementary mechanisms: (1) FAO/IAEA Coordinated Research Projects (CRPs), and (2) Technical Cooperation Projects (TCPs). CRPs are international research networks designed to develop, improve and/or validate methodologies related to the use of nuclear science and its applications. When and where a given technology is validated for use under field conditions, then upon request from Member States, it may be transferred through one or more IAEA or FAO TCPs. From 1995 to 2005, about USD 36 million and USD 5 million worth of assistance was provided to support SIT-based AW-IPM programmes in Member States through TCPs (largely IAEA) and CRPs, respectively.

The assistance given through these mechanisms in areas related to SIT and AW-IPM has been aimed mainly at: feasibility assessments and baseline data collection, development of local capacity in support of implementing AW-IPM programmes with an SIT component, supplying specific equipment, providing training and expertise, promoting international coordination of supranational programmes, and developing improved rearing and quality assurance/management procedures.

The direct and indirect application of these two mechanisms by the organizations to support the implementation of AW-IPM programmes has provided valuable data and experience suitable for analysis and extraction of factors conducive to programme success or failure.

3. Prerequisites for Success and Lessons Learned

The successful implementation of AW-IPM campaigns with an SIT component, depends on complex and effective interactions between its major components, i.e. baseline data collection, pre-release population suppression, mass-rearing of the target insect, sexual sterilization, handling procedures and field release operations, monitoring programme progress, quality control of sterile insects, public relations, etc.

The scale of these programmes can vary from a few square metres (e.g. whitefly control in greenhouses (Calvitti et al. 2000)) to several 100 square kilometres (e.g. the SIT-based AW-IPM programme against the tsetse fly *Glossina austeni* Newstead on Unguja Island, Zanzibar (Vreysen et al. 2000)) or to several million square kilometres (e.g. the New World screwworm campaign in the USA, Mexico and Central America (Wyss 2000)). Considerable funds are usually needed and programme delays or failure cause significant economic losses. Consequently, after defining the strategic approach, e.g. suppression, containment/prevention or eradication (Hendrichs et al. 2005), each campaign requires the development of an appropriate strategy and corresponding thorough and detailed operational planning well before it is initiated.

A critical analysis of various successful and a few troubled AW-IPM programmes involving the SIT has allowed some major technical, managerial, logistical and strategic requirements to achieve success to be identified.

3.1. Technical Requirements

3.1.1. Collection of Baseline Data and Development of an Appropriate Strategy

Before implementing an AW-IPM programme, especially one that integrates the release of sterile insects, essential data on the

distribution, density and dynamics of the target population must be collected. As each pest population in a given target area is unique, control strategies should be developed that are adapted to the ecological, topographic and technical characteristics of each situation. It is therefore unwise to directly transfer control techniques and technologies that have proven their effectiveness under specific conditions to a different geographical area.

In the programme throughout the Okinawa Archipelago of Japan, that successfully implemented the SIT against the melon fly *Bactrocera cucurbitae* (Coquillett), knowledge of population ecology was seen as an essential prerequisite (Koyama et al. 2004). Before the SIT activities were implemented, accurate data collected on the number of wild male melon flies on the target islands were analysed using relatively simple mathematical methods such as the Petersen index, the negative and positive Jackson method or modified versions thereof (Itô 1973, Hamada 1976, Itô and Koyama 1982). These studies were carried out in various vegetation types in view of the extreme differences in the densities of the fly populations, e.g. on average 7.04 wild males per hectare in subtropical rain forests and on average 622 wild males per hectare in villages with cultivated fields (Itô and Koyama 1982). These data were complemented by studies on the mating behaviour of wild and mass-reared strains and on some genetic aspects of the wild population (Koyama et al. 1986, 2004). As a result, accurate data on the total number of fruit flies on each of the islands was available, which was critical to develop a strategy for meeting the suppression, rearing and release requirements (Koyama et al. 1982).

In spite of being an emergency programme, the New World screwworm eradication programme in Libya was preceded by an intensive and detailed planning phase, deemed essential for the development of the control strategy and corresponding detailed operational plans. The field director, D. A. Lindquist commented later that the programme would have never succeeded without this long, often frustrating, but necessary detailed planning (FAO 1992). This generated a sound operational (action) plan consisting of: (1) intensive monitoring to assess the distribution of the infestation and to prevent its spread to neighbouring areas and countries, (2) extensive training to improve local expertise, and (3) employment of foreign consultants and experts to assist with the collection and/or analysis of a variety of data on the New World screwworm in Libya, including mating compatibility studies to assess the possibility of using the strain being mass-reared in Mexico for implementing the SIT (FAO 1992). In both the melon fly programme in Okinawa and the New World screwworm programme in Libya, these preliminary studies and planning exercises were considered to be vital to success.

The availability of a good set of baseline data is, on the other hand, no guarantee that a sound action programme will be developed. At the start of the AW-IPM campaign against the tsetse fly *Glossina pallidipes* Austen in the Southern Rift Valley of Ethiopia (Alemu et al., this volume), entomological data on the spatial and temporal variations in the distribution and density of the tsetse populations were efficiently collected with five field teams over an area of 10 000 square kilometres during a period of one year (Vreysen et al. 1999). Considerable time elapsed, however, between the collection of the data and the development of a strategy during which unavoidable changes occurred in pest population distribution and land use patterns. This highlights the need for a timely analysis of the data after collection to allow proper programme initiation and implementation.

The baseline data collection exercise in the Mediterranean fruit fly programme in Egypt (1984-1986) provides an example of data collected that were never properly used. During the initial years of the programme, an extensive and efficient network of 8000 fly traps that covered 95% of all agricultural areas was set up and data were collected on a weekly basis. After one year, detailed and accurate field data on fly population distribution and

Figure 1. *Field cage to conduct studies on the mating behaviour and mating compatibility of insect pests.*

dynamics were available from most areas. However, its processing and in-depth analysis was never completed; the data were never published and/or made available to the scientific community (Hendrichs 1986).

The New World screwworm eradication programme in Jamaica was initiated in 1999 with little data available on spatial and seasonal fluctuations in the density of the native population. With only historical screwworm case data (Rawlins and Chen Sang 1984) and results from limited ecological or behavioural studies available, the programme started on the premise of the "infallibility of the SIT against New World screwworm". This was understandable in view of a history of decades-long successful use of the SIT in eliminating screwworm populations. The Jamaica programme was considered to be a "turnkey" operation that applied an "off-the-shelf" technology package, but that failed in an important component – effective suppression of the native population through the continuous treatment of wounds. Consequently, protocols such as release densities established and developed for New World screwworm programmes in Mexico and Central America were simply transferred to Jamaica (Grant et al 2000). The key assumptions were that: (1) the New World screwworm population on Jamaica would be similar in many ways to populations on the mainland, and that (2) farmer participation would be adequate to suppress the indigenous population. Pre-programme collection of baseline data on population densities, the importance of feral dogs in the ecosystem as hosts, etc., might have persuaded the managers to develop of a somewhat different, but possibly more efficient, control strategy.

3.1.2. Competitiveness of Sterile Males and Back-Up Strains

Insects tend to lose their field competitiveness after being mass-reared for a number of generations; therefore procedures need to be in place to monitor the extent of relevant changes. Most progress in developing protocols to measure competitiveness of mass-reared males when competing with wild males for wild females has been made with tropical fruit flies. For these species, field cage tests (Fig. 1) have been developed that use standard procedures for assessing the most appropriate parameters (FAO/IAEA/USDA 2003). These tests ensured that the Mediterranean fruit fly strain selected for mass-rearing and release was fully compatible and competitive with the target population in eradication or suppression programmes in, for example Patagonia (Argentina) (Cayol et al. 1999), the Hex River Valley (South Africa) (Barnes et al. 2004) and in the Arava/Araba Valley (Israel/Jordan) (Cayol et al. 2004).

The importance of conducting these studies prior to embarking on an operational programme was exemplified in the case of the South American fruit fly *Anastrepha fraterculus* (Wiedemann). Mating barriers were revealed between strains of this species from different geographical areas in South America indicating that, unlike the Mediterranean fruit fly, different strains of *A. fraterculus* will have to be reared to apply the SIT against different geographical populations (Vera et al. 2006).

In addition to field cage studies, innovative developments such as the introduction of the filter rearing system have been instrumental in improving and maintaining the quality of the male-only strain of the Mediterranean fruit fly. In this approach, a mother colony is main-

tained under "relaxed rearing" and optimized environmental conditions. Eggs are derived from this mother colony on a regular basis for large colony development. This reduces the accumulation of genetic changes induced by mass-rearing over time because all of the mass-reared insects are released and none of these are returned to the mother colony (Fisher and Cáceres 2000, Cáceres et al. 2004).

Prolonged mass-rearing of New World screwworm was found to induce quantitative and qualitative changes in the profile of their cuticular hydrocarbons (Pomonis and Mackley 1985, Pomonis 1989). The exact composition of these chemicals is essential for a New World screwworm male to recognize a female for mating. Rearing-induced changes in this composition can lead to asymmetric mating patterns in which wild males select only wild females, whereas sterile males may mate at random both with wild and sterile females (Hammack 1987). In small-cage mating studies with wild and mass-reared strains of New World screwworm in Cuba and Jamaica, very high mating preferences (96-100%) of wild males for wild females were observed (García 2002). Moreover, recently developed mathematical models have indicated that twice as many sterile insects may be required to compensate for this mating imbalance (Vreysen et al. 2006).

To minimize the risk of rapid reduction in quality, New World screwworm strains for mass-rearing have traditionally been developed from wild material collected in the target zone, and strain replacement was routinely done approximately every two years in New World screwworm programmes in the USA and Mexico (Marroquin 1994). These procedures, crucial for the success of earlier New World screwworm programmes applying the SIT, were neglected for the eradication programme on Jamaica. The programme managers on Jamaica had, however, no influence on the selection of the strain used for mass-rearing and release. This decision was made unilaterally by the provider of the sterile males (outside the programme) and was, understandably, based upon the provider's own needs and priorities. These releases were initiated with a strain originating from Costa Rica that had been mass-reared for seven years, followed by a strain from Panama that had been maintained under laboratory conditions for six years. The development of a Jamaican strain was only taken into consideration in 2003. In addition, the compatibility between the strain selected for release and that of the target population was assessed only in small, overcrowded cages in a laboratory; a method hardly appropriate to predict mating interactions in the field.

3.1.3. Quality Assurance of Sterile Insects

As mentioned before, the capability of sterile insects to successfully compete with wild males for wild females is of prime importance for the success of AW-IPM programmes with an SIT component. When sterile insects are purchased from outside the programme, a contract should therefore be elaborated which includes specifications for quality assurance of the sterile flies, both in the laboratory and in the field. This assessment, using mutually agreed procedures, should be carried out regularly by both the supplier and the end-user and periodically checked by independent experts. A penalty clause should be included in the contract that would be applied in case the quality of the sterile flies is below the agreed standard, or when the required number of sterile insects is not supplied according to the agreed schedule.

In this respect, most progress has been made with tephritid fruit flies and standard quality tests are now used in control programmes worldwide (FAO/IAEA/USDA 2003). This greatly facilitates taking corrective measures in case mean values are obtained which deviate from the expected mean values stated in the manual.

The importance of routinely using standardized quality control tests was exemplified during long-distance shipments of Mediterranean fruit flies from Guatemala to Israel for use in the Arava/Araba programme (Cayol et al. 2004). Due to increasingly strin-

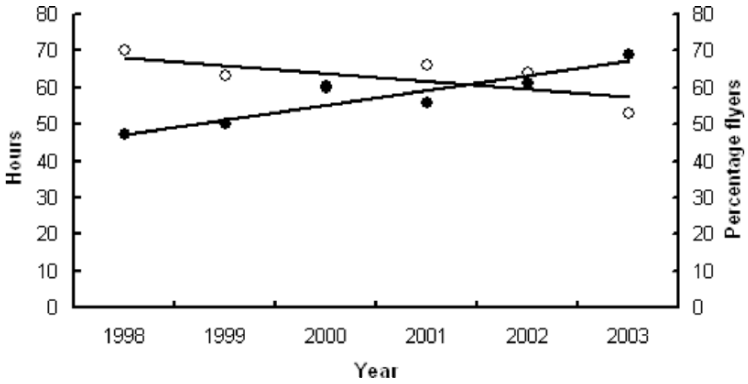

Figure 2. *Quality control data for sterile Mediterranean fruit fly pupae shipped from El Pino, Guatemala to Sapir Centre, Israel, showing a decrease in flight ability (open circles) as a result of increased duration of hypoxia (black circles).*

gent air-freight regulations, the durations of shipments of Mediterranean fruit fly pupae from Guatemala to Israel were greatly extended. Through quality control data routinely collected from samples taken prior to shipment and after the arrival of pupae in Israel, a significant decrease in flight ability of emerged males was identified (Fig. 2).

In the New World screwworm eradication programme in Jamaica, a procurement contract was established between the provider of the sterile male screwworm flies and the Government of Jamaica, but the non-delivery of pupae for extended periods during two labour strikes in the mass-rearing facility (Dyck et al. 2005b), and the unfortunate case of an unirradiated shipment that resulted in the release of fertile New World screwworms were, to the writers' knowledge, never properly considered for compensation. Also, the provider of the sterile flies remained reluctant to support induced sterility and other field studies essential for assessing the quality of the released sterile flies. The programme managers on Jamaica were assured that the assessment of the quality control parameters in the rearing facility and in the dispersal centre in Jamaica would be sufficient indicators of fly quality. However, an analysis of one of the relevant quality control parameters (the average pupal weight), indicated that the quality of the shipped flies decreased significantly with time (Fig. 3).

3.1.4. Monitoring Programme Progress

Careful, systematic and continuous monitoring is an essential component of any AW-IPM programme and certainly one with a SIT component (Vreysen 2005). It has been argued that a control campaign can be carried to success without an intense monitoring programme. This may be true if the programme progresses according to expectations, but this is rarely the case. An efficient monitoring programme enables early detection of problems and their swift rectification before substantial damage occurs or irretrievable time is lost. A combination of suitable parameters needs to be selected, which, in the case of programmes that integrate the release of sterile insects, include the ratio of sterile to wild insects and the percent of insects in the native population with induced sterility (Vreysen 2005).

Examples of excellent monitoring components incorporated into successful AW-IPM campaigns are: (1) the eradication of New World screwworm from Libya, (2) the elimination of the tsetse fly *G. austeni* from Unguja Island, Zanzibar, and (3) the removal of the melon fly from Okinawa, Japan.

In Libya, the monitoring consisted of three components: (1) extensive animal surveil-

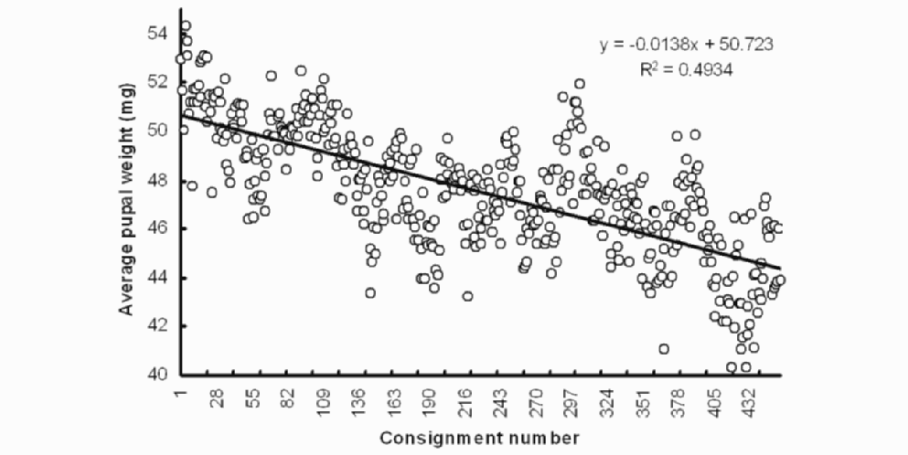

Figure 3. *The average weight of sterile New World screwworm pupae of consignments received on Jamaica from 1999 to 2004.*

lance (Fig. 4, upper, left) by 94 field teams which inspected 16 and 30 million animals for infested wounds in 1990 and 1991, respectively; (2) trapping adult screwworm flies throughout the infested area to assess the degree of uniformity of releases of sterile flies (Fig. 4, lower, right), and to determine the proportion of sterile to wild insects. Particular attention was given to those traps that did not contain sterile flies to assess if the traps were operating properly, if their location was suitable and whether the dispersal of sterile flies was done adequately in that area, and most importantly, (3) the collection of egg masses from deliberately-wounded sentinel animals to determine the ratio of sterile to fertile egg masses, which is the most direct indicator of the SIT's effectiveness (Fig. 4, lower, left) (FAO 1992).

A similarly intensive field-monitoring programme was implemented during the *G. austeni* eradication campaign on Unguja Island of Zanzibar. More than 500 sticky panel traps were maintained permanently at 55 fixed monitoring sites distributed over the entire island in various vegetation types during the main eradication campaign. Fly samples were analysed on a weekly basis, indigenous flies discriminated from sterile flies and all wild female flies dissected to determine ovarian age and to assess whether they had mated with a sterile or a fertile male. This systematic monitoring programme provided weekly data sets on the apparent density of both the wild and sterile fly populations, sterile to wild male ratios at each fixed monitoring site, the rate of induced sterility in the native female population, and the age composition of the female fly population (Vreysen et al. 2000). The entomological component was accompanied by regular monitoring of the incidence of trypanosomes in livestock (Dyck et al. 2000).

A combination of complementary methods was likewise used to evaluate the melon fly eradication programme in Okinawa, Japan, i.e. the trapping of adult flies, the assessment of morphological abnormalities in the testes to distinguish sterile from wild male flies and to determine sterile to wild male ratios, an inspection of host fruits for larvae, and the hatchability of eggs (on an experimental basis because the method is labour intensive) (Koyama et al. 2004).

In all three programmes, the monitoring was executed in strict compliance with the plan, i.e., routinely, rigorously and without interruption. Thus the monitoring component provided programme managers with up-to-date information at all times, which was indis-

Figure 4. *Monitoring and suppression methods in AW-IPM programmes against the New World screwworm: (upper, left) inspection of cattle for screwworm-infested wounds, (upper right) insecticide treatment of wounds, (lower, left) animal with artifical wounds in sentinel pen to collect egg masses to assess the rate of induced sterility in the native screwworm population (Photo from P. Spradbery, reproduced with permission), and (lower, right) a wind-oriented trap to assess the distribution of sterile males and the ratio of sterile to fertile insects.*

pensable for taking timely corrective actions, and overall for the smooth implementation of programme activities.

Consistency in the methods used for data collection before, during and after programme implementation is also very important for analysing programme operations (IAEA 2003). The difficulty created when this rule is violated, is illustrated by trapping in the Mediterranean fruit fly programme in the Araba valley (Rössler et al. 2000). Here Steiner traps were used to collect the baseline data, but the traps were replaced by Jackson, Tephri® and International Pheromone McPhail traps in the later phases of the programme (Cayol et al. 2004), which made the assessment of the effectiveness of the AW-IPM operations in the first year extremely difficult. This was unfortunate because the demonstration of a significant decrease in pest population density is essential to maintain the support of major stakeholders early in a programme.

The New World screwworm programme on Jamaica did not place sufficient emphasis on the systematic collection of various kinds of field data needed to monitor programme progress, and this deficiency was not adequately corrected even though the programme did not advance according to expectations. Programme evaluation was based solely on the number of positive screwworm cases reported, a parameter influenced more by the willingness of the livestock owners to diligently collect larvae from wounds and submit these samples to the programme than by the

effect of the SIT on the ratio of sterile to fertile egg masses. Despite some sporadic sampling of egg masses at the beginning of the programme (Robinson et al. 2000), systematic collection of information on mating frequencies (i.e. the rate of induced sterility) of the sterile males with native female flies was never attempted, even when it became clear that the programme was in dire straits. It was always assumed that the released sterile insects would perform adequately and their potentially inferior competitiveness was not questioned. As a result, by the end of 2004 and after several years of sterile insect releases, the crucial question of whether the released sterile male flies were capable of locating virgin females and transferring their sterile sperm at the desired frequency still remained unanswered.

3.2. Management and Logistical Prerequisites

3.2.1. A Flexible and Independent Management Structure

The organizational structures of most successful large-scale AW-IPM programmes with a SIT component had management components with a high degree of political and financial autonomy, as well as considerable freedom from government regulation and bureaucratic intervention (Dyck et al. 2005b). Good examples include the New World screwworm eradication commissions, which were established initially between Mexico and the USA (Meyer 1994) and subsequently involved the various countries in Central America. The legal authorities and managerial arrangements were based on precedents learned at great cost in the cooperative Mexico-USA programme (1947-1954) to eradicate foot and mouth disease, which had appeared in Mexico and threatened to spread into the USA. Thus, although one high-level representative each of the secretary of agriculture of Mexico and of the USA served on the screwworm commission, the commission had essentially complete responsibility for and control of the eradication campaign. Also, the commission was fully accountable for all financial, physical and human resources. In addition, this legal body was exempt from the usual bureaucratic rules, and the roles and responsibilities of all staff (senior, middle-level, technical, administrative, etc.) were established before the programme was initiated. All staff members were employed full-time. Each major supervisory activity was jointly managed by two professional counterparts, one from each country. As a consequence, the primary ownership of the programme was vested in the commission, rather than in the agricultural ministries of the governments of the countries involved. Nevertheless, the latter were sufficiently involved so that emergency needs for additional finances, personnel, facilities, etc. could be met without costly delays. Also livestock owners from the countries involved were represented on the commission, and their influence was very positive and vitally important.

A different, but similarly efficient management structure was established by the Director General of the FAO for the New World screwworm eradication programme in Libya, i.e. the Screwworm Emergency Centre for North Africa (SECNA). Established to operate outside the formal bureaucracy inherent to an international organization, SECNA received special authority to implement the eradication programme and maintain the flexibility needed to meet unforeseen difficulties. This facilitated rapid decision-making and greatly expedited all operations including procurement, direct communication with donors, countries at risk and other organizations. A director from FAO and a co-director from Libya headed the SECNA field programme and were responsible for all field activities, including management of all resources, programme strategy and its implementation (FAO 1992). This flexible and efficient management unit was essential to the success of the operational programme, and it was able to draw on the strong support of the IAEA. Planning for this campaign had been led by the FAO/IAEA Joint Division of Nuclear Techniques in Food and Agriculture, which also took responsibility for genetic comparison of Libyan and release strains to assure their compatibility,

for other specialized studies, recruitment of experts, etc. (Lindquist et al. 1993). Training of many Libyan personnel in advance of the campaign had been organized and funded by the Technical Cooperation Department of the IAEA. Construction of traps and development of other specialized equipment, procurement of the attractant (swormlure), etc. were accomplished by the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf, Austria.

Other examples of efficient, independent management structures are the Programa Moscamed, a cooperative agreement between Mexico, the USA and Guatemala to contain the Mediterranean fruit fly, and the Arava Medfly Eradication Programme (AMEP) in Israel. The Programa Moscamed is operated jointly by the plant protection organizations of these countries through a special commission. This commission has a management structure that is independent, manages its own budget of ca USD 40 million per year, and retains full authority for all final decisions pertaining to operations. In Israel, AMEP was established with full-time employees in charge of sterile fly handling, release and monitoring. Although the growers largely fund this organization, it is independent in its day-to-day managerial and technical decisions regarding field operations. The Mediterranean fruit fly control programme of Patagonia, largely funded by a private sector organization (Fundación Barrera Zoofitosanitaria Patagónica (FUNBAPA)), is another example of an effective management structure with stakeholder involvement.

In contrast, the Jamaica New World screwworm eradication programme's management structure lacked full-time staff and the same degree of independence enjoyed by the above programmes, and this tended to compromise its effectiveness and efficiency. The Jamaican Ministry of Agriculture operated the programme and staffed it with government civil servants with many other responsibilities and duties within the Veterinary Services Division. Thus, these technical officers were unable to give undivided attention to the programme. In addition, the Ministry of Agriculture's regulations hindered the timely employment of temporary field technicians in accordance with the changing needs of the programme. This constraint was at the root of suboptimal surveillance of the animals and collection of egg masses on the island.

Similarly, the Mediterranean fruit fly programme in Egypt suffered from many administrative problems such as changing leadership, the lack of full-time staff and the establishment of an independent management structure. In addition, the two senior managers of the programme had conflicting views on the programme approach, i.e. one advocated a research approach whereas the other favoured a management and technology transfer approach. Most of the senior programme staff had a research background and lacked the necessary "get-the-job-done" attitude. The programme director was not stationed at the programme headquarters but at a research institute in another city, which resulted in lack of delegation, contradicting orders being issued, and considerable delays in programme implementation. Moreover, several senior staff constantly voiced open doubts about the feasibility of the programme and were convinced that the strategy developed and especially, the use of the SIT, was not the correct approach. All these problems contributed to insufficient support, postponement of important decisions, internal fights for control of funds and ultimately resulted in the premature closure of the project (Hendrichs 1986).

Following the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) initiative, many African nations have or are planning to embark on large-scale area-wide programmes to create tsetse-free zones with the ultimate aim to eliminate the disease trypanosomosis. Most of the current programmes are implemented under existing government ministries and all suffer from restrictive rules and regulations, exhaustive bureaucracies, inflexibility, low staff salaries (and hence, morale), etc.: all contributing to the slow and ineffective implementation of most of these programmes.

3.2.2. Continuity in Implementation of all Essential Programme Components

AW-IPM programmes, especially those that include the release of sterile insects, have several interdependent components; failure to adequately implement one component has drastic implications for the success of the entire programme. Consistency in the implementation of all components is therefore indispensable for success.

In the New World screwworm eradication programme in Libya, the systematic releases of sterile males were combined with an efficient field surveillance programme. Not one interruption occurred in weekly releases of ca 40 million sterile flies per week over the 25 000 square kilometres of infested area, and the field teams travelled daily through their designated zones to inspect all livestock on a 15-21 day-rotation basis (FAO 1992). This regular, uninterrupted inspection of animals facilitated treatment of all wounds (albeit at intervals longer than the 5-7 days required for larvae to mature), and to efficient suppression of the native New World screwworm population. In addition, it allowed the collection of very accurate data on the density and distribution of the screwworm population (see paragraph 3.1.4. on monitoring) (FAO 1992).

In the tsetse programme in Zanzibar, regular aerial releases of sterile male *G. austeni* were initiated in August 1994 and terminated in December 1997. Not one of these regular release flights was omitted, nor was a day of field monitoring activities missed.

On the other hand, between 2001 and 2003, 18 of 51 scheduled shipments of sterile Mediterranean fruit fly pupae from Guatemala to Israel failed to arrive because of the increased rerouting or cancellations of flights in the aftermath of September 11, 2001. This highlighted the lack of alternative production sources of sterile male flies (Cayol et al. 2004) and paved the way for the establishment of a commercial Mediterranean fruit fly rearing facility in the Middle East (Bassi et al., this volume).

Lack of continuity in implementing all programme components can be very detrimental, and this occurred in the New World screwworm eradication programme in Jamaica. Continuity in vital components of the programme was not sustained for any period longer than three months between July 2002 and December 2003. The programme suffered numerous setbacks, especially in 2003 when, in 27 out of 52 weeks, substantial inadequacies occurred in the receipt, quality or dispersal of sterile pupae/flies, or in the field component (Dyck et al. 2005b). Such inconstancy is disastrous in area-wide campaigns against pests, such as the New World screwworm or various fruit flies, which have the capacity to reproduce explosively and disperse widely.

3.2.3. Resources, Manpower and Institutional Capacity

After developing an appropriate intervention strategy, a realistic budget must be prepared to provide sufficiently for contingencies and for essential obligations that must be made before programme operations are initiated. For campaigns anticipated to last two or three years, full funding needs to be available in a dedicated account for all programme components for the entire life of the programme, including salaries, sterile flies, aircraft time, insecticides, monitoring activities, public information, programme review, outside experts, etc. Of course this is not possible for all phases of a major long-term programme such as the complete removal of the screwworm from the USA, Mexico and Central America, which required more than 40 years.

Sufficient expertise in terms of the biology of the target insect, the management of operational AW-IPM programmes and the SIT component, needs to be assembled before embarking on a programme. Gaps in knowledge and in overall institutional capacity need to be identified on a timely basis and remedial training provided. Ample equipment and logistics need to be at the disposal of the programme, including office space, computers, vehicles, traps, sentinel animals, and sufficient back-up systems for all essential programme components (e.g. a back-up fly dispersal chamber, an adequate stock of essential

spare parts, and back-up release aircraft). Skilled personnel with expertise in the proper maintenance and repair of essential programme components must be included in the core team. Contingency plans must be developed to cope with bad weather, hurricanes, and lapses in availability of sterile insects and insecticides, etc. The programme must be shielded from the category of contingencies from which recovery would be difficult or impossible.

An example of a programme that suffered from inadequate back-up systems and contingency plans is the New World screwworm eradication programme in Jamaica. Only one dispersal centre was available that could handle only two shipments of pupae per week. Each week, only 36 hours were available for maintenance and repairs. Consequently, releases were disrupted for six weeks during August-September 2003, when the centre's refrigeration system did not function. In addition, the number of mechanics available for maintenance and repair of the "chill fly unit", the release machines and the facilities were insufficient; these deficiencies proved to be very disruptive for the programme.

Relatively small programmes, such as the Mediterranean fruit fly suppression programme in the Hex River Valley, South Africa and the Arava/Araba Valley, Israel/Jordan have suffered from insufficient funds to allow for adequate back-ups of expensive components of the programme such as the release aircraft. Although regular maintenance was planned well in advance and contingency solutions were put in place (e.g. ground releases of sterile insects and ground bait spray applications), unexpected maintenance needs of the aircraft often caused disruption in sterile fly dispersal for sometimes several weeks. In some cases, these operational bottlenecks allowed the wild fly population to build-up, and this threat required drastic and costly countermeasures.

The budget of the troubled Mediterranean fruit fly project in Egypt proved to be underestimated according to some senior staff. The project director was, however, of a different opinion and consequently, efforts to attract additional funding from the international community never materialized. In addition, there was insufficient provision for operational funds to cover local expenditures, which forced the conversion of convertible currency funds into local currency, depleting further the overall budget (Hendrichs 1986).

3.2.4. The Relevance and Importance of Stakeholders

Before embarking on an area-wide suppression or eradication programme, a firm commitment from all directly affected stakeholders is required. This should be formalized in a document that outlines the specific role and responsibility of each party. In addition, since the collaboration of the beneficiaries (e.g. the livestock holders and growers) is required, a certain degree of organization among these is essential.

The apple and pear growers of the Okanagan-Kootenay Sterile Insect Release (OKSIR) programme against the codling moth in British Columbia, Canada were the initial driving force behind the programme. The federal and provincial governments funded only the initial capital costs of the codling moth rearing facility. Therefore, to cover the yearly operating costs, an SIT parcel tax was levied on commercial apple and pear growers, and a mil rate tax was levied on all rural and urban property owners (Bloem and Bloem 2000).

The AMEP in Israel is funded by the growers of the valley through fees paid to the Plants Production and Marketing Board of Israel, as well as by the Regional Council of the Arava Valley and by the Ministry of Agriculture. AMEP reports regularly to all its stakeholders on the progress made in suppression of the Mediterranean fruit fly and, occasionally, on problems encountered in the field. Corrective measures can therefore be taken for the benefit of the programme with majority support of the stakeholders. Such measures include authorization of AMEP teams to do supplementary bait spray applications in limited areas, and phytosanitary measures to be

implemented by the growers.

The Mediterranean fruit fly control programme of Patagonia was set up in 1997 following a request of growers in the region. The programme is funded by the growers as well as through fees collected for each ton of vegetable and fruit commodities leaving the area under sterile release. Additional fees charged for fumigation of commodities entering the area are also returned to the programme, which retains day-to-day decision making for implementation of the field operations.

The table grape producers of the Hex River Valley in South Africa requested the initiation of the Mediterranean fruit fly suppression programme. It is largely funded through fees charged on exports of Mediterranean fruit fly-free table grapes from the region (Barnes et al. 2004). This budget provides fully for the cost of field operations and personnel. The programme is managed through a partnership of the Pest Management Division of the Agricultural Research Council-Infruitec-Nietvoorbij – a parastatal body with a mandate to conduct research, technology development and transfer – and the Hex Valley Research Services Trust, which represents the deciduous fruit growers. However, since the growers themselves implement field activities, they make technical decisions on day-to-day implementation. In the past, this has resulted in some wrong decisions, which led Barnes (2004) to conclude that:

> *...the overall success of the project would almost certainly have been greater had the project been adequately funded and managed by a single agency.*

A private company is now managing the sterile male production and release (Barnes, this volume).

The livestock producers on Jamaica have an economic stake in the New World screwworm eradication programme, but the programme was mainly started with funding from the USA to eradicate this pest from the last infested areas in the northern hemisphere. The Jamaica Agricultural Society, which represents the farmers, was never deeply involved in the programme, nor did it contribute financially; consequently, it created little programme ownership. A steering committee was established amongst all local and international stakeholders and chaired by the national director of the programme. However, each of the stakeholders involved in the programme always advanced their own interests with inadequate consensus building, and consequently some of the committee's recommendations were not necessarily helpful to the programme.

3.2.5. Data Analysis and Feedback Mechanisms

All field data have to be collected efficiently in accordance with a strict schedule, and transferred in a timely manner to the responsible staff for entry into a database. The data require proper analysis both in space and time, and have to be displayed in formats that allow easy interpretation by programme managers, i.e. tables, graphs and maps. Efficient feedback mechanisms have to be established between programme managers and field teams, the release centre, the release crews and the mass-rearing facility so that the necessary adjustments to the various programme components can be made in a timely manner. Ideally all this information should be made available on a web site, allowing continuous access to all staff and key stakeholders independent of their location. Such a system is operated by the Programa Moscamed in Guatemala and southern Mexico, integrating information from numerous field centres over vast areas and across a border into a consolidated and standardized report that is continuously updated as new data become available.

Another efficient data management system was developed for the programme against *G. austeni* on Unguja Island, Zanzibar. Data from both the field and the rearing facility, including data on quality control assessment of the sterile males, were collated on a weekly basis and made available to all staff and stakeholders. Data were analysed in greater depth every three months. Daily contact between the field and rearing facility was maintained by all possible means of communication, including HF radio. These weekly

and quarterly analysis reports were critical for the decision-making process (Vreysen et al. 2000).

The SIT programme against the New World screwworm in Jamaica relied strongly on the participation of the farmers, who were requested to check their animals for wounds, collect any screwworm larva found in collection tubes, treat the wounds and submit the samples to the local veterinary clinics in the parishes. The programme experienced frequent delays in the submission of samples to the veterinary clinics and in their transfer to the identification laboratory. This delayed data analysis in turn impeded the provision of useful feedback from the management to the field and dispersal crews. Since "reported screwworm cases" was the only data that was systematically collected, proper analysis of the programme's progress was very difficult, if not impossible, and this led to uneducated guesses and wild assumptions.

Problems in SIT-based AW-IPM programmes cannot be solved without collecting data relevant to the problem, and assumptions are rarely an adequate substitute for measurements. The availability of geographical information systems (GIS) makes the spatial analysis, interpretation and display of data easier and their potential should be better exploited in AW-IPM programmes (Cox and Vreysen 2005, Cox, this volume).

3.2.6. Public Relations

AW-IPM programmes require public awareness and education and cannot be conducted in an information vacuum (Bloem and Bloem 2000). They require support and participation of the government, the general public and the farmer community (Dyck et al. 2005c). Bottom-up support from local communities is indispensable. For example, fruit fly and moth control programmes rely on farmers and backyard owners to eliminate infested host material, mosquito control programmes need the support of rural and urban communities to remove any container with stagnant water, and screwworm programmes require the participation of farmers in the surveillance of all animals in the target area.

Increasingly, area-wide programmes must be conducted by the rules of the urban rather than rural settings and meet the concerns of urbanites who lack empathy for agricultural producers and who abhor pesticides applications, destruction of backyard fruit, etc. A sense of outrage can be readily evoked by involuntary exposure to sprays, mandatory fee levies, quarantines, destruction of host trees, plants, or fruit and the right of trespass by programme officials (Sandman 1987). It is therefore critically important to mount a vigorous and thorough public outreach programme well in advance of programme operations to forestall or at least mitigate outrage by well-meaning but ill-informed people (Klassen 1989). Clearly the public relations component is not an option that can be terminated when funds become scarce (Dyck et al. 2005c).

In the New World screwworm eradication programme on Jamaica, public outreach campaigns were funded so inadequately that they could be implemented only sporadically. In addition, at the beginning of the programme an inaccurate message was delivered, i.e. that the sterile flies alone would eliminate the screwworm and there was little need for help from the people. Only later, was more importance given to preventive wound treatment of animals in order to reduce the local wild population.

These public relations campaigns need to solicit support during the entire programme to create constant awareness and understanding among all affected individuals. Insufficient communication between the management of the codling moth suppression programme in British Columbia, Canada and the growers resulted in the latter initially gaining the mistaken perception that the programme was failure from its outset. Insufficient public information during the pre-release phase resulted in high expectations among the growers who did not understand the principle governing the effectiveness of the SIT. Consequently, many growers stopped applying control measures, which resulted in population densities too high for the SIT to cause a downward trend

(Bloem and Bloem 2000). In view of this experience, a very thorough effective public relation effort was developed as a result of which codling moth is now being effectively suppressed (S. Bloem et al., this volume).

During the development of these public relation campaigns, the cultural characteristics and peculiarities of people in local communities need to be taken into account. Evidence that the general public will do little to assist the programme needs to be countered through the employment of additional field staff from these communities to fill that anticipated gap. For example, in screwworm eradication programmes, cultural determinants of the attitudes of the public and farmers towards animals have to be understood and taken into account. In the Jamaica programme, the prevailing behaviour of physically abusing pets and livestock created small wounds, which served as oviposition sites for New World screwworm flies, generated ideal and persistent opportunities for the fly population to sustain itself. The public education activities were largely insufficient to change this attitude. Therefore the employment of more permanent and/or temporary field staff to proactively screen animals and treat all wounds with insecticide – rather than relying heavily on farmer participation – ameliorated this problem.

3.2.7. Independent Programme Reviews

Any pest intervention programme should benefit from regular peer reviews by independent experts to provide constructive criticism, solve disagreements, balance conflicting views between the different stakeholders and keep the programme managers focussed. Any programme manager or staff member closely involved in an operational programme and engulfed in day-to-day problems becomes vulnerable to forming biased, subjective views. Independent external reviews can be instrumental in removing such bias and broadening the perspective of programme staff. Large operational tephritid fruit fly control programmes usually have an international scientific technical advisory committee with members independent of the management of the programmes. This is the case for the Programa Moscamed in Guatemala-Mexico, where a group of international experts reviews all programme components on a regular basis and proposes recommendations or corrective measures relevant to strategic, managerial and technical issues. A similar review mechanism exists for the Mediterranean fruit fly preventive programme in the Los Angeles Basin of California.

4. Conclusions

There are many lessons to be learned from more than 50 years of successfully releasing sterile insects as part of AW-IPM programmes. The analysis provided in this paper indicates that success cannot be taken for granted and that no programme can be fail-proof, despite such a long history of successful SIT implementation. This technology cannot be transferred "off-the-shelf", and thus has to be adapted to the ecology and socio-economics of each situation, as problems encountered are often not related to the SIT *per se*, but rather to the management and logistics of implementation. This is not surprising since AW-IPM programmes that include the sterile insect technique are technically complex and management intensive with several interdependent components that require timely, consistent and thoughtful implementation. Well in advance of mounting an area-wide campaign, both a strategic and more detailed operational plan need to be formulated and the commitment of all stakeholders needs to be assured. To facilitate realistic planning, appropriate baseline data must be collected. The key strategic objective needs to be defined such as suppression or eradication (Hendrichs et al 2005). An analytical mindset is essential both for planning and for leading and managing an ongoing programme. Some key questions are: (1) what needs to happen in order to reach the strategic goal?, (2) are the key processes being monitored and the data being collected that will inform whether the things that need to happen are, in fact, happening?, (3) what

could go wrong that would be very difficult to overcome, and likely result in failure?, and (4) what measures are in place to guard against such potentially disastrous developments?

These key questions should be asked when examining the following technical issues: (1) competitiveness of sterile males and back-up strains, (2) quality assurance of sterile insects, and (3) monitoring programme progress.

Similarly these same questions should be asked when considering the following management and logistical issues: (1) is the programme management structure sufficiently flexible and independent?, (2) is there a high degree of continuity in implementation of all essential programme components?, (3) are available resources, manpower and institutional capacity sufficient in magnitude and quality to assure effective operations for the length of the programme?, (4) does the programme have the support of all stakeholders and firm commitments from those who must bear costs or conduct relevant operations?, (5) are essential high quality data being collected and properly analysed in a timely manner to enable the programme management to provide feedback essential for corrective action by all key programme personnel?, (6) is the public awareness and public education programme of sufficient quality to help shape attitudes and behaviours in support of programme success?, and (7) is the programme benefiting from timely and independent reviews?

Most insects are not distributed at random but are aggregated. Sterile males are so much more efficient in locating virgin females in nature than entomologists or programme managers ever will be (Krafsur 2003), and this makes the SIT such a powerful technique. Several critics have disputed its effectiveness, but the long history of successful programmes against key insect pests largely refutes these criticisms and has demonstrated the benefits these programmes can bring. However, programmes using technologies proven highly effective over long periods of time may still be ineffective or fail in a new ecological and social setting. As more knowledge becomes available on mating behaviour, breeding structures and genetic relationships between populations, more efficient AW-IPM programmes can be developed and implemented in which SIT can play a significant role (Krafsur 1998, Robinson and Hendrichs 2005). The risk of failure can be minimized provided a series of prerequisites, outlined in this paper are met. Especially, in AW-IPM programmes with an SIT component, more efforts have to be undertaken to estimate mating frequencies to assess the impact of the released sterile insects on population suppression.

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