Sustainability in Plant and Crop Protection 14

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Sustainable Management of Invasive Pests in Africa

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This book is dedicated to Dr Jean Nguya Kalemba Maniania (former Head of ICIPE's Arthropod Pathology Unit – APU) in recognition of his outstanding contribution to insect pathology and integrated pest management using entomopathogens.

His scientific qualities have paved the way for many African entomologists and insect pathologists, including three of the co-authors of this present book, namely Dr Sunday Ekesi (Nigeria), Dr Lorna Migiro (Kenya) and Dr Saliou Niassy (Senegal).

We significantly benefited from his scientific rigour and personal qualities, which have been essential for the expansion of insect science in Africa.

Preface

After many years of research in the field of invasive species, we have learnt that these pests can be disastrous to many cropping systems in Africa, especially in a context where countries are not prepared and do not have monitoring and control systems in place. Thousands of organisms can be labelled as invasive. In fact, the list of invasive species is unlimited, yet, within the system itself, some species can become invasive due to habitat change. Unfortunately, the debate amplifies when there are economic or environmental implications that affect livelihoods in general. The topic of invasive species has become viral lately on the continent. The frequent invasions and permanent threats speak for the importance of this topic, especially with the recent occurrences of species such as *Tuta absoluta* and the fall armyworm *Spodoptera frugiperda* within a short interval period. The pattern for managing these pests, when they occur, is almost similar in all African countries. There has been a very poor effort to harmonise or coordinate research, and little willingness to centralise the various experiences or research outputs to facilitate continuous learning among researchers, academics and related regulatory authorities.

In the academic world, invasive species open new areas of research, including modelling and remote sensing, in short, geomatics, which serve as model systems for the development of an understanding of climate change and insect behaviour. These aspects can help in developing monitoring surveillance, climate suitability studies for an invasive species and its natural enemies, and the decision-making processes on the best appropriate tool for the management of the pest.

When invasive species affect essential crops, such as maize and tomato, or other horticultural crops such as citrus and mango, they automatically become a political or business concern. While invasive insects are part of a more prominent thematic area, which contains weeds, mites and fishes, they also feature strongly in applied research aimed at managing them by using chemicals, biopesticides and natural enemies in order to recommend sustainable solutions. Surprisingly, little emphasis has been given by African governments to applied research or the capitalisation of research outcomes to bolster readiness and preparedness.

We believe that there is a wealth of knowledge on invasive species which is available, but this knowledge is loose and difficult to assemble. This prompted our initiative to use the forum of the African Association of Insect Scientists, AAIS, to produce this book to centralise the findings on the management of most common invasive species, such as fall armyworm, *T. absoluta*, fruit flies and others.

We believe that emphasis should be placed on understanding the biology of the invasive species and their ecology. This could be done by borrowing from the experiences of countries where the invasive species originated. We have noticed that the African continent is increasingly lacking expertise in taxonomy and identification, which is a critical discipline that is essential in the recognition and reporting of insects.

We attempted to gather contributions from various areas in Sudan, Kenya, Ethiopia, Nigeria and Rwanda to record the relevant knowledge, so that it does not fade away over time, as it happened with previous researches on the topic. However, this exercise has not been easy at all, considering the various research areas and interests, including chemical ecology, arthropod pathology, technology transfer, legislation, biological control, chemical control and socio-economics.

Hence, this book project might be one of a kind to integrate aspects of invasive species, presenting various facets of the problem. The lack of capacity is worrying, because, without a sound understanding of the pest, there is no basis for adequate decision making.

Thus, we have aimed to fill a gap in the literature and produce a book that will inform, support and strengthen the work of African researchers and policymakers in the management of invasive species. This book covers aspects of pest management, taxonomy, regulation and technology transfer. We believe that much of this material will also be of interest to entomologists more widely, and to very many others on the continent.

The scope of the material is therefore broad and may be a valuable companion to other research, depending on readers' interests. As such, this book will be a useful reference text for the private sector and agricultural extensionists. It should also be a handy text for those who are researching solutions to combat invasive species.

This book would not have come into existence without the support of our respective institutions: the Centre for Agriculture and Biosciences International, CABI, and the International Centre of Insect Physiology and Ecology, ICIPE. The Plantwise Programme provided financial support for most contributors to attend the 22nd AAIS Meeting and Scientific Conference in Sudan in October 2017. Without that support, this project would not have even started.

Dr Segenet Kelemu, Director General of ICIPE, provided generous support for the participation of ICIPE delegates to the Conference. Dr Sunday Ekesi, Director of Research and Partnerships, took on this project, guided the handling and added valuable contributions through consultative meetings and participation in various forums on the topic.

We thank the German Academic Exchange, DAAD, for generously funding the AAIS Conference in 2017.

Colleagues at ICIPE, CABI, other National Agricultural Research Services (NARS), the Consultative Group on International Agricultural Research (CGIAR) centres, and Universities have contributed in diverse ways, including reviewing, reading and commenting on chapters, suggesting references, providing original images, and supplying specimens and photographs that became figures. We are grateful to them for all their insights and commitment to science: Drs Sunday Ekesi, Sevgan Subramanian, Samira Mohamed, Fathiya Khamis, Shifa Ballo, Amanual Tamiru, David Mfuti Kupesa, Washington Otieno, and others. Dr Lorna Migiro provided support and technical information about CABI Plantwise.

We take responsibility for any errors or shortcomings; our intention is to share knowledge, to the best of our ability.

We gratefully acknowledge the permission granted by Springer to reproduce the copyrighted material in this book and the Series Editor, Dr Aurelio Ciancio, for editing all contributions. We apologise for any errors or omissions in the above list. We would be grateful to be notified of any corrections that should be incorporated in future reprints or editions of this book.

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About the Editors

Saliou Niassy is a Senegalese national. He holds an MSc degree in Natural Sciences, a Postgraduate degree in Zoology from Cheikh Anta Diop University in Dakar (UCAD) and a PhD in Zoology from Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Kenya, through the ARPPIS-DAAD-ICIPE programme (2008–2011). Saliou joined the University of Pretoria in 2011, as a Postdoctoral Fellow, to coordinate the Research Network on Climate Change in African Mountains, AfroMont, funded by the FAO and the Mountain Research Initiative (MRI). After establishing the AfroMont Network successfully, Saliou took up a second Postdoctoral Fellowship at the International Centre of Insect Physiology, *ICIPE*, in Nairobi, under an African Union-funded project on Grain legumes in 2013. In late 2014, Saliou was appointed a Research Scientist, Head of the Technology Transfer Unit, under an IFAD funded project, operating in Kenya, Malawi, Mozambique and Rwanda.

Saliou served as the Coordinator of the Land Matrix Initiative, one of the most prominent global and independent land monitoring initiatives in the world, analysing competition on Large-Scale Land Investments between September 2015 and June 2017.

Saliou has now re-joined ICIPE as Head of the Technology Transfer Unit (TTU), as cross-cutting for the centre. Saliou is a pan-Africanist, and he has trained in and is working in Africa. His research interests encompass agriculture and rural development, food security, and poverty alleviation. Currently, Saliou was first the Secretary of the African Association of Insect Scientists (AAIS) between 2013 and 2017 and the President of AAIS since 2017. Saliou speaks French, English, Wolof and Diola fluently, and a little bit of Swahili and German.

Sunday Ekesi is the Director of Research and Partnerships at ICIPE. He is a scientist, research leader and manager with extensive knowledge and experience in sustainable agriculture, which includes microbial control, biological control, habitat management, managing pesticide use, integrated pest management, pesticide management, and biodiversity in Africa and internationally. As Director of Research and

Partnerships, he also manages and provides leadership on the implementation of high-level strategic goals of the various themes of the organization, in line with the institution's vision and strategy.

Sunday has broad perspectives of agricultural research and development issues, with proven experiences of challenges and opportunities in working with smallholder farmers, extension agents, research organizations and the private sector to improve food and nutritional security. He has vast experience on African Fruit Fly Programme and is familiar with the current priorities of sustainable intensification and agricultural transformation agenda. Sunday advises national programmes in more than 21 African countries on the development and implementation of integrated pest management (IPM) programmes for horticulture and staple crops. He sits on various international advisory and consultancy panels, such as the Food and Agriculture Organization (FAO) of the United Nations, The International Atomic Energy Agency (IAEA), The World Bank (WB), IPM Innovation Lab, and regional and national projects on various arthropod pests and climate change-related issues. He has authored more than 200 peer-reviewed publications and has trained 30 graduate students. He is a member of the Editorial Board of *Journal of Insects as Food and Feed* (JIFF), *International Journal of Tropical Insect Science* (IJTIS) and *Life: The Excitement of Biology* (LEB). Sunday is a Fellow of the Royal Entomological Society (FRES) and the African Academy of Sciences (FAAS).

Lorna Migiro is an agricultural entomologist, with 19 years of experience in research, lecturing and project management. She is currently the Programme Support Manager for the award-winning CABI-led Plantwise programme, whose aim is to increase food security and improve rural livelihoods by reducing crop losses in several developing countries across Africa, Asia and the Americas. In previous roles, Lorna worked at both the Technical University of Kenya and Pwani University as a lecturer, was a Postdoctoral Fellow at the Swedish University of Agricultural Sciences in Alnarp Sweden, was an Assistant Regional Manager with the Kenya Plant Health Inspectorate Service (KEPHIS), a Consultant with Dudutech Kenya Limited, a sales representative with Phillips Pharmaceutical Company Limited, and a Technical Assistant at the International Centre of Insect Physiology and Ecology (ICIPE).

Washington Otieno is the Plantwise Programme Executive at CAB International, where he has worked since 2013. At CABI, Washington has led the coordination of Plantwise, a global programme that builds the capacity of developing countries for plant health management through networks of plant clinics, development, and use of pest databases. He has expertise in phytosanitary capacity development, acquired through working with the Secretariat of International Plant Protection Convention and the Kenyan National Plant Protection Organization, and in agricultural research in tea production systems. Washington holds BSc and MSc degrees in agriculture and plant pathology, respectively, from the University of Nairobi, and a PhD from Wageningen University and Research Centre. He has published one book chapter and more than 30 papers in peer-reviewed and institutional journals.

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Chapter 1
Introduction: An Overview of the Impacts of Invasive Insect Species on Agriculture

Saliou Niassy, Sunday Ekesi, Lorna Migiro, and Washington Otieno

Abstract Agriculture plays a vital role in Africa, providing food and income. It contributes significantly to socio-economic development in rural and peri-urban areas. Among the many challenges that affect the productivity of the sector, invasive insect pests hold a ruinous position, as they inflict heavy economic yield losses. Over the past two decades, repeated scenarios of the invasions process have been experienced, characterised by the lack of preventive measures, weak and fragmented phytosanitary systems in terms of resources and infrastructure, policy and regulatory framework, and lack of capacity. This situation partly explains the high frequency of invasions and the high costs of emergency responses. Despite valuable research efforts being made to address incidences of invasive insect species, there is limited visibility of the successes and outcomes. It is imperative to shift from a passive stance and adopt predictive and proactive approaches to tackle these pests. This book, therefore, presents a panoramic view of invasive species research carried out in Africa. It is the fruit of a collaboration between several research entities and projects. It provides a slice of research work in the field of invasive insect pests management in Africa, and calls for a concerted effort to be made to harmonise research activities and promote knowledge exchange. The Volume covers a wide range of topics, which feeds into extension programmes and policy-making processes on the continent.

Keywords Invasive · Insect · Entomology · AAIS · ICIPE · CABI

Agriculture still occupies the majority of African populations, generating food and income, thereby contributing significantly to the nutritional requirements of the continent (Fairhurst 2017). It contributes significantly to socio-economic

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development in the rural areas and in the peri-urban regions of many African cities (FAO 2009; AGRA 2017). Among the many challenges that affect the sector, insect pests, both native and invasive, hold a ruinous position, as they inflict direct damages, while some of them are vectors of destructive viruses (Paini et al. 2016). The term 'invasive' generally refers to alien organisms, particularly for their ability or potential to establish and overcome various barriers to reproduction, dispersal and proliferation in new ecosystems, and their ability or potential to cause harm to humans, plants and the environment (Richardson et al. 2000). This book deals with invasive insect pests that also have economic and environmental impacts.

The economic repercussions arising from invasive species are far-reaching, and losses are estimated at several billion USD (Paini et al. 2016; Pratt et al. 2017). A study conducted in Florida on the estimated costs and regional economic impacts of the outbreak of the Oriental fruit fly *Bactrocera dorsalis* Hendel presented various scenarios of losses, ranging between $4 and 23 million, which is equivalent to 124 to 726 full-time and part-time jobs, and $10.2 million to $58.5 million in industry output. The costs of the response efforts to combat the fruit fly outbreak were estimated at $3,5 M (Alvarez et al. 2016).

Across Africa, fruit flies are estimated to cause annual losses of USD 2 billion in fruit and vegetable production (Ekesi et al. 2016). In West Africa for instance, yield losses are estimated at between 50 and 80% of the production, which translates into economic losses of 9 M Euro – more than one third of the total value of the exports. Fruit fly infestations also cause indirect damage to the economy, by reducing foreign exchange earnings from fruit exports due to quarantine restrictions and the loss of opportunities for export to lucrative global markets (Ekesi et al. 2016).

Outbreaks of *Chilo partellus* Swinhoe, Maize Lethal Necrosis Disease, *Parthenium hysterophorus* Linn, *Liriomyza* spp. and *Tuta absoluta* Meyrick, suffered by mixed maize smallholders in six countries, resulted in current combined annual losses of USD 0.9–1.1 billion and future annual losses (next 5–10 years) of USD 1.0–1.2 billion (Pratt et al. 2017).

Invasive species indirectly constitute a severe threat to biodiversity. They generally displace native and associated species, inducing changes in habitats, in general (McNeely 2001). The populations of native species and their associated organisms are threatened where invasive species have established themselves, as compared with areas where they have not been introduced (Rouget et al. 2016). This was observed during the emergence of *Chilo partellus* Swinhoe, which has a comparative advantage over *Busseola fusca* Fuller (Kfir 1997), and the same is being observed between Fall armyworm (FAW), *Spodoptera frugiperda* J. E. Smith and stemborers.

Biological invasions have three major phases: arrival, establishment, and spread. During the arrival phase, it is possible to eliminate invasive species if there are technically trained personnel available who can detect the pest. During the late establishment phase, when the population starts spreading, eradication becomes costly and unlikely. The ultimate option is to manage the pest locally, and intense effort is required (Alvarez and Solis 2019).

The approaches to invasive species management are highly variable and poorly reported, with costs being rarely quantified (Abate et al. 2000; de Bon et al. 2014). The most commonly used response to invasive pests, in general, is the extensive use of broad-spectrum synthetic insecticides, which not only compromise environmental health, but also negatively affect beneficial ecosystem service providers, as well as consumers (Mutengwe et al. 2016; Porteous 2017). Synthetic pesticides are often adulterated or applied at inappropriate application rates due to illiteracy and inadequate labelling, hence becoming largely ineffective and causing pest resistance. The harmful effects of pesticide residues seriously threaten human and environmental health, and result in market bans being placed on lucrative exports (Lux et al. 2003). The threat posed by these pests interferes with Africa's attainment of several Sustainable Development Goals (SDGs), and the perpetual vulnerability of the agricultural sector. It is, therefore, imperative to identify alternatives that could sustainably prevent and manage these pests, while mitigating their adverse effects on the environment, and ensuring the accessibility of horticultural produce to export markets (Simberloff 2014).

Over the past two decades, Africa has experienced the invasion of the Asian fruit fly *B. dorsalis* in Africa (in 2003 – Lux et al. 2003), the South American tomato moth *T. absoluta* (Tonnang et al. 2015), and most recently, the fall armyworm *S. frugiperda* (Goergen et al. 2016). Notwithstanding these experiences, there seems to be a repeated scenario for the invasion processes, which generally catch national quarantine systems off-guard and smallholder farmers defenceless. The problems posed by invasive insects are complex, costly and time-consuming. On the one hand, an invasive pest may be introduced accidentally or deliberately from outside its natural habitat or area of origin, but without its complex of natural enemies or biocontrol agents, thus making it a threat to livelihoods in the invaded areas (Emerton and Howard 2008). In a context of a weak agricultural setup and lack of capacity to identify, report and prevent invasions, invasive species spread at very high speeds, escaping all attempts at management.

On the other hand, there are weak national phytosanitary systems in terms of infrastructure, policy and regulatory framework. In most African countries, the Research for Development (R4D) continuum is in place, but with policy instruments being outdated, inadequate or unenforced (Cressman 2008, 2013; Tonnang et al. 2015; Early et al. 2018).

The traditional national disaster coordination strategy that is put into effect after a pest has become established consists of declaring a state of emergency, mobilising funds, and the constitution of a task force or multi-institutional technical team. However, most initiatives for the management of invasive pests are being implemented in isolation, resulting in fragmented efforts and limited initiatives and institutional subsidiarity and complementarity. The failure to address the listed areas for improvement results in the symptomatic lack of preparedness, characterised by the slow response to invasions, poor communication and information sharing, and insufficient coordination and cooperation, as well as fragmented control measures being taken, especially in the fight against trans-boundary pests. Factors such as

mobility, the increase in trade between regions, and climate change also need to be considered. Invasive insect pests are generally of global or regional magnitude; therefore, the concerted efforts of actors are required to prevent, prepare and curtail the potential invasions. Since several entities are involved in the development and scaling of solutions, it is critical to adopt a more predictive and proactive approach. These approaches can be formulated through capacity building in effective surveillance systems; cross-border research to develop novel solutions; and the sharing of research experiences and success stories at institutional, national and regional levels, which would enable a more coherent response to be taken to invasive pests.

A body of work has been generated by African entomologists and their collaborators that endeavours to identify sustainable solutions to invasive pests. Indeed, there is no single "silver bullet" to deal with the invasive species problem; various tactics need to be employed that build on a solid understanding of the origin, identity, ecology, and biology of the pest.

CABI, for example, has developed the Plant Protection Compendium and Plantwise, which are accessible to the public and include information on prevention through surveillance, early detection and eradication, deployment and dissemination of technologies through the use of diagnostic tools for rapid identification, use of host resistance, integrated management, coordinating action against emerging threats, networking for surveillance, enhancing human resource skills development, and upgrading infrastructure.

In the same vein, it is encouraging to note that novel initiatives, such as the use of ICT (Mobile Applications such as FAMEWS, NURU, and wefarm), are being promoted for forecasting, scouting, reporting, learning, and knowledge exchange during invasive species outbreaks in Africa.

Valuable contributions have been made in the field of GIS, modelling and remote sensing for the collection, prediction and interpretation of data (Guimapi et al. 2016). GIS, modelling and remote sensing outputs inform the best ways possible to apply management tools most efficiently, while saving resources (Coulibaly et al. 2007; Nwilene et al. 2008).

The IPM strategies (or components) for dealing with most of the essential invasive species are being piloted and scaled across Africa, with high economic and ecological success (Macharia et al. 2005).

Approaches that were initially designed for pest monitoring are being upgraded into management tools, through the adoption of mass-trapping that uses semiochemicals and killing agents, e.g. biopesticides or minimal doses of soft-killing synthetic chemicals. Accordingly, invasive pests such as thrips, fruit flies, stemborer moths, leaf miners, to cite few, can be easily monitored, controlled and eventually suppressed by using an arsenal of IPM technologies, with limited impact on the environment and the consumer.

Studies have shown that wherever semiochemicals are used, there is high compatibility with predators and parasitoids. Often underreported, there have been several success stories of classical biological control programmes that have significantly contributed to the reduction of pest incidences. As an example, biological control contributed to significant yield increase and pesticide reduction in the Kenya high-

lands through the release of the parasitoid *Cotesia vestalis* Haliday and *Diadegma semiclausum* Hellen, active against the diamondback moth *Plutella xylostella* Linnaeus (Macharia et al. 2005).

The use of nets as a physical barrier to protect crops has also been evaluated in many countries for combatting lepidopteran pests such as diamondback moth, Tomato bollworm, and many others in different climatic conditions (Martin et al. 2009). These nets are reusable for 3–5 years, which makes this technology cost-effective and adaptable to small-scale farming systems.

There is an arsenal of approaches for dealing with the sustainably of invasive species (Simberloff 2014). The only challenge that remains is to package and disseminate them as best-bet technologies in the context of the emergence of invasive species in Africa.

This book is, therefore, a compendium of research works on the sustainable management of agricultural insect pests in Africa, both in horticulture and for staple crops. Primarily, the book targets key pests such as *T. absoluta*, Fall armyworm *S. frugiperda*, and fruit flies *B. dorsalis*. This book mainly focuses on the following management techniques: the use of botanicals, resistance varieties, chemical pesticides, biological control strategies, and strengthening agricultural quarantine services and other regulatory bodies to enable active surveillance, behavioural chemical ecology, digital technologies for surveillance, monitoring and emergency response actions to be undertaken (Plantwise). Also considered are the application of scientific knowledge to help small-scale farmers to manage plant health problems, capacity building for plant health management concerning sanitary and phytosanitary standards (SPS), compliance and market access, and the use of innovations, including ICT, in agriculture for combatting invasive species. A few of the chapters deal with farmers' perceptions of the efficacy of management tools.

Chapter 2 looks at the use of botanicals for the management of *T. absoluta*. This is a field of research that has mostly been overlooked, yet has much potential for generating novel management tools. The team investigated the larvicidal potential of two leguminous trees and the effect of their extracts on eggs of *T. absoluta*, with positive results. In the same vein, Chap. 3 explores the seasonal abundance and the susceptibility of some tomato cultivars to *T. absoluta* in the context of developing an IPM package in Sudan. The outcome of the study revealed a significant difference among tomato genotypes and recommended RILG3–162, KHP 5822 and Salama, which are tolerant, for further research. In Nigeria, *T. absoluta* had deeply affected the tomato sector, despite heavy pesticide use. Chapter 4 presents a clear case of resistance development among several populations of the pest after being subjected to lambda-cyhalothrin, deltamethrin, chlorantraniliprole and lambda-cyhalothrin, spirotetramat and flubendiamide. Pest populations showed resistance to all compounds, confirming farmers' observations. This study stressed the need for an IPM strategy, including the use of pheromone trapping techniques and cultural practices (orchard sanitation), among many others, to contain the pest in the country. Chapter 5 reports on the inefficacy of relying on a single solution. The same team in Nigeria went on to narrate the adoption of an IPM package that resulted in a 57.5% reduction in pest damage. The problem with invasive species is

that they keep on increasing their host range in the new environment. The team, led by ARC and the University of Gezira, presents the effects of *T. absoluta* on cowpea *Vigna ungiculata* L., with 25% damage on leaves (Chap. 6). Although data on pods have not been presented, this already showed that the pest is not only confined within the Solanaceae family, but may also attack other unusual crops as well.

Chapter 7 deals with the invasive fruit fly, *B. dorsalis*, which invaded Africa in early 2000. The study is mainly focused on the virulence of the entomopathogen *Metarhizium anisopliae* (Metchn) Sorokin, initially isolated on a Coleoptera on pupae of *B. dorsalis*, with 74% mortality at a dose of 2. 10^7 propagules/ml. These results will be extremely useful in the management of the pest, especially using powder formulations under trees where pupation occurs, since *M. anisopliae* naturally occurs in soil.

Chapter 8 presents the diversity of fruit fly species in Sudan. This chapter is particularly important in the context of developing a national strategy for the management of invasive species. Disciplines such as taxonomy and pest identification are becoming increasingly scarce among African entomologists and need to be revived.

A critical aspect of invasive species research is the characterisation of the relationship between damage and yield. For the case of FAW, for instance, panic and lack of experience led governments to spend astronomic amounts of money to purchase chemicals. However, the after emergency caused many stakeholders to realise that the correlation between leaf damage and yield can be subjected to various factors. In Chap. 9, the authors endeavour to describe that relationship by identifying the ultimate factors or parameters that enable the accurate measurement of the links between injury and yield in maize. Factors, such as time of infestation, cob tunnel length and cob biomass, are shown to follow a function, whereas the length of stem tunnel and cob mass were less reliable as indicators.

Biological control is also an essential component in the management of invasive species. Often, classical biological control is applied because the alien species present themselves in a new environment without their original natural enemies. The local complex of natural enemies often takes time to develop new associations. Classical biological control often requires time to become effective because newly introduced natural enemies often take time to adjust in the new environment, and they need to be produced in numbers. Chapter 10 recounts a success story in East Africa, where the parasitoid *C. vestalis* imported from Southern Africa was introduced into Eastern Africa (Kenya), with satisfactory results in the lowlands of Kenya. The study explored the establishment of the parasitoid in the South Eastern and Coastal regions of Kenya after several releases and recorded the limiting factors to its establishment, confirming the molecular identity of the *Cotesia* specimens and investigating the farming practices in the region. Although it takes much time to confirm the establishment of natural enemies confidently, the introduction needs to be accompanied by specific measures, e.g. reduced chemical use and awareness creation.

Chapter 11 presents a study on maize lethal necrosis disease and thrips in Kenya.

This book on invasive species management would not be meaningful without presenting research on the Fall armyworm, one of the most recent invasive insect

pests attacking cereal crops on the continent. Chapters 12 and 13 present a review of FAW in Rwanda and the farmers' perceptions of the use of chemical pesticides in an emergency context. It is imperative to emphasise that the use of chemical pesticides is a component of IPM, and it generally comes as the first line of defence in a situation where no other tool is available, for instance during the recent FAW invasion. National governments, NGOs and many other actors in the maize value chain have promoted the use of chemical pesticides. Accordingly, Chap. 13 deals with aspects of farmers' perceptions of the efficacy of several chemical pesticides recommended for the management of FAW in Rwanda.

The book also presents research on other key pests, for example the palm green pit scale *Palmapis phoenicis*, which is believed to have reached Africa through imports of shoots. The pest inflicts high yield losses and causes severe economic losses in many Arab countries, including Sudan and Egypt.

Chapter 15, therefore, describes a classical IPM approach, starting from pest identification, biology and ecology, leading to the implementation of management strategies including the use of resistant varieties.

Chapter 16 presents the use of a male annihilation technique for the management of the Mango Fruit flies in Ethiopia.

The occurrence of invasive species also leads to product export bans. This was the case in Ghana when the EU banned the import of chillies, eggplant and ridged gourds from 2012 to 2015, and then extended the ban to 2017. Chapter 18 narrates the process by which quarantine officers conducted trials to identify solutions to uplift the ban by understanding crop protection practices in Ghana. The study revealed that building capacity among the growers, staff members of quarantine inspectors, National Plant Protection Organizations (NPPO), and Plant Protection and Regulatory Services Directories could help them to implement adequate measures that would lead to pest-free produce, fit for export.

CABI's Plantwise project is one of a kind, playing a pivotal role in the detection of pests' status in Africa and beyond. Chapter 19 is dedicated to providing information and extension officer support systems called "Plant doctors". The Plantwise system also plays a crucial role in the early detection of critical pests, their monitoring and noting of changes of status.

Chapter 20 explores the use of management techniques, commonly referred to as "lure and kill", on thrips such as *Megalurothrips sjostedti*, a key pest of grain legumes in Africa. This approach is novel and very relevant in the context of invasive species management, in that it combines several tools, including semiochemicals, coloured traps and entomopathogens. The approach helps to minimise the costs of inoculum and labour, and improves the persistence of biological products.

Chapters 21 and 22 present work on the tomato red spider mite, *Tetranychus evansi*. Chapter 21 looks at the use of natural enemies, such as the predatory mite *Phytoseiulus longipes*, for promoting within-plant pest dynamics. This suggests the use of a combination of this technique with other IPM tools for the management of this pest. Chapter 22 examines the use of endophytes as a means to control mite damage on various tomato varieties.

One key element in combatting invasive pests comprises gaining an understanding of their biology and ecology. Without this, perhaps tedious, exercise, no sound management strategy can be developed. The team of Rida Musa presents a study on the flight behaviour of *T. absoluta* on tomato and different crops in Chap. 23. The male *T. absoluta* flies at low heights, during the early morning hours. This information can contribute significantly to the development of an IPM package for the pest.

1.1 Conclusion

There is an immense repertoire of research studies on invasive insect pests. The sustainable management of invasive species in Africa suggests that enhanced coordination, cooperation, and communication of research should be implemented. This book is a modest contribution to the global effort in managing invasive species. Solutions to counter some of the most devastating invasive species exist, and these have been highlighted in the book.

In addition to being accountable to the farmers who face most of the problems posed by invasive species, there is a need for a trust security fund to be established to support innovation processes and the dissemination of knowledge products to end-users.

References

Abate, T., van Huis, A., & Ampofo, J. K. O. (2000). Pest management strategies in traditional agriculture: An African perspective. *Annual Review of Entomology, 45*, 631–659. https://doi.org/10.1146/annurev.ento.45.1.631.

AGRA. (2017). Africa agriculture status report: the business of smallholder agriculture in Sub-Saharan Africa (Issue 5). Nairobi, Kenya: Alliance for a Green Revolution in Africa (AGRA), Issue No. 5. Alliance for a green revolution in Africa 180. http://hdl.handle.net/10568/42343

Alvarez, S., Evans, E. A., Hodges, A. W. (2016). *Estimated costs and regional economic impacts of the oriental fruit fly outbreak in Miami-Dade county*, Florida. Edis 1–19.

Alvarez, S., & Solis, D. (2019). Rapid response lowers eradication costs of invasive species: Evidence from Florida. *Choices: The Magazine of Food, Farm, and Resource Issues, 33*.

Coulibaly, O., Cherry, A. J., Nouhoheflin, T., et al. (2007). Vegetable producer perceptions and willingness to pay for biopesticides. *Journal of Vegetable Science*. https://doi.org/10.1300/J484v12n03_04.

Cressman, K. (2008). The use of new technologies in desert locust early warning. *Outlooks on Pest Management*.

Cressman, K. (2013). Role of remote sensing in desert locust early warning. *Journal of Applied Remote Sensing*. https://doi.org/10.1117/1.jrs.7.075098.

de Bon, H., Huat, J., Parrot, L., et al. (2014). Pesticide risks from fruit and vegetable pest management by small farmers in sub-Saharan Africa. A review. *Agronomy for Sustainable Development, 34*, 723–736. https://doi.org/10.1007/s13593-014-0216-7.

Early, R., González-Moreno, P., Murphy, S. T., & Day, R. (2018). Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. *NeoBiota, 40*, 25–50. https://doi.org/10.3897/neobiota.40.28165.

Ekesi, S., Mohamed, S. A., & De Meyer, M. (2016). *Fruit fly research and development in Africa – Towards a sustainable management strategy to improve horticulture*. Cham: Springer. https://doi.org/10.1007/978-3-319-43226-7.

Emerton, L., & Howard, G. (2008). A toolkit for the economic analysis of invasive species. *GISP – Global Invasive Species Programme, 110*.

Fairhurst, J. (2017). Agriculture. In *Rural development planning in Africa* (pp. 69–91). New York: Palgrave Macmillan.

FAO – Food and Agricultural Organization of the United Nations. (2009). Global agriculture towards 2050. *High Level Expert Forum-How to feed the world 2050*, 1–4.

Goergen, G., Kumar, P. L., Sankung, S. B., et al. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS One*. https://doi.org/10.1371/journal.pone.0165632.

Guimapi, R. Y. A., Mohamed, S. A., Okeyo, G. O., et al. (2016). Modeling the risk of invasion and spread of *Tuta absoluta* in Africa. *Ecological Complexity, 28*, 77–93. https://doi.org/10.1016/j.ecocom.2016.08.001.

Kfir, R. (1997). Natural control of the cereal stemborers *Busseola fusca* and *Chilo partellus* in South Africa. *Insect Science and its Application, 17*, 61–67. https://doi.org/10.1017/s1742758400022177.

Lux, S. A., Copeland, R. S., White, I. M., et al. (2003). A new invasive fruit fly species from the *Bactrocera dorsalis* (Hendel) group detected in East Africa. *International Journal of Tropical Insect Science.* https://doi.org/10.1017/s174275840001242x.

Macharia, I., Löhr, B., & de Groote, H. (2005). Assessing the potential impact of biological control of *Plutella xylostella* (diamondback moth) in cabbage production in Kenya. *Crop Protection, 24*, 981–989. https://doi.org/10.1016/j.cropro.2005.02.005.

Martin, T., Assogba-Komlan, F., Houndete, T., et al. (2009). Efficacy of mosquito netting for sustainable small holders' cabbage production in Africa. *Journal of Economic Entomology, 99*, 450–454. https://doi.org/10.1603/0022-0493-99.2.450.

McNeely, J. (2001). Invasive species: A costly catastrophe for native biodiversity. *Land Use and Water Resources Research, 1*, 1–10.

Mutengwe, M. T., Chidamba, L., & Korsten, L. (2016). Monitoring pesticide residues in fruits and vegetables at two of the biggest fresh produce markets in Africa. *Journal of Food Protection, 79*, 1938–1945. https://doi.org/10.4315/0362-028X.JFP-16-190.

Nwilene, F. E., Nwanze, K. F., & Youdeowei, A. (2008). Impact of integrated pest management on food and horticultural crops in Africa. *Entomologia Experimentalis et Applicata, 128*, 355–363.

Paini, D. R., Sheppard, A. W., Cook, D. C., et al. (2016). Global threat to agriculture from invasive species. *Proceedings of the National Academy of Sciences of the United States of America, 113*, 7575–7579. https://doi.org/10.1073/pnas.1602205113.

Porteous, O. (2017). Empirical effects of short-term export bans: The case of African maize. *Food Policy, 71*, 17–26. https://doi.org/10.1016/j.foodpol.2017.07.003.

Pratt, C. F., Constantine, K. L., & Murphy, S. T. (2017). Economic impacts of invasive alien species on African smallholder livelihoods. *Global Food Security, 14*, 31–37.

Richardson, D. M., Pyšek, P., Rejmánek, M., et al. (2000). Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and Distributions, 6*, 93–107. https://doi.org/10.1046/j.1472-4642.2000.00083.x.

Rouget, M., Robertson, M. P., Wilson, J. R. U., et al. (2016). Invasion debt – Quantifying future biological invasions. *Diversity and Distributions, 22*, 445–456. https://doi.org/10.1111/ddi.12408.

Simberloff, D. (2014). Biological invasions: What's worth fighting and what can be won? *Ecological Engineering, 65*, 112–121. https://doi.org/10.1016/j.ecoleng.2013.08.004.

Tonnang, H. E. Z., Mohamed, S. F., Khamis, F., & Ekesi, S. (2015). Identification and risk assessment for worldwide invasion and spread of *Tuta absoluta* with a focus on Sub-Saharan Africa: Implications for phytosanitary measures and management. *PLoS One, 10*. https://doi.org/10.1371/journal.pone.0135283.

Chapter 2
Bioassay-Guided Isolation of Active Phytochemicals Against *Tuta absoluta* (Meyrick) from *Turraea floribunda* and *Caesalpinia welwitschiana*

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Abstract *Tuta absoluta* Meyrick is now one of the most harmful insect pests of Solanaceae in various parts of the world. Synthetic pesticides are the most used, current control method, but are associated with several problems including the development of resistance and negative ecological effects. These led to the search for more eco-friendly methods of controlling the pest, such as a search for phytochemicals that show subtle anti-pest properties. In the present study, the effects of the constituents of methanolic extracts associated with *Turraea floribunda* and *T. nilotica* leaves (Meliaceae) and those of *Caesalpinia welwitschiana* and *C. bonduc* roots (Fabaceae-Caesalpiniaceae) were evaluated on second instar larvae of *T. absoluta*. The extract from *T. floribunda* leaves was the most active (LD_{50} = 587.0 ng/µl), followed by *C. welwitschiana* (LD_{50} = 779.1 ng/µl). Bioassay-guided isolation of active compounds from these active extracts, using column chromatographic and preparative HPLC, led to the identification of twenty-two compounds. Their structures were established using spectroscopic techniques, including MS, 1 and 2D-NMR, and also by comparison with reported data. The methanolic leaf extract of *T. floribunda* yielded ten compounds (*β*-sistosterol,

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stigmasterol, ursolic acid**,** betulinic acid, a mixture of *β*-sistosterol-3-*O*-*β*-D-glucopyranoside and stigmastreol-3-*O*-*β*-D-gluco pyranoside, fridelin, lupeol, 11-epi-21-hydroxytoonacilide and 11*β*, 12*α*-diacetoxycedrelone). The methanolic roots extract of *C. welwitschiana* afforded twelve constituents, including apigenin, luteolin, afzelin, quercitrin, epiafzelechin-3-*O*-gallate, Kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, Kaempferol, dipteryxic acid, neocaesalpin L, rutin, methyl gallate, and galic acid. Some of the isolates were tested on *T. absoluta* eggs. They showed varying levels of ovicidal effect, with quercitrin being the most active constituent at 81%. The results of the study showed potential of the phytochemicals of these plants in the management of *T. absoluta.*

Keywords *Turraea* · *Caesalpinia* · *Tuta absoluta* · Terpenoids · Flavonoids · Ovicidal activity

2.1 Introduction

The tomato borer *Tuta absoluta* is an oligophagous and very harmful leaf-mining moth that feeds on Solanaceae crops, particularly tomato, *Solanum lycopersicum* (Campos 1976; Garcia and Espul 1982). Native to Latin America, this pest has recently spread via infested fruits and packaging materials throughout Afro-Eurasia and Middle Eastern countries (Biondi et al. 2018; Desneux et al. 2010; Sylla et al. 2017; Xian 2017). The high speed of this colonization is associated with the ability of the pest to adapt to various climatic conditions and its high biotic potential. After hatching, the larvae (the most destructive stage) can penetrate into the whole plant, but prefer apical buds, tender new leaflets, flowers, and green and ripe fruits, causing yield losses of up to 80–100% (Moreira et al. 2005; Desneux et al. 2010). Currently, the most effective method for control of *T. absoluta* is the use of commercial synthetic pesticides, including organophosphates, pyrethroids, thiocarbamates, diamides and acylurea growth regulators (Bughio and Wilkins 2004; Abbes and Chermiti 2012; Roditakis et al. 2015). However, these pesticides have exhibited low to moderate efficiency due to the cryptic nature of *T. absoluta* (Lietti et al. 2005). This has considerably increased the spraying frequency per crop cycle, which has accelerated the evolution of resistance in the pest (Bughio and Wilkins 2004; Roditakis et al. 2015), and has caused disruption in the natural biological control, as well as serious drawbacks for the environment. Thus, there is an urgent need for finding new eco-friendly tools for the control of *T. absoluta*.

Turraea and *Caesalpinia*, two genera of tropical plants, could play a key role in the search for new insecticidal compounds with low toxicity to warm-blooded mammals and easy biodegradability (Cox 2004; Ben et al. 2010). Plants of these genera are mostly trees, shrubs and liana that are distributed in many parts of the world. In Africa, some species are used in traditional medicines to treat different ailments, and are also used against insect bites (Fraser et al. 1994; Kuria et al. 2001;

Ochieng' et al. 2012). Previous studies on some *Turraea* and *Caesalpinia* spp. have reported the presence of various classes of secondary bioactive metabolites, including limonoids, flavonoids, cassane type-diterpenoids and terpenoids (Ndung'u et al. 2004; Udenigwe et al. 2007; Yuan et al. 2013; Xu et al. 2016). Some of these compounds, as well as extracts, showed interesting biological activities, such as antimalarial (Linn et al. 2005; Irungu et al. 2015) and insecticidal (Zanin et al. 2012; Essoung et al. 2017, 2018) properties.

The objective of this study was to evaluate the insecticides properties of Meliaceae (*Turraea*) and Fabaceae-Caesalpiniaceae (*Caesalpinia*) plant species. Here, we report the larvicidal activity of methanolic extracts of leaves from two *Turraea* species (*T. floribunda* and *T. nilotica*), and from roots of two *Caesalpinia* plants (*C. welwitschiana* and *C. bonduc*). These grow in different agro-ecological zones of Kenya and Cameroon. These species were initially screened on the larvae of *Tuta absoluta*. The most active extracts were then subjected to bioassay-guided isolation and characterisation of constituents. In this chapter, we report the isolation of twenty-two compounds, together with their ovicidal activity.

2.2 Material and Methods

2.2.1 Insects

The insects used for all bioassays originated from a colony reared at the International Centre of Insect Physiology and Ecology (ICIPE), Duduville Campus, Nairobi, Kenya. The original population from pupae and larvae of *T. absoluta* was collected in the field from infected tomato plants in Meru County, Eastern Kenya, without any history of exposure to pesticides. The insects collected were initially maintained under quarantine to identify any parasitized individuals before the establishment of the colony, which was maintained at 26 ± 2 °C. The relative humidity ranged from 60% to 70%, with a photoperiod of 12 h.

2.2.2 Initial Screening of Turraea *and* Caesalpinia *Extracts*

2.2.2.1 Collection and Solvent Extractions

Four plant species were collected in Kenya from the Gede Ruins Museum Forest (*T. nilotica* and *T. floribunda*) in September 2015, and in Cameroon at Betare Oya town, East Region, and Ebodié beach, Kribi, Sud Region (*C. welwitschiana* and *C. bonduc*, respectively), in December 2014. The plants were identified and authenticated by botanist Mr. Simon Mathenge (Department of Botany, University of Nairobi), and by taxonomist Mr. Nana Victor, (National Herbarium Yaoundé, Cameroon), where voucher specimens were deposited: EEFRTN-2015/2 (03° 18′

31 N/40° 01′ 06 E), EEFRTF-2015/3 (03° 19′ 28 N/40° 02′ 08 E), HNC 52547 (2° 34′ 0″ N/ 9° 50′ 0″ E) and HNC 51334 (5° 34′ 55″ N/13° 51′ 33″ E) for *T. nilotica*, *T. floribunda*, *C. welwitschiana* and *C. bonduc*, respectively. The plant materials were dried under shade for three weeks before extraction. The air-dried, powdered leaves (400 g) of *Turraea* species and roots (200 g) of *Caesalpinia* plants were separately extracted with methanol at room temperature with constant shaking (5 L, 72 h × 3 assays each). After each extraction, the solvent was removed using a rotary evaporator (40 °C) (BÜCHI R-205).

2.2.2.2 Larvicidal Effects of Extracts

The effects of different doses (100, 250, 500 and 1000 ng/µl) of each extract in acetone were evaluated using a leaf disc painting method in Petri dishes (90 × 20 mm), as described by Miresmailli et al. (2006). Briefly, 1 ml of each concentration of each test material was painted on the tomato leaf (*L. esculentum*, variety Moneymaker, planted in ICIPE greenhouse without any pesticide application) with a micropipette, and the solvent was allowed to evaporate at room temperature for 15 min prior to the introduction of 20 s instar larvae in each dish. The dishes were then immediately closed and placed in an incubator at 25 ± 2 °C with 75 ± 5% relative humidity, for a photoperiod of 12 h. Acetone alone was used as control on leaf discs. Each dose was replicated four times, and the dead larvae were assessed after 24 h of exposure by prodding the insects with a fine hairbrush. Larvae were presumed dead if they showed no response. The lethal dose (LD_{50}) of each extract was estimated.

2.2.3 *Isolation and Identification of Compounds from* Turraea floribunda *Leaves*

The methanolic extract of *T. floribunda* leaves (23.1 g) was fractionated by medium pressure liquid chromatography (MPLC) on silica gel 230–400 mesh (Buchi MPLC, C-601/C-605 dual pump) eluting with blends of hexane/ethyl acetate/methanol, with increasing amounts of the polar solvent. One hundred and five fractions of 100 ml each were collected and grouped into seven main fractions (A-G), based on their TLC profiles, which were performed using pre-coated aluminium silica gel $60F_{254}$ plates (Merck, 0.25 mm thickness). Spots were visualised under UV light (254 and 365 nm) or by using ceric sulphate reagent. Ehrlich's test ($CHCl_3$, HCl and 4-N,N-dimethylaminobenzaldehyde) and/or the Liebermann-Burchard test ($CHCl_3$, Ac_2O and concentrated H_2SO_4) were carried out. All fractions were bio-assayed on *T. absoluta* larvae, using the method described above. The most potent fractions (C,

4.3 g and D, 3.5 g) of *T. floribunda* were further fractionated by column chromatography (CC) (silica gel, 230–400 mesh, Merck, Darmstadt, Germany) and eluted with hexane – EtOAc – MeOH gradient system, resulting in the isolation of ten known terpenoids constituents. Fraction C was subjected to CC (2.5 × 40.0 cm) over silica gel (230–400 mesh) eluted with a Hex-EtOAc-MeOH gradient system. This resulted in the isolation of *β*-sistosterol (**1**, 0.5 mg), stigmasterol (**2**, 1.1 mg), ursolic acid (**3**, 0.5 mg), betulinic acid (**4**, 1.2 mg), a mixture of *β*-sistosterol-3-*O*-*β*-D-glucopyranoside (**5a**) and stigmastreol-3-*O*-*β*-D-glucopyranoside (**5b**) (1.0 mg). Fraction D was analysed on CC (2.5 × 40.0 cm) over silica gel eluted with a similar gradient system solvent to yield fridelin (**6**, 0.8 mg), lupeol (**7**, 0.6 mg), 1-epi-23-hydroxytoonacilide (**8**, 3.3 mg) and 11*β*, 12*α*- diacetoxycedrelone (**9**, 3.6 mg).

2.2.4 *Isolation and Identification of Compounds from* Caesalpinia welwitschiana *Roots*

The methanolic extract of *C. welwitschiana* roots (13.4 g) was fractionated by MPLC on silica gel (230–400 mesh), eluting with the similar blends of solvents with increasing amounts of the polar solvent. Eighty fractions of 100 ml each were collected and grouped into five fractions ($F_{1–5}$) based on their TLC profiles. Spots were visualised under UV light or by using ceric sulphate reagent. Shinoda (Magnesium, MeOH and concentrated HCl), Molish (EtOH, *α*-naphtol and concentrated H_2SO_4), ferric chloride ($FeCl_3$ and MeOH) and Liebermann-Burchard tests were performed. The most active fractions (F_2, 2.9 g and F_5, 2.5 g) of *C. welwitschiana* were also fractionated, using column chromatography with silica gel and eluted with similar mixture of solvent, yielding twelve known compounds. Fraction F_2 was subjected to CC (2.5 × 40.0 cm) over silica gel 230–400 mesh (Merck) eluted with the Hex – EtOAc – MeOH gradient system, resulting in the isolation of apigenin (**10**, 0.4 mg), luteolin (**11**, 0.6 mg), afzelin (**12**, 4.5 mg) and quercitrin (**13**, 5.0 mg). Three sub-fractions F_{2A-C} were grouped, based on TLC profiles. Sub-fractions F_{2-B} (80.4 mg) were re-chromatographed (250 × 21.2 mm) using preparative HPLC on reverse phase silica gel, eluted with Acetonitrile/H_2O (40: 90) to afford epiafzelechin-3-*O*-gallate (**14**, 2.0 mg) and Kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**15**, 1.7 mg). Fraction F_5 was purified by CC (2.5 × 40.0 cm) on silica gel (230–400 mesh), eluted with similar gradient system solvent and yielded: Kaempferol (**16**, 0.7 mg), dipteryxic acid (**17**, 3.2 mg) and neocaesalpin L (**18**, 4.3 mg). The sub-fraction F_{5-C} (100 mg) was rechromatographed using the HPLC to obtain rutin (**19**, 2.2 mg), methyl gallate (**20**, 6.3 mg) and gallic acid (**21**, 5.2 mg).

All solvents used were of analytical grade (Fisher Chemical Germany Limited, LE11 5RG).

2.2.5 *Structure Elucidation of the Compounds*

All the isolates were identified by their UV, IR and 1 and 2 D- NMR spectra, and by comparison with reported data. UV absorption spectra were recorded on a Water UV 2998 spectrophotometer in the range of 200–500 nm. Electrospray ionisation mass spectrometry (ESI-MS) experiments were performed, using an Agilent 1100 LCF mass spectrometer (Agilent, USA), and EI mass spectra were measured on a Finnigan MAT 95 Spectrometer (70 eV). Fourier transform infrared (FT-IR) spectra were recorded on Shimadzu IRAffinity-1S using KBr pellet. 1D and 2D NMR spectra were run on Bruker UltaShield™ spectrometers, operating at 500 or 600 MHz, where chemical shifts (δ) were expressed in ppm with reference to the solvent signals. The twenty-two compounds were identified, as follows:

***β*-Sistosterol** (**1**): White needles; 0.5 mg; ^{1}H (500 MHz, $CDCl_3$): δ_H 5.37 (br s); 1.03 (s); 0.94 (d, J = 6.4 Hz); 0.85 (m); 0.84 (m); 0.82 (m); and 0.70 (s); ^{13}C (125 MHz, $CDCl_3$) (Table 2.1); EIMS *m/z* int (%) 414 $[M]^+$ (64), 396 (40), 382 (14), 303 (23), 255 (20), 159 (23), 81 (37), 43 (100), $C_{29}H_{50}O$.

Stigmasterol (**2**): White needles; 1.1 mg; ^{1}H (500 MHz, $CDCl_3$): δ_H 5.35 (br s); 5.15 (dd, J = 8.4, 14.6 Hz); 5.02 (dd, J = 8.4, 14.6 Hz); 4.61 (m); 1.02 (s); 0.92 (d, J = 6.4 Hz); 0.87 (m); 0.83 (m); 0.81 (m); and 0.68 (s). ^{13}C (125 MHz, $CDCl_3$) (Table 2.1); EI-MS *m/z* int (%) 414 $[M]^+$ (64), 396 (40), 382 (14), 303 (23), 255 (20), 159 (23), 81 (37), 43 (100), $C_{29}H_{48}O$.

Ursolic acid (**3**): White crystals; 0.5 mg; m.p.: 290–292 °C, $[\alpha]_D^{25}$ = +25° (*c* 0.30, MeOH); ^{1}H (600 MHz, D_2O): δ_H 3.15 (q, 10.0, 5.0); 2.20 (d, 7.5); 1.10 (s); 1.13 (s); 0.95 (s); 0.88 (s); 0.86 (s); 0.79 (s); 0.77 (s). ^{13}C (150 MHz, CD_3CN) (Table 2.1); EI-MS *m/z* 456 $[M^+]$ for $C_{30}H_{48}0_3$.

Betulinic acid (**4**): White needles; 1.2 mg; ^{1}H (500 MHz, C_5D_5N): δ_H 4.93 (br s); 4.76 (br s); 3.52 (m); 3.44 (t, J = 8.0 Hz); 1,78 (s); 1.21 (s); 1.06 (s); 1.04 (s); 0.99 (s); 0.81 (s); 0.77 (m). ^{13}C (125 MHz, C_5D_5N) (Table 2.1); EIMS *m/z* 457.1 for $C_{30}H_{49}O_3$ $[M + H]^+$.

***β*-Sistosterol-3-*O*-*β*-D-glucopyranoside** (**5a**) and **Stigmasterol-3-*O*-*β*-D-glucopyranoside** (**5b**): White amorphous powder, 1.0 mg; ^{1}H (500 MHz, DMSO-d_6): δ_H 5.36 (br s); 5.17 (dd, J = 8.4, 15.6 Hz); 5.03 (dd, J = 8.4, 15.6 Hz); 4.22 (d, J = 7.8 Hz); 3.64 (m); 3.48 (m); 3.43 (m); 3.13 (m); 3.10 (m); 3.02 (m); 2.89 (m); 0.96 (s); 0.90 (m); 0.80 (m); 0.79 (m); 0,78 (m) and 0.65 (s). ^{13}C (125 MHz, DMSO-d_6) (Table 2.1); EIMS *m/z* 572 and 574.8 for $C_{35}H_{60}O_6$ and $C_{35}H_{58}O_6$ respectively; int (%) 414 (5), 396 (63), 381 (24), 255 (77), 159 (39), 81 (99), 55 (100)

Fridelin (**6**): White needles; 0.8 mg; ^{1}H (500 MHz, $CDCl_3$): δ_H 0.71 (s); 0.85 (s); 0.86 (d, J = 6.5 Hz); 0.93 (m); 0.93 (s); 0.98 (s); 0.99 (s); 1.03 (s); 1.16 (s); 1.18 (m); 1.18 (m); 1.19 (m); 1.20 (m); 1.24 (m); 1.25 (m); 1.26 (m); 1.27 (m); 1.32 (m); 1.32 (m); 1.33 (m); 1.37 (m); 1.44 (m); 1.45 (m); 1.46 (m); 1.46 (m); 1.50 (m); 1.50 (m);1.56 (m);1.66 (m); 1.75 (m); 1.94 (m); 2.23 (q, J = 6.5 Hz); 2.36 (m); 2.37 (dq, J = 8.5, 3.5 Hz). ^{13}C (125 MHz, $CDCl_3$) (Table 2.1); ESIMS *m/z* 426.3 for $C_{30}H_{50}O$ $[M]^+$.

Table 2.1 ^{13}C NMR data of the isolated constituents from *Turraea floribunda* and *Caesalpinia welwitschiana*

Carbons	1[a]	2[a]	3[b]	4[c]	5a[d]	5b[d]	6[a]	7[a]
1	37.0	37.0	39.8	39.3	38.3	38.3	22.3	38.8 $_2$
2	28.2	28.2	27.8	28.3	33.3	33.3	41.5	27.4
3	73.6	73.6	79.7	78.1	77.2	77.2	213.3	79.0
4	39.7	39.7	39.9	39.5	41.8	41.8	58.2	38.8
5	139.7	139.7	56.6	55.9	138.5	138.5	42.1	55.3
6	122.5	122.5	17.8	18.8	121.7	121.7	41.3	18.3
7	31.9	31.9	34.3	34.8	31.4	31.4	18.2	34.3
8	31.8	31.8	40.2	41.1	31.3	31.3	53.1	40.8
9	50.0	51.2	40.7	50.9	50.6	50.6	37.4	50.4
10	36.6	36.6	37.9	37.6	36.8	36.8	59.4	37.1
11	21.0	21.0	21.7	21.2	22.6	22.6	35.6	20.9
12	38.1	38.1	126.7	26.1	41.8	41.8	30.5	25.1
13	42.3	42.3	139.5	38.6	45.1	45.1	39.7	38.0
14	56.7	56.7	40.3	42.8	56.1	56.1	38.3	42.8
15	24.3	24.3	29.2	31.2	24.8	24.8	32.7	27.4
16	26.1	26.1	25.3	32.9	27.8	27.8	36.0	35.6
17	56.0	56.0	43.2	56.6	55.4	55.4	30.0	43.0
18	11.8	11.8	54.2	49.7	11.6	11.6	42.8	48.3
19	19.0	19.0	38.0	47.8	19.0	19.0	35.3	48.0
20	36.1	36.1	35.9	151.3	36.1	36.1	28.1	150.9
21	18.8	18.8	31.7	30.3	18.6	18.6	32.4	29.8
22	33.9	138.3	36.8	37.5	35.4	138.0	39.2	40.0
23	29.7	129.3	28.8	28.6	29.2	121.1	6.8	28.1
24	45.8	51.2	16.4	16.3	49.6	49.6	14.7	15.4
25	29.1	28.1	16.1	16.4	28.7	28.7	14.9	16.1
26	19.9	19.8	17.7	16.4	20.6	20.6	20.3	16.0
27	19.3	19.2	24.3	15.0	19.7	19.7	18.7	14.5
28	23.0	23.2	181.6	178.8	23.8	23.8	35.3	18.0
29	12.0	12.1	19.4	109.9	11.7	11.7	32.1	109.3
30			24.3	19.5			31.8	19.3
Glusosyl								
1′					101.2	101.2		
2′					73.4	73.4		
3′					76.7	76.7		
4′					70.1	70.1		
5′					76.7	76.7		
6′					61.5	61.5		

Carbons	8[a]	9[a]	Carbons	10[e]	11[d]	12[d]	13[d]	14[f]	15[e]	16[d]	19[e]
1	152.1	150.1	1								
2	125.4	127.7	2	164.3	163.8	157.5	156.8	78.1	161.3	146.8	155.4
3	204.1	203.2	3	103.4	102.9	134.5	134.5	69.4	134.4	135.6	135.5

(continued)

Table 2.1 (continued)

Carbons	8[a]	9[a]	Carbons	10[e]	11[d]	12[d]	13[d]	14[f]	15[e]	16[d]	19[e]
4	46.1	48.5	4	182.2	181.7	178.0	178.1	26.5	179.3	175.9	179.1
5	44.7	134.7	5	157.8	161.5	161.7	161.7	157.5	163.1	160.7	162.9
6	31.1	140.8	6	99.4	98.8	99.4	99.2	96.4	99.7	98.2	96.1
7	174.1	197.1	7	164.6	164.1	165.5	164.8	157.8	165.6	163.9	161.1
8	35.5	45.9	8	94.5	93.8	94.3	94.1	95.7	94.6	93.5	95.5
9	52.1	41.8	9	161.6	157.3	160.5	157.7	157.1	158.4	156.1	158.7
10	41.9	40.1	10	104.2	103.7	104.2	104.4	98.9	105.9	103.1	105.0
11	70.3	71.3	1′	121.8	121.5	120.9	120.9	130.5	123.1	121.7	123.1
12	76.0	79.2	2′	128.9	113.4	131.0	116.0	128.9	132.1	129.5	117.6
13	44.9	45.4	3′	116.5	145.7	115.8	145.7	115.6	116.1	115.4	145.9
14	72.0	67.5	4′	161.9	149.7	157.0	148.9	157.7	158.5	159.2	150.0
15	58.9	54.9	5′	116.5	116.4	115.8	116.1	115.6	116.1	115.4	116.1
16	32.4	29.9	6′	128.9	118.9	131.0	121.5	128.9	132.1	129.5	123.5
17	40.2	42.5	7′					165.9			
18	12.9	15.5	8′					121.7			
19	20.8	26.8	9′ 11′					109.8			
20	168.1	122.1	10′ 12′					145.9			
21	99.0	140.8	13′					138.7			
22	118.1	110.9	**Glusosyl**								
23	171.2	143.0	1′′						100.2		105.0
24			2′′						80.0		75.7
25			3′′						78.9		78.2
26			4′′						71.8		74.3
27			5′′						78.3		77.2
28	22.6	22.5	6′′						62.6		68.6
29	22.2	24.5	**Rhamnosyl**								
30	121.6	21.3	1′′′			102.1	102.2		102.6		102.4
11-O<u>C</u>OCH$_3$	169.9	169.2	2′′′			71.1	71.0		72.4		72.1
12-O<u>C</u>OCH$_3$	170.0	169.3	3′′′			70.7	70.7		72.3		72.2
11-OCO<u>C</u>H$_3$	20.3	20.9	4′′′			71.5	71.6		74.0		73.9
12-OCO<u>C</u>H$_3$	20.5	20.8	5′′′			70.5	70.3		69.9		67.9
-OMe	52.2		6′′′			17.9	178		17.5		17.9

Carbons	17[f]	18[f]	20[f]	21[e]
1	38.3	71.8	120.1	122.0
2	17.7	23.1	108.5	110.3
3	36.5	32.5	145.1	146.4
4	46.7	40.1	137.8	139.6
5	49.2	78.5	145.1	146.4
6	23.4	74.9	108.5	110.3

(continued)

Table 2.1 (continued)

Carbons	17[f]	18[f]	20[f]	21[e]
7	28.9	75.6	166.7	170.4
8	39.9	49.9		
9	45.5	33.5		
10	35.5	43.9		
11	36.9	37.9		
12	109.6	105.4		
13	173.1	178.1		
14	35.7	74.3		
15	112.9	113.2		
16	169.7	170.3		
17	12.6	20.2		
18	16.9	29.9		
19	178.6	24.9		
20	14.2	16.9		
1-CO-		169.8		
1-OMe		21.5		
6-CO-		170.2		
6-OMe		21.5		
7-CO-		170.4		
7-OMe		22.0	51.0	

[a]^{13}C (125 MHz, $CDCl_3$)
[b]^{13}C (150 MHz, CD_3CN)
[c]^{13}C (125 MHz, C_5D_5N)
[d]^{13}C (125 MHz, DMSO-d_6)
[e]^{13}C (125 MHz, CD_3OD)
[f]^{13}C (125 MHz, Acetone-d_6)

Lupeol (**7**): White powder; 0.6 mg; ^{1}H (500 MHz, $CDCl_3$): δ_H 4.68 (br s); 4.56 (br s); 3.18 (m); 2.37 (m); 1.67 (s); 1.03 (s); 0.94 (s); 0.90 (s); 0.82 (s); 0.69 (s); 0.68 (d, J = 8.9 Hz); 0.67 (s). ^{13}C (125 MHz, $CDCl_3$) (Table 2.1); ESIMS *m/z* 427.1 for $C_{30}H_{51}O$ $[M + H]^+$.

11-epi-21-Hydroxytoonacilide (**8**): White amorphous powder; 3.3 mg; ^{1}H (500 MHz, $CDCl_3$): δ_H 7.40 (d, J = 10.4 Hz); 6.16 40 (d, J = 10.4 Hz); 5.88 (s); 5.81 (s); 5.74 (m); 5.58 (m); 5.37 (br s); 5.29 (br s); 3.89 (br s); 3.71 (s); 2.99 (m); 2.96 (m); 2.89 (m); 2.47 (m); 2.45 (m); 2.31 (m); 2.11 (s); 1.97 (s); 1.87 (m); 1.07 (s); 1.06 (s); 0.97 (s) and 0.95 (s). ^{13}C (125 MHz, $CDCl_3$) (Table 2.1); ESIMS *m/z* 586.2 for $C_{31}H_{38}O_{11}$ $[M + H]^+$.

11*β*,12*α*-Diacetoxycedrelone (**9**): White amorphous powder; 3.6 mg; ^{1}H (500 MHz, $CDCl_3$): δ_H 7.55 (d, J = 1.7 Hz). 7.38 (1H. br d, J = 1.7 Hz). 6.98 (d, J = 10.0 Hz). 6.45 (s, OH. disappears in D_2O); 6.18 (d, J = 10.0 Hz); 6.10 (dd, J = 1.7, 0.7 Hz); 5.56 (br s). 5.29 (br s); 3.89 (br s); 2.92 (s); 2.90 (dd, J = 7.0, 11.3 Hz); 2.29 (dd, J = 7.0. 13.5 Hz); 1.97 (dd, J = 11.3, 13.5 Hz); 2.15 (s); 1.94

(s); 1.55 (s); 1.48 (s); 1.35 (s). 1.27 (s); 0.77 (s). ^{13}C (125 MHz, $CDCl_3$) (Table 2.1); ESIMS *m/z* 539.2 for $C_{30}H_{35}O_9$ $[M + H]^+$.

Apigenin (**10**): Yellowish amorphous powder; 0.4 mg; ^{1}H (500 MHz, CD_3OD): δ_H 12.92 (s); 7.91 (d, J = 10.0 Hz); 6.92 (d, J = 10.0 Hz); 6.73 (s); 6.48 (d, J = 5.0 Hz) and 6.19 (d, J = 5.0 Hz). ^{13}C (125 MHz, CD_3OD) (Table 2.1); ESIMS. *m/z* 271.1 for $C_{15}H_{11}O_5$ $[M + H]^+$.

Luteolin (**11**): Greenish-yellow crystals. 0.6 mg; m.p.: 328–330 °C; ^{1}H (500 MHz, DMSO-d_6): δ_H 6.55 (dd, J = 11.0, 2.6 Hz)' 6.10 (d, J = 9.0 Hz); 6.72 (s); 6.48 (d, J = 5.0 Hz) and 6.19 (d, J = 5.0 Hz) and 5.68 (d, J = 2.62 Hz). ^{13}C (125 MHz, DMSO-d_6) (Table 2.1); ESIMS. *m/z* 287.1 for $C_{15}H_{11}O_6$ $[M + H]^+$.

Afzelin (**12**): Yellowish amorphous powder; 4.5 mg; ^{1}H (500 MHz, DMSO-d_6): δ_H 7.77 (dd, J = 8.4. 2.0 Hz); 6.92 (dd, J = 8.4. 2.0 Hz); 6.37 (d, J = 2.1 Hz); 6.19 (d, J = 2.1 Hz); 5.30 (d, J = 1.5 Hz); 3.97 (m); 3.78 (m); 3.56 (m); 3.42 (m) and 0.78 (d, J = 5.9 Hz). ^{13}C (125 MHz, DMSO-d_6) (Table 2.1); ESIMS *m/z* 433.4 for $C_{21}H_{21}O_{10}$ $[M + H]^+$.

Quercitrin (**13**): Yellowish amorphous powder; 5.0 mg; ^{1}H (500 MHz, DMSO-d_6): δ_H 7.30 (d, J = 2.2 Hz); 7.26 (dd, J = 8.3, 2.2 Hz); 6.86 (d, J = 8.3Hz); 6.38 (d, J = 2.1 Hz); 6.20 (d, J = 2.1 Hz); 3.98 (m); 3.78 (m); 3.55 (m); 3.41 (m) and 0.80 (d, J = 5.8 Hz). ^{13}C (125 MHz, DMSO-d_6) (Table 2.1); ESIMS *m/z* 449.3 for $C_{21}H_{21}O_{11}$ $[M + H]^+$.

Epiafzelechin-3-*O*-gallate (**14**): Orange red powder; 2.0 mg; ^{1}H (500 MHz, acetone-d_6): δ_H 7.38 (dd, J = 8.6. 2.1 Hz); 7.02 (s); 6.77 (dd, J = 8.6, 2.1 Hz); 6.05 (d, J = 2.5 Hz); 6.03 (d, J = 2.5 Hz); 5.52 (m); 5.18 (br s); 3.05 (m); 2.93 (m). ^{13}C (125 MHz, acetone -d_6) (Table 2.1); ESIMS *m/z* 425.1 for $C_{22}H_{17}O_9$ $[M-H]^+$.

Kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**15**): Yellow needles; 1.7 mg; ^{1}H (500 MHz, CD_3OD): δ_H 8.03 (dd, J = 8.4, 2.0 Hz); 6.88 (dd, J = 8.4, 2.0 Hz); 6.36 (d, J = 2.0 Hz); 6.16 (d, J = 2.0 Hz); 5.73 (d, J = 7.7 Hz); 5.22 (d, J = 1.5 Hz); 4.04 (m); .4.00 (m); 3.78 (m); 3.73 (m); 3.62 (m); 3.56 (m); 3.50 (m); 3.33 (m); 3.29 (m); 3.23 (m); 0.95 (d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) (Table 2.1); EIMS *m/z* 595.3 for $C_{27}H_{31}O_{15}$ $[M + H]^+$.

Kaempferol (**16**): Yellowish amorphous powder; 0.7 mg; ^{1}H (500 MHz, DMSO-d_6): δ_H 8.07 (d, J = 9.0 Hz); 6.88 (d, J = 9.0 Hz); 6.38 (d, J = 2.0 Hz); 6.17 (d, J = 2.0 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) (Table 2.1); EIMS *m/z* 287.1 for $C_{15}H_{11}O_6$ $[M + H]^+$.

Dipteryxic acid (**17**): Colourless amorphous powder, 3.4 mg; ^{1}H (500 MHz, acetone-d_6): δ_H 5.73 (s); 2.88 (m); 2.25 (m); 1.69 (m); 1.63 (m); 1.53(m); 1.50 (m); 1.49 (m); 1.48 (m); 1.42 (m); 1.30 (m); 1.18 (m); 1.17 (m); 1.08 (s); 1.04 (d, J = 7.2 Hz); 1.00 (m) and 0.80 (s). ^{13}C (125 MHz, acetone-d_6) (Table 2.1); ESIMS. *m/z* 349.4 for $C_{20}H_{29}O_5$ $[M + H]^+$.

Neocaesalpin L (**18**): Colourless amorphous powder; 4.3 mg; $[\alpha]_D^{22}$ – 69 (c = 0.20. MeOH); ^{1}H (400 MHz, acetone-d_6): δ_H 5.79 (s); 4.66 (t, J = 2.2 Hz); 5.28 (d, J = 9.2 Hz) 5.54 (d, J = 9.2 Hz); 3.62 (s); 2.94 (m); 2.25 (m); 2.14 (m); 1.85–1.72 (m); 1.52 (m); 1.50 (m); 1.49 (m); 1.45 (m); 1.31 (m); 1.19 (m); 1.18 (m) 1.10

(s); 1.08 (d, J = 7.2 Hz) 1.05 (m) and 0.80 (s). ^{13}C (125 MHz, acetone-d_6) (Table 2.1); ESIMS. *m/z* 523.1 for $C_{26}H_{35}O_{11}$ $[M-H]^+$.

Rutin (**19**): Colourless powder; 2.2 mg; m.p.: 196.5–198 °C; $[\alpha]_D^{29}$ +13.82 (*c* 0.2; EtOH); ^{1}H (500 MHz, CD_3OD): δ_H 7.67 (d, J = 2.0 Hz); 7.62 (dd, J = 8.0, 2.0 Hz); 6.85 (d, J = 8.0 Hz); 6.33 (d, J = 2.0 Hz); 6.15 (d, J = 2.0 Hz); 5.05 (d, J = 7.5 Hz); 4.51 (br s); 3.47 (dd, J = 9.0; 7.0 Hz); 3.39 (t, J = 8.5 Hz); 3.27 (t, J = 9.0 Hz); 3.32 (m); 3.80 (dd, J = 10.5, 1.5 Hz); 3.39 (dd, J = 9.0, 6.0 Hz); 3.62 (br d, J = 2.0 Hz); 3.53 (dd, J = 9.5, 3.5 Hz); 3.27 (t, J = 9.0 Hz); 3.42 (q, J = 6.1 Hz); 1.11 (t, J = 6.0 Hz). ^{13}C (150 MHz, CD_3OD) (Table 2.1); ESIMS: 464.9 (M – rham) (18.0), 303.2 (M – rham – glu) (20). *m/z* 611.1 for $C_{27}H_{30}O_{16}$ $[M + H]^+$.

Methyl gallate (**20**): Yellowish crystal; 6.3 mg; m.p.: 195–200 °C; ^{1}H (500 MHz, acetone-d_6): δ_H 8.11 (s) and 7.13 (s). ^{13}C (125 MHz, acetone-d_6) (Table 2.1); ESIMS: *m/z* 185.2 for $C_8H_9O_5$ $[M + H]^+$

Galic acid (**21**): Brunish solid; 5.2 mg; m.p.: 220–223 °C ^{1}H (500 MHz, CD_3OD): δ_H 8.09 (s) and 7.11 (s). ^{13}C (125 MHz, CD_3OD) (Table 2.1); ESIMS: *m/z* 171.2 for $C_7H_7O_5$ $[M + H]^+$

2.2.6 Ovicidal Activity

For the assessment of the ovicidal properties of the isolates, the method described by Yanar et al. (2011) was used. Tomato leaves containing 20 eggs were sprayed with 0.5 ml of each concentrate of each test material. There were five replications for each treatment. The ovicidal percentage was determined for both experimental and control batches of eggs for a period of 8 days after oviposition. Those eggs that did not hatch after this period were regarded as non-viable. The positive control was made with azadirachtin, which was prepared similarly. The treatments were carried out at concentrations of 100 ng/μl and all the experiments were carried out at 25 ± 2 °C with 75 ± 5% relative humidity, for a photoperiod of 12 h.

2.3 Data Analysis

The data of the effects of each extract were analysed using generalised linear models (GLMs) belonging to the binomial family of distribution (Crawley 2007). When significant differences were found between the extracts, multiple comparisons were performed using Tukey's post-hoc test at $P < 0.05$, using the general linear hypothesis test (glht) function from the Multcomp package. The isolated compounds were subjected to two-way Analysis of Variance (ANOVA) to evaluate their ovicidal effects at different doses on *T. absoluta*. Means were compared using the Student-

Neuman-Kuel (SNK) Test ($P < 0.05$). Probit analysis was used to estimate the lethal dose (LD_{50}) of each extract and constituent. All the analyses were implemented using R 3.3.2 software (R Core Team 2015).

2.4 Results

2.4.1 Larvicidal Effect of Plant Extracts

Figure 2.1 below summarises the larvicidal effect of the methanolic extracts of *Turraea* leaves and *Caesalpinia* roots plant species.

The results showed that the mortality of larvae was dose-dependent (Fig. 2.1). The *T. floribunda* leaves extract exhibited the highest activity on *T. absoluta* larvae, with 80% of mortality at 1000 ng/µl and LD_{50} of 587.0 ng/µl after 24 h of exposure, followed by *C. welwitschiana* root extract (53%. LD_{50} = 779.1 ng/µl). The mortality caused by the solvents (acetone alone taken as control) was zero (0.0%) in all bioassays. The activities of all extracts at all doses were significantly different, at a 95% confidence level. Based on these results, the extract of *T. floribunda* leaves and *C. welwitschiana* roots were fractionated for isolation and structure elucidation of their bioactive constituents (Fig. 2.2).

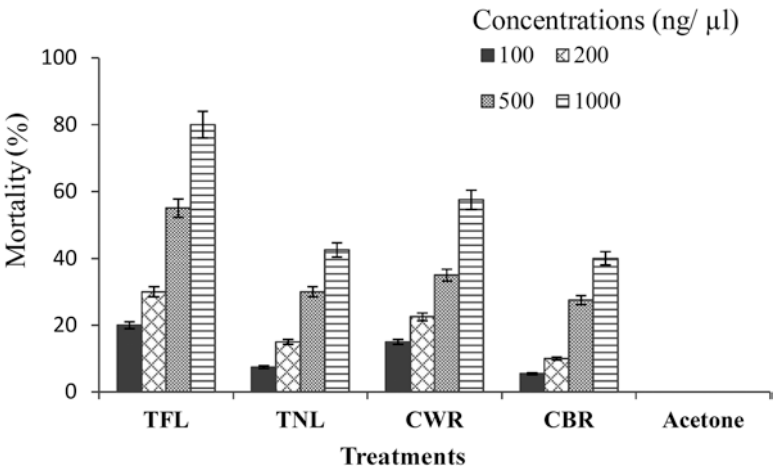

Fig. 2.1 Mortality percentage induced by methanol extracts of *Turraea* and *Caesalpinia* spp. Note: recorded at 100, 250, 500 and 1000 ng/µl against *Tuta absoluta* after 24 h of exposure. Acetone used as control. TFL = *Turraea floribunda* leaves; TNL = *Turraea nilotica* leaves; CWR = *Caesalpinia welwitschiana* roots; CBR = *Caesalpinia bonduc* roots

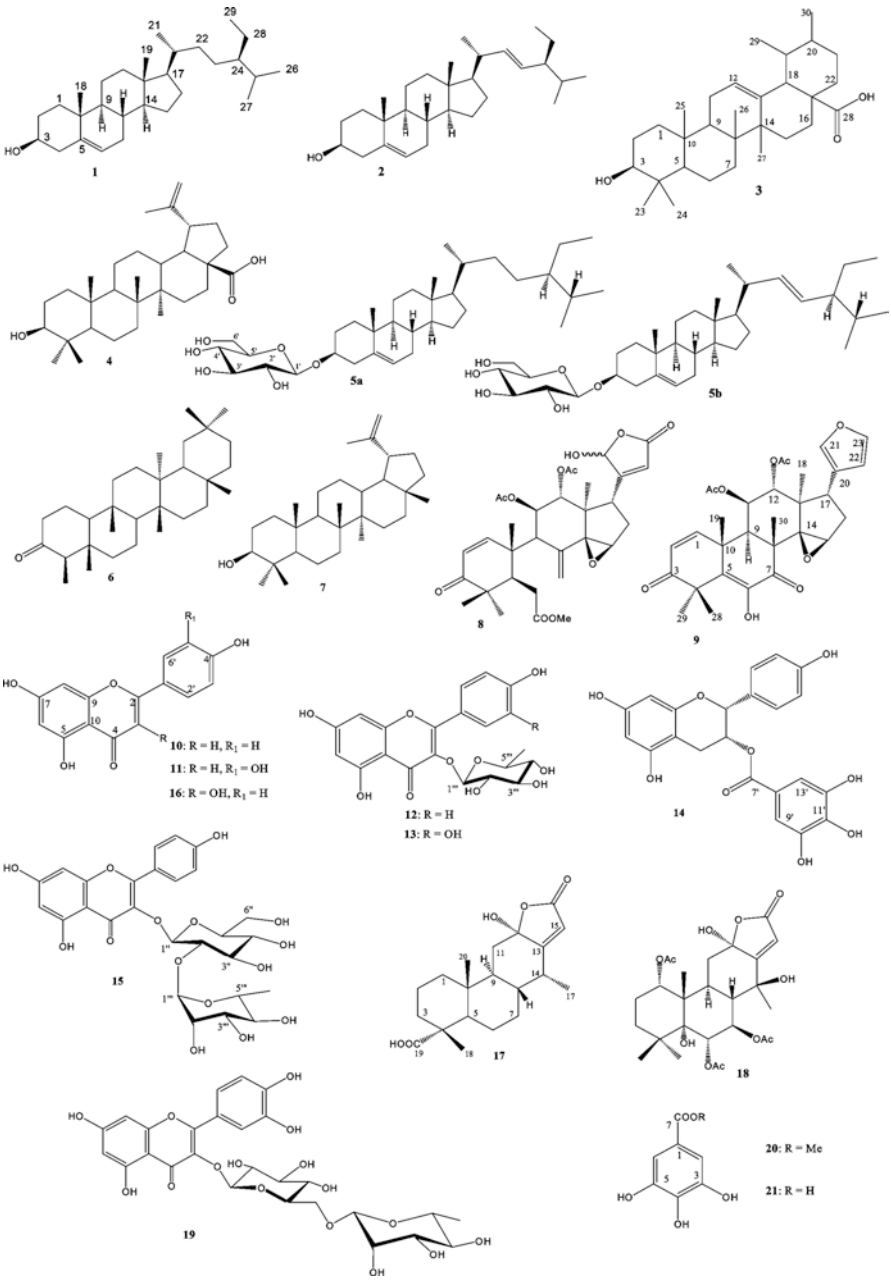

Fig. 2.2 Isolated constituents from *Turraea floribunda* leaves and *C. welwitschiana* roots

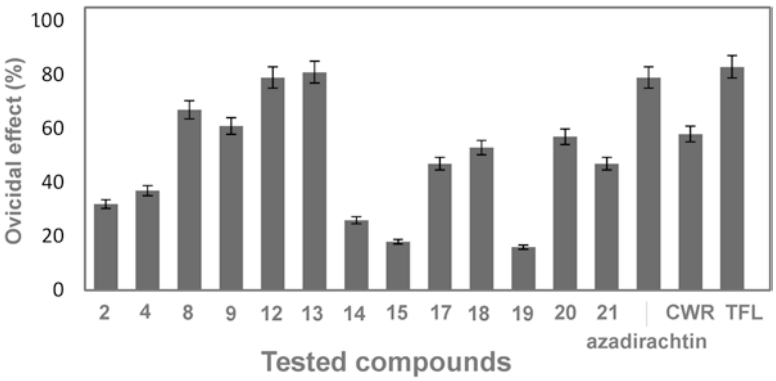

Fig. 2.3 Mean percentage of ovicidal activity of methanolic extracts (TFL and CWR) and some of their isolated constituents. Notes: measured at 100 ng/ µl ($P < 0.05$ Student-Neuman-Keuls test) and azadirachtin as positive control. TFL = *Turraea abyssinica* leaves and CWR = *C. welwitschiana* roots

2.4.2 *Isolation and Structures of Constituents from* T. floribunda *and* C. welwitschiana

The methanolic extract of *T. floribunda* leaves showed an ovicidal activity on *T. absoluta* of 83% (Fig. 2.3). Bioassay-guided fractionation of the crude extract and the active fractions led to the isolation of ten known compounds, including *β*-sistosterol (**1**) (Malebo et al. 2014), stigmasterol (**2**) (Malebo et al. 2014), ursolic acid (**3**) (Moghaddam et al. 2007), betulinic acid (**4**) (Cichewicz and Kouzi, 2003), a mixture of *β*-sistosterol-3-*O*-*β*-D-glucopyranoside (**5a**) and stigmastreol-3-*O*-*β*-D-glucopyranoside (**5b**) (Faizi et al. 2001), fridelin (**6**) (Sousa et al. 2012), lupeol (**7**) (Mahato and Kundu 1994), 11-epi-21-hydroxytoonacilide (**8**) (Cheplogoi and Mulholland 2003), and 11*β*, 12*α*-diacetoxycedrelone (**9**) (Mulholland et al. 1998).

The *C. welwitschiana* root extract exhibited 58% ovicidal effect on *T. absoluta* eggs (Fig. 2.3). The bioassay-guided fractionation of this extract led to the identification of twelve known constituents: apigenin (**10**) (Wagner et al. 1976), luteolin (**11**) (Youssef and Frahm 1995), afzelin (**12**), quercitrin (**13**) (Lee et al. 2014), epiafzelechin-3-*O*-gallate (**14**) (Hashimoto et al. 1987), Kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**15**) (Kazuma et al. 2003), Kaempferol (**16**) (Harborne and Williams 2000), dipteryxic acid (**17**) (Jang et al. 2003), neocaesalpin L (**18**) (Li et al. 2006), rutin (**19**) (Kazuma et al. 2003), methyl gallate (**20**), and galic acid (**21**) (Chaudhuri et al. 2015).

The ^{13}C NMR data of the isolates are listed in Table 2.1.

2.4.3 Ovicidal Activity

The isolated constituents from *T. floribunda* leaves and *C welwitschiana* roots were tested on *T. absoluta* eggs. All of them demonstrated varying levels of ovicidal activity (Fig. 2.3). Analysis of Variance (ANOVA) showed significant differences between the compounds, (F value = 277.9, DF = 15, $P < 0.0001$). The most ovicidal constituent was **13** (81%), which was more potent than that of the control azadirachtin (79%). Compounds **1**, **3**, **5**, **6**, **7**, **10**, **11** and **16** obtained in very small quantity were not bio-assayed.

2.5 Discussion

In the present study, the methanolic extracts of the leaves of two *Turraea* species and the roots of two *Caesalpinia* plants showed varying levels of larvicidal activities against *T. absoluta*, with *T. floribunda* leaves extract being the most active. The age, the geographical location and the parts of the plant, with the phytochemicals present, may play a role in affecting the different levels of activity of the crude extracts (Sukumar et al. 1991). The bioassay-guided isolation of constituents from *T. floribunda* and *C. welwitschiana* extracts led to the identification of twenty-two compounds, with thirteen being tested on *T. absoluta* eggs. Interestingly, except for afzelin and quercitrin**,** the crude extracts were more active than all the tested constituents, which suggest synergic interactions among the different compounds. These data corroborated previous reports on insecticidal properties of limonoids, diterpenes and flavonoids (Ndung'u et al. 2004; Tang et al. 2009; Singh et al. 2014; Essoung et al. 2017, 2018).

Moreover, flavonoid derivatives were found to possess the highest ovicidal activity, followed by limonoids. The difference in activity between limonoids and flavonoids may be attributable to the fact that limonoids act over a long period, with subtle growth-disrupting effects on insect cells (Bilker et al. 2002; Ndung'u et al. 2004). In addition, they were found to be non-toxic to mammal cells (Arona et al. 2004). On the other hand, flavonoids were found to be harmful to mammalian cells (Mukinda 2005). For example, quercetin has been identified as a potent topoisomerase II inhibitor at low concentrations (Skibola and Smith 2000); luteolin has been shown to be slightly more toxic towards isolated rat hepatocytes, and apigenin has been found to have inhibitory effects on Hela tumour cell proliferation (Moridani et al. 2002). It will be interesting to isolate a series of other limonoids from different *Turraea* species and flavonoids from *Caesalpinia* plants, in order to test their activities against the different stages of tomato borer and compare their structures with levels of activities, to identify structural features associated with activity. Further studies could help downstream development of these phytochemicals and their use in the effective control of the pest.

In summary, the present study demonstrates the potential of *Turraea* and *Caesalpinia* species as sources of terpenoids and flavonoids derivatives that have ovicidal effects on *T. absoluta*.

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References

Abbes, K., & Chermiti, B. (2012). Failure of the biological control of *Tuta absoluta* using the predator *Nesidiocoris Tenuis* in a protected tomato crop: Analysis of factors. *IOBC/WPRS Bulletin, 80*(October), 231–236.

Arona, R., Dhaliwal, G. S., Ganapathy, N., Giagnacovo, G., Gopalakrishnan, G., Isman, M. B., Kleeberg, H., et al. (2004). Present concepts of the mode of action of Azadirachtin from neem. In O. Koul & S. Wahab (Eds.), *Neem: Today and in the new millennium*. New York/Boston/Dordrecht/London/Moscow: Kluwer Academic. https://doi.org/10.1007/1-4020-2596-3_3.

Ben, I. C., Ndubuisi, U., & Nnah, M. B. (2010). Comparative studies of effect of garlic (*Allium Sativum*) and ginger (*Zingiber Officinale*) extracts on cowpea insects pest attack. *World Rural Observations, 2*(2), 65–71.

Bilker, O., Shaw, M. K., Jones, I. W., Ley, S. V., Mordue, A. J., & Sinden, R. E. (2002). Azadirachtin disrupts formation of organized microtubule arrays during microgametogenesis of Plasmodium. *Journal of Eukaryotic Microbiology, 49*, 489–497.

Biondi, A., Guedes, R. N. C., Wan, F., & Desneux, N. (2018). Ecology, worldwide spread, and Management of the Invasive South American Tomato Pinworm, Tuta absoluta: Past, present, and future. *Annual Review of Entomology, 63*(September 2017), 239–258.

Bughio, F. M., & Wilkins, R. M. (2004). Influence of malathion resistance status on survival and growth of *Tribolium Castaneum* (Coleoptera: Tenebrionidae), when fed on flour from insect-resistant and susceptible grain rice cultivars. *Journal of Stored Products Research, 40*, 65–75. https://doi.org/10.1016/S0022-474X(02)00077-2.

Campos, R. G. (1976). Control químico del minador de hojas y tallos de la papa (*Scrobipalpula absoluta* Meyrick) en el Valle de Cañete. *Rev Per Entomol, 19*(7), 102–106.

Chaudhuri, D., Ghate, N. B., Singh, S. S., & Mandal, N. (2015). Methyl gallate isolated from *Spondias pinnata* exhibits anticancer activity against human glioblastoma by induction of apoptosis and sustained extracellular signal-regulated kinase 1/2 activation. *Pharmacognosy Magazine, 11*(42), 269. https://doi.org/10.4103/0973-1296.153078.

Cheplogoi, P. K., & Mulholland, D. A. (2003). Tetranortriterpenoid derivatives from *Turraea parvifolia* (Meliaceae). *Phytochemistry, 62*(8), 1173–1178. https://doi.org/10.1016/S0031-9422(03)00028-1.

Cichewicz, R. H., & Kouzi, S. A. (2003). Chemistry, biological activity, and chemotherapeutic potential of betulinic acid for the prevention and treatment of cancer and HIV infection. *Medicinal Research Reviews, 24*(1), 90–114. https://doi.org/10.1002/med.10053.

Cox, P. D. (2004). Potential for using semiochemicals to protect stored products from insect infestation. *Journal of Stored Products Research, 40*(1), 1–25. https://doi.org/10.1016/S0022-474X(02)00078-4.

Crawley, M. J. (2007). *The R book*. Chichester: Wiley.

Desneux, N., Wajnberg, E., Wyckhuys, K. A. G., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., González-Cabrera, J., et al. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. *Journal of Pest Science, 83*(3), 197–215. https://doi.org/10.1007/s10340-010-0321-6.

European Plant Protection (EPPO). (2014). *PQR database*. Paris: European and Mediterranean Plant Protection. http://www.eppo.int/DATABASES/pqr.htm.

European Plant Protection (EPPO). (2016). *Global database*. Paris: EPPO. https://gd.eppo.int/.

Essoung, E. F. R., Chhabra, S. C., Mba'ning, M. B., Mohamed, S. A., Lwande, W., Lenta, B. N., & Hassanali, A. (2018). Larvicidal activities of limonoids from Turraea abyssinica (Meliaceae) on Tuta absoluta (Meyrick). *Journal of Applied Entomology*, (August 2017), 1–9. https://doi.org/10.1111/jen.12485.

Essoung, E. F. R., Mba'ning, M. B., Abuelgasim, M. S., Lwande, W., Ngouela, S. A., Tsamo, E., & Cox, R. J. (2017). Welwitschianalol a and B, two cyclohexene derivatives and other insecticidal constituents of Caesalpinia welwitschiana (Oliv.) *Brenan. Phytochemistry Letters, 22*(August), 81–86. https://doi.org/10.1016/j.phytol.2017.09.010.

Faizi, S., Ali, M., Saleem, R., Irfanullah, & Bibi, S. (2001). Spectral assignments and reference data complete1h and13c nmr assignments of stigma-5-en-3-o-β-glucoside and its acetyl derivative. *Magnetic Resonance in Chemistry, 39*(7), 399–405. https://doi.org/10.1002/mrc.855.

Fraser, L. A., Mulholland, D. A., & Nair, J. J. (1994). Limonoids from the seeds of *Turraea floribunda*. *Phytochemistry, 35*(2), 45545–45548.

Garcia, M. F., & Espul, J. C. (1982). Bioecology of the tomato moth (Scrobipalpula Absoluta) in Mendoza, Argentine Republic. *Revista de Investigaciones Agropecuarias, 17*(2), 135–146.

Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55*, 481–504.

Hashimoto, F., Nonaka, G.-i., & Nishioka, I. (1987). Tannins and related compounds. LVI isolation of four new acylated flavan-3-Ols from oolong tea. *Chemical & Pharmaceutical Bulletin, 35*(2), 611–616.

International Protection Plant Convention (IPPC). (2014). *New Pest in Kenya: Preliminary Surveillance Report on Tuta Absoluta – Preliminary report by Kenya*. IPPC Official Pest Report (No. KEN-01/2). Rome: FAO. https://www.ippc.int/

Irungu, B. N., Adipo, N., Orwa, J. A., Kimani, F., Heydenreich, M., Midiwo, J. O., Björemark, P. M., Håkansson, M., Yenesew, A., & Erdélyi, M. (2015). Antiplasmodial and cytotoxic activities of the constituents of *Turraea Robusta* and *Turraea Nilotica*. *Journal of Ethnopharmacology, 174*, 419–425. Elsevier. https://doi.org/10.1016/j.jep.2015.08.039.

Jang, D. S., Park, E. J., Hawthorne, M. E., Schunke Vigo, J., Graham, J. G., Cabieses, F., Santarsiero, B. D., et al. (2003). Potential cancer chemopreventive constituents of the seeds of *Dipteryx odorata* (Tonka Bean). *Journal of Natural Products, 66*(5), 3–7.

Kazuma, K., Noda, N., & Suzuki, M. (2003). Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochemistry, 62*, 229–237.

Kuria, K. A. M., De Coster, S., Muriuki, G., Masengo, W., Kibwage, I., Hoogmartens, J., & Laekeman, G. M. (2001). Antimalarial activity of *Ajuga Remota* Benth (Labiatae) and *Caesalpinia Volkensii* Harms (Caesalpiniaceae): In vitro confirmation of ethnopharmacological use. *Journal of Ethnopharmacology, 74*(2), 141–148. https://doi.org/10.1016/S0378-8741(00)00367-6.

Lee, S. Y., So, Y. J., Shin, M. S., Cho, J. Y., & Lee, J. (2014). Antibacterial effects of afzelin isolated from *Cornus Macrophylla* on *Pseudomonas Aeruginosa*, a leading cause of illness in immunocompromised individuals. *Molecules, 19*(3), 3173–3180. https://doi.org/10.3390/molecules19033173.

Li, D. M., Ma, L., Liu, G. M., & Hu, L. H. (2006). Cassane diterpene-lactones from the seed of *Caesalpinia minax* HANCE. *Chemistry and Biodiversity, 3*(11), 1260–1265. https://doi.org/10.1002/cbdv.200690128.

Lietti, M. M. M., Botto, E., & Alzogaray, R. A. (2005). Insecticide resistance in argentine populations of *Tuta Absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology, 34*(1), 113–119. https://doi.org/10.1590/S1519-566X2005000100016.

Linn, T. Z., Awale, S., Tezuka, Y., Banskota, A. H., Kalauni, S. K., Attamimi, F., Ueda, J.-y., et al. (2005). Cassane- and norcassane-type diterpenes from *Caesalpinia crista* of indonesia and their antimalarial activity against the growth of *Plasmodium falciparum*. *Journal of Natural Products, 14*(17), 11–15.

Mahato, S. B., & Kundu, A. P. (1994). 13C NMR Spectra of pentacyclic triterpenoids-a compilation and some salient features. *Phytochemistry, 37*, 1517–1575.

Malebo, H. M., Wenziler, T., Cal, M., Swaleh, S. M., Hassanali, A., Machocho, A. K., Sequin, U., et al. (2014). Anti protozoal and structure-activity relationship of chemical constituents of *Acridocarpus chloropterus* Oliver (Malpighiaceae) from Tanzania. *International Journal of Natural Products Research, 4*(2), 58–64.

Miresmailli, S., Bradbury, R., & Isman, M. B. (2006). Comparative Toxicity of *Rosmarinus Officinalis* L. essential oil and blends of its major constituents against *Tetranychus Urticae* Koch (Acari: Tetranychidae) on two different host plants. *Pest Management Science, 62*(4), 366–371. https://doi.org/10.1002/ps.1157.

Moghaddam, F. M., Farimani, M. M., Salahvarzi, S., & Amin, G. (2007). Chemical constituents of dichloromethane extract of cultivated *Satureja Khuzistanica*. *eCAM, 4*, 95–98.

Moreira, G. R., Derly, J., Silva, H., Picanço, M. C., Peternelli, L. A., & Caliman, R. B. (2005). Divergência genética entre acessos de tomateiro infestados por diferentes populações da traça-do-tomateiro. *Horticultura Brasileira, Brasilia, 23*(4), 893–898. https://doi.org/10.1590/S0102-05362005000400006.

Moridani, M. Y., Galati, G., & O'Brien, P. J. (2002). Comparative quantitative structure toxicity relationships for flavonoids evaluated in isolated rat hepatocytes and HeLa tumor cells. *Chemico-Biological Interactions, 139*, 251–264.

Mukinda, J. T. (2005, December). *Acute and chronic toxicity of the flavonoid- containing plant, Artemisia afra in rodents keywords*. University of Western Cape. MSc dissertation, pp. 1–149. http://hdl.handle.net/11394/1604

Mulholland, D. A., Monkhe, T. V., Coombes, P. H., & Rajab, M. S. (1998). Limonoids from *Turraea Holstii* and *Turraea Floribunda*. *Phytochemistry, 49*(8), 2585–2590.

Ndung'u, M. W., Kaoneka, B., Hassanali, A., Lwande, W., Hooper, A. M., Tayman, F., Zerbe, O., & Torto, B. (2004). New Mosquito Larvicidal Tetranortriterpenoids from *Turraea wakefieldii* and *Turraea floribunda*. *Journal of Agricultural and Food Chemistry, 52*(16), 5027–5031. https://doi.org/10.1021/jf049474y.

Ochieng', C. O., Okinda Owuor, P., Mang'Uro, L. A. O., Akala, H., & Ishola, I. O. (2012). Antinociceptive and antiplasmodial activities of Cassane Furanoditerpenes from *Caesalpinia Volkensii* H. Root Bark. *Fitoterapia, 83*(1), 74–80. Elsevier B.V. https://doi.org/10.1016/j.fitote.2011.09.015.

R Core Team. (2015). *R Core Team. R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. ISBN:3-900051-07-0. http://www.R-Project.org/

Roditakis, E., Vasakis, E., Grispou, M., Stavrakaki, M., Nauen, R., Gravouil, M., & Bassi, A. (2015). First report of *Tuta Absoluta* resistance to diamide insecticides. *Journal of Pest Science, 88*(1), 9–16. https://doi.org/10.1007/s10340-015-0643-5.

Singh, H., Dixit, S., Verma, P. C., & Singh, P. K. (2014). Evaluation of total phenolic compounds and insecticidal and antioxidant activities of tomato hairy root extract. *Journal of Agricultural and Food Chemistry, 62*(12), 2588–2594. https://doi.org/10.1021/jf405695y.

Skibola, C. F., & Smith, M. T. (2000). Potential health in pacts of excessive flavonoid intake. *Free Radical Biology & Medicine, 29*, 375–383.

Sousa, G. F., Duarte, L. P., Alcântara, A. F. C., Silva, G. D. F., Vieira-filho, S. A., Silva, R. R., Oliveira, D. M., & Takahashi, J. A. (2012). New triterpenes from *Maytenus Robusta*: Structural

elucidation based on NMR experimental data and theoretical calculations. *Molecules, 17*, 13439–13456. https://doi.org/10.3390/molecules171113439.

Sukumar, K., Perich, M. J., & Boobar, L. R. (1991). Botanical derivatives in mosquito control: A review. *Journal of the American Mosquito Control Association, 7*(2), 210–237.

Sylla, S., Brévault, T., Bal, A. B., Chailleux, A., Diatte, M., Desneux, N., & Diarra, K. (2017). Rapid spread of the tomato leafminer, Tuta absoluta (Lepidoptera: Gelechiidae), an invasive pest in sub-Saharan Africa. *Entomologia Generalis, 36*(3), 269–283. https://doi.org/10.1127/entomologia/2017/0453.

Tang, W., Wei, X., Xu, H., Zeng, D., & Long, L. (2009). 13-deoxyitol a, a new insecticidal isoryanodane diterpene from the seeds of *Itoa orientalis*. *Fitoterapia, 80*(5), 286–289. https://doi.org/10.1016/j.fitote.2009.03.006.

Udenigwe, C. C., Ata, A., & Samarasekera, R. (2007). Glutathione S-transferase inhibiting chemical constituents of *Caesalpinia Bonduc*. *Chemical & Pharmaceutical Bulletin, 55*(3), 442–445. https://doi.org/10.1248/cpb.55.442.

Wagner, H., Chari, V. M., & Sonnenbichler, J. (1976). 13C NMR Spektren Naturlich Vorkommender Flavonoide. *Tetrahedron Letters, 17*, 1799–1802.

Xian, X. (2017). *The potential invasion risk of the tomato leafminer Tuta absoluta in China*. In conference. Ottawa, Canada, 30th August 2017 (pp. 1–37).

Xu, X., Yuan, J., Zhou, X., Li, W., Zhu, N., Wu, H., Li, P., Sun, Z., Yang, J., & Ma, G. (2016). Cassane diterpenes with oxygen bridge from the seeds of *Caesalpinia sappan*. *Fitoterapia, 112*, 205–210. https://doi.org/10.1016/j.fitote.2016.06.005.

Yanar, D., Kadıo, Đ., & Gökçe, A. (2011). Ovicidal activity of different plant extracts on two- spotted spider mite (*Tetranychus Urticae* Koch) (Acari: Tetranychidae). *Scientific Research and Essay, 6*(14), 3041–3044. https://doi.org/10.5897/SRE11.535.

Youssef, D., & Frahm, A. W. (1995). Constituents of the Egyptian Centaurea scoparia; III. Phenolic constituents of the aerial parts. *Planta Medica, 61*, 570–573. https://doi.org/10.1055/s-2006-959378.

Yuan, C.-M., Tang, G.-H., Wang, X.-Y., Zhang, Y., Cao, M.-M., Li, X.-H., Li, Y., et al. (2013). New steroids and sesquiterpene from *Turraea Pubescens*. *Fitoterapia, 90*, 119–125. Elsevier B.V. https://doi.org/10.1016/j.fitote.2013.07.006.

Zanin, B. J. L., De Carvalho, B. A., Salles Martineli, P., Dos Santos, M. H., Lago, J. H. G., Sartorelli, P., Viegas, C., & Soares, M. G. (2012). The genus *Caesalpinia* L. (Caesalpiniaceae): Phytochemical and pharmacological characteristics. *Molecules, 17*(7), 7887–7902. https://doi.org/10.3390/molecules17077887.

Chapter 3
Tuta absoluta (Meyrick): Seasonal Abundance and Susceptibility of Some Tomato Genotypes in Gezira State, Sudan

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Abstract *Tuta absoluta* (Meyrick) is a key agricultural pest threatening tomato production in Sudan. The objective of this study was to evaluate the seasonal abundance of this pest and the susceptibility of different tomato genotypes. Experiments were carried out on the experimental farm of the University of Gezira, during the years 2015–2017, arranged in a randomised complete block design, with three replicates. Three parameters (infested leaves, % of infested fruits, and yield ton/fed) were used as indicators for the susceptibility of the tomato genotypes. Seasonal abundance reflected peaks during March, April and May. Genotype Rioking recorded the highest level of infestation. However, in the second year (2017), there were significant differences among genotypes. Salama and RILG3- 162 recorded the lowest levels of leaf infestation. Moreover, both Salama and RILG3-162 recorded lowest fruit infestations, followed by Rioking. RILG3-162, KPH 5822 and Salama were tolerant to insect infestation and could be recommended for further studies.

Keywords *T. absoluta* · Tomato genotypes · Seasonal abundance · Temperature · Wind velocity

3.1 Introduction

Over the last century, tomato *Solanum lycopersicum* has become an important vegetable crop and has attained a tremendous popularity as it can be grown in most places all over the world, in open fields, greenhouses, net houses and under tree

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shade. In Asia and Africa, tomato accounts for more than 65% of global production. In Africa, tomatoes are produced by small- and medium-scale farmers for home consumption and as a source of income (Daniel and Bajarang 2017). The tomato crop is grown and used for both fresh markets and processing, and is an adaptable crop. Over the past 25 years, the demand for tomato has shown high records in both production and consumption. In the Sudan, the main production areas are concentrated in the northern part of Gezira scheme, in the southern part of Khartoum State (El Assi 2001). Nevertheless, tomato is grown all around the country as a winter crop (main season) and as an off-season crop during summer and autumn. It is the second most important vegetable after onion. It is produced around large cities and towns along the Nile, and on seasonally flooded plains.

Tomato production is hindered by many obstacles and the most serious of these comprise insect pest infestation and inappropriate insect pest control. Tomato is one of the crops that receives the most prevalent but unwise use of pesticides. The target pests are whitefly, *Bemisia tabaci*; leaf miner, *Liriomyza trifolii*; fruit borer, *Helicoverpa armigera*; and recently, the invasive and devastating tomato leaf miner, *Tuta absoluta* (EPPO 2005).

Tuta absoluta originates from Peru or Chile in South America (Urbaneja et al. 2007) and has only recently been introduced to the Mediterranean region. However, since then, it has spread very quickly along the Mediterranean basin and to other Central and Northern European countries (Potting 2009; Desneux et al. 2010). It is mainly carried from place to place through wind currents, because it has a limited flight capability (Desneux et al. 2010). The larvae are the damage-causing agents. They penetrate the leaves, fruits and stems, and feed on them. This creates conspicuous voids which may be invaded later by secondary pathogens, leading to fruit rot (EPPO 2005), thereby directly reducing crop value.

The use of insecticides to control *T. absoluta* is proving to be an unsustainable management option in South America, where insecticide resistance has been recorded for several products. However, *T. absoluta* specimens have a short generation time and high biotic potential characteristics that have increased their likelihood of developing resistance to the insecticide used (Michereff-Filho et al. 2000; Ferrara et al. 2001).

T. absoluta was reported in Sudan in 2010 (Mohamed et al. 2012) and since then, a number of insecticides have been tested, but *T. absoluta* is not yet under control. No classical control approaches to cater for *T. absoluta* have been practised, such as mass trapping of males using a sex pheromone. This is a technique that can be used for pest detection, population monitoring, and mass trapping and/or mating disruption. Moreover, some insects may be controlled by a combination of practices that are each not fully effective when used separately. *Tuta absoluta* is one of those insects requiring more than one practice to be used at a time, to attain successful control (Daniel and Bajarang 2017). The contribution of tomato pest-resistant varieties among these practices used for control of *T. absoluta* is meagre. This may be due to the character of the tomato plant, which has been claimed to have a narrow genetic base (Zekeya et al. 2017). However, the development of pest-resistant tomato varieties has been intensively pursued since the early 1990s, particularly in

Brazil. Sources of *T. absoluta* resistance were readily identified, and the leaf density of glandular trichomes (mainly of type VI) was the initial focus of attention (Ecole et al. 1999; Lietti et al. 2005). Various tomato genotypes and cultivars were grown under field conditions during winter periods to give yielding tomatoes in March, so as to coincide with the peak of *T. absoluta*, a fact that was known from a preliminary seasonal abundance study of the insect. The objective of this study, then, was to determine the peak of the pest population through techniques of seasonal abundance, using pheromone-baited traps, and then to ascertain a sowing date that would lead to crops fruiting within the peak of pest population, under which various tomato cultivars and genotypes were tested.

3.2 Materials and Methods

3.2.1 Site of the Experiments

Experiments were conducted at the Experimental Farm, University of Gezira, Faculty of Agricultural Sciences, which is located at Latitude 14.44°N, Longitude 33.39°E and Altitude 407 m above sea level.

3.2.2 Seasonal Abundance of Tuta absoluta

Pheromone-baited water traps were used in this experiment. The trap was a plastic bowl of a round shape, with a radius of 15 cm and a volume of 3.5 l. The bowls were made of red plastic material, baited with 'Tua optima' sex pheromone of 0.8 mg loaded onto a grey rubber septa (produced by Russell IPM, England, imported by Star Chemical Ltd). The pheromone was stored in a refrigerator until time of use in the field. The pheromone rubber septa was renewed every 10 weeks. The grey rubber septa impregnated with pheromone was secured in a small porous, translucent, plastic vial (vol. 50 ml). The base of the vial and the screw cover were perforated for a tender wire to pass through longitudinally and fixed in the centre of the round shape by knotting the ends of the tender wire at the rim of the bowl.

The bowl was filled with water to a level just below the vial. The water surface tension was broken by drops of liquid soap. All bowl traps were deployed upwind of the tomato crop at 6:00 pm and the captured males (if any) were collected at 9:00 am, the next day. The study continued for two years, 2015 and 2016. The bowls, tender wire, liquid soap and others necessary items were purchased at local grocery stores. Temperatures and wind velocities (m/s), were recorded. The average numbers of males captured per month, recorded against temperatures and wind velocities, were shown on graphs using Microsoft's Excel program.

3.2.3 Tomato Cultivation Practices

A disc plough was used for land preparation, followed by a disc harrow to break the soil clods, after which the soil was levelled before making plant beds at a width of 160 cm each. The area under the crop was pre-watered to allow weeds to grow before using an herbicide (Glyphosate), at a rate of 1.5 l per feddan (1 feddan = 0.42 ha), equivalent to 615 g active ingredient (a.i.).

Tomato plants were directly seeded at the line of water seepage on the top of the bed, at a distance of 30 cm between holes, and thinned to 2–3 plants, 3 weeks after sowing. Nitrogen in the form of urea was applied at equal doses, 4 weeks after first irrigation and one month later, to give a total dose of 2 N (80 kg urea per feddan). Foliar fertiliser (Aminogen) was applied twice, at a rate of 500 ml/feddan. The first dose was applied 12 weeks after planting and the second dose was applied one week later, after the first dose. Weeding of the plots was done when needed. Irrigation, depending on temperature, was applied every 7 to 10 days. No pesticides were used, except the seed dresser Gaucho 70 WP at the time of sowing, which was applied at the rate of 7 g (4.9 g a.i.) per kg of seeds, that were locally produced. A fungicide (sulphur) was also applied as a protective chemical to control powdery mildew, 9 weeks after planting.

3.2.4 Layout

Tomato plants were grown for two years, 2016 and 2017. For the first year, tomato was planted on January 25, 2016, and for the second year, it was planted on November 10, 2016. For the first year, the following tomato genotypes and cultivars were tested:

1. Peto 86
2. Amani
3. Rioking
4. Castlerock
5. Salama
6. GS-12F1
7. RILG3-162
8. KPH 5822

For the second year, the genotypes GS-12 F1 and KPH 5822 were replaced by Strain B and Ahlam cultivars, due to lack of seeds.

The tomato genotypes and cultivars were arranged into a randomised complete block (RCB) design of 24 plots, and each treatment was replicated thrice into plots. The plot size was prepared into beds of 5.0 broad ridges (1.6 m width), each ridge with a length of 7.0 m.

3.2.5 Data Collection

Data were collected for the tomato leaf miner, *T. absoluta*; leaf miner, *Liriomyza trifolii*; and mealybug, *Phenacoccus solenopsis.* For the tomato leaf miners, the upper-third portion of the plant was observed closely, while a similar observation was made for leaf miners in the bottom-third portion of the plant. For the mealybug, the whole plant was observed for the presence or absence of the insect. Moreover, in each plot, 5 plants were checked for tomato leaf miner and leaf miner. In each plant, 10 tomato leaflets were checked: 4 leaflets in the upper portion, 2 in the middle, and 4 at the bottom. For mealybug, 10 plants were checked in each plot. Plants were chosen randomly. For yield estimation and damage, the fruits were harvested periodically when their colour changed from green to pink. Five plants were tagged per plot, and the harvested fruits were counted and sorted into damaged and sound groups at the field. These were taken to the laboratory for weighing, where the non-fully ripened fruits were allowed to ripen for 3.0 days and then sorted into damaged and sound groups.

3.2.6 Data Analysis

The data were transformed to appropriate transformation if needed, before being subjected to analysis of variance using the Statistx 8 program. The significant means were separated using Duncan's Multiple Range Test (DMRT, $p \leq 0.05$).

3.3 Results and Discussion

3.3.1 Seasonal Abundance of Tuta absoluta

The numbers of *T. absoluta* males captured in pheromone traps are shown in Figs. 3.1, 3.2, 3.3 and 3.4. The results reflect that peaks in the numbers of captured males occurred in March, April and May, which are considered as favourable months for this insect. However, June reflected a higher temperature and wind velocity, while a lower wind velocity was recorded in November 2015 and October 2016. A lower temperature was reported during December to January for the two years during the study, and no correlation was found between seasonal moth abundance and both temperature and wind velocity.

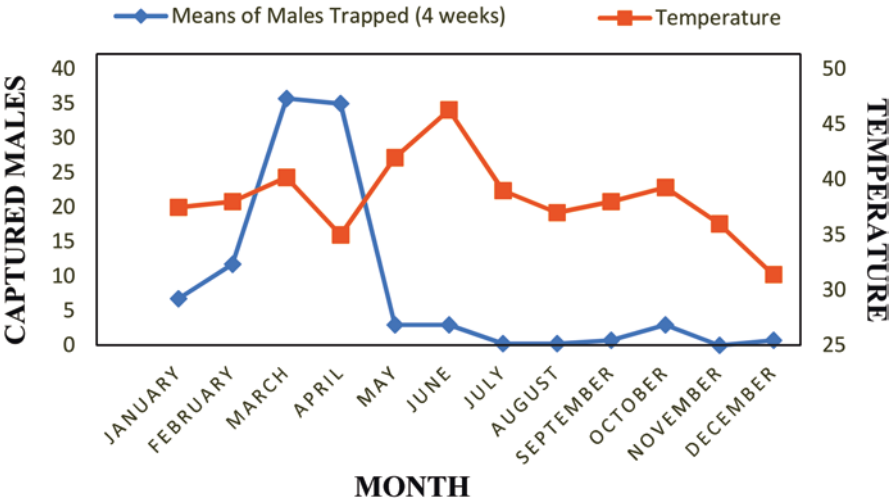

Fig. 3.1 Captured males of *Tuta absoluta*, and temperature data, 2015

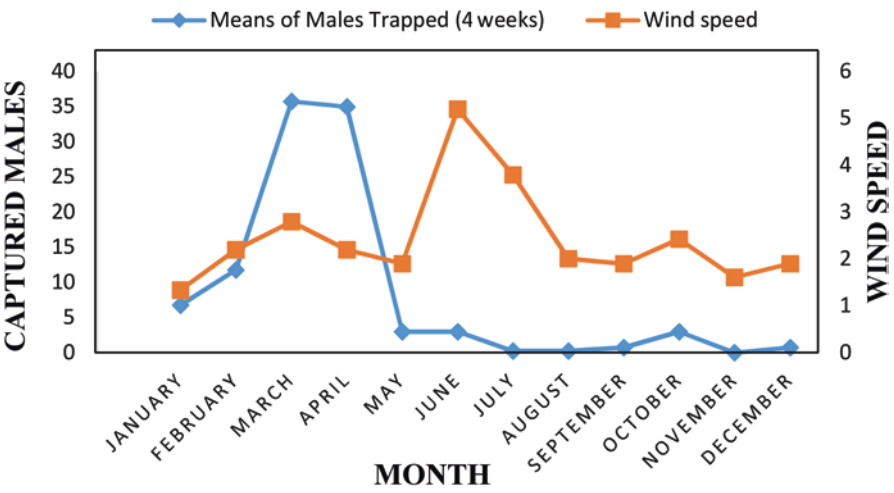

Fig. 3.2 Captured males of *T. absoluta*, and wind velocity, 2015

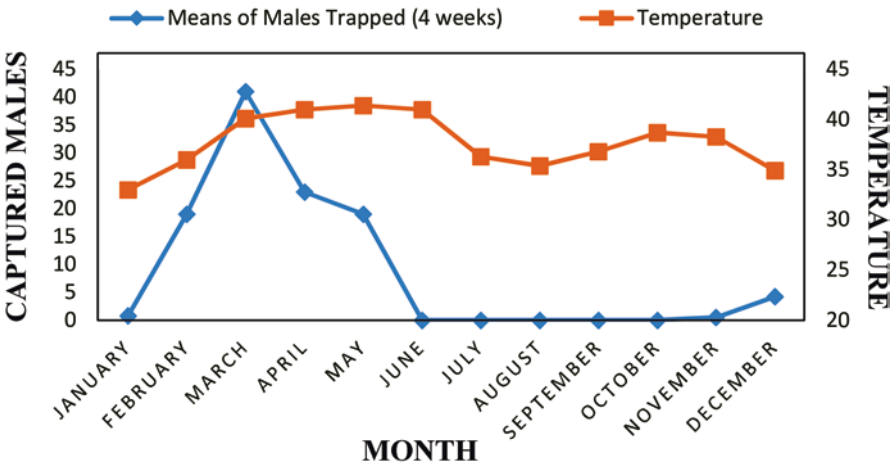

Fig. 3.3 Captured males of *T. absoluta*, and temperature data, 2016

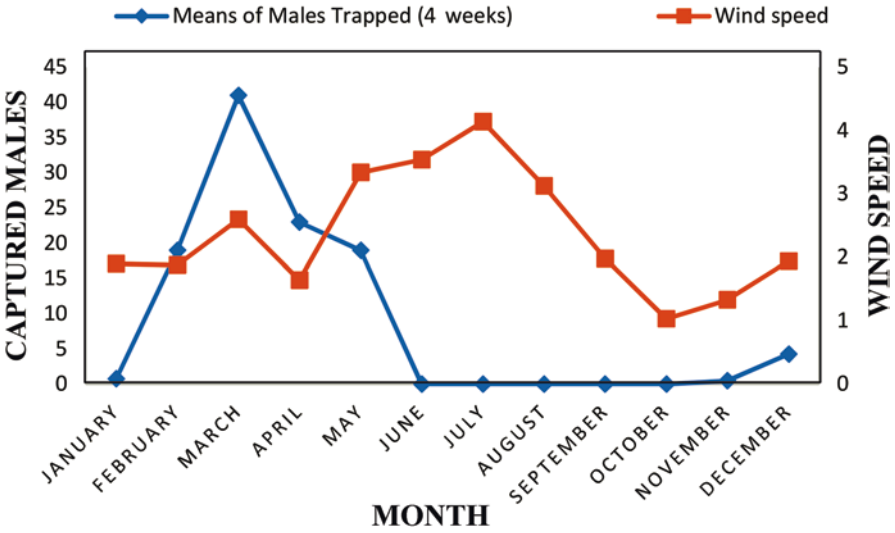

Fig. 3.4 Captured males of *T. absoluta* and wind velocity, 2016

3.3.2 Leaf Infestation Level

Tomato Leaf Miner, *Tuta absoluta*

Table 3.1 shows that the infestations ranged between 28% and 41% during 2016, while in 2017 the range was between 13% and 28%. However, in both seasons, the genotype RILG3-162 reflected some resistance to tomato leaf miner infestation. Nevertheless, the genotype KPH 5822, tested for one season,

also showed performance similar to that shown by genotype RILG3-162. The genotypes RILG3-162, KPH 5822 and GS-12F1 were significantly different from most of the tested cultivars (p = 0.05). On the other hand, all tested cultivars, whether for one or for two seasons, showed high susceptibility to tomato leaf miner infestations, which were significantly different (p = 0.05), except cultivar Salama, which showed sporadic infestation in the two seasons. This cultivar was as good as genotype RILG3-162 in terms of resistance to leaf infestation, and was significantly different from all cultivars under test in season 2017 (p = 0.05).

There is a need to investigate if the resistance in genotypes RILG3-162, KPH 5822 and GS-12 F_1 is due to antixenosis or antibiosis (Ataide et al. 2017). However, all cultivars under test were proved to be susceptible to tomato leaf miner, except the Salama cultivar which proves more appropriate to be utilised as a component of integrated pest management (Vendramim and Nishikawa 2001).

Leaf Miner, *Liriomyza trifolii*

Table 3.2 shows that infestation of the leaf miner, *Liriomyza trifolii*, was very erratic in 2016, as reflected by the high coefficient of variation (32.8). Nevertheless, the leaf infestation was in the range of 8 – 14%, while the leaf infestation in 2017 was in the range of 13 – 28%. Again, genotypes RILG3-162, KPH5822 and GS-12F1 reflected lower infestations. However, cultivar Salama showed a resistant pattern as good as the one shown by resistant genotype RILG3-162.

Table 3.1 Mean percentage leaf infestation by tomato leaf miner *Tuta absoluta* on tomato cultivars and genotypes during 2016 and 2017

	Infestation (%)	
Treatment	2016∗	2017
Ahlam	–	21.0 a
Peto 86	39 a	16.0 ab
Amani	35 ab	16.0 ab
Rioking	41 a	17.0 ab
Strain B	–	17.0 ab
Castlerock	36 ab	14.0 b
Salama	35 ab	11.0 bc
GS-12 F1	32 ab	–
RILG3 162	28 b	8.0 c
KPH 5822	28 b	–
SE±	4.07	2.9
CV%	21	23.3

∗Means followed by the same letter(s) are not significantly different according to DMRT ($p \leq 0.05$)

Table 3.2 Mean percentage leaf infestation by leaf miner *Liriomyza trifolii* on tomato cultivars and genotypes during 2016 and 2017

	Infestation (%)	
Treatment	2016∗	2017
Peto 86	(14.3)3.7 a	26 a
Amani	(11.3) 3.2 a	25 a
Rioking	(12) 3.3 a	26 a
Castlerock	(10) 3.1 a	26 a
Strain B	–	28 a
Ahlam	–	27 a
Salama	(12) 3.4 a	16 b
GS-12 F1	(8) 2.8 a	–
RILG3 162	(9) 2.9 a	13 b
KPH 5822	(8.3) 2.6 a	–
SE±	0.5939	2.7
CV%	32.8	14

∗Means followed by the same latter(s) were not significantly different according to DMRT at ($p \leq 0.05$). Actual data between parentheses data transformed to $\sqrt{x + 0.5}$

The degrees of leaf infestation, whether shown by the tomato leaf miner or the leaf miner, had a great similarity for resistance on both insects (Ataide et al. 2017).

3.3.3 Plant Infestation

Mealybug, *Phenacoccus solenopsis* **(Tinsley)**
Tomato infestation with mealybug, *Phenacoccus solenopsis*, took place as in the classical manner of members of Malvaceae, for which all plants were infested, with the pests showing special preference for the growing tips (twigs). It was shown that the most vulnerable plants to infestation were the tested cultivars, with RILG3-162 genotype as most infested. However, the most resistant plant was genotype KPH5822. The result revealed that the source of resistance in RILG3-162 worked on chewing insects – tomato leaf miner, *T. absoluta*, and leaf miner, *Liriomyza trifolii* – while it did not work on the sucking mealybug, *Phenacoccus solenopsis.* However, for KPH5822, the source of resistance worked on both sucking and chewing insects. This might be an indication that the source of resistance was antibiosis in one genotype, and antixenosis in the other (Vitta et al. 2016) (Table 3.3).

Table 3.3 Mean percentage plant infestation by Mealybug, *Phenacoccus solenopsis* on tomato cultivars and genotypes during the season 2015/2016

Treatment	Infestation (%)∗
RILG3 162	72 a
CASTLEROCK	69 a
PETO 86	67 a
AMANI	66 a
RIOKING	60 ab
SALAMA	58 ab
GS-12 F1	40 bc
KPH 5822	35 c
SE±	7.3
CV%	21.6

∗Means in the same column followed by the same letter (s) are not significantly different according to DMRT ($p \leq 0.05$)

3.3.4 Fruit Infestation

Tuta absoluta

In the 2015/2016 season no fruit was harvested due to a shortage of irrigation resources to apply at fruit setting. However, in 2017 the yields and fruit infestations by tomato leaf miner were monitored. Tomato fruit damage due to infestation by tomato leaf miner was not observable at harvesting time, but it became clearly visible when fruits were kept under laboratory condition for a period of 3 days. Some of the fruits become very watery, while others conspicuously showed the bores (exit holes), taken as a parameter for damage due to tomato leaf miner.

Table 3.4 shows that the fruit damage was in the range of 20–40%. The resistant cultivars and genotypes were found to be Salama and the RILG3-162 genotype. However, the most susceptible cultivars were Peto 86, Strain B and Amani. In the literature, instances of 80 to 100% fruit damage have been recorded (Daniel and Bajarang 2017). The medium to low fruit damage, in addition to the characters (traits) of cultivars and genotypes, may be undetectably assisted by the native natural enemies found in nature (Ferracini et al. 2012).

The highest yields of marketable fruits were recorded by Salama and Amani (see Table 3.5). However, the lowest tomato production was shown by cultivar Ahlam. The productivity was in the range 8.7–20.0 tons per feddan. In Sudan, the national production was 8–10 tons per feddan at farmer level and 12–18 tons per feddan at Gezira, while it was 25–35 tons per feddan at the River Nile State, at the experimental level (Mamoon 2008). World productivity was 36 tons per feddan (Dias et al. 2013)

Table 3.4 Mean percentage fruit infestation by *T. absoluta* during 2017

Treatment	Infestation (%)∗
Peto 86	40 a
Strain B	39 a
Amani	39 a
CastleRock	35 a
Rioking	35 a
Ahlam	34 ab
Salama	27 bc
Rilg3 162	20 c
SE±	3.9
CV%	14.1

∗Means in the same column followed by the same letter (s) are not significantly different according to DMRT at ($p \leq 0.05$)

Table 3.5 Yields of tomato genotypes and cultivars during 2017

Treatment	Yield (ton/feddan)∗
Salama	20.0 a
Amani	18.0 ab
CastleRock	12.0 bc
Rioking	13.7 abc
Rilg3 162	10.0 c
Peto 86	9.7 c
Ahlam	8.7 c
Strain B	10.7 c
SE±	3.2
CV%	30.2

∗Means in the same column followed by the same letter (s) are not significantly different according to DMRT Test at ($p \leq 0.05$)

3.4 Conclusion

The results of this study indicated the following:

- The tomato leaf miner showed the lowest infestation levels on genotypes Coded RILG3-162, GS-12F1, Salama and KPH5822
- The cultivar Salama recorded the highest yield, followed by the cultivar Amani.
- These results suggest that cultivars and genotypes of tomato, Salama, RILG3-162 and KPH5822, have resistance traits to tomato leaf miner, and that this pest can be successfully tackled in future breeding programmes.

- The genotypes RILG3-162 and KPH5822 were resistant to the mesophilic leaf feeders, *T. absoluta* and *L. trifolii*. However, RILG3-162 was susceptible to mealybug, *P. solenopsis*, which is a plant sap feeder, while genotype KPH5822 was resistant. This might indicate that the mechanisms of resistance in the two genotypes are different (antixenosis and antibiosis).
- The damage attributable to tomato leaf miner on tomato fruits, when harvested at the stage of colour change (pink fruits) was negligible, but became remarkably noticeable when stored for an extra 3 days at room temperature.

References

Ataide, S. M. L., Juliana, N. C., Clare, A., & Lima, E. (2017). Flight behavior and oviposition of Tuta absoluta on susceptible and resistant genotypes of Solanum lycopersicum. *Arthropod-Plant Interactions, 11*, 567–575.

Daniel, T. I., & Bajarang, B. S. (2017). Control and management of Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae). *Journal of Applied Chemistry, 10*(6), 14–22.

Desneux, N., Wajnberg, E., Wyckhuys, K. A., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., et al. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science, 83*, 197–215.

Dias, D. M., Resende, J. T., Faria, M. V., Camargo, L. K., Chagas, R. R., & Lima, I. P. (2013). Selection of processing tomato genotypes with high acyl sugar content that are resistant to the tomato pinworm. *Genetics and Molecular Research, 12*, 381–389.

Ecole, C. C., Picanço, M., Jham, G. N., & Guedes, R. N. C. (1999). Variability of *Lycopersicon hirsutum* f. *typicum* and possible compound involved in its resistance to *Tuta absoluta*. *Agricultural and Forest Entomology, 1*, 249–254.

El Assi A. O. G. (2001). *Environmental and economic impact of pesticides and fertilizers use on tomato production in Gezira Scheme*. Faculty of Agriculture. MSc thesis, University of Gazira, 183 pp.

EPPO. (2005). *Tuta absoluta.* Data sheets on quarantine pests. *EPPO Bulletin, 35*, 434–435.

Ferracini, C., Ingegno, B. L., Navone, P., Ferrari, E., Mosti, M., Tavella, L., & Alma, A. (2012). Adaptation of indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. *Journal of Economic Entomology, 105*, 1311–1319.

Ferrara, F. A. A., Vilela, E. F., Jham, G. N., Eiras, A. E., Picanco, M. C., Attygale, A. B., & Meinwald, J. (2001). Evaluation of the synthetic major component of the sex pheromone of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Journal of Chemical Ecology, 27*, 907–917.

Lietti, M. M. M., Botto, E., & Alzogaray, R. A. (2005). Insecticide resistance in argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology, 34*(1), 113–119.

Mamoon, B. M. (2008). *Sudanese General Agricultural Engineers Union. Guidance to agricultural engineers for vegetables production*. Khartoum: National Bookshop.

Michereff-Filho, M., Vilela, E. F., Jham, G. N., Attygall, A., Svatos, A., & Meinwald, J. (2000). Initial studies of mating disruption of the tomato moth, *Tuta absoluta* (Lepidoptera: Gelechiidae) using synthetic sex pheromone. *Journal of the Brazilian Chemical Society, 11*(6), 621–628.

Mohamed, E. S. I., Mohamed, M. E., & Gamiel, S. E. (2012). First record of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Sudan. *EPPO Bulletin, 42*, 325–327.

Potting, R. (2009). Pest risk analysis, *Tuta absoluta*, tomato leaf miner moth. *Plant Protection Service of the Netherlands*, *24*. www.minlnv.nl

Urbaneja, A., Monton, H., & Molla, O. (2007). Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. *Journal of Applied Entomology, 133*(4), 292–296.

Vendramim, J. D., & Nishikawa, M. A. N. (2001). Melhoramento para Resistencia a insetos. In L. L. Nass, A. C. C. Valois, I. S. Melo, & M. C. Valadaris-Inglis (Eds.), *Recursos genéticos e melhoramento: plantas* (pp. 737–781). Fundação: Rondonópolis.

Vitta, N., Estay, P., & Chorbadjian, R. A. (2016). Characterization of resistance expression in genotypes of *Solanum* Section *Lycopersicon* against *Tuta absoluta* (Lepidoptera: Gelechiidae). *Ciencia e Investigacion Agraria, 43*, 366–373.

Zekeya, N., Chacha, M., Ndakidemi, P. A., Materu, C., Chidege, M., & Mbega, E. (2017). Tomato leaf miner (*Tuta absoluta* Meyrick 1917) threat to tomato production in Africa. *Journal of Agriculture and Ecology Research International, 10*, 1–10.

Chapter 4
Evaluation of Resistance and Toxicity of Different Insecticides on *Tuta absoluta* Meyrick Populations in Major Tomato Growing States of Nigeria

O. A. Oke, A. A. Oladigbolu, and H. S. Hamisu

Abstract The sudden invasion of the tomato leaf miner, *Tuta absoluta* Meyrick into tomato crops in Nigeria in 2015 has led to the extensive use of common insecticides, together with the introduction of a new insecticide referred to as "Tuta solution", as only control method. After two years the farmers in major tomato producing states of Nigeria (Kaduna, Kano and Katsina States) reported that most of the insecticides applied were no longer effective in controlling Tuta. This led to the investigation of the toxicities of common insecticides lambda-cyhalothrin (MoA group 3A) and deltamethrin (MoA group 3A), and of the newly introduced formulations of chlorantraniliprole + lambda-cyhalothrin (MoA group 28 + 3A) and spirotetramat + flubendiamide (MoA group 23 + 28). These insecticides were tested on larvae obtained from populations collected from major tomato-growing states (Bomo and Giwa in Kaduna State; Beriberi and Funtua in Katsina State; and Bagauda, Watari and Samawa in Kano State) and a susceptible laboratory population of the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. These populations were subjected to concentration mortality bioassays, according to susceptibility test method No. 022, devised by the Insecticide Resistance Action Committee (IRAC). Resistance to chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin, and deltamethrin were observed in all the populations and compared with that of the susceptible NIHORT population. The resistance ratios obtained within the 7 populations ranged from 4.09 to 16.97 for chlorantraniliprole + lambda-cyhalothrin, 2.66 to 7.88 for lambda-cyhalothrin, and 3.23 to 6.24 for deltamethrin. However, resistance to spirotetramat + flubendiamide was not observed in all the 7 populations, with resistance ratio value of 1.05 as this combination was only introduced in 2017 for the control of *T. absoluta* in Nigeria and differently from the others, has not yet been abused. The sole dependence on, and indiscriminate usage of insecticides

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by farmers due to reckless recommendations, without regard for Integrated Pest Management, resulted in the widespread, higher levels of resistance observed in chlorantraniliprole + lambda-cyhalothrin, a product recently introduced into Nigeria in 2015.

Keywords Resistance Ratio Value · Insecticide Resistance Action Committee (IRAC) · Integrated Pest Management (IPM)

4.1 Introduction

In 2015 Nigeria experienced an unprecedented invasion by the tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) which completely ravaged tomato farms all over the nation, leaving farmers with almost zero yields. It crumbled the National tomato value chain, which is ranked the largest in Sub-Saharan Africa, leading to importation of tomato from neighbouring countries. *Tuta absoluta* is a very challenging pest to control because it attacks tomato plants in all developmental stages, damaging the stems, apices, flowers and fruits, as well as mining the leaves (Miranda et al. 1998). Since its invasion in Nigeria, excessive insecticides applications have been the main method of control. Guedes et al. (1994) reported that some of the compounds recommended for *T. absoluta* control are apparently not providing the desired effect. Excessive applications of the insecticides commonly applied to tomato crops during a single cultivation period (sometimes up to 36 sprays) could have led to the evolution of resistant pest populations, as well as the elimination of their natural enemies, thus leading to additional occupational hazards (Castelo and Franca 1992; Gonçalves et al. 1994; Picanço et al. 1995). The existence of resistance to organophosphates and pyrethroids in Chile (Salazar and Araya 1997, 2001) and to abamectin, cartap, methamidophos and permethrin in Brazil (Siqueira et al. 2000, 2001) have been reported. Bassi et al. (2012) stated that reliance on insecticides alone will not provide the flexibility and sustainability required for a rational insect resistance management scheme, as part of an integrated pest management (IPM) scenario: a reduction of the abuse of insecticides and the adoption in parallel of IPM principles will be mandatory, in order to mitigate directional selection of resistance in *T. absoluta* populations.

Chlorantraniliprole + lambda-cyhalothrin (Ampligo®[1]) was the first insecticide introduced, as a "Tuta solution", and extensively used all over Nigeria for the control of *T. absoluta.* However, shortly after farmers realized that a solution had been found, they began to report that the insecticide had become inefficient. This compelled us, for the first time, to determine the resistance of *T. absoluta* to the most commonly applied insecticides, for its control in Nigeria.

[1] Registered trademark of Syngenta Group Company.

The objective of this work was, therefore, to evaluate the toxicity of chlorantraniliprole + lambda-cyhalothrin, spirotetramat + flubendiamide, lambda-cyhalothrin and deltamethrin to *T. absoluta* populations in the major tomato-producing locations in Nigeria. Main goal was to ascertain the claim of resistance development by farmers, which will guide us in designing resistance management strategies.

4.2 Materials and Methods

The *Tuta absoluta* populations used for analysis were collected from commercial tomato fields in March 2017, from 7 local government areas of the 3 major tomato-producing states in Nigeria. A population provided from the laboratory colonies of NIHORT, reared since 2016 without exposure to insecticide, was used as susceptible population (Table 4.1). Informations on insecticides used on each of the fields were obtained from farmers. Fourth instar larvae collected from each of the sampled farms were reared individually on tomato plants in cages, without insecticide exposure, in the laboratory. The emerged adults were reared on tomato plants in cages to obtain second instar larvae, which were used in the bioassays.

4.2.1 Insecticides

The insecticides used in this study were lambda-cyhalothrin (MoA group 3A, Karate®[1]) and deltamethrin (MoA group 3A, Decis®[2]), which have been mostly used for control of various pests on tomato for over 10 years, as well as chlorantraniliprole + lambda-cyhalothrin (MoA group 28 + 3A, Ampligo®[1]) and spirotetramat + flubendiamide (MoA group 23 + 28, Tihan®[2]), which were introduced for control of *T. absoluta* during its recent invasions into Nigeria in 2015 and 2017.

Table 4.1 The origin of the collected population of *Tuta absoluta*

Location of farm	Local government area	State
Bomo	Sabon gari	Kaduna
Giwa	Giwa	Kaduna
Beriberi	Faskari	Katsina
Funtua	Funtua	Katsina
Bagauda	Bebeji	Kano
Watari	Bagwai	Kano
Samawa	Garun Mallam	Kano
NIHORT	Ibadan North-West	Oyo

[2] Registered trademark of Bayer Crop Science.

4.2.2 Bioassays

The insecticide bioassays were conducted according to the susceptibility test method No. 022 formulated by the Insecticide Resistance Action Committee (IRAC). Young, tender, non-infested, untreated tomato leaves were collected and kept in sealed plastic bags to prevent wilting. For each bioassay, 7 different insecticide concentrations, including a control treatment of distilled water, were applied. Collected leaflets were dipped singly in the diluted concentrations for 3 s, with gentle agitation to ensure total submergence. The treated leaves were dried on wire net, with the upper leaf surface facing up. The dried treated leaflets were placed singly on moistened filter paper in Petri dishes (9 cm diam. × 1.5 cm height). A leaf square was cut around a 2nd instar larva with a scalpel and lifted with a brush on to the treated leaflet in the Petri dish and covered. The Petri dishes were arranged on a laboratory working bench at 29 ± 2 °C, under a photoperiod of 13:11 (L:D). Larval mortality was assessed after 72 h of treatment by prodding larvae with a hair brush. Larvae were considered dead if they were unable to move.

4.2.3 Data Analysis

Concentration mortality data were subjected to probit analysis (Proc Probit, SAS Institute 1997).

4.3 Results

There was a significant variation in the insect populations resistance to chlorantraniliprole + lambda-cyhalothrin. The lethal dose (LD_{50}) of the susceptible population (0.321) was significantly smaller than those from the other 7 population due to a failure of 95% confidence level (CL) to overlap (Table 4.2). The slopes of the concentration mortality curve, showing the homogeneity of insect response to the 8 populations, differed. Resistance to chlorantraniliprole + lambda-cyhalothrin was observed in all the 7 populations when compared with the susceptible NIHORT population. The resistance ratio ranged from 4.09 to 16.97 times, with populations from Beriberi having the highest resistance ratio (Table 4.2).

There was no variation in the resistance of the insect populations to spirotetramat + flubendiamide, as the 95% CL for all the 8 populations overlapped with relatively similar slopes of the concentration mortality curve, showing the homogeneity of insect response to the 8 populations (Table 4.3).

There was significant variation in the resistance of the insect populations to lambda-cyhalothrin.

Table 4.2 Comparative resistance of *T. absoluta* population to chlorantraniliprole + lambda-cyhalothrin (MoA group 28 + 3A)

Population	n	Slope ± SE	LD_{50} (95% CL)	Resistance ratio[a]
NIHORT	282	0.232 ± 0.05	0.321 (0.122–0.52)	
Bomo	279	0.180 ± 0.03	1.526 (1.311–1.741)	4.75
Giwa	280	0.178 ± 0.03	1.314(1.115–1.513)	4.09
Beriberi	328	0.614 ± 0.01	5.447 (4.708–6.186)	16.97
Funtua	289	0.246 ± 0.02	3.478 (3.048–3.908)	10.83
Bagauda	311	0.605 ± 0.01	5.086 (4.414–5.758)	15.84
Watari	321	0.393 ± 0.02	4.695 (4.092–5.298)	14.63
Samawa	290	0.243 ± 0.01	3.548 (3.108–3.988)	11.05

[a]Resistance ratio = LD_{50} field population/ LD_{50} NIHORT population

Table 4.3 Comparative resistance of *T. absoluta* population to spirotetramat + flubendiamide (MoA group 23 + 28)

Population	n	Slope ± SE	LD_{50} (95%CL)	Resistance ratio[a]
NIHORT	192	0.437 ± 0.02	0.947 (0.796–1.028)	
Bomo	258	0.424 ± 0.02	0.990 (0.879–1.058)	1.05
Giwa	203	0.422 ± 0.02	0.990 (0.878–1.058)	1.05
Beriberi	275	0.456 ± 0.02	0.999 (0.884–1.071)	1.05
Funtua	192	0.464 ± 0.02	0.999 (0.884–1.071)	1.05
Bagauda	223	0.482 ± 0.03	0.998 (0.884–1.071)	1.05
Watari	240	0.422 ± 0.02	0.998 (0.884–1.071)	1.05
Samawa	200	0.461 ± 0.02	0.998 (0.884–1.071)	1.05

[a]Resistance ratio = LD_{50} field population/ LD_{50} NIHORT population

The LD_{50} of the susceptible population (0.491) was significantly smaller than those from the other 7 population due to a failure of 95% CL to overlap (Table 4.4). The slopes of the concentration mortality curve, showing the homogeneity of insect response to the 8 populations, were relatively similar. Resistance to lambda-cyhalothrin was observed in all the 7 populations when compared with the susceptible population from NIHORT. The resistance ratio ranged from 2.66 to 7.88 times, with populations from Bagauda showing the highest ratio (Table 4.4).

There was significant variation in the resistance of the insect populations to deltamethrin. The LD_{50} of the susceptible population (0.307) was significantly smaller than those from the other 7 population due to a failure of 95% CL to overlap (Table 4.5). The slopes of the concentration mortality curve, showing the homogeneity of insect response to the 8 populations, were relatively similar. Resistance to deltamethrin was observed in all the 7 populations when compared with the susceptible population from NIHORT. The resistance ratio ranged from 2.23 to 6.24, with populations from Giwa having the highest resistance ratio (Table 4.5). Among all the 4 insecticides tested, the highest resistances of 15.84 and 16.97 were recorded in Bagauda and Beriberi populations, respectively, on chlorantraniliprole + lambda-cyhalothrin.

Table 4.4 Comparative resistance of *T. absoluta* population to lambda-cyhalothrin (MoA group 3A)

Population	n	Slope ± SE	LD_{50} (95%CL)	Resistance ratio[a]
NIHORT	192	0.104 ± 0.022	0.491 (0.037–0.061)	
Bomo	205	0.187 ± 0.012	1.976 (1.694–2.189)	4.02
Giwa	235	0.181 ± 0.012	2.044 (1.810–2.321)	4.16
Beriberi	205	0.192 ± 0.013	1.981 (1.607–2.255)	4.03
Funtua	192	0.133 ± 0.014	1.306 (1.058–1.576)	2.66
Bagauda	195	0.212 ± 0.010	3.868 (3.654–4.082)	7.88
Watari	195	0.207 ± 0.013	1.868 (1.627–2.100)	3.80
Samawa	192	0.195 ± 0.013	1.903 (1.672–2.153)	3.88

[a]Resistance ratio = LD_{50} field population/LD_{50} NIHORT population

Table 4.5 Comparative resistance of *T. absoluta* population to deltamethrin (MoA group 3A)

Population	n	Slope ± SE	LD_{50} (95%CL)	Resistance ratio[a]
NIHORT	180	0.199 ± 0.021	0.307 (0.473–0.651)	
Bomo	190	0.131 ± 0.013	1.386 (1.272–1.430)	4.51
Giwa	191	0.197 ± 0.013	1.916 (1.667–2.283)	6.24
Beriberi	195	0.147 ± 0.013	1.502 (1.320–1.745)	4.89
Funtua	195	0.186 ± 0.015	1.532 (1.434–1.632)	4.99
Bagauda	190	0.165 ± 0.013	1.821 (1.380–1.543)	5.93
Watari	188	0.150 ± 0.018	0.992 (1.298–1.521)	3.23
Samawa	191	0.144 ± 0.014	1.303 (1.201–1.427)	4.24

[a]Resistance ratio = LD_{50} field population/ LD_{50} NIHORT population

Table 4.6 Insecticides manufacturers' recommendations

Insecticide	Commercial name	Product recommendations/season
Lambda-cyhalothrin (MoA group 3A)	Karate	400 ml/ha. 3 applications between 7 and 10 days
Deltamethrin (MoA group 3A)	Decis	500 ml/ha. 3 applications between 7 and 10 days
Chlorantraniliprole + Lambda-cyhalothrin (MoA group 28 + 3A)	Ampligo	400ml/ ha. Apply at 1st sign of infestation of larvae in the leaves, or 1st signs of leaf damage. 3 applications at14–21 days interval
Cpirotetramat + flubendiamide (MoA group 23 + 28)	Tihan	400 ml/ha. Treat at the beginning of infestation. 3 applications within 14 days

The total number of insecticide applications between 2015, when the Tuta invasion was first recorded in Nigeria, and 2017 differed among the 7 populations, the 4 tested insecticides and from the manufacturers recommendations (Table 4.6).

Chlorantraniliprole + lambda-cyhalothrin recorded the highest application number range from 10–29, while spirotetramat + flubendiamide had the lowest application number range from 2–5 (Tables 4.7, 4.8, 4.9 and 4.10). Beriberi conse-

Table 4.7 Number of chlorantraniliprole + lambda-cyhalothrin applied to the 7 populations within 3 planting seasons of 2015–2017

Population	Feb–Apr 2015	Feb–Apr 2016	Feb–Apr 2017	Subtotal application
Bomo	2	8	0	10
Giwa	3	8	0	11
Beriberi	5	19	5	29
Funtua	3	10	2	15
Bagauda	6	16	5	27
Watari	5	17	4	26
Samawa	3	12	2	17
Total	**27**	**90**	**18**	**135**
Mean	**3.9**	**12.9**	**2.6**	**19.3**

Table 4.8 Number of spirotetramat + flubendiamide applied to the 7 populations within 3 planting seasons of 2015–2017

Population	Feb–Apr 2015	Feb–Apr 2016	Feb–Apr 2017	Subtotal application
Bomo	0	0	2	2
Giwa	0	0	2	2
Beriberi	0	0	2	2
Funtua	0	0	3	3
Bagauda	0	0	3	3
Watari	0	0	4	4
Samawa	0	0	5	5
Total	**0**	**0**	**21**	**21**
Mean	**0**	**0**	**3**	**3**

Table 4.9 Number of lambda-cyhalothrin applied to the 7 populations within 3 planting seasons of 2015–2017

Population	Feb–Apr 2015	Feb–Apr 2016	Feb–Apr 2017	Subtotal application
Bomo	10	3	2	14
Giwa	12	5	3	17
Beriberi	10	3	2	15
Funtua	6	2	1	8
Bagauda	16	2	6	24
Watari	6	3	1	10
Samawa	6	3	1	10
Total	**66**	**21**	**16**	**98**
Mean	**9.4**	**3**	**2.3**	**14**

quently contributed the highest chlorantraniliprole + lambda-cyhalothrin application numbers of 29 (Table 4.7).

There was a significant linear relationship between the total number of applications and the resistance ratios of the insecticides to *T. absoluta* in the 7 populations (Figs. 4.1 and 4.2), except for spirotetramat + flubendiamide.

Table 4.10 Number of deltamethrin applied to the 7 populations within 3 planting seasons of 2015–2017

Population	Feb–Apr 2015	Feb–Apr 2016	Feb–Apr 2017	Subtotal application
Bomo	8	3	7	18
Giwa	13	6	8	27
Beriberi	8	5	6	19
Funtua	10	4	7	21
Bagauda	12	5	4	21
Watari	8	1	2	11
Samawa	8	3	5	16
Total	**67**	**27**	**39**	**133**
Mean	**9.6**	**3.9**	**5.6**	**19**

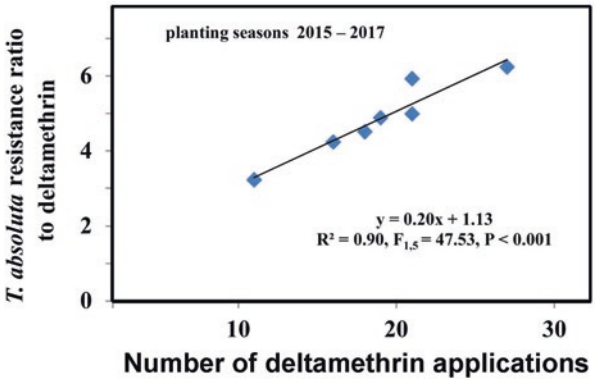

Fig. 4.1 Relationship between total number of chlorantraniliprole + lambda-cyhalothrin applications and resistance ratio of *Tuta absoluta* on the 7 populations within 3 planting seasons of 2015–2017

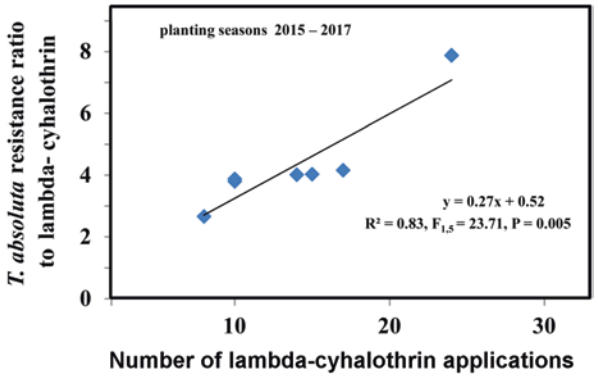

Fig. 4.2 Relationship between total number of lambda-cyhalothrin applications and resistance ratio of *T. absoluta* on the 7 populations within 3 planting seasons of 2015–2017

4.4 Discussion

This is the first time that a resistance study was reported on *T. absoluta* in Nigeria since the invasion in 2015. Immediately after its invasion, chlorantraniliprole + lambda-cyhalothrin was introduced recklessly as a solution for the insect control. As a result, sole and high dependence by farmers, led to consecutive and increased applications and abuse, despite manufacturers recommendations. As a consequence, on-farm inefficacy of chlorantraniliprole + lambda-cyhalothrin from farmers in Watari, Bagauda and Beriberi was reported. These locations had the highest numbers of applications of chlorantraniliprole + lambda-cyhalothrin, which resulted in the most resistant populations of *T. absoluta*.

The significant variations recorded among the populations in resistance to chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin and deltamethrin revealed the presence of a genetic diversity underpinning *T. absoluta* resistance. Such variability in resistance levels indicates the occurrence of differential selection pressures, and/or a genetic diversity in the resistance mechanisms among the insect populations (Kerns and Gaylor 1992). The relative differences in the populations responses to chlorantraniliprole + lambda-cyhalothrin may be attributed to the significant variations in the application rates among the populations. Picanço et al. (1995) reported greater resistance levels for *T. absoluta* populations to abamectin and cartap, likely due to a higher selection pressure provided by the more intensive use of these insecticides in Brazil. Bassi et al. (2012) stated that, among the factors that could favour resistance to any insecticide MoA, the intensity of usage is the main parameter that has an overriding influence.

During the first year of invasion in 2015, when farmers were completely ignorant about *T. absoluta* infestation, lambda-cyhalothrin and deltamethrin were applied frequently because both insecticides were among the most commonly applied for the control of tomato major pests. The combination chlorantraniliprole + lambda-cyhalothrin was introduced during this time of invasion, with the lowest application rate, because it had not gained popularity among farmers and rotational application with lambda-cyhalothrin and deltamethrin. Nevertheless, the few farmers who used it reported its efficacy in all locations. In the subsequent planting year of 2016, its application rate became the highest due to the sole and high dependence on it, which was based on the efficacy report of previous year (2015). A drastic reduction in the application rate of chlorantraniliprole + lambda-cyhalothrin was recorded in 2017 due to its scarcity and high cost in the country, which was predicated by its high demand for the control in the country of another invasive pest, armyworm *Spodoptera frugiperda* (J.E. Smith) on maize. The few farmers that desperately purchased it, at high cost, expecting complete relief from the infestation were shockingly disappointed as chlorantraniliprole + lambda-cyhalothrin failed to reduce infestation due to the rapid development of resistance by *T. absoluta*, leading to a general outcry and reports of inefficacy. There was also a drastic reduction in the application rates of lambda-cyhalothrin and deltamethrin, and although there was scarcity of chlorantraniliprole + lambda-cyhalothrin, this reduction was the result of the introduction of an indigenous water + light Tuta trap tray (NIHORT-TTtray), designed

by the first author to trap the adult *T. absoluta* in these locations. The NIHORT-TTtrays, which were set up every night, trapped between 2673 and 4872 adult *T. absoluta* daily across 48 farms in the 7 locations, thereby reducing the infestation massively.

The significant correlations between the rate of application and resistance ratio suggest that the variations in resistance of *T. absoluta* populations to chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin and deltamethrin resulted from the variations in usage in the different locations. The unavailability of correlations between rate of application and resistance ratio of *T. absoluta* populations to spirotetramat + flubendiamide was reflected in the similarity of usage in the 7 locations and the fact that it had only been introduced in Nigeria against *T. absoluta* in 2017 , therefore it is yet to be abused like the others.

Lambda-cyhalothrin (MoA group 3A, Karate®[1]) and deltamethrin (MoA group 3A, Decis®[2]) should not have been used together because they both belong to the same mode of action, group 3A. The application of both insecticides together in the same seasons within 3 years increased the application of the active ingredient pyrethroids, which accelerated the development of resistance to both insecticides. Bassi et al. (2012) reported that the abuse of a single insecticidal mode of action (MoA) in commercial agriculture can lead to insect resistance in as little as 5 to 6 years, from the date of commercial introduction.

4.5 Conclusion

Populations of *T. absoluta* from the 7 major tomato-producing locations in Nigeria showed resistance to chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin and deltamethrin, confirming the farmers reports. The sole and high dependence and indiscriminate usage of insecticides by farmers due to reckless recommendations, without any adoption of IPM rules, have resulted in the widespread, higher levels of resistance observed in chlorantraniliprole + lambda-cyhalothrin, a product only introduced into Nigeria in 2015. Therefore, a rotational application, based on the manufacturer's recommendations, of insecticides that have different modes of action, utilised in combination with cultural and/or trapping methods and the preservation of natural enemies, must be adopted for the development of a sound IPM strategy to control *T. absoluta* on tomato.

References

Bassi, A., Roditakis, E., & Wilbert, F. G. (2012). The first cases of diamide-resistant *Tuta absoluta* (Meyrick) and the alternation of the insecticidal modes of action as a key IPM practice for sustainable control. http://www.irac-online.org/documents. Accessed 15 Nov 2016.

Castelo, B. M., & Franca, F. H. (1992). Avaliação da Susceptibilidade de três Populações de Scrobipalpuloides absoluta Cartap. *Horticultura Brasileira, 10*, 33–34.

Gonçalves, D. M. H. R., Picanço, M. C., Ribeiro, L. J., & Campos, L. O. (1994). Seletividade de quatro inseticidas a *Polybia* sp.2 (Hymenoptera: Vespidae) predador de *Scrobipalpuloides absoluta* (Lepidoptera: Gelechiidae). *Horticultura Brasileira, 2*, 81.

Guedes, R. N. C., Picanço, M. C., Matioli, A. L., & Rocha, D. M. (1994). Efeito de inseticidas e sistemas de condução do tomateiro no controle de *Scrobipalpuloides absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Anais Da Sociedade Entomológica Do Brasil, 23*, 321–325.

IRAC. (2012). *Tuta absoluta* IRAC susceptibility test method No. 022: version 3 October 2012. https://www.irac-online.org/methods/tuta-absoluta-larvae/

Kerns, D. L., & Gaylor, M. J. (1992). Insecticide resistance in field populations of the cotton aphid (Homoptera: aphididae). *Journal of Economic Entomology, 85*, 1–8.

Miranda, M. M. M., Picanço, M., Zanuncio, J. C., & Guedes, R. N. C. (1998). Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Biocontrol Science and Technology, 8*, 597–606.

Picanço, M. C., Guedes, R. N. C., Leite, G. L. D., Fontes, P. C. R., & Silva, E. A. (1995). Incidência de *Scrobipalpuloides absoluta* (Meyrick) (Lepidoptera: Gelechiidae) em tomateiro sob diferentes sistemas de tutoramento e controle químico de pragas. *Horticultura Brasileira, 13*, 180–183.

Salazar, E. R., & Araya, J. E. (1997). Detección de resistencia a insecticidas en la polilla del tomate. *Simiente, 67*, 8–22.

Salazar, E. R., & Araya, J. E. (2001). Respuesta de la polilla del tomate, *Tuta absoluta* (Meyrick), a insecticidas en arica. *Agricultura Técnica, 61*, 429–435.

SAS Institute. (1997). *SAS user's guide: Statistics, Version 6.12*. Cary: SAS Institute.

Siqueira, H. A. A., Guedes, R. N., & Picanço, M. C. (2000). Cartap resistance and synergism in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Applied Entomology, 2*, 223–238.

Siqueira, H. A. A., Guedes, R. N., Fragoso, D. B., & Magalhaes, L. C. (2001). Abamectin resistance and synergism in brazilian populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *International Journal of Pest Management, 47*, 247–251.

Chapter 5
The First Effort at Adopting Integrated Pest Management IPM to Contain the Infestation of the *Tuta absoluta* in Nigeria

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Abstract *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), a devastating pest of tomato *Lycopersicum esculentum* L. native of South American, was reported for the first time in Kano, Nigeria, in June, 2015 and was found to have spread and become established in Abeokuta, Ogun State in September 2015, where it infested plants of the screenhouse tomato project Kotopo, at Ogun State Agricultural Development Programme (OGADEP). An account of the first attempt in the application of Integrated Pest Management (IPM) for control of *T. absoluta* in Nigeria is given herein. The application of a systemic insecticide, thiamethoxam (MoA group 4A) actara® (Registered trademark of Syngenta Group Company) (Syngenta™), at an increased frequency of 10 applications within 5 weeks, could not by itself prevent the complete ravaging of 3 screen houses by *T. absoluta* to a zero yield. The application of IPM, which commenced with setting up of indigenous water + light traps in the nights in the remaining 3 infested screen houses, resulted in trapping adult moths, ranging in numbers between 13,882 and 14,576 per screenhouse, within 6 weeks. The integration and incorporation of many control measures involved gaining knowledge of the pest's behaviour and life cycle, indigenous water and light traps, hygiene, and 6 rotational applications of insecticides with different mode of actions (MoA). These insecticides comprised imidacloprid (MoA group 4A) imi force® (Registered trademark of Jubaili Agrotec Limited) (Jubaili™) (systemic insecticide) and lambdacyhalothrin (MoA group 3A) Karate® (Registered trademark of Syngenta Group Company) (Syngenta™), acetamiprid + lambdacyhalothrin

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(MoA group 4A + 3A) prodal 130EC® (Registered trademark of Dizengoff West Africa (Nigeria) Limited) (Dizengoff™) and novaluron + bifentrin (MoA group 15 + 3A), rimon fast 100SC® (Registered trademark of Dizengoff West Africa (Nigeria) Limited) (Dizengoff™) (contact + stomach insecticides). These measures resulted in a 57.52% reduction in total damage within 6 weeks. Therefore, the IPM strategy proved to be an effective option for achieving a sustainable management of *T. absoluta* on tomato.

Keywords *Tuta absoluta* · Water + light traps · Hygiene · Insecticides · Screen house

5.1 Introduction

Tuta absoluta Meyrick (Lepidoptera: Gelechiidae), the tomato leaf miner, is native of South America, where it is a serious pest of tomato, *Lycopersicum esculentum* L. It is also known to attack cultivated solanaceous plants such as eggplant *Solanum melongena* L. and potato *S. tuberosum* L. (Ferracini et al. 2012). *Tuta absoluta* was observed for the first time in Europe (Spain) in 2006. It then was found in Italy, France and Greece and then in the Mediterranean Basin and, currently, in several other European countries (Desneux et al. 2010). It appeared in the North African states of Algeria, Morocco, and Tunisia in 2007 (Potting et al. 2009; Roditakis et al. 2010). It was found in East Africa: Kenya and Ethiopia in 2012, West Africa: Senegal in 2013 (Pfeiffer et al. 2013) and in Kano, Nigeria in June, 2015. Since then, it has spread to all the tomato-producing states in Nigeria: Kaduna, Katsina, Jigawa, Zamfara, Bauchi, Gombe, Taraba, Benue, and the Plateau States including Lagos, Oyo and Abeokuta, Ogun State. It completely ravaged tomato farms, leaving farmers with absolutely zero yields.

Tuta absoluta is a very challenging pest to control. A chemical approach is difficult because of the mine-feeding behaviour of it larvae. Moreover, a chemical approach quickly results in a build-up of insecticide residues on tomato fruits and in the environment (Walgenbach et al. 1991). It also leads to rapid development of insecticide resistance to many conventional insecticides and side effects for other organisms that are useful in IPM programmes (Siqueira et al. 2000a; Lietti et al. 2005; Cabello et al. 2009; IRAC 2011).

Resistance development has been reported against abamectin, cartap, methamidophos and permethrin in Brazil (Siqueira et al. 2000a, b) and against deltamethrin and abamectin in Argentina (Lietti et al. 2005). Thus, in order to quickly contain the menace of *T. absoluta* and to avoid the development of resistant biotypes, a careful management of the infestation that uses a rotational application of insecticides with different MoAs, together with other control measures, is desirable. Therefore, a team of researchers from the National Horticultural Research Institute (NIHORT, Ibadan, Nigeria), incorporated many control measures to contain the economic

damage, prevent the development of resistance to insecticides, and prevent the increase in insecticide residues in the food chain.

5.2 Materials and Method

The tomato variety 'Eva' was raised on 16th Sept. 2015 in the screenhouse nursery of the Ogun State Agricultural Development Programme (OGADEP). The tomato project was located at Kotopo (Abeokuta, Nigeria). Four weeks after planting, seedlings were transplanted into 12-litre nylon pots filled with sterilized soil, totalling 480 pots per screen house. The commercial screenhouse was a new farmers' kit, supplied by Dizengoff Nigeria (24 m × 8 m × 4.5 m), with one single door and 1 m wide side nets, with 4 mm holes. Each of the 6 screenhouses was supported with drip irrigation and trellis. Immediately after *T. absoluta* infestation was noticed for the first time, on 31st Oct. 2015, thiamethoxam (MoA group 4A) Actara® (Syngenta™) was sprayed twice weekly, until 8th Dec. 2015. On the 9th Dec. 2015, a team of researchers from NIHORT immediately applied the following IPM strategies to arrest the rapid multiplication of the moth and to contain its damage on plants.

1. All the staff of the Ogun State Agricultural Development Programme (OGADEP), at the screen house tomato project, were shown how to identify eggs of *T. absoluta* laid on the under-side of tomato leaves and around the sepals of fruits; the larvae buried within a leaf; the frass that indicates larval entry holes; the pupae on the soil and within tomato leaves; and the adult moth. They were enlightened about the larvae that does the most damage to tomato.
2. Hygiene: During daily inspections of screenhouses, infested leaves and fruits were immediately removed, bagged in plastic bags, and burnt.
3. Four (4) indigenous water + light traps were set up per screen house every night. The tray was 45 cm diam. × 5 cm depth, filled with 5 litres of water and 1 tablespoon of detergent added and stirred gently to prevent lather. A stone was placed in the centre of the tray on which to sit a rechargeable lamp. Trays were elevated off the ground, but not more than 35 cm from the ground. Adult moth catches per screen house were counted daily and recorded.
4. Weekly rotational application followed using insecticides with different MoAs: imidacloprid (MoA group 4A) imi force®2 (Jubaili™) (systemic insecticide) and lambdacyhalothrin (MoA group 3A), Karate®1 (Syngenta™), acetamiprid + lambdacyhalothrin (MoA group 4A + 3A), prodal 130EC®3 (Dizengoff™) and novaluron + bifentrin (MoA group 15 + 3A), rimon fast 100SC®3 (Dizengoff™) (contact + stomach insecticides), at recommended dosage. Yields per screenhouse were recorded and monetary values calculated.

Statistical Analysis The variances, averages and standard deviation were calculated and analysed by the test of Studentized Newman Keuls (SNK) (P 0.05).

5.3 Results

At exactly 49 days after the infestation was noticed in all the screenhouses, 3 of them were completely ravaged to zero yields by the infestations, despite the 10 applications of thiamethoxam over 5 weeks (Table 5.1).

Because the plants were completely destroyed with the fruits after infestation, even with the increased frequency of 10 applications of only thiamethoxam, no monetary value was realised (Table 5.2).

Table 5.1 Yield obtained after *Tuta absoluta* infestation with application of thiamethoxam only and IPM control measure

Screen house (Sh) no	Yield after infestation with application of thiamethoxam only/Sh (kg)	Total no of adult *Tuta absoluta* trapped/Sh within 6 weeks	Yield after infestation with application of IPM within 6 week/Sh (kg)
1	Completely ravaged	NA	NA
2	Completely ravaged	NA	NA
3	Completely ravaged	NA	NA
4	NA	14576	443
5	NA	13882	359
6	NA	14036	190
Total		**42494**	**992**
Mean		**14164.7**	**330.7**
Stdev		**364.5**	**128.9**

Table 5.2 Yield and monetary value realized before and after *T. absoluta* infestation with application of thiamethoxam only and IPM control measure

Screen house (Sh) no	Yield before infestation/ Sh (kg)	Amount realized			Percentage of damage reduction after application of IPM for 6 weeks/Sh (%)
		before infestation/ Sh at (₦450/ kg)	after infestation + thiamethoxam for 5 weeks/Sh (₦)	after infestation + IPM for 6 weeks/Sh at (₦450/kg)	
1	959.1	431,595.00	0	NA	NA
2	802.6	361,170.00	0	NA	NA
3	719.6	323,820.00	0	NA	NA
4	714.7	321,615.00	NA	199,350.00	57.52
5	700.6	315,270.00	NA	161,550.00	46.61
6	697.3	313,785.00	NA	85,500.00	24.67
Total 4620.9		**₦2,067,255**	**0**	**₦446,400**	
USD equivalent (USD1 @ ₦160)		**$12,920.3**		**$2790**	
Mean 770.15		**₦344,542.5**	**0**	**₦148,800**	
USD equivalent (USD1 @ ₦160)		**$2153.4**		**$930**	

Note: *Sh* Screen house, *USD* United States dollar, *NA* Not applicable

After the set up of the indigenous water + light traps in the remaining 3 infested screenhouses in the nights, the total numbers of adult moths trapped per screen house within 6 weeks ranged between 13,882 and 14,576 adults (Table 5.1). It was observed that the indigenous water + light trap only trapped the adult moths effectively when the trays were not elevated above 35 cm from the ground.

The application of the pest behaviour and life cycle, integrated with the indigenous water + light trap, hygiene, and 6 applications of 2 systemic insecticides and 2 contact + stomach insecticides resulted in tomato yields ranging from 190 to 443 kg. This gives a total monetary value of four hundred and forty six thousand four hundred (446,400) naira equivalent to ($2790) (Table 5.1) and a consequent reduction of 57.52% in total damage within 6 weeks (Table 5.2).

5.4 Discussion

The mine symptom on leaves resulting from larvae feeding on the leaf mesophyll reduced the leaves photosynthetic surface, leaving the upper and lower epidermis intact, which serves as protection against predators or insecticide sprays, making the most commonly used control 'insecticide' method ineffective. The exercise of properly identifying the symptoms of *T. absoluta* infestation by farmers is most necessary for making an accurate distinction between *T. absoluta* and *Liriomyza* spp., so as to inform the appropriate decision-making by farmers, regarding the applicable control measure.

The immediate removal of the infested leaves and fruits during daily inspections prevented larvae from completing their life-cycle and increasing infestations. The bagging of infested leaves and fruits in plastic bags and burning prevented the re-infestation of other sites and establishment of breeding sites. InfoAgro Systems (2009) also advised that plants and plant parts infested by *T. absoluta* should be removed, especially at the beginning of cultivation, and residues should be carefully disposed of, ensuring that they are kept stored in sealed containers until they are sent to a waste management facility.

Tuta absoluta completely ravaged 3 screenhouses to zero yield within 5 weeks after first detection, despite the 10 applications of thiamethoxam. This confirms the report of Derbalah et al. (2012) which stated that the pest is capable of causing a yield loss of 100%. The application of a single insecticide, thiamethoxam, even at an increased frequency, was not adequate to reduce the infestation, revealing that there is no single magic solution to contain the menace of *T. absoluta* infestation. Moreover, increased usage could also result in the development of resistance biotypes, as reported for resistance against abamectin, cartap, methamidophos and permethrin in Brazil (Siqueira et al. 2000a, b) and against deltamethrin and abamectin in Argentina (Lietti et al. 2005). Accordingly, a diversification in control tactics should be implemented, with the minimum use of chemicals. Insecticides should be applied only when needed, and only used as the last form of control. When insecticides are applied, the way in which they are used should be rationalised and

optimised to exploit the full diversity of synthetic chemicals and natural products, and should mostly be used on a rotational basis (Mohamed and Hajji 2012). In practice, the alternation, sequence, and rotation of compounds with different MoAs will usually provide a sustainable and effective approach to managing insecticide resistance (IRAC 2009).

The indigenous water + light trap effectively trapped adults, as suggested by Bolkmans (2009) and Rodrigues de Oliveira et al. (2008) who noted that light traps can be used to capture adult males and females of *T. absoluta* during sundown. Although the adult population declined drastically in the screenhouses due to trapping, it was observed that adults moths from outside the screenhouses migrated into the screenhouses through the 4 mm hole mesh of the 1 m wide side net, which allowed the moths entry, especially during the night when they were attracted to the trap lights set in the screen house. Moreover, the single door at the entrance was not adequate to keep the adults out during entry; therefore, the structural defects in the design of the commercial screenhouse (a new farmers' kit by Dizengoff Nigeria), facilitated re-infestation. Installing double self-closing doors, covering windows and other openings with 1.6 mm (or smaller) insect mesh, can prevent the entry or exit of adult *T. absoluta* in greenhouses (CFR 7-319 2009; InfoAgro Systems 2009).

The high effectiveness for trapping adult moths exhibited by the indigenous water + light trap, elevated below 35 cm above the ground, imply that the moths prefer to restrict their activities to the lower plant parts. This supports the findings by Real IPM (2015) which stated that the correct placement of the traps is critical for success: traps placed at 15 –20 cm above the ground captured 5 times as many *T. absoluta* adults than those placed at 1 m above the ground.

The integration and incorporation of many control measures with the minimum number of insecticide applications (6 applications) within a short period of 6 weeks was more effective to reduce the devastation of *T. absoluta* than the increased frequency of 10 applications, of only thiamethoxam, within 5 weeks

5.5 Conclusion

This study was the first endeavour made in the IPM application for control of *T. absoluta* in Nigeria. The integration and incorporation of many control measures, which involved gaining an understanding of the insect's behaviour, using indigenous water + light trap, hygiene practices, and 6 rotational applications of 4 insecticides with different modes of action, proved to be the more effective option in achieving sustainable, effective control of *T. absoluta*. It is therefore of necessity to shift the current sole dependence on insecticides, to switch towards IPM as a preferred pest management practice in Nigeria.

References

Bolkmans, K. (2009). Integrated pest management of the exotic invasive pest Tuta absoluta. In *Paper presented at the 4th annual biocontrol industry meeting international biocontrol manufacturers association, Lucerne, Switzerland*.

Cabello, T., Gallego, J. R., Vila, E., Soler, A., Del Pino, M., Camero, A., et al. (2009). Biological control of the South American tomato pinworm, *Tuta absoluta* (Lepidoptera: Gelechiidae), with releases of *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae) in tomato greenhouses of Spain. *IOBC WPRS Bulletin, 49*, 225–230.

CFR 7-319. (2009). U.S.A. code of federal regulations, title 7, part 319. *Foreign Quarantine Notices, 56*, 523–556.

Derbalah, A. S., Morsey, S. Z., El-Samahy, M., et al. (2012). Some recent approaches to control *Tuta absoluta* in tomato under greenhouse conditions. *African Entomology, 20*, 27–34.

Desneux, N., Wajnberg, E., Wyckhuys, K. A. G., Burgio, G., Arpaia, S., Narvaez-Vasquez, C. A., et al. (2010). Biological invasion of European tomato crops by *Tuta absoluta* ecology geographic expansion and prospects for biological control. *Journal of Pest Science, 83*, 197–215.

Ferracini, C., Ingegno, B. L., Navone, P., Ferrari, E., Mosti, M., Tavella, L., Alma, A., et al. (2012). Adaptation of indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. *Journal of Economic Entomology, 105*, 1311–1319.

InfoAgro Systems. (2009). La Polilla del Tomate (*Tuta absoluta*). InfoAgro Systems, Inc. Madrid.. http://www.infoagro.com/hortalizas/polilla_tomate_tuta_absoluta.htm.

IRAC. (2011). *Tuta absoluta the tomato leafminer or tomato borer*. Recommendations for sustainable and effective resistance management insecticide. http://www.iraconline.org/wp-content/uploads/2009/12/Tuta_brochure_print-version_11Oct11.pdf

IRAC Insecticide Resistance Action Committee. (2009). *IRAC mode of action classification*. http://www.irac-online.org/documents/MoA%20classification_v6.3.3_28july09.pdf. Accessed 7 Jan 2010.

Lietti, M. M., Botto, E., & Alzogaray, R. A. (2005). Insecticide resistance in argentine populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Neotropical Entomology, 34*, 113–119.

Mohamed, B., & Hajji, L. (2012). *Management of Tuta absoluta (Lepidoptera, Gelechiidae) with insecticides on tomatoes, insecticides – Pest engineering* (F. Perveen, Ed.). ISBN:978-953-307-895-3. InTech. Available at: http://www.intechopen.com/books/insecticides-pest-engineering/managementof-tuta-absoluta-lepidoptera-gelechiidae-with-insecticides-on-tomatoes

Pfeiffer, D. G., Muniappan, R., Dienaba, S., Diongue, A. P., & Dieng, E. O. (2013). First record of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Senegal. *Florida Entomologist, 96*, 661–662.

Potting, R., Van der Gaag, D. J., Loomans, A., van der Straten, M., Anderson, H., MacLeod, A., et al. (2009). *Tuta absoluta, tomato leaf miner moth or South American tomato moth*. Ministry of Agriculture, Nature and Food Quality (LVN) Plant Protection Service of the Netherlands. http://www.minlnv.nl/cdlpub/servlet/CDLServlet?p_file_id=42402. Accessed 16 Dec 2009.

Real IPM. (2015). *Limited (Kenya) real insights on Tuta absoluta*. Available from: www.realipm.com

Roditakis, E., Papachristos, D., & Roditakis, N. E. (2010). Current status of the tomato leafminer *Tuta absoluta* in Greece. *OEPP/EPPO Bulletin, 40*, 163–166.

Rodrigues de Oliveira, A. C., Veloso, V. R. S., Barros, R. G., Fernandes, P. M., & Barboza de Souza, E. R. (2008). Captura de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) com armadilha luminosa na cultura do tomateiro tutorado. *Pesquisa Agropecuaria Tropical, 38*, 153–157.

Siqueira, H. A. A., Guedes, R. N., & Picanço, M. C. (2000a). Cartap resistance and synergism in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Applied Entomology, 124*, 233–238.

Siqueira, H. A. A., Guedes, R. N., & Picanço, M. C. (2000b). Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forestry Entomology, 2*, 147–153.

Walgenbach, J. F., Leidy, R. B., & Sheets, T. J. (1991). Persistence of insecticides on tomato foliage and implications for control of tomato fruitworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology, 84*, 978–986.

Chapter 6
New Record of *Tuta absoluta* on Cowpea *Vigna unguiculata*, Gezira State, Sudan

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Abstract *Tuta absoluta* is a threatening pest for tomato production in Sudan. It has been reported on other solanaceous plants. Cowpea grains and branches constitute an important source of protein for people and their animals. In Sudan, cowpea is produced under subsistence farming conditions, in rainfed sectors. The University of Gezira has been planning to introduce cowpea production into the irrigated sector. Cowpea genotypes were planted during the rainy season (summer time) and after that (winter season). Various insects were monitored during the budding, flowering and podding stages. *Tuta absoluta* was recorded on these genotypes as infesting leaves at a range of 3–8% during the summer time and 1–5% during winter, depending on genotypes. *Tuta absoluta* males were trapped using a pheromone (Tua optima 0.8 mg) baited water traps, when deployed upwind of cowpea field. The numbers of trapped males were higher on cowpea (68%) than on cotton (32%), during October–December. However, the tomato crop was a powerful attractant to males (59%), as compared to cowpea (25%) and cotton (16%). *Tuta absoluta* infested leaves of cowpea, requiring future work with more emphasis on pod damage.

Keywords *Tuta absoluta* · Cowpea genotypes · Pheromone

6.1 Introduction

Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) is known under so many common names like South American tomato leafminer (Biondi et al. 2013) being native to South America (Urbaneja et al. 2007). The insect was reported in East Spain in

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2006 (Judit and Rosa 2010), then spread to Afro-Asian countries (Desneux et al. 2010), and was reported in Sudan in 2010 (Mohamed et al. 2011). It is often detected on members of the plant family Solanaceae (Caparros Megido et al. 2014) with feeding preference for tomato, *Solanum lycopersicum* (Bawin et al. 2014). Solanaceous volatiles in fact attract the insect (Caparros Megido et al. 2014). Males were found to be attracted to tomato plants (70%), as compared with miscellaneous crops of cowpea, sorghum and cotton (30%) (Rida et al. 2018). Males captures start in December, and reach a peak in March, April and May. Subsequently their numbers declines, reaching the minimum during the period of June to November (Hamed 2018).

The threats on non-solanaceous crops has never been evaluated before. Cowpea, *Vigna unguiculata*, has been historically grown in Sudan by smallholder farmers under subsistence farming, which show no agrochemicals use, except in a limited experimental area (Abdalla et al. 2015). However, in other African countries, cowpea is the main source of plant proteins and the use of chemicals and bioinsecticides is a routine practice to control various types of insects, including chewing and sucking pests (Oparaeke et al. 2000; Udo and Akpan 2012; Nta et al. 2013; Singh and Singh 2015; Ashigar and Umar 2016; Swathi et al. 2017). In the literature on *T.absoluta* and cowpea, *Vigna unguiculata*, no work was found in relation to *T. absoluta* infestation on this crop.

6.2 Materials and Methods

6.2.1 Experimental Site

The experiment was conducted on the University of Gezira Farm, Faculty of Agricultural Sciences, at lat. 14.44 N° longit. 33.39 E° at 407 m above sea level.

The crops under study comprised cotton, cowpea and tomato. Each crop was grown on a plot measuring 0.5 feddan (1 feddan = 0.42 ha).

The sowing date for cotton was June 20, 2017. Cowpea genotypes were sown on July 20, 2017 and October 20, 2017 in 13 lines, while tomato was directly planted on November 02, 2017. All crops were grown under irrigation. The usual cultural practices for all crops were followed, as recommended by Agricultural Research Corporations.

6.2.2 Data Collection

6.2.2.1 Leaves Damage

At the outset, the damage to leaves was recorded in September 2017, with the damage causal agent then being unknown. The damaged leaves, at various stages, were taken to the laboratory and each leaf was confined in a plastic Petri dish (90 mm).

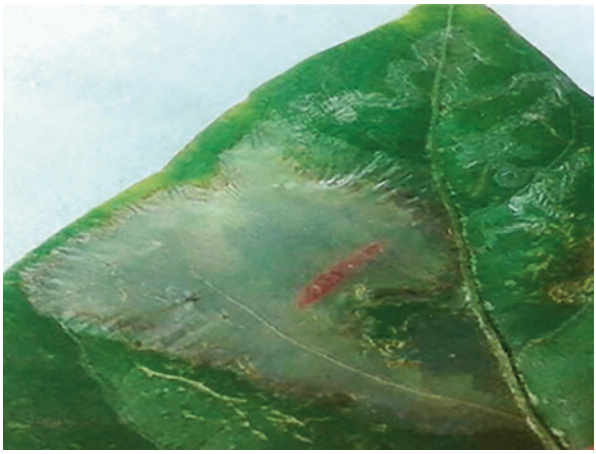

Fig. 6.1 Cowpea leaf collected from the field and containing a pupa of *Tuta absoluta*

The Petri dishes were stored in a desiccator that had moist cotton placed at the base to prevent dehydration of the leaves, while waiting for the damage causal agent to emerge. Adults of *T. absoluta* emerged from damaged leaves and the identification was confirmed by the insect museum of the Agricultural Research Corporation (Sudan). Thereafter, the leaf damage caused by *T. absoluta* was monitored on cowpea following the two sowing dates. The leaves at the base of the upper-third portion of the plant vine were monitored in 10 plants per plot, with 5 leaves for each plant. Characteristic insect damage that removed the mesophilic tissue of the leaf (Fig. 6.1) was considered. The data were recorded every week, for 2 months, for each sowing date.

6.2.2.2 Males Trapping

Water-bowl traps, with a radius of 15 cm and a volume of 3.5 litres, were used in these experiments. The bowls were made of red plastic material, and baited with "Tua optima" sex pheromone of 0.8 mg loaded onto a grey rubber septum (produced by Russell IPM England, imported by Star Chemical Ltd). The pheromone was stored in a refrigerator until the time of use in the field. The pheromone grey rubber septa were renewed every 10 weeks. Each septum, impregnated with pheromone, was secured in a small porous, translucent, plastic vial (vol. 50 ml). The base of the vial and the screw cover were perforated for a tender wire to pass through longitudinally and fixed in the centre of the bowl void by knotting the ends of the tender wire at the rim of the bowl.

The bowl was filled with water to a level just below the vial. The water surface tension was broken by drops of liquid soap. All bowl traps were deployed upwind of the crop concerned.

6.2.2.3 Statistical Analysis

The captured males were numbered per month, while the leaf infestations were taken as percentages. Appropriate statistical transformations were done when needed. Then, the data were analysed using ANOVA. Significant differences between means were separated using Duncan's Multiple Range Test (DMRT) (P = 0.05).

6.3 Results and Discussion

6.3.1 Leaves damage

The damages to leaves are shown in Tables 6.1 and 6.2. During September, the percentages of leaf damage ranged between 2 and 6%, while in October, the percentages of damage ranged between 3 and 8%. A similar trend in the percentage of leaf infestation was shown during December (2–6%), while the infestation percentage during January was low (1–5%). Some cowpea genotypes showed erratic performance regarding susceptibility. For example, the genotype Land race 2 was susceptible when grown in summer (rainy season), yet it was resistant in the winter season (Tables 6.1 and 6.2). On the other hand, genotypes B301 and IFE brown were resistant in the summer growing season, yet they were susceptible in the winter growing season. However, genotypes T183D-442, T100K-1263 and T185F-867-5 showed steady performance in resistance, during the two seasons. Similar results of an erratic nature, characterised by a lack of consistency in cowpea genotype performances, were reported by Tanzubil et al. (2008) in Ghana.

6.3.2 Male Trapping

Generally, more males were attracted towards pheromone-baited water traps upwind of cowpea, than upwind of cotton. However, the attraction towards traps deployed upwind of tomato caught a higher number of males than those for cotton and cowpea, collectively (Table 6.3). Before the tomato cropping, the total number of males captured in cowpea traps was 68%, compared with the 32% of males captured in cotton traps. However, after the tomato plants were cropped during January and February, the total number of males captured in cowpea was 25%, and the total number of captured males in cotton was 16%. In tomato crop, the total number of captured males was 59%. This result was consistent with previous data, where 70% of captured males were recorded in traps deployed upwind of a tomato field, as compared with lower percentages for traps deployed upwind of miscellaneous crops (Rida et al. 2018).

Table 6.1 Percentage of cowpea leaves infested by *Tuta absoluta* (n = 10 plants) – summer season

Cowpea line	% Infestation*	
	September	October
T183D-442	2.1 ab (5.0)	2.3ab (5.3)
Land race 1	2.1 ab (4.4)	2.4 ab (6.0)
T199K-214-2	1.9 ab (4.0)	2.4ab (6.2)
T198K-409-4	2.5 a (6.1)	1.7b (3.0)
T199K-573-2-1	2.1 ab (4.2)	2.1ab (4.3)
T100K-1263	1.9 ab (4.0)	1.6 b (3.0)
IFE brown	1.8 ab (3.4)	1.6b (3.0)
T100K-901-6	1.8ab (3.2)	2.1 ab (5.0)
T185F-867-5	2.1 ab (4.1)	1.7b (3.0)
B301	1.4 b (2.0)	2.1ab (4.3)
T182E-18	1.6 ab (3.0)	1.8 ab (3.3)
T197K-499-35	1.6 ab (3.0)	1.8 ab (3.3)
Land race2	1.8 ab (3.4)	2.7 a (8.0)
SE±	0.2	0.1
CV%	20.5%	21.9%

*Data transformed to square root. Actual data between parentheses. Means followed by the same letter(s) do not differ significantly according to DMRT (P = 0.05)

6.4 Conclusions

Tuta absoluta infests cowpea plants. The infestation, so far, was verified on leaves.

The insect males were captured on cotton plants, but with no observation of damage on them.

The damage by *T. absoluta* to cowpea leaves occurred in greater degree in crops grown during summer (rainy season), than in crops grown in the winter season.

Table 6.2 Percentage of infested cowpea leaves by *T. absoluta* (n = 10 plants) – winter season

	% Infestation*	
Cow pea line	December	January
T183D-442	2.2 ab (5.0)	1.1 b (1.2)
Land race1	1.9 ab (4.0)	1.5 ab (2.4)
T199K-214-2	2.4 ab (6.2)	1.8 ab (3.4)
T198K-409-4	1.9 ab (4.2)	1.7 ab (3.2)
T199K-573-2-1	1.7 ab (3.0)	1.5 ab (2.3)
T100K-1263	2.2 ab (5.0)	1.6 ab (2.9)
IFE brown	2.7 a (3.2)	1.7 ab (3.2)
T100K-901-6	2.1 ab (4.0)	1.4 ab (2.0)
T185F-867-5	2.3 ab (6.0)	1.6 ab (2.5)
B301	1.9 ab (3.8)	1.3 ab (1.7)
T182E-18	2.1 ab (4.8)	2.2 a (5.0)
T197K-499-35	1.7 ab (3.1)	2.1 ab (5.2)
Land race2	1.2 b (2.0)	1.7 ab (3.2)
SE±	0.4	0.2
CV%	32.5%	29.5%

∗Data transformed to square root. Actual data between parentheses. Means followed by the same letter(s) do not differ significant according to DMRT (P = 0.05).

Table 6.3 Total number of captured males of *T. absoluta* using pheromone (Tua optima 0.8 mg) baited water trap on various crops

Month	Cowpea	Cotton	Tomato
October	1	1	–
November	28	4	–
December	7	12	–
Total number	36 (68%)	17 (32%)	
January	18	9	32
February	29	22	82
Total number	47 (25%)	31 (16%)	114 (59%)

References

Abdalla, A., Elgailani, A., Osman, K., Maki, M. A., Nur, F. M., Ali, S. B., & Aune, H. B. (2015). The response of sorghum, groundnut, sesame and Cowpea to seed priming and fertilizer microdosing in South Kordofan State, Sudan. *Agronomy, 5*, 476–490.

Ashigar, M. A., & Umar, K. M. (2016). Biology of *Maruca vitrata* (Lepidoptera: Crambidae) a serious pest of cowpea and other legume crops: A review. *Annals of Experimental Biology, 4*, 33–37.

Bawin, T., De Backer, L., Dujeu, D., Legrand, P., Caparro Megido, R., Francis, F., & Verheggen, F. (2014). Infestation level influences oviposition site selection in the tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae). *Insects, 5*, 887–884.

Biondi, A., Zappala, L., Stark, J. D., & Deneaux, N. (2013). Do biopesticides affect the demographic traits of a parasitoid wasp and its biocontrol services through sublethal effects? *PloS One, 8*(9), e76548. https://doi.org/10.1371/journal.pone.0076548.t001

Caparros Megido, R., De Backer, L., Ettaib, R., Brostaux, Y., Fauconnier, M. L., Delaplace, P., Lognay, G., et al. (2014). Role of larval host plant experience and Solanceous plant volatile emission in *Tuta absoluta* (Lepidoptera: Gelechiidae) host finding behavior. *Arthropods-Plant Interaction, 8*, 293–304.

Desneux, N., Wajnberg, E., Wyckhuys, K. A., Burgio, G., Arpaia, S., Narvaez-Vasquez, C. A., et al. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science, 83*, 197–215.

Hamed, A. G. M. (2018). *Tuta absoluta (Meyrick). Seasonal abundance and susceptibility of some tomato genotypes, Gezira State, Sudan*. MSc dissertation, Faculty of Agricultural Sciences, University of Gezira.

Judit, A., & Rosa, G. (2010). Controlling Tuta absoluta, a new invasive pest in Europe, Training in Integrated Pest Management – No.5 IRTA Cabrils, Spain.

Mohamed, E. S. I., Mohamed, M. E., & Gamiel, S. A. (2011). First record of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Sudan. *EPPO Bulletin, 42*, 325–327.

Nta, A. I., Ibiang, Y. B., Uyoh, E. A., Edu, N. E., Ekanem, B. E., & John, Q. E. (2013). Insect pest damage to leaves of cowpea (*Vigna unguiculata* L. Walp): comparative effects of aqueous extracts of *Piper guineensis*, *Allium sativum* and *Myristica fragrans*. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 3*, 17–20.

Oparaeke, A. M., Dike, M. C., & Amatabi, C. I. (2000). Field evaluation of extracts of five nigerian spices for control of post-flowering insect pests of cowpea, *Vigna unguiculata* (L.) Walp. *Plant Protection Science, 41*, 14–20.

Rida, M. M. M., Hassan, A. E. M., & Hussein, A. A. E. (2018). *Flight behaviour and activity time of Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) males trapped on tomato and miscellaneous crops, Gezira State, Sudan*. MSc dissertation, Faculty of Agricultural Science, University of Gezira.

Singh, C., & Singh, N. N. (2015). Bioefficacy and economics of different insecticides against legume pod borer (*Maruca vitrata* Fabricius) infesting cowpea (*Vigna unguiculata* Walpers). *Indian Journal Agricultural Research, 49*, 358–362.

Swathi, Y. K., Pandya, H. V., Shiva, S. K., & Srasvan, G. K. (2017). Efficiency of different combination of insecticides against cowpea pod borer in cowpea (*Vigna unguiculata* (L.) Walp.). *International Journal of Current Microbiology and Applied Sciences, 6*, 2030–2037.

Tanzubil, P. B., Zakariah, M., & Alem, A. (2008). Integrating host plant resistance and chemical control in the management of cowpea pests. *Australian Journal of Crop Science, 2*, 115–120.

Udo, I. O., & Akpan, E. A. (2012). Evaluation of local spices as biopesticides for the control of *Ootheca mutabilis*, Shalbera and *Clavigrallato mentosicollis* (Stal.) on cultivated cowpea (*Vigna unguiculata* L.) in Nigeria. *Journal of Agricultural Science, 4*, 1916–9760.

Urbaneja, A., Monton, H., & Molla, O. (2007). Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. *Journal of Applied Entomology, 133*(4), 292–296.

Chapter 7
Efficacy of a Sudanese Strain of Entomopathogenic Fungus, *Metarhizium anisopliae* Met. Sorokin on Puparia of *Bactrocera dorsalis* Hendel, Under Laboratory Conditions

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Abstract Fruit flies (Diptera: Tephritidae) are among the main constraints that limit horticultural production in Sudan. The country has an enormous potential for horticultural production, over its wide range of climatic conditions and diverse ecosystems. However, it is threatened by an invasion of exotic fruit flies over its long borders with neighbouring countries, and by its weak interception and quarantine procedures. Although entomopathogenic fungi are known to attack fruit flies, very few efforts have been exerted to confirm their efficacy in integrated pest management (IPM) strategies against fruit flies. The fungus *Metarhizium anisopliae* Sorokin has been found on the dead body of a larva of white grub, *Schizonycha* sp. (Coleoptera: Melolonthidae), at Kenana Sugarcane Farm. A laboratory experiment was undertaken by applying four different concentrations (20×10^6, 10×10^6, 5×10^6 and 2.5×10^6) of the fungus on the pupae of *Bactrocera dorsalis* in order to inhibit adult emergence. The results showed that adult emergence from the pupae of *B. dorsalis* was inhibited by the tested concentrations of *M. anisopliae*. The inhibition increased with increasing concentrations. LC_{50} and LC_{90} values for the fungus were 10×10^6 and 18×10^6 conidia/ml, respectively. The mentioned fungus can be incorporated in fruit fly management strategy.

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Keywords Fruit flies · Diptera · Tephritidae · Entomopathogenic fungi

7.1 Introduction

Mango (*Mangifera indica* L.) is believed to have been cultivated for about 6,000 years, with its likely native home in Eastern Asia. This species is now grown worldwide, in both hemispheres. The fruit is a good source of fibres and vitamins, and is described as the most favourite and valuable fruit in the world market. Moreover, the fruit is rich in antioxidants and therefore reduces the risks of cardiac disease, and has anti-cancer and anti-viral properties. In Sudan, mango is an important horticultural crop for local consumption and export (Ministry of Industry and Trade 2019). It has economic importance, being produced almost all the year round in different parts of the country (Ministry of Industry and Trade 2019). It also has regional and international demand in markets, being a beloved fruit with great nutritive value and palatable taste. The area cultivated for mango in Sudan is estimated to be about 2,814,000 ha, and its production is estimated at 635,000 tons, annually (Fatima and Dawoud 2017).

Mango is commercially grown in every state – South Kordofan, Sinnar, Blue Nile, West Darfur, South Darfur and Kassala (Ministry of Industry and Trade 2019). Fruit flies of the family Tephritidae are among the most destructive agricultural pests in the world (De Meyer et al. 2001). Because of their widespread agricultural impacts and rapid expansion, tephritid fruit flies are the subject of quarantine and control efforts, worldwide (White and Elson Harris 1992). There are about 450 genera and more than 4,300 described species within Tephritidae, making it one of the largest families within the order Diptera.

Anastrepha Schiner, *Bactrocera* Macquart, *Ceratitis* MacLeay, *Dacus* Fabritius and *Rhagoletis* Loew are the most economically important genera because many of their species are frugivorous. They infest almost every cultivated area and have wide bio-climatic adaptation potential (Bateman 1972). Thus, with the increasing movement of people and produce, they have the potential to invade other territories.

Sudan has enormous potential for horticultural production, over its wide range of climatic conditions and diverse ecosystems (Mahmoud 2011). There are several methods of fruit fly suppression, including the application of bait spray, male annihilation techniques, sterile insect techniques, and the use of biological control agents such as parasitoids and pathogens (White and Elson Harris 1992). The fungus *Metarhizium anisopliae*, a naturally occurring antagonist isolated from soil, is used in ICIPE to kill mature maggots and puparia of fruit flies (Ekesi and Billah 2006).

Social and environmental problems are currently associated with insecticide use for fruit fly control whether by aerial or ground applications on foliage against adults, or to soil for larvae or newly emerged adult control. Humans, domestic animals, and beneficial insects (natural enemies, pollinators etc.) can be affected by the

insecticides. The application of the same type of insecticide for a long period against particular insects may lead to lineages developing resistance. Certain pesticides provoke phytotoxic effects on various crops or varieties, especially under extreme climatic conditions in Sudan (Schmutterer 1969). Such problems have motivated the search for biological control alternatives, including entomopathogenic fungi.

This study aims at evaluating the efficacy of the fungus *M. anisopliae* against the pupae of *Bactrocera dorsalis* Hendel.

7.2 Materials and Methods

This experiment was carried out at the laboratory of the Plant Pathology Centre and the Biology Laboratory, Faculty of Agricultural Sciences, University of Gezira. The fungus, *M. anisopliae* Met. Sorokin, was isolated from a cadaver of a white grub, *Schizonycha* sp. (Coleoptera: Melolonthidae), on Kenana Sugarcane Farm by Elnour et al. (2009).

A laboratory assay was undertaken by applying four different concentrations (20×10^6, 10×10^6, 5×10^6 and 2.5×10^6) of *Metarhizium anisopliae* Sorokin on the pupae of *Bactrocera dorsalis* in order to test the potential to inhibit adult emergence.

7.2.1 Culture of the Fungus

Sabouraud Dextrose Agar (SDA, 32.5 g in 500 ml of distilled water) was dissolved by slightly heating on a hot plate and then autoclaved at 121 °C for 15 min under 15 psi. Next, the medium was cooled at room temperature and poured into 9-cm sterilized Petri dishes under aseptic conditions (Elnour et al. 2009). For isolation, a needle loop was used for intercepting the conidia on the cuticle of the insect host cadaver to inoculate the medium. After that, the culture was placed in an incubator at 27±0.5 °C for 3 weeks to get fungus ready for further multiplication and testing on other fruit flies.

7.2.2 Harvesting of Conidia

To harvest the spores, the surfactant emulsifier Tween 20% was added to Petri dishes containing the sporulating fungus. Conidia were suspended in the bathing media by stirring with a sterilised glass rod. The conidial suspension was discharged into a sterilised glass beaker, sieved through double-layer sterilised cloth in order to get rid of hyphal fragments. Other matter found within the conidia in the suspension was removed by washing, transferring the suspension to test tubes, for a 5 min centrifugation at 3000 rpm. This procedure was carried out in order to wet and disperse

the conidia. The supernatant or floating material was decanted and replaced by an aliquot of the washing solution. The conidia were then re-suspended using a vortex mixture, repeating this procedure four times. The concentration of the harvested conidia was then determined using a Neubauer ruled counting chamber (haemocytometer). The required spore concentrations were then prepared by dilution in sterilised distilled water (Hassan 1983; Elnour et al. 2009).

7.2.3 Application of the Fungus on Fruit Fly Pupae

Ripe and fallen fruits were collected from orchards, placed on ventilated bags and transferred to laboratory where they were distributed to small containers in equal numbers (5 fruits each). A sheath of sand had been previously placed at the bottom of the containers for pupation since the 3rd larval instar had begun springing. Fine cloth was added to the top of the containers for aeration purpose. After the larvae changed into pupae, the latter were placed into small vials (10 cm long, 5 cm internal diam.), with 10 pupae in each vial which contained a sheath of sand.

Four concentrations of the harvested conidia, 20×10^6, 10×10^6, 5×10^6 and 2.5×10^6 conidia/ml and the untreated control, were applied as treatments in 1 ml topically on the pupae of the invasive fruit fly *B. dorsalis*, using a micropipette. Treatments were arranged in a completely randomised design (CRD) with 3 replicates. Observations were taken daily on the pupal infestiation by the fungus and on adult emergence.

Data were recorded and the percentage mortality was taken, and subjected to Probit analysis using the Statistical Package for Social Sciences (SPSS). LC^{50} and LC^{90} were calculated in this experiment.

7.3 Results

Four different concentrations (20×10^6, 10×10^6, 5×10^6 and 2.5×10^6 conidia/ml) of *M. anisopliae*, were directly applied to the pupae of the oriental fruit fly, *B. dorsalis*, during this study. The four concentrations were effective in inhibiting the emergence of adults from the pupae, but in different degrees. Adult emergence from the pupae of *B. dorsalis* was inhibited by the tested concentrations of *M. anisopliae*, and the inhibition progressed as the concentration increased.

The relationship between dose concentrations and inhibition of emergence was in a direct proportion, as the inhibition of adult emergence increased with increasing doses of concentration, i.e. dose-dependent, while the log-dose Probit regression line was:

$$Y = 4.069X - 22.10.$$

Table 7.1 Inhibition of *Metarhizium anisopliae* of adult emergence of *Bactrocera dorsalis*

Concentration (10^6 conidia/ml)	No. of pupae	Observed response	Expected response	Probability
Untreated control	10	0	0	0
2.5	10	3	2.620	0.262
5	10	4	4.168	0.417
10	10	5	5.858	0.586
20	10	8	7.402	0.740

The mortality percentages of *B. dorsalis* pupae treated by *M. anisopliae* were 0%, 30%, 40%, 50% and 80% for doses: 0 (control), 2.5×10^6, 5×10^6, 10×10^6, 20×10^6, respectively.

The LC_{50} and LC_{90} values for the fungus were 10^6 and 18^6 conidia/ml, respectively. The heterogeneity factor ($\chi^2 = 0.576$), concentration, log concentration, tested response, corrected response, and tabulated Probit are shown in Table 7.1. The slope of the Ld-p line was stated in the equation above, and corresponded to 4.069.

7.4 Discussion

The entomopathogenic fungus *Metarhizium anisopliae* reproduces by mitotic spores (conidia). When these come into contact with the body of an insect host, they germinate and the emerging hyphae penetrate the cuticle. The fungus then develops inside the body, eventually killing the insect after a few days. The cuticle of the cadaver often becomes red. If the ambient humidity is high enough, a white mould then grows in the cadaver and soon turns green as conidia are produced (Freimoser et al. 2003).

The purpose of this study was to evaluate the efficacy of *M. anisopliae* on the pupae of the oriental fruit fly *B. dorsalis.* The relationship between dose concentrations and inhibition of adult emergence was in direct proportion: the inhibition of adult emergence increases with increasing dose concentration, in a dose-dependent relationship. The results of this study agreed with those of Musa (2013), who applied two concentrations, of 6.5×10^{10} conidia/ml and 4.3×10^8 conidia/ml, on *B. bassiana* and *M. anisopliae*, respectively, on two *Dacus* spp. The pure formulation of the fungi showed adult mortality between 42 and 90% and 29 and 77% in the two species, respectively.

7.5 Conclusion

Metarhizium anisopliae appeared suitable to control *B. dorsalis* as it affected the pupae with a relationship linking the applied dose concentration and the inhibition of adult emergence, in a direct proportion, in a classical dose-dependent relation-

ship. The LC_{50} and LC_{90} values for the fungus were 10×10^6 and 18×10^6 conidia/ml, respectively. *Metarhizium anisopliae* proved to be an effective bio-agent against the pupae of the oriental fruit fly and may be recommended to be used in suitable formulation for control.

Although the mentioned findings suggest the fungus can be incorporated in a fruit fly management strategy, field studies on the application of *M. anisopliae* on immature and adult stages of fruit flies are, however, required.

References

Bateman, M. A. (1972). *The Ecology of Fruit Flies. C.S. I. R., School of Biological Sciences*, University of Sydney, N. S .W. 2006, Australia.

De Meyer, M., et al. (2001). Taxonomic notes on the Afrotropical subgenera *Ceratitis* (Acroptromma) Bezzi and C. (Holpolophomiya) Bezzi (Diptera: Tephritidae). *Cimbebasia, 17*, 77–84.

Ekesi, S., & Billah, M. K. (2006). *A field guide to the management of economically important Tephritid fruit flies in Africa*. Nairobi: The International Centre of Insect Physiology and Ecology (ICIPE) Science Press.

Elnour, A. E., Ahmed, E. M., Rudwan, M. K., Yousif, F. M., & Bernard, V. (2009). Effects of temperature and substrate on vegetative growth of the entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin. *Gezira Journal of Agricultural Sciences, 7*, 124–137.

Fatima, A. A., & Dawoud, H. D. (2017). *Mango cultivation and production in Sudan* (pp. 17–18). Ministry of Agriculture and Forestry, Agricultural Research Corporation, Horticulture Crop Research Centre.

Freimoser, F. M., Screen, S., Bagga, S., Hu, G., & St. Leger, R. G. (2003). EST analysis. *Microbiology, 149*, 239–247.

Hassan, A. E. M. (1983). *The interaction of entomopathogenic fungus, Metarhizium anisopliae (Sorokin) and the insecticide diflubenzuron on Manduca sexta (Johnson)*. PhD thesis, University of Bath, United Kingdom.

Mahmoud, M. E. E. (2011). *Population dynamic, mass trapping and molecular characterization of major fruit flies (Diptera: Tephritidae) in Khartoum, Kassala and Kordofan States (Sudan)*. PhD thesis, Department of Zoology, Faculty of Sciences, University of Khartoum, Sudan.

Ministry of Industry and Trade, Sudan. (2019). *Sudan trade point: mango*. www.tpsudan.gov.sd.

Musa, I. O. (2013). *Effects of NeemAzal 1% and two entomopathogenic fungi on some cucurbit fruit flies (Diptera: Tephritidae)*. PhD thesis, Faculty of Agriculture, Department of Crop Protection, University of Gezira.

Schmutterer, H. (1969). *Pests of crops in Northeast and Central Africa, with particular reference to the Sudan*. Stuttgart: Gustav Fisher Verlag. 257 pp.

White, I. M., & Elson Harris, M. M. (1992). *Fruit flies of economic significance: Their identification and bionomics* (601 pp). Wallingford: CAB International.

Chapter 8
Taxonomic Keys to Economic Fruit Flies (Diptera: Tephritidae) of the Sudan

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Abstract Fruit flies (Diptera: Tephritidae) are among the main constraints that limit the horticultural production in Sudan. The country has an enormous potential for horticultural production, over its wide range of climatic conditions and diverse ecosystems, but is threatened by an invasion of exotic fruit flies over its borders, due to weak interception and quarantine procedures. Thus, the correct identification of the pest is the first step in a management strategy for fruit flies. This study was carried out in order to produce identification keys for the economic fruit flies found in the Sudan. Intensive searching in the data of the National Insect Collection was carried out to ascertain the taxonomic status of the family Tephritidae (the true fruit flies) and its species of economic importance, among families of the order Diptera (the true flies). Specimens of fruit flies were identified morphologically at the National Insect Collection Unit at the Agricultural Research Corporation (ARC) and Faculty of Agriculture Biology Laboratory. The morphological identification of African tephritid fruit flies largely depends on the use of classical single-entry (dichotomous) keys. Taxonomic keys for the family Tephritidae and its genera were prepared, following the fruit fly taxonomic keys of White and Harris (Fruit flies of economic significance: their identification and bionomics. CAB International, Wallingford, 601 pp, 1992), De Meyer and Copeland (Taxonomic notes on the Afrotropical subgenera *Ceratitis* (*Acroptromma*) Bezzi and C. (Holpolophomiya) Bezzi (Diptera: Tephritide). Cimbebasia 17:77–84, 2001), and Billah (2005). As a result, six taxonomic keys for fruit flies were prepared: an introductory key to the family Tephritidae; and a key to the economic genera of Tephritidae, including *Ceratitis* spp., *Dacus* spp., *Bactrocera* spp. and *Carpomya* spp. from Sudan.

Keywords Fruit flies · Diptera · Tephritidae · Taxonomic key

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8.1 Introduction

Fruit flies of the family Tephritidae are among the most destructive agricultural pests in the world (De Meyer and Copeland 2001). Because of their widespread, agricultural impact and rapid expansion, tephritid fruit flies are the subject of quarantine and control efforts, worldwide (White and Elson Harris, 1992). There are about 450 genera and more than 4300 described species within Tephritidae, making it one of the largest families within the order Diptera.

Anastrepha Schiner, *Bactrocera* Macquart, *Ceratitis* MacLeay, *Dacus* Fabritius and *Rhagoletis* Loew are the most economically important genera, because many of their species are frugivorous. They infest almost every cultivated area and have wide bio-climatic adaptation potential (Bateman 1972). Thus, with the increasing movement of people and produce, they have the potential to invade other territories.

Sudan has enormous potential for horticultural productions over its wide range of climatic conditions and diverse ecosystems (Mahmoud 2011), but fruit flies are the main limiting constraints. More than 39 fruit fly species have been sampled in the Insect Museum of the Agricultural Research Corporation since early nineteenth century. Studies from 2008 to 2017 reported 19 species. The situation regarding fruit flies became exacerbated after the invasion of the country by the alien invasive species *Bactrocera dorsalis* in 2005 and by *B. zonata* in 2012. Competitive displacement between *B. dorsalis* and species of the genus *Ceratitis* MacLeay was noted, and was later reported between *B. zonata* and *B. dorsalis*, mainly on mango and guava. Fruit flies are reported as being the main insect pests that cause severe losses to fruit production, exceeding 80% for guava and 30–50% for the Abu Samaka mango variety, from 2005 to 2008 (Gesmalla et al. 2014). According to El Tahir and Taha Yousif (2004), in addition to the already existing problem of indigenous species in Sudan – *Ceratitis capitata*, *C. cosyra*, *Dacus vertebratus* and *D. ciliatus* – the country is threatened by invasions of many exotic pests.

Sidahmed et al. (2014), reported in their assessment of farmers' knowledge of fruit flies and their management, that only 12% of the interviewed farmers had high experience of fruit flies, and that 52% had received extension services and information about plant protection. 43% of the respondents use methyl eugenol to control fruit fly, with only 17% applying the correct dose. Several 'training of trainers' programmes (TOT) were held for plant protection directorate members, teaching staff, and extension and plant quarantine officers. In 2006, the International Center of Insect Physiology and Ecology held one TOT at the Agricultural Research Corporation, Wad Medani. More than 15 PhD and 25 MSc degrees were awarded by several Sudanese universities on various topics regarding basic studies or applied control of fruit flies. However, there is still a need for capacity building efforts to combat fruit flies, especially in taxonomy (Mahmoud et al. 2019).

The family Tephritidae has a history that is complicated by revisions in classification and taxonomy. Its taxonomic status has been repeatedly revised and positions of related groups have undergone complicated changes (White and Elson Harris 1992). Thus, there is a need to develop unified taxonomic keys to help in identifying

flies easily so as to facilitate their control since an appropriate identification is one of the major tools in integrated pest management.

This study was carried out in order to create taxonomic keys for the economic fruit flies found in the Sudan in order to facilitate their identification by entomology students and researchers.

8.2 Materials and Methods

Intensive searching in the data of the National Insect Collection was done to ascertain the taxonomic status of the family Tephritidae (the true fruit flies) and of species of economic importance among the families of the order Diptera (the true flies).

Specimens of fruit flies were identified morphologically at the National Insect Collection Unit, at the Agricultural Research Corporation (ARC), and at Faculty of Agriculture Biology Laboratory.

The morphological identification of tephritid fruit flies largely depends on the use of classical single-entry (dichotomous) keys. Taxonomic keys for the family Tephritidae and its genera were prepared following the fruit fly taxonomic keys of White and Elson Harris (1992), De Meyer and Copeland (2001), White (2006) and Billah (2005). The species accounts include basic nomenclature details referring to White (2006). The keys, to the level of genera, were revised by Dr. De Meyer, The Royal Museum of Central Africa, Belgium.

Species groups were named by the type species name, with an initial letter as established by White (2006). Besides collecting fruit flies from orchards, four types of male lures (para-pheromones) were used: Cue lure (CUE) for *Bactrocera* and *Dacus* spp.; methyl eugenol (ME) for *Bactrocera* spp.; and terpinyl acetate (TA) and Trimedlure (TM) for *Ceratitis* spp. After transporting the captured flies to the laboratory, they were examined in taxonomic studies. A stereo binocular, a camel-hair brush, forceps, brushes and Petri dishes were used for this purpose.

8.3 Results and Discussion

Diptera represents one of the largest insect orders, comprising 43 families arranged in 300 genera that include about 700 species. Among these families, Tephritidae (the true fruit flies) is regarded as one of the largest families. The Tephritidae comprise 45 species, arranged in 21 genera; *Bactrocera* Macquart, *Carpomya* Costa, *Carpophthoromyia* Austen, *Celidodacus* Hendel, *Ceratitis* MacLeay, *Chelyophora* Rondani, *Coelotrypes* Bezzi, *Dacus* Fabricius, *Elaphomyia* saunders, *Ensina* Robineau-Desvoidy, *Euribia* Hendel, *Isoconia* Munro, *Leucotaeniella* Bezzi, *Myiopardalis* Bezzi, *Paroxyna* Hendel, *Platensina* Enderlein, *Rhabdochaeta* de Meijere, *Sphenella* Kützing, *Sphenicomyia* Bezzi, *Tephrella* Marston Bates, *Trupanea* Schrank and *Zeugodacus* Hendel.

The first three genera constitute the most concerning fruit flies that attack economically important fruit trees and vegetables. The most disastrous species are:

1. *Bactroceradorsalis* Hendel
2. *B. zonata* Saunders
3. *B. oleae* Rossi
4. *Ceratitis capitata* Wiedemann
5. *C. cosyra* Walker
6. *C. quinaria* Bezzi
7. *C. anonae* Graham
8. *Carpomya incompleta* Becker
9. *Dacus ciliatus* Loew
10. *D. frontalis* Becker
11. *D. bivittatus* Bigot
12. *D. vertebratus* Bezzi
13. *Zeugodacus cucurbitae* Coquillett

Six taxonomic keys of fruit flies were prepared during this study: an introductory key to the family Tephritidae; a key to the economic genera of Tephritidae found in the Sudan; and keys to *Ceratitis* spp., *Dacus* spp., *Bactrocera* spp. and *Carpomya* spp. in Sudan. These keys were compiled with reference to the efforts of White and Harris (1992), De Meyer and Copeland (2001), White (2006) and Billah (2005) who prepared reports on the taxonomic status of various fruit fly groups. They were prepared based on the distinctive characteristics of the economically harmful fruit flies. In these keys, there is an opposition of two traits and grading to others, until reaching the target group.

8.3.1 An Introductory Key to Family Tephritidae

1. Vein Sc abruptly bent forward at nearly 90° at the 3rd subcostal break. Dorsal side of vein R_1 with setulae. Wing is patterned by coloured bands. Wing basal cell cup with an acute extension.

TEPHRITIDAE

- Vein Sc not abruptly bent forward. Dorsal setulae on vein R_1 and frontal setae are absent; there is no any wing patterning and wing cell cup without an acute extension.

Non-Tephritidae

8.3.2 Key to Genera of Tephritidae of Economic Importance in Sudan

1. Wing cell cup is very narrow with very long extension. The 3rd segment of antennae is at least 3 times as long as broad. Head and thorax with reduced chaetotaxy; lacking ocellar, postocellar, dorsocentral and katepisternal setae.

   2

   Wing cell cup is broader than half depth of cell bm. Abdominal tergites are 5 with a pair of slightly depressed areas or ceromata.

   3

2. Abdomen with all tergites fused into a single plate with smooth transverse lines along boundaries. There is no overlapping in sclerites.

   DACUS

- Abdomen with all tergites separate with overlapping sclerites.

  BACTROCERA

3. Scutellum is usually convex and patterned with yellow and black areas. Head with 2 pairs of frontal setae.

   4

   Scutellum is concave and not patterned with yellow and black areas

   Non-target genus

4. Head without or with very small ocellar setae. Cell cup extension is about one-third as long as vein $A_1 + CuA_2$.

   CAPPARIMYIA

   Head with longer ocellar setae similar in length and strength to orbital setae. Cell cup extension is longer about half as long as $A_1 + CuA_2$.

   5

5. Scutellum with yellow areas, wing with pre-apical cross band on dm-cu which is isolated from the rest of the wing pattern. Basal cells of wing (c, br, bm and cup) with spot and fleck-shaped marks giving a reticulate appearance.

   CERATITIS

   Scutellum is flat, convex and patterned with yellow and black areas. Cell cup extension is short. Head with 3-4 pairs of frontal setae. Ocellar setae are very small, only as long as distance between anterior and posterior ocelli.

   CARPOMYA

8.3.3 Key to Ceratitis *Species of Economic Importance in Sudan*

1. Scutellum with yellow areas meeting margin, such that each apical scutellar is based in or adjacent to a yellow stripe

   2

   Scutellum with small black areas. Male mid tibia without stout setae arranged in such a way as to give a feathered appearance

   Ceratitis* (*Certalaspis*) *quinaria

2. Wing with apex of vein M not covered by diagonal coloured band. Yellow wavy band runs across base of scutellum. Male anterior pair of orbital setae is black with a sharp end to spatulate section.

   C.* (*Ceratitis*) *capitata

   Vein M may not be covered by diagonal coloured band. Yellow wavy band on scutellum not on across base. Male anterior orbital setae are not sharp to spatulate.

   3

3. Yellow band runs down to apex of scutellum dividing it to 3 dark spots. Wing banding brown to black, with a small break in costal band between costal break and R_1 or across R_1.

   4

   Wing banding more yellowish with no break in costal band. Scutellum also with 3 dark spots.

   5

4. Male with thick feathering on both mid femur and tibia along most of inner edge of femur with no gap in feathering.

   C.* (*Ceratitis*) *anonae

   Male with thick feathering restricted only to mid tibia.

   Non-target species

5. Scutum predominately yellow or pale brown with pattern of brown to black spots. Fore femur yellow on both sides. Postpronotal spot relatively big with an anterior seta. Costal band continuous.

   C.* (*Certalaspis*) *cosyra

8.3.4 *Key to* Bactrocera *Species of Economic Importance in the Sudan*

1. Scutellum with or without yellow areas, preapical crossband absent or not isolated. Basal cells of wing with consistent colour and no reticulate appearance.

   2

   Wing without a distinct costal band; cell sc often yellow, and apex of vein R_{4+5} with a brown spot. Scutellum with a triangular black mark.

   3

2. Scutum with high degree of variations, from dark brown to black. Postpronotal lobe yellow. Scutellum yellow except a narrow black band at the base. Aggression of microtrichia in cell br. All femora yellow and tibiae dark with hind tibiae darker. Abdominal tergites 3-5 with black T-shape mark. Anatergite and katatergite both yellow. Males with pectin.

   Bactrocera* (*Bactrocera) dorsalis* = *B. invadens

- Scutum with both lateral and medial yellow stripes. Scutellum with 2 marginal setae. Wing with cross vein dm-cu covered by infuscate area which is separate from other parts of the wing pattern.

  Zegodacus* (*Bactrocera*) *cucurbitae* = *B.* (*Zeugodacus*) *cucurbitae

3. Scutum and abdomen are pale orange-brown to red-brown. Scutum with only lateral yellow stripes. Presence of anterior supra-alar setae, prescutellar acrostichal setae. All the femora yellow, fore tibiae dark and hind tibiae with lighter middle part. No conspicuous black T-shape mark on abdomen.

   B.* (*Bactrocera*) *zonata

- Scutum without any yellow or orange stripes, although specimens with a distinct black area on the scutum may be red brown laterally, giving the appearance of lateral stripes. Area of wing close to apex with a dense patch of microtrichia.

  B.* (*Daculus*) *oleae

8.3.5 *Key to* Dacus *Species of Economic Importance in the Sudan*

1. Scutum with lateral and/or medial yellow or orange stripes.

   2

   Scutum without any yellow or orange stripes.

   3

2. Wing with very broad costal bands which extends below R_{4+5}, almost reaching vein M. postpronotal lobe pale at top and brown down.

Dacus* (*Dacus*) *bivittatus

Wing without such a broad costal band; combined depth of cells r_1 and r_{2+3} at r-m crossvein about equal to half-length of the r-m crossvein.

4

3. Both anatergite and katatergite with some yellow marking.

5

Yellow marking confined to only anatergite and katatergite brown.

6

4. General body colour orange-brown. Posterolateral area of thorax with a diagonal yellow stripe below the scutellum which extends across both katatergite, in front of the halter base, and anatergite.

D.* (*Dacus*) *punctatifrons

General body colour black. Posterolateral area of thorax with a yellow spot in front of the halter base which is confined to katatergite.

Non-target species

5. Yellow spot in anatergite separated from scutellum by its own diameter. Mid-femur yellow or orange, almost apical half slightly darker than basal half. Narrow costal band, with a small apical spot at tip. Two dark spots on lower scutum and a 3rd one on upper end which extends to a line. Abdominal tergite 3 with 2 dark spots on each side.

D.*(*Didacus*) *ciliatus

Postpronotal area of thorax with a diagonal yellow stripe below scutellum which extends across both katatergite and anatergite; stripe only separated from the scutellum by about 1/3 of its length.

6

6. Yellow spot in posterolateral not confined to katatergite, but extends across both katatergite and anatergite forming stripe. Yellow stripe separated from scutellum only by 1/3 of its length. All femora yellow in basal half and orange in apical one.

D.*(*Didacus*) *vertebratus

A predominately orange species. Basal parts of scutum with 2 black rounded spots on either sides of abdominal tergite 3. Most parts of anatergite covered by a yellow stripe. Apical half of mid femur darkened; no darkening in fore and hind femora. Apical spot of costal band extends more than 1/2 way between veins R_{4+5} and M. Anterior supra-alar and prescutellar acrostichal setae present.

D.* (*Didacus*) *frontalis

8.3.6 *Key to* Carpomya *Species of Economic Importance in the Sudan*

1. Scutum consistently reddish-yellow. Scutellum regularly pale yellow. Wing with 3 slight cross-bands; without an apical cross-band.
Carpomya* (*Trypeta*) *incompleta
Scutum and scutellum bright yellow with black patches. Wing with 4 distinct cross-bands; apical cross-band joined to pre-apical one.
Carpomya* (*Myiopardalis*) *pardalina

8.4 Conclusion

The six taxonomic keys to fruit flies of economic importance in Sudan herein provided will facilitate their identification for students and researchers of Entomology and related disciplines. The taxonomic keys of the family Tephritidae, economic families, genera and species are recommended to be used in identifying fruit flies. Since taxonomy is a dynamic field in which names of different groups usually change and follow the species. Work in taxonomic keys on economic fruit flies should be continued to facilitate the identification process, which is the first step for fruit fly management.

References

Bateman, M. A. (1972). The ecology of fruit flies. *Annual Review of Entomology, 17*, 493–517.

De Meyer, M., & Copeland, R. S. (2001). Taxonomic notes on the Afrotropical subgenera *Ceratitis* (*Acroptromma*) Bezzi and C. (Holpolophomiya) Bezzi (Diptera: Tephritide). *Cimbebasia, 17*, 77–84.

El Tahir, I. M., & Taha Yousif, M. (2004). Indigenous melons (*Cucumis melo* L.) in Sudan: A review of their genetic resources and prospects for use as sources of disease and insect resistance. *Plant Genetic Resources Newsletter, 138*, 36–42.

Gesmalla, A., Bahir, E., Hassan, N., & Assad, Y. A. (2014). *Identification, damage, host range, seasonal abundance and chemical control of mango fruit fly species (Diptera:Tephritidae) in Sennar State, Sudan*. Third conference on Pests Management in Sudan,3–4 February 2014, ARC, Sudan.

Mahmoud, M. E. E (2011). *Population dynamic, mass trapping and molecular characterization of major fruit flies (Diptera: Tepritidae) in Khartoum, Kassala and Kordofan States (Sudan)*. Ph. D. Thesis, Department of Zoology, Faculty of Sciences, University of Khartoum, Sudan.

Sidahmed, O. A., Abdalla, I., Allagabo, A. H., & Taha, A. K. (2014). *Famer knowledge about fruit flies problem and their control in guava and mango orchards in three states in Sudan*. Third conference on Pests Management in Sudan, 3–4 February 2014, ARC, Sudan.

White, I. M. (2006). *Taxonomy of the Dacina (Diptera: Tephritidae) of Africa and the Middle East*. Entomology Society of Southern Africa, African Entomology Memoir No. 2.

White, I. M., & Elson Harris, M. M. (1992). *Fruit flies of economic significance: Their identification and bionomics*. Wallingford: CAB International. 601 pp.

Chapter 9
Injury and Yield Losses Due to the Maize Stem Borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) on Smallholder Farms

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Abstract The present study aims at investigating damage and yield losses due to *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), which is one of the most important insect pests of maize in Kenya. A precise sampling scheme was applied to study the incidence of damage in maize caused by this insect pest on selected small-scale farms, on which pest control measures and fertilisers had not been applied. During the crop-growth stages and harvesting, plant and cob geometrical features were recorded, together with the length of tunnels bored by the insect. It is demonstrated that cob mass is an adequate variable for understanding yield losses caused by this pest. Although the number of plants damaged characterised by stem tunnels was greater than those with cob tunnels, the damages inflicted in the ears have the most considerable impact for yield losses. The recorded yield losses ranged from 35.96% to 48.19%, corresponding to 56.85 to 133.48 Kg/ha in terms of average cob-mass reduction. In general, the cob tunnel and the time of infestation were linearly correlated, while cob tunnel length and cob biomass were linked by a cubic nonlinear function. The observed yield losses at harvest of the maize crop suggest that control measures should be applied continuously, throughout the whole growing season.

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9.1 Introduction

Kenya is among the Sub-Saharan countries of Africa with a higher consumption rate of maize per person (Ranum et al. 2014). The cropping of this staple food in Kenya is significantly constrained by insect pests such as lepidopteran stem borers (De Groote 2002; Kfir et al. 2002). Reported maize yield losses are attributed to infestations by stem borers, which can damage more than half of the total production as a result of leaf feeding, stem tunnelling and grain damage (Kfir et al. 2002; Polaszek 1998).

The assessment of yield losses in maize caused by stem borers is usually done by collecting data such as the mass of plant stem, cob or grains (Herbert 2000; Walker 1981). The relevance of the knowledge gained from the data depends on the resources available for sampling and the accuracy needed for the purposes of assessment (Herbert 2000; Walker 1983b). After obtaining data on the different degrees of reduction in masses of maize grain, the yield losses can be assessed by comparing the features of un-attacked and attacked plants (Herbert 2000; Walker 1983b).

The stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is one of the most important stem borer species that attacks maize in Sub-Saharan Africa, and Kenya in particular (Harris and Nwanze 1992; Kfir et al. 2002; Ong'amo et al. 2006). The injuriousness of *B. fusca* has been assessed by various authors over the decades (Ingram 1958; Walker 1960; Harris 1962; Usua 1968; van Rensburg et al. 1988b; Macfarlane 1990; van den Berg et al. 1991; Cardwell et al. 1997; Ebenebe et al. 1999; Ndemah et al. 2001; Ndemah and Schulthess 2002; Chabi-Olaye et al. 2005). These studies emphasised that the major factors affecting yield losses are: the destruction of the growing part of the plant (dead heart), the number of larvae per plant, stem tunnelling, ear damage, and positions of the attacks on the stem.

Estimating the link between pest damages and yields losses at the field scale by empirical models can be useful for predicting and managing the pest (Bardner et al. 1974; Herbert 2000; Madden et al. 1995; Walker 1983a). Most of the relationships between the level of damage caused by *B. fusca* and yield are decreasing functions (Cardwell et al. 1997; Ndemah et al. 2001; Ndemah et al. 2003a; Walker 1960). In addition to regression analysis, the estimate of the overall percentage of yield losses can be useful to policymakers and farmers for gaining a better perception of the incidence of pest damages (Ajala and Saxena 1994; Ampofo 1988; Chabi-Olaye et al. 2005; Chabi-Olaye et al. 2006; Chabi-Olaye et al. 2008; Cugala et al. 2006; De Groote 2002; Walker 1983a, b; Zadoks 1985).

While yield-loss estimates comprise key information for use in managing stem borers, the estimates of yield losses in existing studies are ambiguous. On one hand, it seems to be overestimated, while on the other hand, the methods and techniques for estimations are either not clear or unknown (Calatayud et al. 2014; Harris and Nwanze 1992; Kfir et al. 2002; Walker 1987). Links between pest damages and yields have been established, but their extrapolation is difficult because the studies have been carried out using artificial infestations, on insecticide-protected fields, or the data collected focused on a few samples, randomly selected in the field (Ingram 1958; Walker 1960; Harris 1962; Usua 1968; van Rensburg et al. 1988b; Macfarlane 1990; van den Berg et al. 1991; Cardwell et al. 1997; Ebenebe et al. 1999; Ndemah et al. 2001; Ndemah and Schulthess 2002; Chabi-Olaye et al. 2005). Additionally, rigorous selections of plant physical traits used to study damage incidence and associations of ear mass to different damage types have not been made.

Although the perceptions of African farmers of the impact of lepidopteran stem borers are still high (Bonhof et al. 2001; Ebenebe et al. 2001; Oben et al. 2015), it is noticed that the values of yield losses due to *B. fusca* currently reported in literature have not been updated (Calatayud et al. 2014; Harris and Nwanze 1992; Kfir et al. 2002; Walker 1987).

The purposes of this study are: (i) to gain an understanding of the current trends in damage incidences attributable to lepidopteran stem borers on small-scale maize farms; (ii) to link these to yield losses, (iii) to describe the incidence of infestation dynamics on the yield, and (iv) to establish empirical relationships between damage types and yield losses.

9.2 Materials and Methods

9.2.1 Study Site and Data Collection Protocol

The trial was conducted at Murang'a located between Nyeri and Thika (00° 43′ 00″ S, 37° 09′ 00″ E, 1255 m above sea level) in Kenya. As a result of the varying altitudes, Murang'a can get quite cold from May to Mid-August and can experience hail.

Murang'a has a tropical climate classified as Equatorial savannah with dry winter (Aw) (Kottek et al. 2006). In contrast with winters, the summers have considerable rainfall. The average annual temperature and precipitation are 20.0 °C and 1195 mm, respectively. The driest month is September, with 19 mm of rainfall. Most precipitations here fall in April, averaging 310 mm. The warmest month of the year is November, with an average temperature of 22.5 °C. August is the coldest month, with temperatures averaging 18.1 °C. The difference in precipitation between the driest and the wettest month is 291 mm. Temperatures vary by 4.4 °C, throughout the year.

Table 9.1 Description of plots

Plots	Row length	Row spacing	Dimensions (length × width)[a]	GPS coordinates	
				Latitude	Longitude
1	21.00	0.60	21.00 × 17.17	−0.922244964	37.15198829
2	21.00	0.60	21.00 × 17.17	−0.922477751	37.15234651
3	18.60	0.60	23.40 × 20.70	−0.923219997	37.14361341
4	18.60	0.60	18.60 × 10.80	−0.919620737	37.13853050
Plots	Total number of rows	Total number of plants per row	Space between plants in row	Total number of quadrants during sampling at harvest	Total number of maize plants
1	36	60	0.30	4	2160
2	36	60	0.30	4	2160
3	40	70	0.30	4	2800
4	32	37	0.30	4	1184

[a]Dimensions given in m. The plots were located within a radius of 0.7 Km

A total of four sites were selected from the farmer's field. Then, each plot was divided into four quadrants of equal size. Land preparation was done one month before the onset of rains. Plant residues from the previous season were removed from the experimental plot. Oxen ploughing was carried out on the plots. A commercial maize variety, DUMA-4, commonly grown by Kenyan farmers in mid-altitude areas, was used throughout this study. Planting activities were done by manual labour, at a spacing of 30:70 cm.

The seeds provided to farmers were planted in four plots, as described in Table 9.1. The plots were submitted to identical management and exposed to natural infestation by *B. fusca*. No integrated pest management (IPM) control measure and no fertiliser were applied during the whole of plant growing period. Planting was conducted on 3rd March 2014. After germination, only two healthy plants were left to continue growing in each cluster hole until harvest, which occurred during July 2014. The data collection started on 16th April 2014, approximately three weeks after plant germination, in order to give time for the laid eggs to hatch and the larvae to start feeding on the young plants, and ended on 25th July 2014.

Data collection protocol was identical for all plots. In each plot, all plants were monitored weekly to detect infested maize individuals. The incidence of damage was determined by visual observation of all plants within the plots. Infected plants were tagged with coloured plastic materials having a unique set of numbers and letters which served as an identifier. The types of damages assessed were leaf damage, exit hole, and dead heart. Observations were done in one-week intervals, beginning on 16th April 2014. Plants were examined *in situ* without uprooting. Damage level, taken as the tunnel length bored into the stem and the ear by *B. fusca* individuals that successfully colonised the plants, was assessed at harvest. This was conducted by dissecting (opening by vertical splits) the ears and stems of infested plants.

During harvest, all infested plants were uprooted for proper inspection. An additional 25 non-infested plants were randomly harvested in each quadrant within the experimental plots.

Plants variables, such as stem length and diameters, and plant dry mass (leaves and stem without the cob) were recorded. Physical characteristics of unshelled maize cobs, with corn silk and husk removed were collected. Cob lengths, mass and diameter from both infested and non-infested plants were also recorded. The total number of plants observed in each experimental plot is given in Table 9.1.

9.2.2 Data Analyses

Data were subjected to a one-sample Kolmogorov-Smirnov (K-S) normality test. The mean values of physical features of stems and ears from both non-infested and infested plants were compared, using Welch's two-sample Student t-test for pairs of normally distributed data. Wilcoxon's two-sample test was used for pairs of non-normally distributed data. The Fligner-Killeen (F-K) test was used to assess variance homogeneity between plots (Conover et al. 1981; Fligner and Killeen 1976). All the statistical tests were considered as less significant for p-values ≥ 0.05.

To determine yield loss, only the cob masses were considered (Cardwell et al. 1997; Ebenebe et al. 1999; Ndemah et al. 2001, 2003b). Yield loss is frequently expressed as the fraction (percentage) of the attainable yield lost because of pest injuries (De Groote 2002; Walker 1983a; Zadoks 1985). It is then called relative yield loss (*RYL*), and is computed as: $RYL = 100 \times [(Y - Y_i)/Y]$ (Ajala and Saxena 1994; Ampofo 1988; Chabi-Olaye et al. 2005, 2006, 2008; Cugala et al. 2006; De Groote 2002; Walker 1983a, b; Zadoks 1985). Yield loss was expressed as a difference in mean cob masses between the un-infested (Y) and infested plants (Y_i). In addition, the total losses per hectare were estimated by summing the difference between the average value cob masses from non-infested plants and individual values of cob masses from infested cobs, after which the result was divided by the plot surface value and expressed in hectares.

The estimate of the parameters of the damage functions that link *B. fusca* tunnel length and corresponding cob masses was conducted through nonlinear least squares, using the Levenberg-Marquardt method (Marquardt, 1963). The goodness of fit and selection of the candidate nonlinear functions was operated with the Akaike Information Criteria (AIC) (Akaike 1974) and the R-squared. The linear link between the evaluation of yield losses and the mean cob tunnelling was calculated by using Person's correlation coefficient (PCC). All analyses were conducted with statistical software R (R Core Development Team 2013).

9.3 Results

Data collected at harvest show that plants infested by *B. fusca* outnumbered the proportion containing the crambid *Chilo partellus* (Swinhoe) and the noctuid *Sesamia calamistis* Hampson. The relative percentages of the tunnelled maize

Table 9.2 Number of larvae found in the plants during field sampling

	Busseola fusca inside tunnels				*Chilo partellus* inside tunnels				*Sesamia calamistis* inside tunnels			
	Bf/S	Bf Total in Stem	Bf/C	Bf Total in Cob	Cp/S	CP Total in Stem	Cp/C	Cp Total in Cob	Sc/S	Sc Total in Stem	Sc/C	Sc Total in Cob
Plot 1	1.25	45	1	7	1	1	0	0	1	5	1.66	5
Plot 2	1.15	37	1	1	0	0	0	0	1.5	3	1	3
Plot 3	1.38	63	1.33	8	0	0	0	0	1	1	0	0
Plot 4	1.07	29	1	2	0	0	0	0	0	0	0	0
	Total of plants tunnelled		Total of cob tunnelled		Total of plants tunnelled		Total of cob tunnelled		Total of plants tunnelled		Total of cob tunnelled	
Plot 1	40		7		1		0		5		3	
Plot 2	32		1		0		0		2		3	
Plot 3	47		6		0		0		1		0	
Plot 4	27		2		0		0		0		0	

Bf/S, Cp/S, and Sc/C = mean number of *B. fusca* (Bf), *C. partellus* (Cp) and *S. calamistis* (Sc) per maize stem (S). Bf/C, Cp/C, and Sc/C = the mean number of *B. fusca, C. partellus* and *S. calamistis* per maize cob (C)

plants were 92.57%, 0.57% and 6.87% for *B. fusca, C. partellus* and *S. calamistis*, respectively (Table 9.2).The majority of larvae were found in stem tunnels and less in cob tunnels (Table 9.2). The ratio (%) of the total number of larvae inside stem tunnels-cob tunnel was 90.63–9.37 for *B. fusca*, 52.94–47.06 for *S. calamistis.* Unique *C. partellus* larvae were found in stem tunnel. The average number of larvae per plant and cob did not exceed 2 (Table 9.2).

The comparison between average physical traits of infested and un-infested maize plants is depicted in Fig. 9.1. Only the cob masses between non-infested and infested plants were differed significantly across all plots. The variance homogeneity among cob masses of infested plants across all plots was less significant ($p\text{-val} < 0.05$) compared with all other factors.

The relative yield losses in term of average cob mass reductions were estimated for each plot as 40.79%, 43.14%, 48.19% and 35.96% for plots 1, 2, 3 and 4, respectively. Given the plant density across plots ranging from 58111 to 58940 plants/ha, with an average of one cob per plant, infestations from *B. fusca* inflicted losses ranging from 56.85 to 133.48 kg/ha. Total yield losses due to stem and cob tunnelling were 42.86% and 62.52%, respectively.

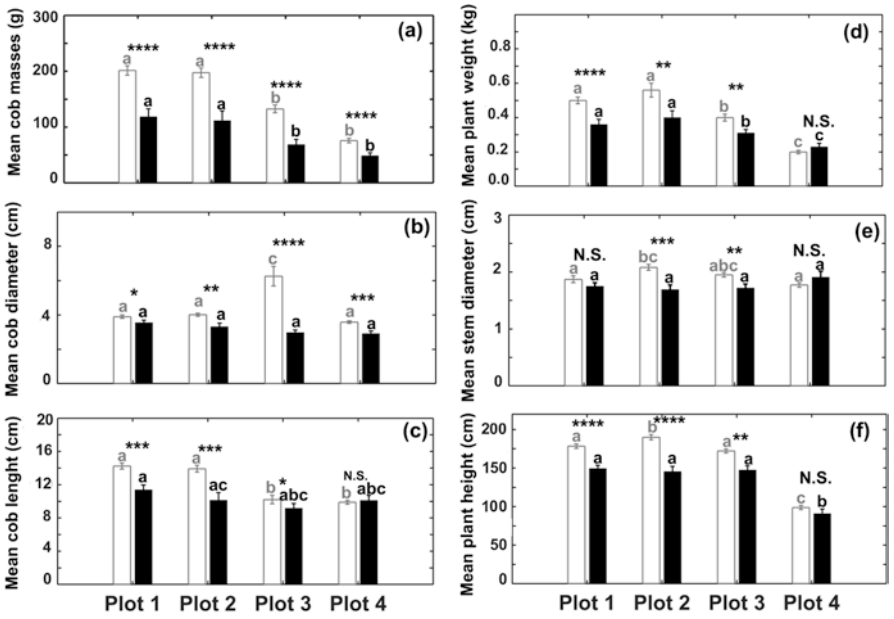

Fig. 9.1 The black and white bars represent infested and non-infested sets of plants; the star is the significance level between means.
The symbols are: N.S. (Not Significant) for $P > 0.05$, ∗for $P < 0.05$, ∗∗for $P < 0.01$, ∗∗∗for $P < 0.001$, ∗∗∗∗for $P < 0.0001$. Bars with the same color letter are not significantly different ($P > 0.05$)

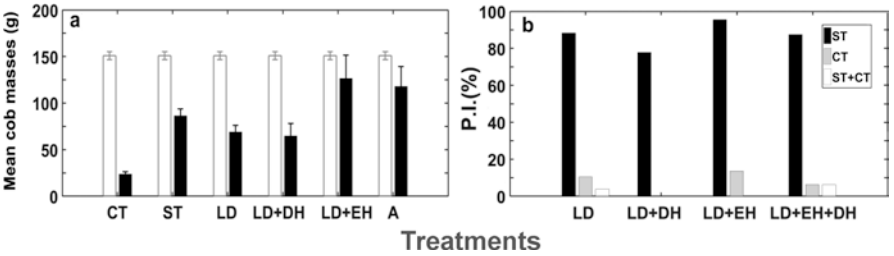

Fig. 9.2 Effect of the first infestation on the mean cob biomass reduction (**a**, A = LD+DH+EH. The black and white bars represent infested and non-infested sets of plants). Proportion (**b**) of plants infested (P.I.), plant with stem tunnelling (ST) and cob tunnelling (CT) according to the type of infestation such as leaf damage (LD), dead heart (DH), exit hole (EH)

Busseola fusca was able to bore, on average, 60.23% and 15.18% of the cob and plant stem lengths, respectively. An investigation of damage severity in cobs, according to the type of infestation such as leaf damages (LD), dead heart (DH), exit hole (EH), was conducted. It was observed that all infested plants have, on average, a lower cob mass compared with the non-infested ones (Fig. 9.2a). The cob tunnelling induced the lowest cob masses, directly followed in terms of incidence by

LD+DH (Fig. 9.2b). It can be noticed that a high proportion (>70%) of infested plants had tunnelled stems. A lower number of infected plants showed cob tunnelling, and it was also noticed that a reduced proportion (<20%) of plants were damaged by stem tunnelling and cob tunnelling, simultaneously. Additionally, 100%, 24.67%, 19.48% and 6.49% of infested plants with LD, LD+EH, LD+EH+DH, and LD+DH, respectively, had a cob at the time of harvest.

Yield-loss patterns attributable to different damages with time are depicted in Fig. 9.3. When the damages are taken separately, a particular trend of variation in yield, according to the week of the first infestation, was not observed. No yield losses for plants with LD at week 11 was noticed while for weeks 4, 7 and 10, no new damages were recorded (Fig. 9.3a). The time of infestation was not related to leaf damage incidence. The EH graph (Fig. 9.3b) did not show any particular trend. For the DH (Fig. 9.3c), the time factor appeared important, and when this damage occurred at the initial stages of maize development, the yield loss is high. However, when DH occurred later in the plant life cycle (after the 7th week of planting), yield losses were lower.

In general, the maize variety selected showed that, after 5 weeks, almost half of the plants in each plot had managed to generate cobs. When the analysis was conducted by pooling data without distinguishing the type of infestations, the losses decreased with the time period of infestation (Fig. 9.3d). The linear regression between the yield losses (y) and the time (t) of infestation in week ($y = at + b$) had slope a = −4.28 ± 0.02 and intercept b = 60.48 ± 0.17 (P-val > 0.05, $R^2 = 0.31$), with Pearson correlation coefficient $R = -0.56$. This suggests that the linear function was not representative for that data set. However, the linear regression between the average cob tunnel (y) and the time of infestation (t) in week ($y = at + b$) had slope a = 0.50 ± 0.13 with intercept b = 3.14 ± 0.11 (P-val < 0.05, $R^2 = 0.78$) and Pearson

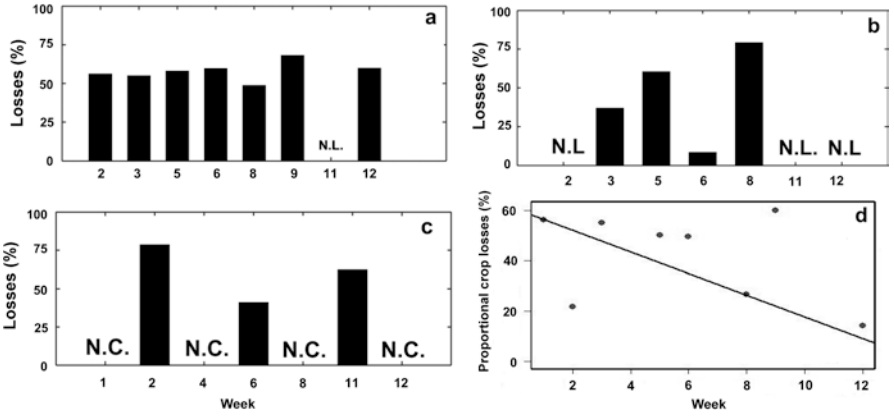

Fig. 9.3 Temporal patterns of yield losses for plant with leaf damage (**a**), exit hole (**b**), and dead heart (**c**), N.L. and N.C. = no losses and no cob, respectively. Linear regression (**d**) between the week of infestation and the yield losses

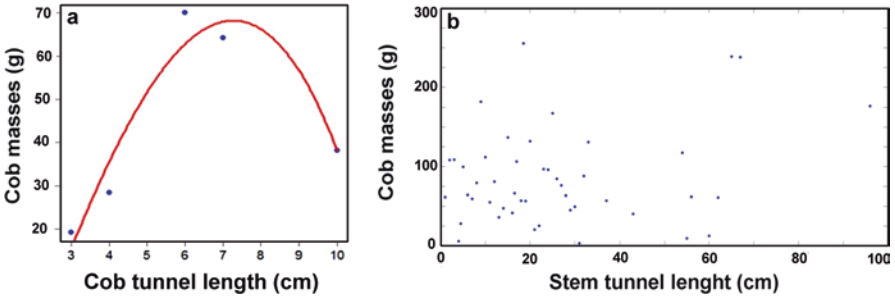

Fig. 9.4 Relationship (**a**) between length of cob tunnel and cob mass. The the red curve is the estimated function. Cob mass (**b**) as a function of cob tunnel

correlation coefficient $R = 0.88$, showing a linear tendency for mean cob tunnelling increases with time.

Analysis of the link between the cob masses and severity of damages on the stem and cob is shown in Fig. 9.4. After several trials to select a bell-shaped nonlinear function using the AIC, it was observed that the link between cob mass (y) and the cob tunnel length (x) follows a trend represented by a cubic function (Fig. 9.4a). The analytical expression is: $y = a + bx + cx^3$ and the coefficients are: $a = -54.46 \pm 19.70$, (P-val > 0.05), $b = 26.13 \pm 4.99$ (P-val < 0.05) and $c = -0.16 \pm 0.03$ (P-val < 0.05); $R^2 = 0.92$. We were not able to find any function linking the cob mass and the tunnel length (Fig. 9.4b). This result implies that, while the average value of cob tunnel length increases linearly with time of infestation, the cob masses of infested plants follow a pattern depicted by a cubic functional curve.

9.4 Discussion

9.4.1 Overview

In regard to the yield losses and damages attributable to *B. fusca* on maize farms, all recorded studies have been based on different methodological approaches, yielding a high variability in results (Ingram 1958; Walker 1960; Harris 1962; Usua 1968; van Rensburg et al. 1988b; Macfarlane, 1990; van den Berg et al. 1991; Cardwell et al. 1997; Ebenebe et al. 1999; Ndemah et al. 2001; Ndemah and Schulthess 2002; Chabi-Olaye et al. 2005). For comparison purposes, the approach, as well as a synthesis of the methodologies used in literature, applied to study yields losses attributable to *B. fusca* on maize is provided in Table 9.3. Lack of standardisation of experimental methods makes any comparison of results difficult or subjective. This causes a serious problem in the selection of the information concerning the damaging ability of *B. fusca* that may be given to farmers and decision makers. In this work, a simple, realistic and accurate approach has been implemented to study this problem.

Table 9.3 A summary of methods used in literature to study damage and yield losses due to *Busseola fusca* attacking maize

Country	Use of insecticide∗	Use of fertilizer	Artificial infestation	Random harvest of infested plants	References
Kenya	✗	✗	✗	✗	This study
South-Africa	✓	✓(c), ✗(a, b),	✓(a, c), ✗(b)	✓	a: van Rensburg et al. (1988a). b: van Rensburg et al. (1988b). c: van Rensburg et al. (1988c).
Nigeria	✗	✓	✓	✗	Usua (1968)
Cameroon	✓	✓(a), ✗(b, c)	✗	✓	a: Ndemah and Schulthess (2002). b: Chabi-Olaye et al. (2005). c: Chabi-Olaye et al. (2008).
Lesotho	✓	✗	✗	✓	Ebenebe et al. (1999).

Damages and yield losses

Country	Damages considered				Plant parts considered to measure yield losses		Link between damages level and yield losses		Yield losses estimates		References	
	LD	EH	DH	ST	CT	Cob mass	Seed mass	L.	N.L.	N.E.		
Kenya	✓	✓	✓	✓	✓	✓				✓	35.96–48.19%	This study
South-Africa	✓	✓	✓			✓(b)	✓(a, c)			✓	N.E.	a: van Rensburg et al. (1988a). b: van Rensburg et al. (1988b). c: van Rensburg et al. (1988c)
Nigeria	✓	✓	✓			✓	✓			✓	N.E.	Usua, (1968)
Cameroon				✓			✓	✓ (N.S)			0.4–41.00%	Chabi-Olaye et al. (2005); Chabi-Olaye et al. (2008); Ndemah and Schulthess (2002)
Lesotho						✓	✓			✓	0.4–36.60%	Ebenebe et al. (1999)

∗Symbols ✓and ✗ refer to 'yes' and 'no', respectively. The abbreviated names of damages are: leaf damage (LD), exit hole (EH), dead heart (DH), plant stem tunnelling (ST) and cob tunnelling (CT). The relationship between the yield losses and the damages are grouped into two categories such as linear (L.), non-linear (N.L.); not estimated (N.E.) means that authors did not estimate a link between damages and yield losses or that the overall yield losses are not estimated. N.S. = not significant. Only studies where a clear establishment of a link between damages intensity and yield losses are reported in this table

9.4.2 *Methodology of Sampling*

In our work, all plants in the field were considered, in contrast to other studies available in the literature (Cardwell et al. 1997; Chabi-Olaye et al. 2005; Ebenebe et al. 1999; Harris 1962; Ingram, 1958; Macfarlane 1990; Ndemah et al. 2001; Ndemah and Schulthess 2002; Usua 1968; van den Berg et al. 1991; van Rensburg et al. 1988; Walker 1960). Focusing on only a few samples can originate a considerable bias, which reduces the accuracy of results because the selected plants not always provide a valid representation of the whole farm. The majority of methods used in the literature for studying yield losses attributable to lepidopteran stem borers comprise visual-damage rating, by which grain or cob masses are assessed (Ingram 1958; Walker 1960; Harris 1962; Usua 1968; van Rensburg et al. 1988b; Macfarlane 1990; van den Berg et al. 1991; Cardwell et al. 1997; Ebenebe et al. 1999; Ndemah et al. 2001; Ndemah and Schulthess 2002; Chabi-Olaye et al. 2005). In contrast, we appled weekly visual ratings and measurements of cob masses at harvest.

9.4.3 *Plant Physical Trait to Study* B. fusca *Injury*

Although several studies about the link between *B. fusca* damaging factors and yields losses have been conducted over decades (Cardwell et al. 1997; Chabi-Olaye et al. 2005; Ebenebe et al. 1999; Harris, 1962; Ingram, 1958; Macfarlane 1990; Ndemah et al. 2001; Ndemah and Schulthess 2002; Usua 1968; van den Berg et al. 1991; van Rensburg et al. 1988; Walker 1960), it is noticed that a rigorous selection of the plant physical features suitable for studying incidence of the pest has not been done. Comparing features of infested and uninfested plants has been suggested as a systematic method for assessing yield losses in cereals (Walker 1983b). However, studies have considered cob mass (infested versus non-infested) directly as an indicator to assess the pest impact, but without much justification (Cardwell et al. 1997; Ebenebe et al. 1999; Ndemah et al. 2001, 2003b). In this study, a statistical comparison of the plants' physical features has been conducted in order to select cob masses as the key factor.

9.4.4 *Revisiting Yield Losses in Maize Due to* B. fusca

The study of the incidence of pest damage provides crucial data for decision makers that would enable them to allocate meaningful resources for research and management (Reddy and Walker 1990; Savary et al. 2006; Walker 1983a; Zadoks 1985). Therefore, making decisions about controlling lepidopteran stem borers should rely on an accurate estimate of damage incidences and yield losses. However, the values of the overall yield losses in maize attributable to *B. fusca* reported in the literature vary greatly from one country to another, and between different agro-ecological

zones (Kfir et al. 2002). Losses reported ranged from 10% to 100% in South Africa (Kfir et al. 2002), 0.4% to 36% in Lesotho (Ebenebe et al. 1999), 0.4% to 41% in Cameroon (Cardwell et al. 1997; Chabi-Olaye et al. 2005; Chabi-Olaye et al. 2008; Ndemah and Schulthess 2002), and 17% in Zimbabwe (Walker 1987). In Kenya and Tanzania, 12% in yield reductions for every 10% of plants attacked have been reported (Walker 1960) and, later on, yield losses of 14% were reported by Walker (1987). All the methods for estimating the yields losses were carried out either by comparing grain or cob masses in a few infested plants, in the field (Cardwell et al. 1997), or by comparing grain masses in a few samples from fields that were protected and unprotected by insecticide (Walker, 1960, 1987; Ebenebe et al. 1999; Ndemah and Schulthess 2002; Chabi-Olaye et al. 2005; Chabi-Olaye et al. 2008). The present research opted to sample all the damaged plants on naturally infested farms. Average yield-loss estimates in this study range between 35.96% and 48.19%.

9.4.5 Injuriousness of Busseola fusca

Several studies have been conducted to identify the types of damage that imply higher yield losses due to *B. fusca* (Cardwell et al. 1997; Ebenebe et al. 1999; Ingram 1958; Ndemah et al. 2001; Ndemah et al. 2003b; van Rensburg et al. 1988). Because of the various experimental approaches adopted in these different studies, it was difficult to specify the types of damages (LD, DH, EH, ST or internode attacks) that have the most significant effect in the reduction of maize yields. Concerning tunnelling, *B. fusca* was reported to tunnel from 15% to 30% of the length of the stem (Cugala et al. 2006), a range almost similar to our results. In this study, although the stem tunnelling considerably reduced the cob masses, the results obtained suggest that cob tunnelling by *B. fusca* has a greater effect on yield.

Busseola fusca is reported to prefer attacking young plants (Calatayud et al. 2014; Harris and Nwanze 1992; Kfir et al. 2002), and this is a primary reason leading IPM practitioners to apply control measures at the early stage of development of maize crops (Kfir et al. 2002). However, the results obtained in this study suggested that all the plants with leaf damages (primary infestation damage from *B. fusca*) had a cob at the end, in contrast to those having secondary and ternary damages like dead hearts and exit holes, respectively. Therefore, control measures should be applied continuously during the maize life cycle.

9.4.6 Temporal Pattern of Infestation

Much information on the temporal patterns of stem borer infestations has been reported in the literature (Ebenebe et al. 1999; Kfir et al. 2002; Ndjomatchoua et al. 2016; Reddy et al. 1991; van Rensburg 2001; van Rensburg and van den Berg 1992). It is demonstrated that *C. partellus* infestation on younger plants causes a higher

incidence of yield losses than infestation on older plants does. Additionally, it is reported that the intensity of leaf damage is significant in younger plants and that stem tunnelling is significantly correlated to yield losses in older plants (Reddy et al. 1991). In the conditions of this study, we did not observe any particular trend in yield losses according to the leaf damage and exit holes made by *B. fusca*. For the dead heart, the time of infestation appears important: when it happens within a two-week interval from planting, yield losses are high. Furthermore, the decrease in yield losses, with the time of infestation observed in this study, is similar to what has been reported in the literature (van Rensburg et al. 1988).

9.4.7 Empirical Link Between Infestation and Yield

Numerous studies have attempted to link the stem tunnelling damages and the cob masses (Cardwell et al. 1997; Ebenebe et al. 1999; Ndemah et al. 2001; Ndemah et al. 2003b). A linear link was presented with little significance in one study (Ndemah et al. 2001). We also failed to establish a link between these two variables in this study. This implies that others factors should be considered while linking yield losses and stem tunnelling, or that no link might exist between these two variables. We went beyond existing studies by estimating the type of link between the cob tunnelling and the cob masses. In general, the yield loss due to a pest on a crop is reported to have a linear or a non-linear function, decreasing with pest-damage intensity (Bardner et al. 1974; Herbert 2000; Madden et al. 1995; Walker 1983a). This trend was observed in maize in the case of *B. fusca* (Cardwell et al. 1997; Chabi-Olaye et al. 2005; Ndemah et al. 2001, 2003a; Walker 1960) and *C. partellus* (Mgoo et al. 2006; Reddy and Walker 1990; Reddy et al. 1991). In this study, a different tendency was observed while analysing cob mass function of the length of the tunnel in a cob, which is a cubic function.

9.5 Conclusions

The important role of cob damage caused by *B. fusca* infestation is emphasised. The results confirm that both early and late infestations can be important causes of maize yield losses during the plant life cycle. There is a real need for a standardisation of experimental approaches to be formulated for investigating damages and yield losses attributable to *B. fusca* in order to ensure the replication of studies and facilitate comparisons among results from different authors. The important yield losses recorded in this study on small-scale farms in Central Kenya indicate that *B. fusca* remains a major pest of maize, in spite of the many methods deployed for its control over decades (Bruce et al. 2009; Kfir et al. 2002). Our results suggest that the current control methods, such as male disruption (Critchley et al. 1997), cultural control (Van den Berg et al. 1998), and habitat management (Kfir et al. 2002) should be

improved and intensified. Moreover, innovative new control methods, such as dissemination of entomopathogenic fungi (Maniania et al. 2011) and more specific natural enemies (Branca et al. 2011), should be deployed.

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References

Ajala, S. O., & Saxena, K. N. (1994). Interrelationship among *Chilo partellus* damage parameters and their contribution to grain yield reduction in maize (*Zea mays* L.). *Applied Entomology and Zoology, 29*, 469–476. https://doi.org/10.1303/aez.29.469.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control, 19*, 716–723. https://doi.org/10.1109/TAC.1974.1100705.

Ampofo, J. K. O. (1988). Assessment of on-farm losses in maize production due to insect pests. *International Journal of Tropical Insect Science, 9*, 687–690. https://doi.org/10.1017/S1742758400005543.

Bardner, R., Flecher, K. E., & Fletcher, K. E. (1974). Insect infestations and their effects on the growth and yield of field crops: A review. *Bulletin of Entomological Research, 64*, 141–160. https://doi.org/10.1017/S0007485300027061.

Bonhof, M. J., Van Huis, A., Kiros, F. G., & Dibogo, N. (2001). Farmers' perceptions of importance, control methods and natural enemies of maize stemborers at the Kenya Coast. *International Journal of Tropical Insect Science, 21*, 33–42. https://doi.org/10.1017/S1742758400020026.

Branca, A., Le Ru, B. P., Vavre, F., Silvain, J. F., & Dupas, S. (2011). Intraspecific specialization of the generalist parasitoid *Cotesia sesamiae* revealed by polyDNAvirus polymorphism and associated with different Wolbachia infection. *Molecular Ecology, 20*, 959–971. https://doi.org/10.1111/j.1365-294X.2010.04977.x.

Bruce, Y. A., Schulthess, F., Mueke, J., & Gohole, L. (2009). Egg parasitoids of noctuid stemborers on maize in Kenya. *International Journal of Biodiversity Science, Ecosystem Services & Management, 5*, 174–180. https://doi.org/10.1080/17451590903244810.

Calatayud, P. A., Le Ru, B., van den Berg, J., et al. (2014). Ecology of the African Maize Stalk Borer, Busseola fusca (Lepidoptera: Noctuidae) with special reference to insect-plant interactions. *Insects, 5*, 539–563. https://doi.org/10.3390/insects5030539.

Cardwell, K. F., Schulthess, F., Ndemah, R., & Ngoko, Z. (1997). A systems approach to assess crop health and maize yield losses due to pests and diseases in Cameroon. *Agriculture, Ecosystems and Environment, 65*, 33–47. https://doi.org/10.1016/S0167-8809(97)00056-X.

Chabi-Olaye, A., Nolte, C., Schulthess, F., & Borgemeister, C. (2005). Relationships of intercropped maize, stem borer damage to maize yield and land-use efficiency in the humid forest of Cameroon. *Bulletin of Entomological Research*. https://doi.org/10.1079/BER2005373.

Chabi-Olaye, A., Nolte, C., Schulthess, F., & Borgemeister, C. (2006). Relationships of soil fertility and stem borers damage to yield in maize-based cropping system in Cameroon. *Annales de la Société Entomologique de France, 42*, 471–479. https://doi.org/10.1080/00379271.2006.10697481.

Chabi-Olaye, A., Schulthess, F., & Borgemeister, C. (2008). Effects of nitrogen and potassium combinations on yields and infestations of maize by *Busseola fusca* (Lepidoptera: Noctuidae) in the humid forest of Cameroon. *Journal of Economic Entomology, 101*, 90–98. https://doi.org/10.1093/jee/101.1.90.

Conover, W. J., Johnson, M. E., & Johnson, M. M. (1981). A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. *Technometrics, 23*, 351–361. https://doi.org/10.1080/00401706.1981.10487680.

Critchley, B. R., Hall, D. R., Farman, D. I., et al. (1997). Monitoring and mating disruption of the maize stalkborer, Busseola fusca, in Kenya with pheromones. *Crop Protection, 16*, 541–548. https://doi.org/10.1016/S0261-2194(97)00026-4.

Cugala, D., Schulthess, F., Ogol, C. P. O., & Omwega, C. O. (2006). Assessment of the impact of natural enemies on stemborer infestations and yield loss in maize using selected insecticides in Mozambique. *Annales de la Société Entomologique de France, 42*, 503–510. https://doi.org/10.1080/00379271.2006.10697485.

De Groote, H. (2002). Maize yield loss from stem borers in Kenya. *International Journal of Tropical Insect Science, 22*, 89–96. https://doi.org/10.1017/S1742758400015162.

Ebenebe, A. A., Van den Berg, J., & Van der Linde, T. C. (1999). Effect of planting date of maize on damage and yield loss caused by the stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in Lesotho. *South African Journal of Plant and Soil, 16*, 180–185. https://doi.org/10.1080/02571862.1999.10635007.

Ebenebe, A. A., Van Den Berg, J., & Van der Linde, T. C. (2001). Farm management practices and farmers' perceptions of stalk-borers of maize and sorghum in Lesotho. *International Journal of Pest Management, 47*, 41–48. https://doi.org/10.1080/09670870150215586.

Fligner, M. A., & Killeen, T. J. (1976). Distribution-free two-sample tests for scale. *Journal of the American Statistical Association, 71*, 210–213. https://doi.org/10.1080/01621459.1976.10481517.

Harris, K. M. (1962). Lepidopterous stem borers of cereals in Nigeria. *Bulletin of Entomological Research*, 169–171. https://doi.org/10.1017/S0007485300048021.

Harris, K. M. M., & Nwanze, K. E. E. (1992). *Busseola fusca (Fuller), the African maize stalk borer: A handbook of information*. Patanchem: International Crops Research Institute for Semi-arid Tropics.

Herbert, D. A. (2000). *Biotic stress and yield loss*. Boca Raton: CRC Press.

Ingram, W. R. (1958). The Lepidopterous Stalk Borers associated with Gramineae in Uganda. *Bulletin of Entomological Research, 49*, 367–383. https://doi.org/10.1017/S0007485300053682.

Kfir, R., Overholt, W. A., Khan, Z. R., & Polaszek, A. (2002). Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology, 47*, 701–731. https://doi.org/10.1146/annurev.ento.47.091201.145254.

Kottek, M., Grieser, J., Beck, C., et al. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorol Zeitschrift, 15*, 259–263. https://doi.org/10.1127/0941-2948/2006/0130.

Macfarlane, J. H. (1990). Damage assessment and yield losses in sorghum due to the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in Northern Nigeria. *Tropical Pest Management, 36*, 131–137. https://doi.org/10.1080/09670879009371456.

Madden, L. V., Nutter, J. F. W., & Nutter, F. W. (1995). Modeling crop losses at the field scale. *Canadian Journal of Plant Pathology, 17*, 124–137. https://doi.org/10.1080/07060669509500703.

Maniania, N. K., Ouna, E., Ahuya, P., et al. (2011). Dissemination of entomopathogenic fungi using *Busseola fusca* male as vector. *Biological Control, 58*, 374–378. https://doi.org/10.1016/j.biocontrol.2011.05.012.

Marquardt, D. W. (1963). An algorithm for least-squares estimation of nonlinear parameters. *Journal of the Society for Industrial and Applied Mathematics, 11*, 431–441. https://doi.org/10.1137/0111030.

Mgoo, V. H., Makundi, R. H., Pallangyo, B., et al. (2006). Yield loss due to the stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) at different nitrogen application rates to maize. *Annales de la Société Entomologique de France, 42*, 487–494. https://doi.org/10.1080/00379271.2006.10697483.

Ndemah, R., & Schulthess, F. (2002). Yield of maize in relation to natural field infestations and damage by lepidopteran borers in the forest and forest/savanna transition zones of Cameroon. *Insect Science and Its Application, 22*, 183–192.

Ndemah, R., Schulthess, F., Korie, S., et al. (2001). Distribution, relative importance and effect of lepidopterous borers on maize yields in the forest zone and mid-altitude of Cameroon. *Journal of Economic Entomology, 94*, 1434–1444. https://doi.org/10.1603/0022-0493-94.6.1434.

Ndemah, R., Schulthess, F., Korie, S., et al. (2003a). Factors affecting infestations of the stalk borer *Busseola fusca* (Lepitoptera: Noctuidae) on maize in the forest zone of Cameroon with Special reference to scelionid egg parasitoids. *Environmental Entomology, 32*, 51–60. https://doi.org/10.1603/0046-225X-32.1.51.

Ndemah, R., Schulthess, F., Korie, S., et al. (2003b). (Lepitoptera: Noctuidae) on maize in the forest zone of Cameroon with special reference to scelionid egg parasitoids. *Environmental Entomology, 32*, 51–60. https://doi.org/10.1603/0046-225X-32.1.51.

Ndjomatchoua, F. T., Tonnang, H. E. Z., Plantamp, C., et al. (2016). Spatial and temporal spread of maize stem borer Busseola fusca (fuller) (Lepidoptera: Noctuidae) damage in smallholder farms. *Agriculture, Ecosystems and Environment, 235*, 105–118. https://doi.org/10.1016/j.agee.2016.10.01.

Oben, E. O., Ntonifor, N. N., Kekeunou, S., & Abbeytakor, M. N. (2015). Farmers knowledge and perception on maize stem borers and their indigenous control methods in south western region of Cameroon. *Journal of Ethnobiology and Ethnomedicine, 11*, 77. https://doi.org/10.1186/s13002-015-0061-z.

Ong'amo, G. O., Le Rü, B. P., Dupas, S., et al. (2006). Distribution, pest status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. *Annales de la Société Entomologique de France, 42*, 171–177. https://doi.org/10.1080/00379271.2006.10700620.

Polaszek, A. (Ed.). (1998). *African cereal stem borers economic importance, taxonomy, natural enemies and control*. Wallingford: CTA/CABI.

R Core Development Team. (2013). R: A Language and Environment for Statistical Computing.

Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences, 1312*, 105–112. https://doi.org/10.1111/nyas.12396.

Reddy, K. S., & Walker, P. T. (1990). A review of the yield losses in graminanous crops caused by Chilo SPP. *Insect Sci Appl, 11*, 563–569. https://doi.org/10.1017/S1742758400021123.

Reddy, K. V. S., Sum, K. O. O. S., Seshu, R. K. V., & Sum, K. O. O. S. (1991). Determination of economic injury level of the stem borer, Chilo partellus (Swinhoe) in maize, Zea Mays L. *International Journal of Tropical Insect Science, 12*, 269–274. https://doi.org/10.1017/S1742758400020786.

Savary, S., Teng, P. S., Willocquet, L., & Nutter, F. W. (2006). Quantification and modeling of crop losses: A review of purposes. *Annual Review of Phytopathology, 44*, 89–112. https://doi.org/10.1146/annurev.phyto.44.070505.143342.

Usua, E. J. (1968). Effect of varying populations of Busseola fusca larvae on the growth and yield of maize. *Journal of Economic Entomology, 61*, 375–376. https://doi.org/10.1093/jee/61.2.375.

van den Berg, J., van Rensburg, J. B. J., & Giliomee, J. H. (1991). The effect of plant density on the injuriousness of Busseola fusca (Fuller) (Lepidoptera: Noctuidae) in grain sorghum. *South African Journal of Plant and Soil, 8*, 85–87. https://doi.org/10.1080/02571862.1991.10634585.

Van den Berg, J., Nur, A. F., & Polaszek, A. (1998). Cultural control. In A. Polaszek (Ed.), *African cereal stem borers: Economic importance, taxonomy, natural enemies and control* (pp. 333–347). Wallingford: CABI.

van Rensburg, J. B. J. (2001). Larval mortality and injury patterns of the African stalk borer, Busseola fusca (Fuller) on various plant parts of Bt -transgenic maize. *South African Journal of Plant and Soil, 18*, 62–68. https://doi.org/10.1080/02571862.2001.10634405.

van Rensburg, J. B. J., & van den Berg, J. (1992). Stem borers in grain sorghum: I. Injury patterns with time after crop emergence. *South African Journal of Plant and Soil, 9*, 73–80. https://doi.org/10.1080/02571862.1992.10634607.

van Rensburg, J. B., Walters, M. C., Giliomee, J. H., et al. (1988a). Plant population and cultivar effects on yield losses caused by the maize stalk borer, Busseola fusca (Lepidoptera: Noctuidae). *South African Journal of Plant and Soil, 5*, 215–218. https://doi.org/10.1080/02571862.1988.10634989.

van Rensburg, J. B. J., Giliomee, J. H., & Walters, M. C. (1988b). Aspects of the injuriousness of the maize stalk borer, Busseola fusca (Fuller) (Lepidodtera: Noctuidae). *Bulletin of Entomological Research, 78*, 101–110. https://doi.org/10.1017/S0007485300016102.

van Rensburg, J. B. J., Walters, M. C., & Giliomee, J. H. (1988c). Response of maize to levels and times of infestation by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Journal of the Entomological Society of South Africa, 51*, 283.

Walker, P. T. (1960). The relation between infestation by the stalk borer, Busseola fusca, and yield of maize in East Africa. *The Annals of Applied Biology, 48*, 780–786. https://doi.org/10.1111/j.1744-7348.1960.tb03579.x.

Walker, P. T. (1981). The relation between infestation by Lepidopterous stem borers and yield in maize: Methods and results 1. *EPPO Bulletin, 11*, 101–106. https://doi.org/10.1111/j.1365-2338.1981.tb01774.x.

Walker, P. T. (1983a). The assessment of crop losses in cereals. *International Journal of Tropical Insect Science, 4*, 99–104. https://doi.org/10.1017/S1742758400004082.

Walker, P. T. (1983b). Crop losses: The need to quantify the effects of pests, diseases and weeds on agricultural production. *Agriculture, Ecosystems and Environment, 9*, 119–158. https://doi.org/10.1016/0167-8809(83)90038-5.

Walker, P. T. (1987). Losses in yield due to pests in tropical crops and their value in policy decision-making. *International Journal of Tropical Insect Science, 8*, 665–671. https://doi.org/10.1017/S1742758400022761.

Zadoks, J. C. (1985). On the conceptual basis of crop loss assessment: The threshold theory. *Annual Review of Phytopathology, 23*, 455–473. https://doi.org/10.1146/annurev.py.23.090185.002323.

Chapter 10
Establishment of an Exotic Parasitoid *Cotesia vestalis* in Coastal Areas of Kenya as Biological Control Agent of *Plutella xylostella*

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Abstract The aims of this study were to follow up on the establishment of *Cotesia vestalis* in the coastal regions of Kenya, assessing its contribution in the management of the diamondback moth (*Plutella xylostella*), parasitism rates, and cultural practices affecting its establishment. Post-release surveys were carried out in five counties in Kenya, Kitui, Kajiado, Kwale, Machakos and Makueni, between 2015 and 2016. The results showed that the overall parasitism rate of *C. vestalis* in 2015 ranged between 0% and 37.86% while that in 2016 ranged from 0% to 32.19% in the different counties. Farmers carry out routine sprays, either weekly or fortnightly, with different synthetic insecticides. Pyrethroids (60.56%) constituted most of the insecticides used, while only 3.18% of the products used were plant or microorganism-based. Farmers did most of their cabbage production during the rainy season, with

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production being greatly reduced during the dry seasons. The sampled diamondback moths from Kajiado, Kitui, Makueni, Kwale and Malawi had ≥98% similarity to *Cotesia vestalis*, as shown by bioinformatics analyses using PCR amplified products of 700 bp, obtained for the mitochondrial COI gene. Alignment showed highly conserved regions, and the phylogenetic analysis revealed two close lineages corresponding to *Cotesia vestalis* (Genbank accession: FJ154897) and *Cotesia* spp. (acc. HM430398). Data provide a clear indication that the parasitoid became established in its release sites in Kenya, although parasitism rates are still low probably due to heavy pesticide use and climatic factors.

Keywords *Plutella xylostella* · *Cotesia vestalis* · Biological control · DNA barcoding · PCR

10.1 Introduction

Cruciferous vegetables, such as kales and cabbages, are widely grown in Africa for subsistence purposes, as well as for income generation (Ayalew et al. 2002; Lohr and Kfir 2004; Macharia et al. 2005; Grzywacz et al. 2010). The annual global production is estimated at 29 tonnes/ha, while productions in Malawi and Kenya reach approximately 20.7 and 30.9 tonnes/ha, respectively (FAOSTAT 2014).

Crucifer production, worldwide, is often hindered by insect pests which include the diamondback moth (*Plutella xylostella* L.), cabbage aphids (*Brevicoryne brassicae* L.), leaf miners (*Liriomyza brassicae* L.), thrips (*Thrips tabaci* L.), and cabbageworms (*Pieris rapae* L.) (Hines and Hutchison 2001; Bjorksten et al. 2005; Munthali 2009). Among these insect pests, *P. xylostella* is the most destructive one in Eastern Africa (Nyambo and Pekke 1995; Badenes-Perez and Shelton 2006). In instances where no management strategies are put in place, the small larvae of *P. xylostella* can cause losses between 90% and 98% (Sandur 2004; Macharia et al. 2005). Worldwide losses of crucifer vegetables by *P. xylostella* are estimated at US$ 1.4 billion (Zalucki et al. 2012), incurring US$ 4–5 billion in management costs (Furlong et al. 2013). In Kenya, annual losses of US$ 7.9 million, due to *P. xylostella* infestations, have been reported (Macharia et al. 2005).

Management of *P. xylostella* in Kenya has mainly focused on the use of pesticides, with reported cases of overuse resulting in increases in production costs, health hazards, development of resistance, and destruction of natural enemies (Badenes-Perez and Shelton 2006; Cooper 2009; Macharia et al. 2013). Biological control has thus been promoted as an alternative (Rowell et al. 2005; Kahuthia-Gathu 2012). The parasitoids of *P. xylostella* mainly found in the East Africa region are *Diadegma mollipla* Holmgren and *Oomyzus sokolowskii* Kurdjumov. However, their parasitism rates have been reported to be below 15% in Kenya, Tanzania,

Malawi and Ethiopia (Lohr and Kfir 2004). One of the most effective parasitoids used in the management of *P. xylostella* is *Cotesia vestalis* Haliday, with reports of 78–88% parasitism rates (Smith and Villet 2001; Rowell et al. 2005). Among all the *C. vestalis* biotypes, the South African biotype is the most effective, owing to its predominance in both low and high altitudes (Talekar and Shelton 1993; Kfir 1997; Verkerk and Wright 1997; Mosiane et al. 2003), high thermal tolerance (Talekar and Yang 1991), and high parasitism rates (Waladde et al. 2001; Smith 2002).

Once introduced into Uganda, *C. vestalis* became established (ICIPE, unpublished data). It has been detected on the Kenyan side of Lake Victoria. Additional releases of *C. vestalis* were carried out in Kajiado, Machakos, Kitui and Makueni counties (Nyambo et al. 2008). However, repeated releases in the eastern region of Kenya resulted in very low parasitism rates (0.5–26.9%) (Nyambo et al. 2008; Kahuthia-Gathu 2012). Although the establishment of *C. vestalis* at the release sites was confirmed by other studies, most of them relied on morphological identification. A recent release of *C. vestalis* in Kwale County in 2013 resulted in a very low parasitism rate (unpublished data). This prompted the need to investigate the cultural practices that preclude the successful establishment of the parasitoid in Kenya, particularly in the coastal area. This study also aimed at confirming the molecular identity of the specimens that prevail in those counties and comparing them with those of Southern Africa biotypes, to ascertain their virulence and parasitism rates, in the various regions.

10.2 Materials and Methods

10.2.1 Study Sites

Surveys for the *P. xylostella* and its parasitoid, *C. vestalis*, were carried out in five counties: Kwale (Matuga, Diani and Lungalunga), Kajiado, Kitui, Makueni and Machakos (Fig. 10.1). Kajiado, Kitui, Makueni and Machakos counties represented *C. vestalis* post-release sites in the arid regions at mid-altitude (882–1918 m asl) of Kenya, while Kwale County represented the humid lowlands (3–416 m asl).

The surveys for *P. xylostella* and *C. vestalis* in Kwale County were conducted in the Lungalunga, Matuga and Diani regions. Kwale County experiences a bi-modal rainfall distribution, with long rains expected from March to June, and short rains from October to December. The annual rainfall range is 400–1680 mm per year. The soils are generally sandy loam, while some parts are richer in claye and fertile. On the other hand, Kitui, Kajiado, Makueni and Machakos counties are semi-arid areas, which are generally hot and dry. The rainfall distribution is bimodal: long rains are expected between March and May, while short rains are usually expected between October and December. The annual average rainfall ranges between 500 and

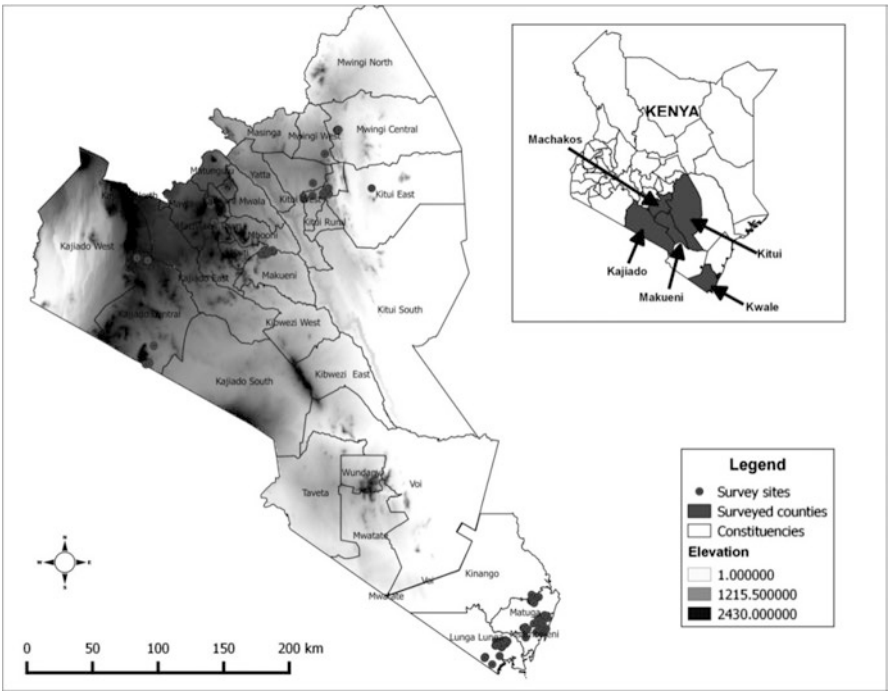

Fig. 10.1 Locations of the survey sites in selected counties of Kenya for recovery of *Cotesia vestalis*

1300 mm, and soils are generally sandy with low fertility. Cabbage and kales are the main cruciferous vegetables produced for subsistence use, and for commercial production to a smaller extent.

10.2.2 Sampling for Plutella xylostella *and Parasitoids on Cultivated Crucifers*

Two surveys were carried out in Kwale County's during the rainy (June 2015, August 2015) and dry season (January 2016). Two surveys were also carried out in the eastern region, during the rainy (November 2015) and dry season (March 2016). The farms sampled were those on which cabbage is produced, and were at least 1 km apart. Ten (10) farms per region in each county were surveyed during each visit, and the same fields were visited on every survey. In cases where a field was not under crucifer crop production, it was replaced by another farm nearby. However, fewer farms in some regions of Kwale County were surveyed, due to a huge decline

in the number of farms on crucifer production because of dry weather conditions in January 2016, since farmers without access to irrigation could not produce vegetables during that period. Ten (10) randomly selected cabbage plants per farm were sampled and thoroughly inspected for *P. xylostella* larvae, pupa and parasitoid cocoons on the leaves. The samples collected were put into plastic containers fitted with a cloth mesh at the top to allow for ventilation, which were lined with a paper towel at the bottom to prevent condensation. The containers were labelled with the field numbers. The types and numbers of samples collected, other pests found on the crops, field numbers, host plants, and collection dates were recorded and the samples taken to the laboratories. The farms sampled were geo-referenced using a Global Positioning System (GPS, model Magellan® Triton™ 400).

Sample processing was conducted at the International Centre of Insect Physiology and Ecology (ICIPE), Duduville Campus, Kasarani, Nairobi. The samples collected were kept at room temperature (23 ± 2 °C), 50–70% relative humidity, and at a photoperiod of 12:12 h (Light: Darkness). Fresh cabbage leaves were provided daily to the *P. xylostella* larvae. The emergence of either *P. xylostella* or parasitoid adults was checked daily until no further emergence was observed. All the *P. xylostella* and different parasitoid samples that emerged were sexed, identified, and recorded. Identification and sexing of the parasitoids collected was carried out using a Leica EZ4D microscope at a magnification of 10× to enable viewing of the parasitoid genitalia. Moreover, the presence or absence of detailed features that identify *C. vestalis* were used, based on IntKey (Dallwitz et al. 1999). Parasitoids from each county were reared in Perspex cages measuring 20 × 20 × 20 cm (external dimensions) in the laboratory to obtain a larger number for molecular work. Rearing of *C. vestalis* was carried out by exposing them to 2nd and 3rd instar larvae of *P. xylostella* on cabbages for 24 h, removal of the plants and putting the larvae into lunch boxes (11.5 cm diam., 6 cm high), and daily feeding on cabbages until emergence. The *C. vestalis* were fed daily on honey droplets on a paper strip.

10.2.3 Survey on Pest Management Practices

Household interviews were conducted to collect information on cultural practices on cabbage farming using structured questionnaires (See Appendix A below). The information collected included: the type of cruciferous crop cultivated, variety grown, planting time, harvesting intervals, intercropped farms, soil type, type of manure and fertiliser applied and their application period, irrigation type and rainfed farms, the pest management strategies applied by farmers, reason for pesticide application, frequency of their application, last application dates, and the change in the frequency on use of the management strategies over time, in addition to the farmers' knowledge on use of natural enemies in management of *P. xylostella*.

10.2.4 Molecular Identification of Cotesia vestalis

The *Cotesia* spp. samples were used for molecular work. Additional samples similar to those released in East Africa (South African biotype) were obtained from the Department of Agricultural Research Services (DARS) in Malawi. All the samples were preserved in 95% ethanol and stored at −20 °C while awaiting genomic DNA extraction. Prior to molecular characterisation, adults were morphologically identified according to the various features used for the identification of *Cotesia* species of economic importance, as described by IntKey (Dallwitz et al. 1999). Lateral, ventral and dorsal images of the sample specimens were taken with a Leica EZ4D microscope, using LAS EZ software ver. 3.0.0. This was followed by surface sterilisation in 3% sodium hypochlorite, rinsing thrice in distilled water, and placing the samples in labelled 1.5 mL tubes. DNA extraction was performed using ISOLATE II Genomic DNA Kit (Bioline, UK) following the manufacturer's protocol. Amplification of the target COI gene was done using universal barcode primers; LCO-1490 (5′ GGTCAACAAATCATAAAGATATTG G 3′) and HCO-2198 (5′ TAAACTTCAGGGTGACCAAAAAATA 3′) (Folmer et al. 1994). The PCR was carried out in a total reaction volume of 20 µL containing 5× My *Taq* Reaction Buffer (5 mM dNTPs, 15 mM $MgCl_2$, stabilisers and enhancers), 10 µmole of each primer, 0.5 mM $MgCl_2$, 0.25 µL My *Taq* DNA polymerase (Bioline, UK) and 15 ng/µL of DNA template. The reaction was set up in the Nexus Mastercycler gradient (Eppendorf). The following cycling conditions were used: initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 40 s annealing at 50.6 °C, and 1 min at 72 °C, then a final elongation step of 10 min at 72 °C.

The amplified PCR products were resolved through a 1.2% agarose gel stained with 10 mg/mL ethidium bromide. DNA bands on the gel were analysed and documented using a KETA GL imaging system trans-illuminator (Wealtec Corp). Successively amplified products were excised and purified using Isolate II PCR and Gel Kit (Bioline, UK) following the manufacturer's instructions. The purified samples were shipped to Macrogen Inc. Europe Laboratory, the Netherlands, for bidirectional sequencing.

10.2.5 Sequence Analysis

The chromatograms were examined with Chromas ver. 2.5.1 (Hall 1999), and when ambiguous sites were found, they were corrected to produce two alternative sequences, corresponding to high and low peaks, respectively, and a sequence was created. The consensus sequences generated from both strands were compared to those available at GenBank, using the built-in BLAST utility (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignment was done with ClustalX version 2.1 (Thompson et al. 1997). jModeltest 2.1.7 program was used to provide an estimate

of the best-fit model selection (Darriba et al. 2012). The sequences were then run in RAxML v8.2.0 (Stamatakis 2014) to get the best-scoring Maximum likelihood tree, with a bootstrap test of 1000 replications. The tree was constructed using Fig Tree v. 1.4 (Rambaut 2012).

The sequences obtained were submitted to the Barcode of Life Database (BOLD) and deposited in GenBank (Accession numbers: ABZ6416 and ADL5635). DNA voucher specimens were stored at ICIPE Molecular Pathology Lab., Arthropod Pathology Unit (APU).

10.2.6 Data Analysis

The parasitism rate of *P. xylostella* by the solitary parasitoid, *C. vestalis* was calculated as follows:

$$\%\,parasitism = \frac{\text{Sum of } \textit{Cotesia vestalis}}{\text{total adults}\left(\text{parasitoids} + \text{DBM}\right)} * 100$$

Calculations of parasitism excluded samples that died before emergence (Nofemela and Kfir 2005). The data on the *P. xylostella* and *C. vestalis* densities were checked for normality using the Shapiro-Wilk test, and log-transformed to correct for overdispersion, while proportional data were arcsine transformed. The data for each survey period were then subjected to Analysis of Variance (ANOVA). Means were separated using Tukey's Honest Significant Difference (HSD) test at 5% level of significance. R Studio software (ver. 2.15.1) was used for all analyses (http://www.rstudio.org/).

10.3 Results

10.3.1 Incidence of Plutella xylostella *and Parasitism Rates by* Cotesia vestalis *in Kwale County in the Coastal Region*

The P. *xylostella* densities for the June 2015 survey (12.22 ± 1.88 per farm, mean ± SD) was over ten times the densities in Jan 2016 (1.18 ± 0.67 per farm) (Fig. 10.2). On the other hand, the *C. vestalis* densities for Jan 2016 (0.65 ± 0.37 per farm) were more than twice the densities in June 2015 (0.31 ± 0.11 per farm) (Fig. 10.2). Parasitism rates by *C. vestalis* in Jan 2016 (7.35 ± 3.523%) were over twice the parasitism in June (2.83 ± 1.02%) (data not shown). The comparison of the *P. xylostella* densities between the two survey periods showed significant differences ($F = 43.11$; $df = 1,74$; $P < 0.05$), while the *C. vestalis* densities ($F = 0.982$; $df = 1,74$; $P = 0.325$) were not significantly different.

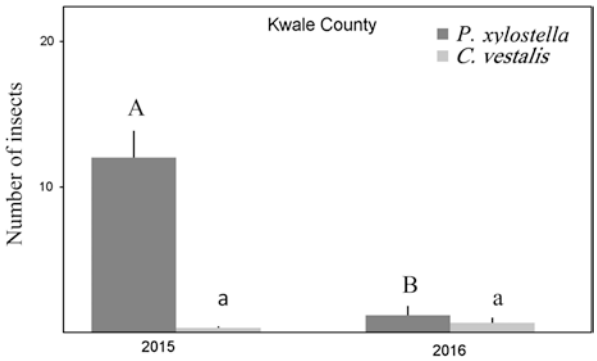

Fig. 10.2 The number of *Plutella xylostella* per farm and its parasitoid *C. vestalis* recorded on cabbage in Kwale County in 2015 and 2016

10.3.2 Incidence of Plutella xylostella *and Parasitism Rates by* Cotesia vestalis *in the Eastern Region*

During the November 2015 survey, the density of *P. xylostella* in Kitui was fivefold (1 ± 0.39 per farm), compared with that recorded in Makueni (0.2 ± 0.13 per farm). In the same survey, the densities of *C. vestalis* in Makueni were 2.25-fold (1.8 ± 0.80 per farm) higher than in Kitui (0.8 ± 0.33a per farm) (Fig. 10.3). There were no significant differences observed between *P. xylostella* (F = 3.358; df = 1, 18; p = 0.0835) and *C. vestalis* densities (F = 0.548; df = 1,18; p = 0.469) in the two counties. The parasitism rates in Makueni (37.86 ± 1 3.88%) were almost twice those in Kitui (20.9 ± 0.92%).

In March 2016, the *P. xylostella* density in Kitui (2.30 ± 1.76 per farm) was over three times higher than in Makueni (0.6 ± 0.50 per farm) (Fig. 10.3 below). The densities of *P. xylostella* (F = 1.119; df = 1,18; p = 0.304) and *C. vestalis* (F = 3.6; df = 1,18; p = 0.074) were not significantly different. The parasitism rate by C. *vestalis* in Kitui (32.19 ± 12.64%) was more than twice the rate recorded in Kitui (15 ± 10.67%). There was no significant difference between *P. xylostella* (F = 0.228; df = 1,38; p = 0.636) and *C. vestalis* (F = 2.896; df = 1,38; p = 0.0969) densities during the 2015 and 2016 surveys, in the Eastern Region.

10.3.3 Survey on Pest Management Practices

A baseline survey of pest management practices indicated that 89.25% of the farm households interviewed at the coastal region were using insecticides. The farmers used a total of 18 different active ingredients of synthetic insecticides under differ-

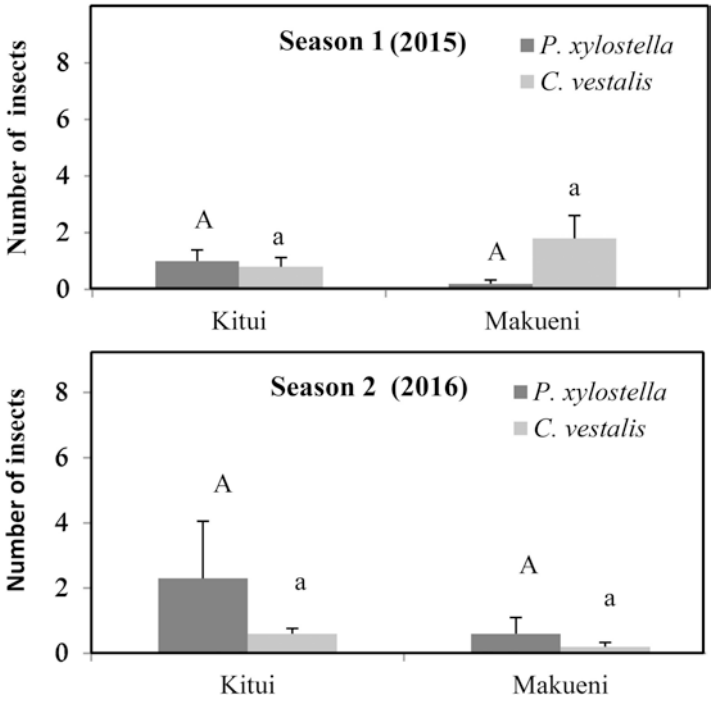

Fig. 10.3 The densities of *P. xylostella* and *C. vestalis* for the different survey periods in Eastern region counties of Kenya

ent application regimes. The majority of the insecticides used in the Coastal region were pyrethroids, constituting 75.47% of the total pesticides, followed by neonicotinoids (8.49%) and carbamates (5.66%) (Table 10.1).

Based on the household data collected in Kitui, Makueni and Machakos counties, 78.57% of the farmers were spraying their vegetables with insecticides. The most commonly used insecticides were pyrethroids (46.16%), with a usage threefold higher than that of organophosphates (15.55%). Additionally, only 5.5% of the products used were micro-organism derived, while 1.1% were plant-derived products (Table 10.1).

The proportion of farmers that practised intercropping in the Coastal region ranged between 9.09% and 51.16%, compared to 0–2.5% in the Eastern region, depending on the growing period. On the other hand, the proportion of farms under irrigation in the coastal region was 78.75%, whereas it was 73.12% in the Eastern region (Table 10.2).

The common pests, other than *P. xylostella,* based on pest densities and frequency of occurrence were cabbage aphids, bollworms, whiteflies, thrips, cabbage loopers, cutworms and leafminers. Bollworms were the most abundant in the Coastal region in both 2015 (65.05%) and 2016 (41.18%). In the Eastern region,

Table 10.1 Use of different insecticide active ingredients in crucifer crops at the Coastal and Eastern semi-arid region of Kenya

Active ingredient	Substance group	Usage frequency (%)	
		Coast	Eastern Region
Lambda-cyhalothrin	Pyrethroid	41.51	16.67
Alphacypermethrin	Pyrethroid	24.53	13.33
Deltamethrin	Pyrethroid	3.77	1.11
Beta-Cyfluthrin	Pyrethroid	3.77	3.33
Cypermethrin	Pyrethroid	1.89	12.22
Mancozeb	Carbamate	2.83	1.11
Carbosulfan	Carbamate	0.94	0
Methomyl	Carbamate	0.94	5.56
Propamocarb	Carbamate	0.94	2.22
Chlorpyrifos	Organophosphate	0	12.22
Dimethoate	Organophosphate	0	1.11
Diazinon	Organophosphate	0	2.22
Thiamethoxam	Neonicotinoid	3.77	4.44
Imidacloprid	Neonicotinoid	2.83	3.33
Acetamiprid	Neonicotinoid	1.89	2.22
Hydrochloride	Neonicotinoids	0	2.22
Lufenuron	Benzoylurea	3.77	3.33
Metalaxyl	Phenylamide	2.83	0
Fluopicolide	Benzamide	0.94	0
Carbendazim	Benzimidazole	0.94	0
Pyridaben	Pyridazinone	0.94	0
Sulphur	Fluoride	0.94	0
Hexaconazole	Triazole	0	2.22
Flubendiamide	Benzenedicarboxamide	0	2.22
Azoxystrobin	Strobilurin	0	1.11
Diafenthiuron	Thiourea	0	1.11
Emamectin benzoate	Micro-organism derived	0	1.11
Abamectin	Micro-organism derived	0	3.33
Azadirachtin	Plant derived	0	1.11
Bt	Microbial pesticide	0	1.11

Table 10.2 Farming practices adopted by farmers in the regions surveyed

Farming practices						
	Coast region			Eastern region		
Survey period	2015	2016	Mean	2015	2016	Mean
Intercropping (%)	30.125	29.41	29.76	2.5	0	1.25
Irrigation (%)	65.855	88.24	77.04	50	97.5	73.75

Table 10.3 Ranking of other pests attacking crucifers in the surveyed regions

	Coastal region		Eastern region	
Pests	2015	2016	2015	2016
Bollworms	65.05%	41.18%	6.67%	15%
Whiteflies	2.17%	23.53%	26.67%	75.0%
Aphids	12.58%	–	43.33%	67.5%
Cabbage loopers	3.03%	11.76%	3.33%	5%
Thrips	–	–	6.67%	35%
Leafminers	–	–	6.67%	5%
Cutworms	–	–	3.33%	–
Other caterpillars	1.515	5.88%	6.67%	35%

Fig. 10.4 1% Agarose gel electrophoretic image of the *C. vestalis* PCR products amplified using the HCO/LCO primers. Lanes and samples are: 1–10 (Kajiado), 10–19 (Kitui), 20–28 (Kwale), 29–38 (Makueni), 39–48 (Malawi), L = 100 bp DNA ladder (Bioline)

cabbage aphids (43.33%) were ranked as the most common pest in 2015, while whiteflies (75.0%) were the most abundant in 2016 (Table 10.3). In regard to farmers' knowledge on parasitoids, only 41.8% of the farmers in the Coastal region and 30% in Eastern region were well-informed about the use of parasitoids for management of *P. xylostella.*

10.3.4 Quantification of DNA and PCR Amplification

The purity of the DNA, based on the ratio of absorbance at $260/280_{nm}$ for all samples, ranged from 1.92 to 2.72, while the nucleic acid concentration of the samples ranged between 30.8 and 146.3 ng/μL. A total of 48 samples (from Kitui, Kwale, Makueni, Kajiado and Malawi) were amplified by PCR, with a band size of approximately 700 base pairs (Fig. 10.4).

10.3.5 Bioinformatics Analysis

BLAST results for the 48 samples from Kajiado, Kitui, Kwale, Makueni and Malawi indicated the complete mitochondrion genome as best hit (E-Value 0.0), with ≥98% similarity to *Cotesia vestalis* (Genbank accession: FJ154897). The only exception was Kw7 from Kwale that recorded the cytochrome oxidase subunit 1 (COI) gene as best hit (E-value 0.0), with 98% similarity to a *Cotesia* spp. (Genbank accession: HM430398). All the COI-aligned sequences showed a high degree of conserved residues among the 48 samples. One sample from each group was used to represent samples from the five regions (Fig. 10.5).

10.3.6 Phylogenetic Analysis

The *C. vestalis* sequences grouped into two clusters. The first one had only one sample (Kw7) collected from Kajiado County, which corresponded to *Cotesia* spp. The second cluster included the remaining samples, which corresponded to *Cotesia vestalis*. The second cluster was further separated into several groups. The cluster with the highest bootstrap value (99%) had two samples (Mw11 and Mw23) from Malawi. Most of the samples were supported by low bootstrap values.

Fig. 10.5 Multiple sequence alignment of *C. vestalis* sequences produced by ClustalX. Conserved regions are marked with asterisks above the sequences. The sequences were sampled from five regions denoted as follows: *Kj* = Kajiado, *Kt* = Kitui, *Kw* = Kwale, *Mw* = Malawi, *Mk* = Makueni (see Table 10.4 for samples details)

Table 10.4 Details of the sequenced samples and their corresponding Genbank accession numbers

Species name	Sample ID	Location	Country	Genbank Accession No.
Cotesia vestalis	Kj6-Kj10, Kj21, Kj22, Kj24, Kj25	Kajiado	Kenya	ABZ6416
Cotesia vestalis	Kt6-Kt10, Kt26-Kt30	Kitui	Kenya	ABZ6416
Cotesia vestalis	Kw8-Kw10, Kw22-Kw25	Kwale	Kenya	ABZ6416
Cotesia vestalis	Kw7			ADL5635
Cotesia vestalis	Mk1-Mk10	Makueni	Kenya	ABZ6416
Cotesia vestalis	Mw11-Mw15, Mw21-Mw25	Malawi	Malawi	ABZ6416

Table 10.5 Estimates of evolutionary divergence over sequence pairs between groups (generated by Mega 6)

Sample[a]	Kt	Mw	Kj	Mk	Kw
Kt	–				
Mw	0.008	–			
Kj	0.010	0.010	–		
Mk	0.011	0.012	0.014	–	
Kw	0.014	0.012	0.014	0.018	–

[a]*Kt* Kitui, *Mw* Malawi, *Kj* Kajiado, *Mk* Makueni, *Kw* Kwale

10.3.7 Genetic Distances and Distance Summary

The divergent distances between the five groups of sequences were estimated by pairwise genetic distances estimated from COI sequences, based on a Kimura-two-parameter algorithm (Tamura et al. 2013). The highest nucleotide distance was between Kw and Mk, with a value of 0.018 (Table 10.5). The distance summary, shown in bold, indicated a within-species mean distance of 23.51%, with maximum distance as 75%, and a minimum distance as 0%. The alignment option used was the Kimura-2-parameter.

10.4 Discussion

The study of *P. xylostella* dynamics in the semi-arid and coastal areas of Kenya showed much of variability with the planting period in both coastal and eastern regions. Despite several releases, *C. vestalis* parasitism rates in the coastal region and eastern regions were below 14% and 38%, respectively. Misuse of pesticides could be one of the factors underlying the poor establishment of *C. vestalis* in both regions. Moreover, farmers continue spraying on a calendar basis, using one or more broad-spectrum synthetic insecticides either weekly or fortnightly, not only to manage *P. xylostella*, but also other pests including aphids, whiteflies and bollworms. There are reports that the fields with minimum pesticide usage or the

organically managed ones have higher densities of parasitoids than those that are frequently sprayed (Ayalew et al. 2002; Kfir 2004; Rowell et al. 2005). Moreover, some products used by farmers, such as spinosad, have detrimental effects on *C. vestalis* (Oliveira et al. 2011), thus slowing down their increase and reducing the parasitoid efficacy. Overreliance on rainfall for vegetable production necessitated broken and discontinuous productions, and could have been a factor behind the poor establishment of *C. vestalis* in the release regions. According to the farmers, their frequency of pesticide usage was on the rise, compared with the previous years. They attributed this to the reduced efficacy of the pesticides that they were using. Several studies have shown that *P. xylostella* developed resistance to abamectin, lufenuron, methomyl and emamectin (Pu et al. 2010; Santos et al. 2011), yet these pesticides are still widely used by farmers.

In the Philippines lowlands, an IPM technology which involved augmentative parasitoid releases and judicious spraying using selective insecticides with strong support from extension efforts, resulted in the successful establishment of *C. vestalis*, reduced *P. xylostella* densities, increased yields and reduced production costs (Morallo-Rejesus et al. 1997; Rowell et al. 2005; Jankowski et al. 2007). The same strategy can be adopted in Kenya to increase its efficacy of as a biological control agent. Moreover, stronger links between research and extension would be very helpful in informing the farmers on the use of safe insect management measures against pests and would go a long way in creating farmer awareness of parasitoids. Furthermore, the adapting of IPM programmes that help in management of other common pests such as aphids, bollworms and whiteflies would greatly reduce spraying, since farmers currently continue spraying, even in absence of *P. xylostella*, and this eventually affect its parasitoids. In a bid to achieve better pest management within the existing cropping system, smallholder farmers need to have access to information on effective IPM technologies through participatory technology transfer approaches. Molecular analysis of the samples collected from Kajiado, Kitui, Kwale, Makueni and Malawi showed that the specimens from the surveyed areas had ≥98% similarity to Cotesia vestalis. The DNA quantification and amplification confirmed that the target gene was present in all samples. Furthermore, bioinformatics showed that all samples had the complete mitochondrion genome as best hit, except one. Some of the samples from different regions clustered together, an indicator that they shared ancestry, while other samples from the same location fell under different clusters, which is an indicator of the occurrence of divergence among the species, over time. The results suggest that there is very close similarity in all the samples, regardless of where they were collected from. This shows that the South African biotype of C. vestalis that was released in the previous years was active in the surveyed areas. The COI gene is highly conserved, as revealed by multiple sequence alignment, and it can be used as a viable marker in confirming Cotesia establishment upon release in the study sites. Similar studies have reported successful use of COI gene in determining the origin of Cotesia flavipes in Ethiopia (Assefa et al. 2008).

In conclusion, this study and the molecular results conclusively show that *C. vestalis* has established in and around the different release sites in Kenya. However, its contribution in management of *P. xylostella* is still low, and this could mainly be attributed to the excessive use of broad-spectrum insecticides. Moreover, the COI

gene greatly improved the identification of the *C. vestalis* without much dependence on morphological characters.

Appendix

Farmer questionnaire used for *Cotesia vestalis* post-release Surveys (Kenya-Kajiado, Kitui, Machakos, Makueni).

County _____ Sub-County _____ Division _____
Location_____ Village_____
Farmer's name _____
Farmer's no_____ Collection no _____ Field no _____
Date _____
Latitude _____ Longitude _____ Altitude_____
Crop variety _____ Planting date _____
Crop stage _____ Harvesting interval _____
Pesticides used to control DBM _____
Last application _____ Application frequency _____
Pesticide application frequency in 2014/season _____
Current pesticide application frequency/season _____
Pesticides used to control aphids _____
Last application _____ Application frequency _____
Management _____ Approximate farm acreage on kale/cabbage _____
Irrigation type used _____ Soil type _____
Manure used _____ Last application _____
Fertilizer used _____ Last application _____
Fertilizer application interval _____
Other related crucifers/intercrop_____
Farmer's knowledge on natural enemies
_____
_____
_____

References

Assefa, Y., Mitchell, A., Conlong, D. E., & Muirhead, K. A. (2008). Establishment of Cotesia flavipes (Hymenoptera: Braconidae) in sugarcane fields of Ethiopia and origin of founding population. *Journal of Economic Entomology, 101*, 686–691.

Ayalew, G., Lorh, B., Baumgartner, J., & Ogol, C. (2002). Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and its parasitoids in Ethiopia. In *Proceedings of the international symposium*, pp. 21–24.

Badenes-Perez, F. R., & Shelton, A. M. (2006). Pest management and other agricultural practices among farmers growing cruciferous vegetables in the Central and Western highlands of Kenya and the Western Himalayas of India. *The International Journal of Pest Management, 52*, 303–315.

Bjorksten, T. A., Robinson, M., & La Salle, J. (2005). Species composition and population dynamics of leafmining flies and their parasitoids in Victoria. *Australian Journal of Entomology, 44*, 186–191.

Cooper, J. (2009). *Pest management in horticultural crops; an integrated approach to vegetable pest management with the aim of reducing reliance on pesticides in Kenya*. Final technical report.

Dallwitz, M. J., Paine, T. A., & Zurcher, E. J. (1999). User's guide to the DELTA Editor. https://www.delta-intkey.com/

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods, 9*, 772–772.

de Oliveira, A. C., de Siqueira, H. Á. A., de Oliveira, J. V., da Silva, J. E., & Michereff, F. M. (2011). Resistance of Brazilian diamondback moth populations to insecticides. *Science in Agriculture, 68*, 154–159.

FAOSTAT. (2014). Food and agriculture organisation, United Nations. http://www.fao.org/faostat.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294–299.

Furlong, M. J., Wright, D. J., & Dosdall, L. M. (2013). Diamondback moth ecology and management: Problems, progress, and prospects. *Annual Review of Entomology, 58*, 517–541.

Gichini, G., Löhr, B., Rossbach, A., Nyambo, B., & Gathu, R. (2008). Can low release numbers lead to establishment and spread of an exotic parasitoid: The case of the diamondback moth parasitoid, *Diadegma semiclausum* (Hellén), in East Africa. *Crop Protection, 27*, 906–914.

Grzywacz, D., Rossbach, A., Rauf, A., Russell, D. A., Srinivasan, R., & Shelton, A. M. (2010). Current control methods for diamondback moth and other brassica insect pests and the prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa. *Crop Protection, 29*, 68–79.

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. In *Nucleic acids symposium series* (pp. 95–98).

Hines, R. L., & Hutchison, W. D. (2001). Evaluation of action thresholds and spinosad for lepidopteran pest management in Minnesota cabbage. *Journal of Economic Entomology, 94*, 190–196.

Jankowski, A., Mithöfer, D., Löhr, B., & Weibel, H. (2007). Economics of biological control in cabbage production in two countries in East Africa. In *Utilisation of diversity in land use systems: Sustainable and organic approaches to meet human needs*. Proceedings of the Conference on International Agricultural Research for Development 'Tropentag.

Kahuthia-Gathu, R. (2012). Seasonal incidence of *Plutella xylostella* (Lepidoptera: Plutellidae) and its associated natural enemies in major crucifer growing areas of Kenya. *Journal of Applied Bioscience, 46*, 3103–3112.

Kfir, R. (1997). Parasitoids of *Plutella xylostella* (Lep.: Plutellidae) in South Africa: An annotated list. *Entomophaga, 42*, 517–523.

Kfir, R. (2004). Effect of parasitoid elimination on populations of diamondback moth in cabbage. In *The management of diamondback moth and other crucifer pests: Proceedings of the 4th international workshop* (pp. 197–205). Gosford: The Regional Institute Ltd.

Lohr, B., & Kfir, R. (2004). Diamondback moth Plutella xylostella (L.) in Africa. A review with emphasis on biological control. *Improving Biocontrol Plutella Xylostella, 2*, 5707.

Löhr, B., Gathu, R., Kariuki, C., Obiero, J., & Gichini, G. (2007). Impact of an exotic parasitoid on *Plutella xylostella* (Lepidoptera: Plutellidae) population dynamics, damage and indigenous natural enemies in Kenya. *Bulletin of Entomological Research, 97*, 337–350.

Macharia, I., Löhr, B., & De Groote, H. (2005). Assessing the potential impact of biological control of *Plutella xylostella* (diamondback moth) in cabbage production in Kenya. *Crop Protection, 24*, 981–989.

Macharia, I., Mithöfer, D., Waibel, H., et al. (2013, September 22–25). Health effects of pesticide use among vegetable farmer in Kenya. In *AAAE fourth international conference*, Hammamet, Tunisia. African Association of Agricultural Economists (AAAE).

Morallo-Rejesus, B., Inocencio, E., Eusebio, J., & Barroga, S. (1997). Technology transfer of Cotesia-based IPM for diamondback moth on lowland crucifers in the Philippines. In *The Management of Diamondback Moth and other crucifer pests*. Proceedings of the Third International Workshop (pp. 267–275).

Mosiane, S. M., Kfir, R., & Villet, M. H. (2003). Seasonal phenology of the diamondback moth, *Plutella xylostella* (L.),(Lepidoptera: Plutellidae), and its parasitoids on canola, *Brassica napus* (L.), in Gauteng province, South Africa. *African Entomology: Journal of the Entomological Society of Southern Africa, 11*, 277–285.

Munthali, D. C. (2009). Evaluation of cabbage varieties for resistance to the cabbage aphid. *African Entomology: Journal of the Entomological Society of Southern Africa, 17*, 1–7.

Nofemela, R. S., & Ktir, R. (2005). The role of parasitoids in suppressing diamondback moth, *PIuteIIa xylostella* (L.) (Lepidoptera: Plutellidae), populations on unspraved cabbage in the North West Province of South Africa. *African Entomology: Journal of the Entomological Society of Southern Africa, 13*, 71–83.

Nyambo, B., & Pekke, A. (1995). *Brassica planning workshop for East and Southern Africa region, Lilongwe-Malawi*. GTZ-IPM Horticulture Nairobi Kenya.

Nyambo, B., Gichini, G., Obiero, J., & Njumwa, G. (2008). Re-distribution of ex-South African strain of *Cotesia plutellae* Kurdjumov (Hymenoptera, Braconidae) in Africa for control of diamondback moth. In *Proceedings of the XXII international congress of entomology*, pp. 6–12.

Pu, X., Yang, Y., Wu, S., & Wu, Y. (2010). Characterisation of abamectin resistance in a field-evolved multiresistant population of *Plutella xylostella*. *Pest Management Science, 66*, 371–378.

Rambaut, A. (2012). FigTree v1. 4 2006–2012. Institute of Evolutionary Biology, The University of Edinburgh, Edinburgh, UK.

Rowell, B., Bunsong, N., Satthaporn, K., Phithamma, S., & Doungsa-Ard, C. (2005). Hymenopteran parasitoids of diamondback moth (Lepidoptera: Ypeunomutidae) in northern Thailand. *Journal of Economic Entomology, 98*, 449–456.

Sandur, S. (2004). *Implications of Diamondback moth control for Indian cabbage farmers*. A report produced for the CIMBAA. http://www.cimbaa.org/downloads/cimbaa_indiandbm_reportfinal-%20apr05.pdf. Last Accessed 17 september 2008.

Santos, V. C., de Siqueira, H. A. A., da Silva, J. E., & de Farias, M. (2011). Insecticide resistance in populations of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), from the state of Pernambuco, Brazil. *Neotropical Entomology, 40*, 264–270.

Shi, Z., Guo, S., Lin, W., & Liu, S. (2004). Evaluation of selective toxicity of five pesticides against *Plutella xylostella* (Lep: Plutellidae) and their side-effects against *Cotesia plutellae* (Hym: Braconidae) and *Oomyzus sokolowskii* (Hym: Eulophidae). *Pest Management Science, 60*, 1213–1219.

Smith, T.J. (2002). *The diamondback moth, Plutella xylostella (L.), (Lepidoptera: Plutellidae) and its biological control in the eastern cape province, South Africa.*

Smith, T.J., & Villet, M.H. (2001, November 26–29). Parasitoids associated with the diamondback moth, *Plutella xylostella* (L.), in the Eastern Cape, South Africa. In *The management of diamondback moth and other crucifer pests*. Proceedings of the fourth international workshop, p. 249.

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics, 30*, 1312–1313.

Talekar, N. S., & Shelton, A. M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology, 38*, 275–301.

Talekar, N. S., & Yang, J. C. (1991). Characteristics of parasitism of diamondback moth by two larval parasites [*Apanteles plutellae*, *Diadegma eucerophaga*]. *Entomophaga, 36*, 95.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*, 2725–2729.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25*, 4876–4882.

Verkerk, R. H. J., & Wright, D. J. (1997). Field-based studies with the diamondback moth tritrophic system in Cameron highlands of Malaysia: Implications for pest management. *International Journal of Pest Management, 43*, 27–33.

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294–299.

Waladde, S. M., Leutle, M. F., & Villet, M. H. (2001). Parasitism of *Plutella xylostella* (Lepidoptera, Plutellidae): Field and laboratory observations. *South African Journal of Plant and Soil, 18*, 32–37.

Zalucki, M. P., Shabbir, A., Silva, R., Adamson, D., Shu-Sheng, L., & Furlong, M. J. (2012). Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera: Plutellidae): Just how long is a piece of string? *Journal of Economic Entomology, 105*, 1115–1129.

Chapter 11
Enhancing Monitoring Efficiency and Management of Vectors of Maize Lethal Necrosis Disease in Kenya

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Abstract This study was carried out to determine the best monitoring strategy to follow as part of management practice for vectors of maize lethal necrosis disease (MLND). The trial was carried out in a randomised complete block design, with four replications in two seasons, from 1st April 2015 to 8th April 2016, in Bomet County, Kenya, the epicentre of the disease. Blue and yellow sticky traps were tested for trapping efficiency when placed at four angles (90°, 120°, 150°, and 180°). Blue and yellow sticky rolls were also tested for their trapping efficiency at different installation times (at germination, and 1 and 2 weeks after germination),and using a control without sticky rolls. Further trials included sampling and planting orientation. Traps positioned at 90° significantly ($P \leq 0.001$) trapped more corn thrips, as compared with the other positions. Blue sticky rolls trapped one-fold more corn thrips than yellow rolls. In contrast, yellow sticky rolls trapped one-fold more corn leaf aphids than the blue rolls ($P \leq 0.001$). Corn thrips infestation was better estimated by sampling along the maize rows than sampling across the rows, by almost half-fold ($P \leq 0.001$). Maize planted along a north–south orientation became more infested by corn thrips than plants along an east–west orientation ($P \leq 0.04$). The findings strongly suggest that sticky traps and sticky rolls should be included in the management of vectors of MLND-causing viruses. The role of maize-row orientation requires further investigation as part of cultural practice in managing the disease. Sampling along maize rows will better estimate the vector population, allowing

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for the deployment of appropriate measures at the earliest time. It is recommended that these effective tools be promoted for farmers to gain from their use.

Keywords Maize · MLND · Kenya · monitoring

11.1 Introduction

In Kenya, maize lethal necrosis disease (MLND) was first reported in Bomet County in September 2011, as well as in Naivasha (Wangai et al. 2012). The disease is caused by co- infection by maize chlorotic mottle virus (MCMV) with sugarcane mosaic virus (SCMV), or any other Potyviridae virus (Makumbi and Wangai 2013; Adams et al. 2014). The disease caused extensive yield losses and complete crop losses (Samson et al. 2014; Wangai et al. 2012). It then rapidly spread to other maize-growing areas in the Rift Valley and other neighbouring countries such as Uganda, Tanzania, Rwanda, and Ethiopia (Adams et al. 2014; Makumbi and Wangai 2013).

Frankliniella williamsi Hood and *Rhopalosiphum maidis* Fitch are the main vectors of MCMV and SCMV, respectively, (Cabanas et al. 2013). They have been sampled in areas with MLND and determined to be the main vectors of the viruses causing this disease (Wangai et al. 2012). MCMV is transmitted by the adult corn thrips, *F. williamsi*. The thrips semi-persistently transmits the virus that is acquired from infected plants, inoculating it into healthy plants (Cabanas et al. 2013). The thrips transmit MCMV immediately after acquisition and can only retain the virus particles for a few days, without latent periods (Chen et al. 2011; Uzest et al. 2007). In addition, they suck plant sap, leading to scarification of the plant and depletion of its nutrients (Ssemwogerere et al. 2013).

SCMV is not new in Kenya and previous studies showed that it has little effect on maize and can be ignored, as long as it infects plants alone (Louie and Darrah 1980). Studies by Singh et al. (2005) show that *R. maidis* efficiently transmits SCMV, even without pre-acquisition fasting. Hence, low numbers of vectors can be as virulent as higher counts of aphids, if they acquire the inoculum.

One of the essential components of integrated pest management is the monitoring of the pest or vector population, to provide an early warning system that helps farmers to take control measures or alter them, in cases they proved unsuccessful (Zayed et al. 2015). Blue and yellow coloured sticky traps have been used to monitor thrips population and map their spread within a given area (Pearsall 2002; Broughton and Harrison 2012). Blue sticky traps have been used successfully to monitor the flower thrips *F. occidentalis*. According to Palumbo (1998), yellow sticky traps were successfully used to monitor the movement of aphids into fields, especially if they are properly used by placing them within the field, near the upwind edges. The sticky traps provided an early indication at the beginning of the crop economic colonisation impact. The use of sticky traps in combination with proper sampling methods can increase the attractiveness and sensitivity required for the

capture of the vectors, as well as provide farmers with an early warning system (Zayed et al. 2015). Accuracy in mapping the population densities of these vectors will lead to the implementation of intervention and mitigation measures, and eventually provide appropriate control. Sticky traps of different colours (blue, yellow and white) have been used by various researchers to control both thrips and aphids (e.g. Muvea et al. 2014; Harbi et al. 2013. However, there is no recorded evidence on their use to monitor and control vectors of MLND, *F. williamsi* and *R. maidis*.

Maize lethal necrosis disease was recently discovered in Kenya and little information exists on the dispersal and movement patterns of the vectors, as well as on their population dynamics in maize crops. Limited data is available on monitoring and management methods for the two vectors, that play a key role in the transmission of the MLN viruses. In Kenya, most farmers only become aware of the damage when the MLND symptom starts to manifest in crops, leading to massive losses. This study was therefore carried out to investigate the utility of sticky traps, sticky rolls, and sampling patterns as tools for monitoring and managing the vectors. Different aspects of sticky trap direction, angle inclination, planting orientation and sampling patterns were used to determine their effectiveness in monitoring and scouting for vectors.

11.1.1 Study Site

The study was conducted in farmers fields in Bomet County, Kenya, during two seasons, from November 2014 to October 2016. The area lies at an altitude of 1900–2100 m above sea level, with a bimodal rainfall pattern amounting to 1200–1400 mm per year. The long rainfall season is experienced from March to June, while the short rain season occurs from November to December (Mutie et al. 2006). The maximum temperature is usually 28–32 °C, with a minimum of 13 °C (Jaetzold and Schmidt 1982; Mutie et al. 2006; Jaetzold et al. 2012). Farmers mainly grow maize as a food crop and a cash crop. Maize is mainly grown as a monocrop, but also as an intercrop with beans. A few farmers grow potatoes and a wide range of vegetables, on a small-scale level (Jaetzold et al. 2012).

11.2 Materials and Methods

For the sticky trap, sampling, and planting orientation aspects, field-based trials were carried out from 1st April 2015 to 18th August 2015, and repeated from 27th October 2015 to 8th April 2016. Plots measuring 3.75 × 5 m were planted with Olerai-500-22A maize variety at 75 × 25 cm spacing. Four factors were examined, comprising: (1) Blue and yellow sticky cards; (2) Sticky traps orientated at 90°, 120°, 150° and 180° angles; (3) Row orientations, where maize was planted along east-to-west contours, and along north-to-south contours; and (4) Sampling patterns, with samples being taken along the rows and across the rows. The treatments

for each factor were arranged in a randomised complete block design and replicated four times. All the plots were separated from each other by a (one) 1-m path. Monitoring was done on a fortnight basis from 1 week after germination up to 7 weeks after germination. The sticky traps were placed at the edges of the four sides of each plot. After 24 h, they were packed in transparent polythene bags and taken to the laboratory for counting the corn thrips and corn leaf aphids trapped under a dissecting light microscope. For the planting orientations and sampling patterns, destructive sampling was carried out at each replicate, with nine plants being sampled from each plot, across the season. The samples were placed in well-labelled transparent sampling bags and transported to the laboratory for vector counting.

For the sticky roll aspect, field-based trials were carried out from 27th October 2015 to 8th April 2016, and repeated from 15th April 2016 to 1st September 2016. There were seven treatments: blue and yellow sticky rolls installed during germination, and at 1 week and 2 weeks after germination, together with a control where no sticky rolls were installed. The treatments were arranged in a randomised complete block design and replicated four times. Each sticky roll was 30 cm wide and was tied around the plots which were separated from each other by 2 m. The sticky rolls were installed to align with the canopy of the crop. Their placement above the ground was adjusted upwards to fit the crop canopy height. Blue and yellow sticky rolls were commercially obtained from Dudutech Ltd. at Naivasha. Furthermore, hand lens-magnifying glasses were used to monitor the number of vectors trapped on sticky rolls at four randomly selected regions on the sticky traps, measuring 10 cm^2. Another sample of the maize was collected 3 and 7 weeks after germination, to determine the viral load using the DAS ELISA and indirect ELISA for MCMV and SCMV, respectively.

11.2.1 Data Analyses

Data was analysed using Gen Stat, 17th edition. Analysis of variance was performed at a 95% level of confidence limit. Where necessary, skewed vector data was transformed by square root and geometrical means taken. Post-hoc analyses were carried out using the Fishers Protected Least Significance Difference Test (LSD), where significance was noted. A t-test was performed to determine the significance and differences in the vectors trapped between two groups of blue and yellow sticky cards. Thereafter, significant means were separated using the LSD.

11.2.2 Results on Sticky Roll, Sampling and Planting Orientation

The blue sticky card captured a highly significant mean number ($P = 0.023$, $t = 2.64$) of corn thrips, as compared with the yellow ones. However, there was no significant difference among the corn leaf aphids trapped by the blue and yellow colours (Table 11.1).

Table 11.1 Mean number of corn thrips and leaf aphids trapped by blue and yellow sticky traps

Sticky card colour	Corn thrips	Corn leaf aphids
Yellow	8.5 b	2.8
Blue	20.7 ab	1.4
P value	0.023	0.16
t-value	2.64	1.43

∗Means within column followed by the same letter are not significantly different at $P \leq 0.05$

Table 11.2 Mean number of corn thrips and corn leaf aphids trapped by selected angle inclinations of sticky traps in Bomet county of Kenya between May 2015 and January 2016

Season	Sticky trap angle	Corn thrips	Corn leaf aphids
Season 1	90°	6.9 a	1.4
	150°	5.2 a	1.3
	120°	3.6 ab	1.2
	180°	3.3 b	1.2
	P value	0.01	0.4
	SE	1.8	0.2
Season 2	180°	2.6	1.5
	150°	2.4	1.6
	120°	1.3	1.1
	90°	1.4	1.1
	P value	0.2	0.6
	SE	0.75	0.5
Seasons 1and 2	90°	4.5 a	0.9
	150°	3.8 a	1
	180°	2.8 ab	0.9
	120°	2.3 b	0.8
	P value	0.002	0.2
	SE	1.7	0.2

∗Means within column followed by the same letter are not significantly different at P = 0.05

During the first season, the sticky trap that were inclined at 90° captured a significantly higher ($P \leq 0.01$) number of corn thrips, as compared with those inclined at 120°and 180°. Corn thrips trapped by sticky cards inclined at 150° had no significant differences from those captured at 90° (Table 11.2). The sticky traps at angles of 90°, 120°, 150° and 180° did not significantly differ in the capture of corn leaf aphids. Pooled data from both seasons show similar results as the first season ($P \leq 0.002$), although the second season showed no difference in the vectors captured by sticky cards, inclined at all the angles (Table 11.2).

During the first and second seasons, corn thrips infestations were significantly lower ($P \leq 0.001$) when the crop had one to two leaves {1 week after germination (WAG)}. At 5 (mid whorl) and 7 (late whorl) WAG, the corn thrips captured showed

Table 11.3 Mean number of vectors of MLND sampled at different planting orientation of maize in Bomet County, Kenya∗

	Planting orientation	Corn thrips	Corn leaf aphids
Season1	North to South	13.9a	15.64
	East to West	11.4b	15.07
	P value	0.04	0.8
	t- value	2	0.2
Season 2	East to West	4.8	0.8
	North to South	3.9	0.5
	P value	0.8	0.7
	t-value	0.18	0.5
Season 1&2	East to West	7.5b	7.1
	North to South	8.5a	7.3
	P value	<.001	0.8
	t- value	1.6	0.16

∗Means within column followed by the same letter are not significantly different at $P \leq 0.05$. Vectors trapped for a period of 24 h

a significantly higher ($P \leq 0.001$) abundance than in any other growth stage. Moreover, the number of corn leaf aphids increased significantly ($P \leq 0.001$) during the late whorl stage (just before tasselling) at 7 WAG, as compared with the other stages. Generally, the mean number of corn thrips was significantly higher than that of corn leaf aphids trapped across all the crop stages, for all seasons. However, the number of corn leaf aphids was significantly higher than the corn thrips during the second season at 1 WAG. Both vectors had significantly ($P \leq 0.001$) higher numbers during the late whorl stage (7 WAG), and this coincided with the disease manifestation in the crops.

When the crop was grown along the north–south orientation, it attracted significantly ($P \leq 0.04$, $t = 2.0$; $P \leq 0.001$, $t = 1.6$) more corn thrips, as compared with that grown along the east–west orientation during season 1 and combined season, respectively. However, this relationship ws only significant for corn thrips (Table 11.3).

Sampling along the rows recorded significantly higher ($P \leq 0.001$ $t = 5.32$; $P \leq 0.001$, $t = 5.14$) numbers of corn thrips, than across the rows, during season 1 and combined season, respectively (Table 11.4). A similar trend was recorded for corn leaf aphids, although it was not significant (Table 11.4).When the crop was grown in an east-to-west orientation, infestation was best explained by sampling along the rows, both for corn thrips and leaf aphids (Table 11.4). Additionally, when the crop was grown in a north-to-south orientation, infestation of both vectors was likewise best estimated by sampling along the rows (Table 11.4).

Table 11.4 Mean numbers of vectors of MLND causing Viruses sampled along and across rows at Bomet County, Kenya

	Sampling pattern	Corn thrips	Corn leaf aphids
Season1	Along the rows	16.0 a	17
	Across the rows	9.3 b	13
	P value	< 0.001	0.16
	t- value	5.32	1.4
Season 2	Along the rows	4.82	0.76
	Across the rows	3.92	0.51
	P value	0.08	0.5
	t-value	1.72	0.7
Season 1and 2	Along the rows	9.8a	8.1
	Across the rows	6.3b	6.3
	P value	< 0.001	0.16
	t value	5.14	1.4

Table 11.5 Mean number of vector infestation levels on maize protected by sticky rolls installed at different times

Sticky roll colour	Time of installation	Corn thrips	Corn leaf aphids	Thrips damage	MLND severity
No protection	Control	34.3	39.88	3.3125	3.763a
Blue	At germination	25.5	31.63	3.2188	2.737 c
Blue	1 WAG	28.8	57	3.2188	2.938 bc
Blue	2WAG	33.1	44.38	3.1875	3.013ab
Yellow	At germination	25.4	30.63	3.1562	3.2 ab
Yellow	1 WAG	28.9	42.69	3.2188	2.875 bc
Yellow	2WAG	27.3	35.4	3.1333	3.312ab
P value		0.06	0.8	0.9	0.02
Se		3.4	18.4	0.28	0.29

11.2.3 Results on Sticky Roll

The installation times for the sticky rolls had no effects on thrips damage levels, on the corn thrips and on corn leaf aphid infestation levels on maize (Table 11.5). However, the MLND severity was significantly ($P \leq 0.02$) higher in plots that were not protected by sticky rolls. However, this did not differ significantly with the disease severity in plots that were installed with yellow sticky rolls at germination, and 2 weeks later (Table 11.5).

The time of installation had a significant effect ($P \leq 0.001$) on the numbers of corn thrips and corn leaf aphids trapped by the blue and yellow sticky rolls. Blue sticky rolls installed immediately and 1 or 2 weeks after germination trapped significantly more corn thrips, as compared with the yellow sticky rolls, at all regimes The yellow sticky rolls trapped more corn leaf aphids than the blue ones (Fig. 11.1).

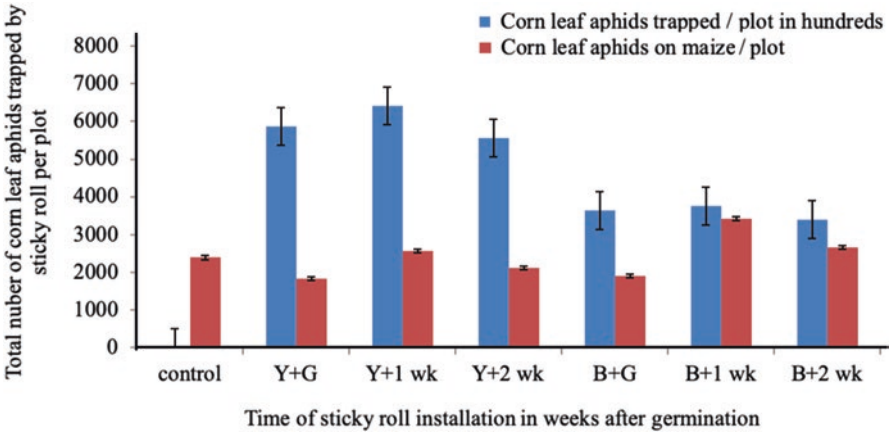

Fig. 11.1 Mean number of vectors trapped on various sticky roll regimes per plot, during 2 months. Control = no sticky roll; Y + G = Yellow sticky roll installed immediately after germination; B + G = blue sticky trap installed immediately after germination; B + 1wk = Blue sticky roll installed 1 week after germination; Y + 1wk = Yellow sticky roll installed 1 week after germination; B + 2wk = Blue sticky roll installed 2 weeks after germination; Y + 2wk = yellow sticky roll installed after germination

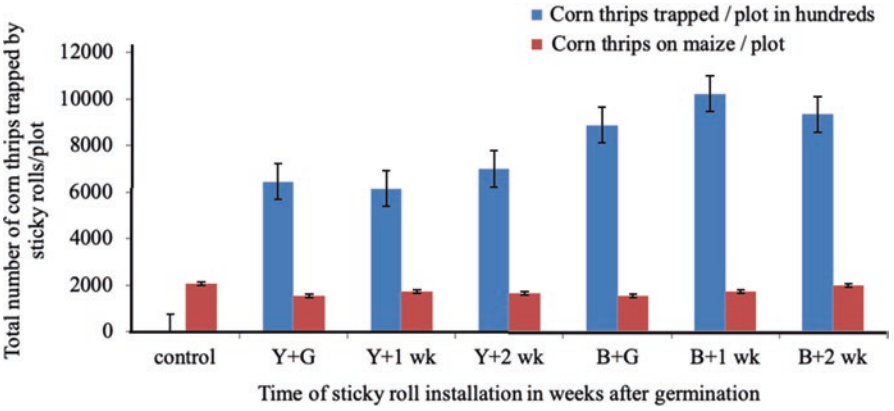

Fig. 11.2 Total number of vectors on maize and sticky roll for 2 months period

More vectors were trapped by the sticky rolls, compared with the ones found on maize (Fig. 11.2). This indicated that the sticky traps were able to protect maize from the massive infestation by the corn thrips and leaf aphids (Fig. 11.2). Correlation analysis shows a high, significant and negative relationship between MLND severity and trapped corn thrips ($P \leq 0.02$, $r = -0.84$), as well as with trapped corn leaf aphids ($P \leq 0.001$, $r = -0.86$). Additionally, corn thrips infestation on maize had a significant high and negative correlation with corn thrips trapped by

Table 11.6 Correlation in between vectors of MLND causing viruses and yield characteristics various sticky roll installation regimes on maize in Bomet County of Kenya

	1	2	3	4	5	6	7	8	9	10	11
1	–										
2	0.6231	–									
3	−0.1543	0.6055	–								
4	0.6848	0.3556	−0.4208	–							
5	−0.3223	0.029	0.6305	−0.8247∗	–						
6	−0.3919	−0.8657∗∗	−0.7404∗∗	0.0332	−0.434	–					
7	0.9067∗∗∗	0.8243∗∗	−0.1373	0.8497∗∗	0.532	−0.346	–				
8	−0.8241∗	−0.844∗	−0.2196	−0.505	0.086	0.481	−0.6942∗	–			
9	−0.4646	−0.5674	−0.0291	−0.7755∗	0.467	0.398	−0.6647	0.4123	–		
10	−0.465	−0.5678	−0.0294	−0.7753∗	0.467	0.399	−0.6647	0.4129	1	–	
11	−0.4649	−0.5674	−0.0289	−0.7755∗	0.467	0.399	−0.6648	0.4126	1	1	–

1. MCMV viral load, 2. MLND severity 3. SCMV severity, 4. Corn thrips damage, 5. Corn leaf aphids on maize, 6. Corn leaf aphids trapped, 7. Corn thrips on maize, 8. Corn thrips trapped, 9. Fresh weight per plot, 10. Weight per cob in g, 11. Weight per cop per plot

∗ = Significant at $P < 0.05$, ∗∗ = Significant at $P < 0.01$, ∗∗∗ = Significant at $P < 0.001$

sticky rolls ($P \leq 0.04$, $r = -0.69$). However, the corn thrips infestation on maize had a significant high and positive correlation with MLND severity ($P \leq 0.02$, $r = 0.82$) (Table 11.6).

11.2.4 Correlation Between Virus Vectors of MLND and Yield Characteristics in Maize Protected by Sticky Rolls

There was a high and negative significant correlation between the MCMV viral load and the corn thrips trapped by the sticky rolls ($r = -0.82$, $P \leq 0.003$) (Table 11.6). In addition, the MCMV viral load showed a strong and high positive correlation with thrips infestation on maize ($r = 0.91$, $P \leq 0.001$). Similarly, a high and negative significant correlation was found between MLND severity and the trapped corn leaf aphids ($r = 0.86$, $P \leq 0.01$) (Table 11.6). Corn leaf aphids and SCMV viral loads showed a high, significant, negative correlation ($r = -0.74$, $P \leq 0.02$). A similar trend was observed between the corn leaf aphids and thrips damage ($r = -0.82$, $P \leq 0.03$). Corn thrips infestation on maize has a significant high and negative correlation with fresh weight per plot, weight of cobs per plot, and weight per seed ($r = -0.77$, $P \leq 0.02$) (Table 11.6).

11.3 Discussion

The finding that blue sticky traps are better in trapping corn thrips than yellow traps constitutes a first report globally, and provides important information about the monitoring and control of this vector. These findings are similar to what has been reported for other thrips infesting other crops, such as the cowpea and French beans (Tang et al. 2016; Muvea et al. 2014; Kasina et al. (2009). Other studies have reported on the efficacy of both blue and yellow sticky traps in monitoring thrips population numbers and mapping their spread in a given area (Thongjua et al. 2015; Broughton and Harrison 2012).

Sticky traps oriented at 90° (positioned vertically to the ground) attracted more corn thrips than other orientations. Tian-Ye et al. (2004) reported the efficacy of vertically positioned yellow fluorescent traps, which attracted more thrips than other placements. Vectors trapped at various phenological stages of maize varied significantly with the mid- and late-whorl stages (when the collars of the eighth and twelfth leaves were visible, respectively), attracting more vectors than the other stages. It is presumed that these stages of maize development provide appropriate cues that lure greater numbers of vectors. The different hues of colours exhibited in the various maize stages, as well as those of the trap materials, together with the reflectance of the sticky trap, might play some part in the attraction of higher numbers during the mid- and late-whorl stages (Kaas 2005). The fewer corn thrips trapped at the one- to two-leaf stages (1 WAG) coincided with those reported on onions, where infestations at 12 weeks after transplanting were 15 times higher than on young plants, at 4 weeks after transplanting (Ibrahim and Adesiyun 2009). The numbers of corn leaf aphids were significantly higher during the late whorl stage. This is likely to be the optimal time when the sugarcane mosaic virus interacts with the maize chlorotic mottle virus to cause MLND. These kinds of vector movement and trends provide the best approaches for initiating control strategies that prevent population explosions and reduce the interaction opportunities of the MCMV and SCMV viruses.

Sampling is critical for estimating the infestation level of the vectors in the field. Therefore, the choice of sampling orientation is very important. In this study, sampling alongside the maize rows provided the best estimates of corn thrips infestation levels. Albrecht (2010) reported similar results when he used the same pattern to sample for turf grass grubs. Planting along the north–south orientation favoured the movement and spread of corn thrips, as compared with planting along the east–west slopes. The behavioural pattern of the corn thrips in this manner should be investigated further and exploited by farmers as an intervention measure to adopt to reduce the spread of MCMV and eventually of MLND.

The results obtained from the sticky roll study show the potential of blue and yellow sticky rolls trapping greater numbers of thrips and of corn leaf aphids, respectively. This is the first report on sticky roll efficiency in managing and controlling the vectors. Previous studies by Sampson et al. (2013) showed that blue sticky rolls are highly effective in controlling western flower thrips *Frankliniella occidentalis* Pergande, on strawberry. In addition, Idris et al. (2012) and Mwangi

(2015) similarly reported that yellow coloured sticky traps have the potential to reduce the numbers of the black bean aphid, *Aphis fabae*. Moreover, Baoyu et al. (2012) demonstrated the efficiency of yellow sticky traps in controlling the tea aphid *Toxoptera aurantii* Boyer de Fonscolombe. The facility ability of the blue sticky rolls for trapping greater numbers of corn thrips, demonstrated in this study, is similar to that reported by Tang et al. (2016), who found that greater numbers of the thrip *Megalurothrips usitatus* Bagnall were attracted to the blue sticky traps than to the other colours. Green spruce aphids are more attracted to yellow sticky rolls than to other colours, as reported by Nigel et al. (2011). This coincides with results from this study, where greater numbers of corn leaf aphids were attracted to the yellow sticky rolls. It is also important to note that, whether the sticky rolls were installed at germination, or at 1 or 2 weeks after germination, the vector trapping did not significantly vary within the regimes for both the blue and yellow sticky rolls. This indicates that the sticky rolls do not lose their trapping efficiency as time elapses.

The significant ($P \leq 0.05$) and negative correlation between the corn thrips trapped versus MCMV viral load and MLND severity on maize could explain the absence of MCMV from plots protected by blue sticky rolls. It is also possible that most of the corn thrips trapped by the sticky rolls were the infected ones. A vector needs enough time to acquire and semi-persistently transmit the virus from an infected plant to a healthy one (Nelson et al. 2011; Cabanas et al. 2013). These results show that the maize plants under the sticky rolls were protected from mass infestations of the corn thrips and leaf aphids. However, it seems that the blue and yellow sticky rolls prevented inoculated corn thrips and corn leaf aphids from infecting the crop, whether with MCMV or SCMV, by trapping the vectors (Mwangi 2015). The trapped corn thrips and corn leaf aphids showed a significant and negative correlation with MLND severity. This simply means that the possibility of transmission of MCMV by corn thrips and SCMV by corn leaf aphids so as to cause MLND was likely to be lower when both vectors were trapped by sticky rolls in greater numbers. Similarly, the significant and negative correlation between the corn thrips trapped by sticky rolls and those found infesting maize clearly indicates that sticky rolls protected maize from the vector infestation.

11.4 Recommendations

- Blue sticky traps that are oriented at 90° should be used by farmers for monitoring and scouting for corn thrips due to their potential for capturing greater numbers of vectors.
- Blue sticky traps can equally predict the corn leaf aphid infestation levels, similar to the yellow sticky traps.
- Farmers and researchers should sample corn thrips and leaf aphids alongside the rows, to best estimate vector infestation levels in maize.
- Farmers should adopt the use of blue and yellow sticky rolls for mass trapping of corn thrips and leaf aphids, as well as for prevention of MLND.

References

Adams, I. P., Harju, V. A., Hodges, T., Hany, U., Skelton, A., Rai, S. J., & Ngaboyisonga, C. (2014). First report of maize lethal necrosis disease in Rwanda. *New Disease Reports, 29*, 22–22.

Albrecht, M. K. (2010). *An integrated approach to insect management in turf grass: White grubs*. Cooperative Extension Fact sheet FS1009. New Jersey Agricultural Extension Service, Rutgers University. https://njaes.rutgers.edu/FS1009/

Baoyu, H., Qing-He, Z., & John, A. B. (2012). Attraction of the tea aphid, *Toxoptera aurantii*, to combinations of volatiles and colors related to tea plants. *Entomologia Experimentalis et Applicata, 144*, 258–269.

Broughton, S., & Harrison, J. (2012). Evaluation of monitoring methods for thrips and the effect of trap colour and semiochemicals on sticky trap capture of thrips (*Thysanoptera*) and beneficial insects (Syrphidae, Hemerobiidae) in deciduous fruit trees in Western Australia. *Crop Protection, 42*, 156–163. https://doi.org/10.1016/j.cropro.2012.05.004.

Cabanas, D., Watanabe, S., Higashi, C. H. V., & Bressan, A. (2013). Dissecting the mode of maize chlorotic mottle virus transmission (Tombusviridae: Machlomovirus) by *Frankliniella williamsi* (Thysanoptera: Thripidae). *Journal of Economic Entomology, 106*, 16–24.

Chen, A. Y. S., Walker, G. P., Carter, D., & Ng, J. C. K. (2011). A virus capsid component mediates virion retention and transmission by its insect vector. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 16777–16782.

Harbi, A., Elimem, M., & Chermiti, B. (2013). Use of a synthetic Kairomone to control *Frankliniella occidentalis* Pergande (Thysanoptera; Thripidae) in protected pepper crops in Tunisia. *African Journal of Plant Science and Biotechnology, 7*, 42–47.

Ibrahim, N. D., & Adesiyun, A. A. (2009). Effect of age and height of onion (*Allium cepa* L.) plants on infestation of Thrips, *Thrips tabaci* L. Thysanoptera; Thripidae in Sokoo, Nigeria. *African Journal of Agricultural Research, 4*, 76–84.

Idris, A. B., Khalid, S. A. N., & Mohamad-Roff, M. N. (2012). Effectiveness of sticky trap designs and colours in trapping alate whitefly, *Bemisiatabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Pertanika Journal of Tropical Agricultural Science, 35*, 127–134.

Jaetzold, R., & Schmidt, H. (1982). *Farm management handbook of Kenya* (Vol. II, pp. 397–400). Nairobi: Ministry of Agriculture.

Jaetzold, R., Hornetz, B., Shisanya, C. A., & Schmidt, H. (2012). *Farm management handbook of Kenya* (Vol. I–IV). Nairobi: Ministry of Agriculture. (Western, Central, Eastern, Nyanza, Southern Rift Valley, Northern Rift Valley, Coast).

Kaas, J. P. (2005). Vertical distribution of thrips and whitefly in greenhouses and relative efficiency of commercially available sticky traps for population monitoring. *Proceedings of Netherlands Entomological Society Meeting, 16*, 109–115.

Kasina, M. (2009). Within plant distribution and population dynamics of flower thrips (Thysanoptera: Thripidae) infesting French beans (*Phaseolus vulgaris* L.) in Kenya. *Spanish Journal of Agricultural Research, 7*, 652–659.

Louie, R., & Darrah, L. L. (1980). Disease resistance and yield loss to sugarcane mosaic virus in East African-adapted maize. *Crop Science, 20*, 638–640.

Makumbi, D., & Wangai, A. (2013). *Maize lethal necrosis (MLN) disease in Kenya and Tanzania: Facts and actions*. CIMMYT-KARI. http://www.cimmyt.org/en/where-we-work/africa/item/maize-lethal-necrosis-mln-disease-in-kenya-and-tanzania-facts-and-actions

Mutie, S. M., Mati, B., Home, P., Gadain, H., & Gathenya, J. (2006). Evaluating land use change effects on river flow using USGS geospatial stream flow model in Mara river basin, Kenya. In *Proceedings 2nd workshop, 123 EArsel SIG on land use and land cover*. Center for remote sensing of land surfaces, Bonn, pp. 28–30.

Muvea, A. M., Waiganjo, M. M., Kutim, H. L., Osiemo, Z., Nyasani, J. O., & Subramanian, S. (2014). Attraction of pest thrips (Thysanoptera: Thripidae) infesting French beans to coloured sticky traps with Lurem-TR and its utility for monitoring thrips populations. *International Journal of Tropical Insect Science, 34*, 197–206.

Mwangi, N. (2015). *Evaluation of botanical pesticides and colored sticky insect traps for management of insect pests (thrips, white flies and aphids) in french beans (Phaseolus vulgaris)*. MSc thesis, Nairobi University.

Nelson, S., Brewbaker, J., & Hu, J. (2011). Maize chlorotic mottle virus. *Plant Disease, 79*, 1–6.

Nigel, A. S., David, D. W., & Green, J. (2011). Influence of sticky trap color and height above ground on capture of Alate Elatobiumabietinum (Hemiptera: Aphididae) in Sitka spruce plantations. *Environmental Entomology, 40*, 120–125.

Palumbo, C. J. (1998). *Management of aphids and thrips on leafy vegetations vegetable report*. College of Agriculture, The University of Arizona, Tucson, Arizona, 85721. http://ag.arizona.edu/pubs/crops/az1011/az1101_2.html

Pearsall, I. A. (2002). Daily flight activity of the western flower thrips (Thysanoptera: Thripidae) in nectarine orchards in British Columbia, Canada. *Journal of Applied Entomology, 126*, 293–302.

Sampson, C., Kirk, J., & William, D. (2013). Can mass trapping reduce thrips damage and is it economically viable? Management of Western flowerthrips in strawberry. *PLoS One, 8*, 80–87.

Samson, M. M., Dickson, M., & Evans, B. (2014). Impact of maize lethal necrosis disease on maize yield: A case of Kisii, Kenya. *International Journal of Agricultural Extension*. ISSN: 2311-6110 (Online), 2311–8547 (Print). http://www.escijournals.net/IJAE

Singh, M., Ashutosh, S., Upadhyaya, P., & Rao, G. P. (2005). Transmission studies on an Indian isolate of sugarcane mosaic Potyvirus. *Sugar Technology, 7*, 32–38.

Ssemwogerere, C., Nyaburu, M. K., Ssemakula, O., & Karungi, J. (2013). Special composition and occurrence of thrips on tomato and paper as influenced by farmer management practices in Uganda. *Plant Protection Research, 53*, 2.

Tang, L. D., Zhao, H. Y., Fu, B. L., Han, Y., Liu, K., & Wu, J. H. (2016). Colored sticky traps to selectively survey thrips in cowpea ecosystem. *Neotropical Entomology, 45*, 96–101.

Thongjua, T., Thongjua, J., Sriwareen, J., & Khumpairun, J. (2015). Attraction effect of thrips (Thysanoptera: Thripidae) to sticky trap color on orchid greenhouse condition. *Journal of Agricultural Technology, 11*, 2451–2455.

Tian-Ye, C., Chang-Chi, C., Glenn, F., Eric, T., Natwick, T., & Henneberry, J. (2004). Trap evaluations for thrips (Thysanoptera: Thripidae) and hoverflies (Diptera: Syrphidae). *Environmental Entomology, 33*, 1416–1420.

Uzest, M., Gargani, D., Drucker, M., Hebrard, E., Garzo, E., Candresse, T., et al. (2007). A protein key to plant virus transmission at the tip of the insect vector stylet. *Procedure of National Science academy Science, U S A, 104*, 17959–17964.

Wangai, A. W., Redinbaugh, M. G., Kinyua, Z. M., Miano, D. W., Leley, P. K., Kasina, M., et al. (2012). First report of maize chlorotic mottle virus and maize lethal necrosis in Kenya. *Plant Disease, 96*, 1582–1583.

Zayed, S. A., Betha, P. J. G., Katherine, J. F., James, W. D. T., Martyn, W., & Butt, T. M. (2015). A new attractant for monitoring western flower thrips, *Frankliniella occidentalis* in protected crop. *Springerplus, 4*, 89. https://doi.org/10.1186/s40064-015-086.

Chapter 12
Outbreak of Fall Armyworm (*Spodoptera frugiperda*) and Its Impact in Rwanda Agriculture Production

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Abstract Agriculture is the major economic activity of the people of Rwanda, providing employment to about 86% of total population and contributing up to 47% of domestic goods and exports. Actual threats include outbreak of a new invasive insect pest, the fall armyworm *Spodoptera frugiperda*. In this review we describe the fall armyworm outbreak in African countries, particularly in Rwanda. An overview is given on available control options, management of the fall armyworm outbreak, and its implications in Rwanda. The information gathered will assist in controlling fall armyworm in newly invaded regions.

Keywords New invasive insect pest · Maize · Pest

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12.1 Introduction

According to FAO (2003), food security is defined as a "situation that exists when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life". In Rwanda, agriculture plays an important role in the contribution to food security, but it provides insufficient quantities to meet the needs of the country, which leads to the importation of food products to complement local productions. For example, the Rwanda food imports were reported at 18.25% in 2015 (WB 2017). The low production in Rwanda, leading to insufficient food security, is associated with the prevalence of a number of constraints that are abiotic, biotic, management, and socio-economic in nature. According to Reynolds et al. (2015), moisture scarcity, nutrient limitation, and biotic stresses are the major constraints in cereal crops such as wheat and rice. The shortage of quality seeds, drought, soil degradation, and poor soil fertility are the main challenges for maize production. In banana crops, *Xanthomonas* wilt (BXW) and *Fusarium* wilt, also known as Panama disease, are major challenges for production (Nkuba et al. 2015). Biotic stresses and post-harvest losses are the major constraints in root and tubers such as potato, cassava and sweet potato, while viral diseases and shortages of resistant varieties constitute one of the major constraints for cassava production (Nduwumuremyi et al. 2016). The production of many crops in Africa, and particularly in Rwanda, has declined due to pest and disease outbreaks (Goldman 1996; Tadele 2017). Insects are major pests in Africa and they cause crop yield losses, estimated between 30 and 60% (Oerke 2006). Furthermore, viral and bacterial diseases cause considerable damage to crops cultivated in different agro-ecological zones. Currently, cereals in Rwanda are threatened by an outbreak of a new invasive insect pest, the fall armyworm, *Spodoptera frugiperda* (*Lepidoptera*: Noctuidae). This study is aimed at reviewing the life cycle of fall armyworm, its outbreak in African countries and particularly in Rwanda, the available control options, the status of current management, and the implications of its outbreak in the country.

12.2 Review of Fall Armyworm

Spodoptera frugiperda is an important pest of members of family *Poaceae* that include major food crops such as corn, sorghum, rice and wheat, and diverse pasture grasses (Pashley 1996; Prowell et al. 2004). This polyphagous insect is native to and widely distributed in the tropical and subtropical regions of America, and known as an occasional serious pest of small grains and corn (Sparks 1979; Buntin et al. 2001; Murúa et al. 2009; Malo et al. 2013). Its invasion into Africa was reported for the first time in January 2016 in Nigeria. Since then, it has become an epidemic pest in several central and southern African countries (Goergen et al. 2016). Due to the

importance of maize crop in these regions, the fall armyworm has become one of the most serious problems for the continent (Murúa et al. 2009).

In Central and South America, farmers face three or more generations of fall armyworm every year, and occasionally severe outbreaks occur as early as mid-April (Flanders et al. 2011). Every year, fall armyworm moths, carried by air currents, spread into the southern and central parts of the USA. The size and timing of the initial moth flights are two factors that influence the outbreak potential of this pest (Flanders et al. 2011). The infestation outbreak is sudden, because the larger fall armyworms sometimes quickly invade an uninfected area in search of food, once an adjacent field has been defoliated. Large fall armyworms frequently disappear almost as suddenly as they appeared, by either dropping onto the ground to pupate, or moving on in search of food (Flanders et al. 2011). The infestations usually develop first in fields of small grains or other grass-cover crops. In conventional tillage systems, larvae can migrate into crop fields such as corn, wheat and sorghum. The damage is usually first noticeable around the field margins adjacent to these areas (Barfield et al. 1980). The name "armyworm" in fact arose from its behaviour of migrating in large numbers into fields, similar to invading armies (Flanders et al. 2011). Cool and wet weather usually favours fall armyworm development, but they are susceptible to cold, and are unable to survive even the mildest winter (Sparks 1979; Flanders et al. 2011).

12.2.1 Life Cycle of Fall Armyworm

The life cycle of the fall armyworm passes through four main stages (moth, egg, larvae, and pupae). The adult fall armyworm is a dark, brownish-grey, mottled moth with oblique markings near the centre of the front wing, and an irregular white or grey patch, near the wing tips. Female moths are darker than males. The back wing is white in females, with a narrow, opaque brown edge. The wingspan of the fall armyworm is approximately 3–4 cm (Bohmfalk et al. 2011; Flanders et al. 2011). Moths have an average life span of 11–14 days, become active at nightfall, and feed on nectar (Flanders et al. 2011). The female moths lay eggs at night, in masses of up to several hundred eggs, on light-coloured surfaces, such as fence rails, tree trunks, and the undersides of tree leaves. These eggs are light grey and are covered with greyish hair from the female's body. The mass of eggs becomes dark with age. All the eggs within a mass hatch at about the same time, within 3–5 days (Flanders et al. 2011). Patterns of newly hatched worms are commonly hard to differentiate. The newly hatched larva of the fall armyworm is white, with a black head. As feeding progresses, the larva becomes darker. As the larvae mature, they turn greenish-brown, with a white line below the top of the back, usually a brownish-black stripe above the midline, and a pale stripe with reddish-brown traces below. Mature larvae are about 3–4 cm long, with a prominent white inverted "Y" on the front of the head. Fall armyworm larvae can also be distinguished from other armyworms by the presence of black hairs on the body (Bohmfalk et al. 2011; Flanders et al. 2011). The

development from egg to fully-grown larva requires about 14–28 days. At this stage, larvae hole into the soil and form pupae. The moths emerge in about 7–14 days (Flanders et al. 2011). In one growing season, three and more generations of fall armyworms can be produced. Therefore, when eliminating fall army worms from leaves in the plantation, another generation is preparing to emerge from the soil to replace them (Flanders et al. 2011). In regions with insignificant winters, fall armyworms will stagnate as eggs and pupae beneath the soil, to become active as climate gets warmer.

12.2.2 Damages Due to Fall Armyworms

Caterpillars of the fall armyworm harm crops and grasses by chewing plant tissues (Flanders et al. 2011). They are mostly active early in the morning and late in the afternoon, although on taller, unsheared grasses, they can be observed feeding on the foliage during the day. On grazed or recently cut pasture and hayfields, fall armyworm larvae spend the warmer hours of the day deep in the turf (Flanders et al. 2011). The larvae of fall armyworm attack a variety of crops as well as grasses, sometime moving *en masse* as an army on the march, and consuming about everything in their way to new areas (Koehler and Short 1979). This assault is mostly aerial, with the grey moths usually arriving under cover of darkness to lay eggs (Koehler and Short 1979). When armyworms are numerous, small corn plants may be completely eaten and destroyed (Koehler and Short 1979). Fall armyworm damage often seems to appear overnight. This damage is mainly caused by the oldest caterpillars, which eat more than all other ages put together. It has been reported that the damage due to young armyworms is insignificant because they do not eat much. Therefore, an infestation may have been present, but not detected, because of the small size of the caterpillars (Flanders et al. 2011). The damage also varies in appearance and severity, according to the type of grass and management practices. In closely grazed fields, the grass may seem to thin out and develop brown spots, while in hayfields or in pastures where there has been substantial growth accumulation, almost all tender green material may be removed, leaving only tough stems a few centimetres long, protruding from the soil surface (Flanders et al. 2011). Fall armyworm larvae hole into the growing point of plants, such as buds, whorls, and others, destroying the growth potential of plants, or clip the leaves. In maize, they also burrow into the ear and feed on kernels in the same way as the corn earworm (*Helicoverpa zea* Boddie) (Sparks 1979).

Armyworms primarily feed on grasses but, under hunger stress, they will also attack some legumes and other plants (Koehler and Short 1979). It has been shown that although maize is one of the major primary host of fall armyworm, the pest is capable of causing extensive damage to an array of crops, including wheat, rice, sorghum, millets, cotton, rice, groundnut, cowpea, sesame, and cassava (Flanders et al. 2011). The expansion of fall armyworm into important cereals and other major food crops of Africa will negatively impact on the livelihoods and well-being of

millions of smallholder farmers, as maize is the main staple food crop in Eastern, Central and Southern Africa.

New plantations of maize are mainly damaged by caterpillars originating in a nearby small-grain field. The poorly managed small-grain cover crops appear to be a frequent source of caterpillars (Koehler and Short 1979). A newly cultivated maize plantation is rarely damaged by fall armyworms. Outbreaks are only experienced infrequently in cultivated fields that had small-grain cover crops growing in the previous season (Ali et al. 1989; Cruz et al. 1999). On a pasture, however, fall armyworms can reach densities as intense as 1000 caterpillars per square meter. This causes quick and severe damage to the pasture and neighbouring crops (Koehler and Short 1979).

In Northern Argentina, the fall armyworm is the most important pest of maize, causing yield losses ranging from 17 to 72% (Murúa et al. 2009). Damage is caused by the loss of photosynthetic area due to foliar feeding, structural damage due to feeding in the whorl, lodging due to cut stems, and direct damage to grains due to feeding larvae. Severe infestations are uncommon, however, and most plants recover from partial foliar feeding. Under severe infestation, the complete defoliation of maize plant is possible. Damage is most severe when worms cause direct damage to the ear. Under severe infestation, larvae are frequently observed migrating in large numbers to new fields. Late-planted maize and advanced growth stages are more vulnerable to damage. Under severe infestation, yield losses ranging from 25 to 50% have been documented. Fall armyworm densities that are as low as 0.2–0.8 larvae per plant during the late whorl stage may be sufficient to reduce yields by 5–20% (Marenco et al. 1992). These observations of damages attributable to fall armyworms reveal the gravity of its outbreak in African countries.

12.2.3 Outbreak of Fall Armyworms in African Countries and Particularly in Rwanda

The fall armyworm was reported for the first time in January 2016 in Nigeria (Goergen et al. 2016). Afterwards, its epidemic proportions were reported in several African countries, including Togo, Ghana, Zambia, Zimbabwe, South Africa, Malawi, Mozambique, Namibia, Kenya, Rwanda, and Tanzania (Abrahams et al. 2017). There is much speculation as to how the fall armyworm arrived in Africa. Scientists believe that fall armyworms crossed the Atlantic on container ships ferrying grain imports from South America. Biological invasions such as fall armyworm threaten the function of the natural equilibrium, agricultural ecosystems, biodiversity, and food security. Any biological invasive species seriously affects Sub-Saharan Africa because this part of the continent relies mainly on agriculture (Kruger 2017).

The spread of a biological species is limited by barriers such as forests, mountains, and oceans. However, international business and travel has critically facilitated biological invasions in recent years. For example, the larger grain borer,

Prostephanus truncatus Horn, another species native of the Americas, was unintentionally introduced into Tanzania in the 1970s. This insect spread rapidly through infested, imported shipments of maize and dried cassava, and invaded numerous countries since its first introduction into Africa (Kruger 2017). The cassava brown streak Virus (CBSV) was noticed in Rwanda after cuttings were imported in 2007 (unpublished results). Maize leaf necrosis virus disease (MLND) was first detected in 2013 (Adams et al. 2014) on maize plants grown from seed imported by seed companies in Rwanda, after which the disease became epidemic in different Rwandan agro-ecologies (unpublished results).

There is disagreement about how the fall armyworm reached Africa. It was thought that it arrived through food products imported from the Americas. This could happen when insects cross borders with infested plant materials. The possibility of this having happened is very high, because some insect species have been detected several times on shipments destined to Europe (Kruger 2017).

It is also possible that the pest arrived in Africa on wind currents. It is known that the adult fall armyworm month can travel long distances. Moreover, this would not be the first insect species that crossed the Atlantic in this way: the monarch butterfly (*Danaus plexippus* L.) is a well-known species that crossed the Atlantic from America to the United Kingdom (Kruger 2017).

Fall armyworms were first detected in West Africa in 2016, before making their way to southern, eastern and central African countries. According to Goergen et al. (2016), the presence of at least two distinct haplotypes of fall armyworm within samples collected on maize in Nigeria and São Tomé suggests multiple introductions into the African continent. The armyworm is able to expand from its endemic area to other areas more than 2000 km away (Pair et al. 1986). The high-spreading performance of fall armyworm, its large reproductive capacity, absence of dormancy, and wide host-plant range will favour its colonisation of tropical Africa (Johnson 1987; Murúa and Virla 2004). Therefore, there is an urgent need for developing ecologically sustainable, economically profitable, and socially acceptable IPM programmes to mitigate its impact in Africa (Goergen et al. 2016).

The establishment of fall armyworm in African countries will have consequences for their economies, agricultural production and access to foreign markets (Goergen et al. 2016). According to EUROPHYT (2017), there is an increase in the rates of quarantine interceptions of fall armyworm caterpillars on fresh vegetables and living plants found at European entry points. It has been reported that the assessment of the status of *S. frugiperda* in 2015 categorised this pest for A1 quarantine on the list of the European and Mediterranean Plant Protection Organization (EPPO 2017). With its new range in extension, it is anticipated that the fall armyworm will shortly be included in the list of quarantine pests of other regional plant protection organisations (Goergen et al. 2016).

The invasion of fall armyworm into Southern Africa countries and its establishment in maize, sorghum, cotton, some vegetables, and sunflower has been reported. In February 2017, the pest had been recorded in many South Africa provinces, including Limpopo, Northwest, Gauteng, Free State, Mpumalanga, Northern Cape, the Western Cape, KwaZulu-Natal, and the Eastern Cape (CropLife 2017). The

Government of Zambia has already spent 3 million USD in an endeavour to control the fall armyworm that has affected approximately 130,000 hectares of crops (Ogolla et al. 2017). As a result, the high severity of fall armyworm outbreak forced farmers to replant their crops. Around 17,000, 50,000 and 130,000 hectares of crops have been affected by fall armyworm in Malawi, Namibia and Zimbabwe, respectively (FAO 2017). In Uganda, this pest has been confirmed to exist in at least twenty districts, damaging maize, sugarcane and pasture grasses (Halima 2017). The Minister of Agriculture and Animal Resources informed the Cabinet Meeting, held on 5th April 2017, that an outbreak of fall armyworms was then destroying grain crops in Rwanda. This outbreak has, so far, been reported in 108 Sectors in 23 Districts, where the pests infected 15,699 ha of maize and sorghum crops (Mugabo 2017). Because of the complexity of the fall armyworm infestation and gaps in technical abilities, countries are still struggling to assess the damage that has been caused so far. Pest identification services are also inadequate in some of the countries. This has a negative impact in making decisions and recommendations on response actions (FAO 2017).

Outbreaks in African countries of other armyworms, different from fall armyworms, have been identified in previous reports. East Africa countries (Tanzania, Kenya and Ethiopia) faced outbreaks of the African armyworm (*S. exempta* Walker) in 1994, 1996 and 1999. The 1994 armyworm outbreak in Ethiopia was the most serious in the experience of crop protection officials since 1984 (Borton 1999). The control of this outbreak required aid from the governments of Japan, Sweden, Korea, Switzerland, Norway and the Netherlands for covering the costs incurred in acquiring and air lifting pesticides to infected regions (Gary 1994). Large-scale African armyworm outbreaks were reported in both Rwanda and Burundi in April 1999. This outbreak affected about 100,000 hectares of cropland and 400,000 hectares of pasture in Rwanda. In spite of heavy rains and rapid interventions from the Government and FAO, the next generation spread to many new areas (Borton 1999). The African armyworm was again reported in Rwanda in May 2008, but its impact on crop production was insignificant (Nambi 2008). In January 2013, an outbreak of African armyworm was reported in Lesotho. Out of 10 districts, 8 were reported to be infected by African armyworm and about 35,000 hectares, representing 25% of the estimated planted area in 2012–2013, were affected. In this outbreak, the pest density ranged from 50 to 250 larvae per square metre (Noko 2013). These perennial outbreaks of the African armyworms in the same locality lead to a prediction of the outbreak of the new coming pest, the fall armyworms.

12.3 Management Options for Fall Armyworm

Management of the fall armyworm involves the integration of several approaches, including the use of insecticides, host plant resistance, and biological control. All these approaches depend on several characteristics of the agro-ecosystems involved (Altieri et al. 1978). Control is based mainly on the use of chemical insecticides.

However, other approaches, such as biological control, the use of resistant varieties and genetically modified varieties harbouring the Bt gene, and agriculture practices, have been reported as alternatives to insecticide spraying (Murúa et al. 2009). Interventions, based on pest-incidence thresholds, have been suggested to better protect young plants and the reproductive stages of the crop (Altieri et al. 1978).

Control of fall armyworm infestations requires fields to be checked to identify the treatment threshold, prior to the use of insecticidal control. Scouting the crop field or pasture assists in identifying fall armyworm infestations, prior they cause economic damage. A simply detectable sign of fall armyworms is the occurrence of groups of birds, feeding in crop fields or pastures. Through a careful exploration of field or pasture sites where many birds are seen feeding, particularly around areas with dead grass in established pastures, or at the base of the plants in the straw, the presence of the larvae and their excrements, in the form of green pellets, can reveal the first evidence of a fall armyworm infestation (Abrahams et al. 2017). Field scouting during the day should focus on looking for leaf feeding and presence of caterpillars in the whorl, where they hide. Fall armyworm moths are difficult to track because they are active during the night, and can move long distances on seasonal winds. Therefore, the setting up of insect net traps during the early morning or later afternoon has been suggested for use in tracking the presence of fall armyworms (Flanders et al. 2011). The use of pheromone traps, using a synthetic female hormone to attract males of fall armyworm, is an approach for tracking and predicting fall armyworm infestation (Kinyua 2017). The trapped male moths are counted each morning, and 30 or more moths trapped per day indicate that an outbreak will be eminent within 7–10 days, followed by a second-generation outbreak within a month, which can be severe (Capinera 2014). The speed, wide dispersal, and damage of fall armyworm infestation is difficult to imagine, and once established, it is difficult to control because there are sudden and urgent needs to acquire control equipment and pesticides, throughout a very large geographical area.

Moth populations can be sampled with black light traps and pheromone traps, of which the latter are more efficient. Pheromone traps should be suspended at canopy height, preferably in maize during the whorl stage. Catches are not necessarily good indicators of density, but do indicate the presence of moths in an area. Once moths are detected, it is advisable to search for eggs and larvae. A sample of 20 plants in five locations, or 10 plants in 10 locations, is generally considered to be adequate for assessing the proportion of plants infested. However, the numbers of individuals captured are not directly related to damage levels in a field. This is especially true of regional trapping (Capinera 2014). Cruz et al. (2012) found that the pheromone trap approach is the best, compared with others, for deciding on the time for insecticide applications in cases of fall armyworm outbreaks. The use of pheromone traps facilitates the identification of insect performance causing 90% of larvae mortality.

Control efforts are generally not economical to undertake, unless 10% or more of the crop plantation is infested. A number of insecticides can be used as rescue treatments (Gary 1994). The decision to undertake insecticide spraying depends on the developmental stage of the fall armyworms and the intended use of the crop and forage. A population of three or more fall armyworms per one square metre is a

judicious treatment threshold. In the case of pasture or hayfields, mowing is the best option for salvaging a plantation. With this approach, insecticide application is not necessary (Flanders et al. 2011). In the management of pest and diseases, time is very important. If infestations are detected, the damage may already have been done. It is known that small fall armyworms are much easier to eliminate than the larger ones, and that some insecticides will never control large larvae. Therefore, it has been suggested to spray with appropriate insecticides, at the right time (Gary 1994). Without application of insecticides in a maize plantation, a yield reduction up to 39% has been recorded (Cruz et al. 2012).

The chosen insecticide is applied early or late in the day, when fall armyworm larvae are most active (Flanders et al. 2011). Their control in tall or dense stands of pasture grasses may be difficult to achieve. Grazing of an infected area before insecticide spraying has been suggested (Flanders et al. 2011). The pest is more virulent in favourable conditions. Consequently, its control with one type of pesticide is difficult, particularly when it has reached an advanced larval development stage (Gary 1994). A very low density and wide dispersal of armyworms in areas makes spraying operations uneconomical. This requires other approaches, or waiting for a certain degree prior to commencing control with insecticides. The spray of a natural horticultural oil such as neem oil can be applied on plants that show signs of armyworm infestation. This oil showed beneficial combatting effects on various stages of the larvae (Randall and Joey 2016). In some cases, resistance to insecticides may be developed and widely spread in fall armyworm populations, and this complicates their control process. Insects can develop resistance to an insecticide through behavioural, penetration, metabolic and altered target-site capacities. The use of very little amounts of insecticide, a rotation of different chemicals, a mixture of insecticides, and spraying two insecticides in a mosaic have been reported to delay the evolution of insecticide resistance (Mallet 1989). All these practices, preventing the development of resistance to insecticides, should be considered during insecticide application to control fall armyworm. Recommended insecticides for controlling fall armyworm are detailed in Flanders et al. (2011). However, their availability and use depends on the ministerial order establishing the list of registered and prohibited agrochemicals in the respective country.

The date of planting and type of agriculture system used has revealed significant effects in crop and interactions with pests such as leafhoppers and beetles. A reduction of 66% of leafhoppers (*Empoasca kraemeri* Ross & Moore) on beans was observed when maize was planted 30 and 20 days earlier than beans, as compared with simultaneous planting, while the maize damage due to the fall armyworm was reduced up to 88% when beans were planted 20–40 days earlier than maize (Altieri et al. 1978). The adult populations of *E.kraemeri* and of th beetle *Diabrotica balteata* LeConte were 26 and 45%, respectively, fewer in number in the intercrop of bean and maize, as compared with monoculture of these crops. In the same culture, the *S. frugiperda* incidences and infestations in maize was reduced by 14 and 23%, respectively, in polycultures (Altieri et al. 1978). Nevertheless, the regulation mechanisms are not fully understood, some factors which condition a lower pest incidence in polycultures than in monocultures include natural enemies, microclimatic

gradients and chemical interactions. These factors may function together as an associational resistance. The intensive application of intercropping systems by farmers in tropical regions appears to be a suitable pest and diseases management strategy. It is well known that a greater stability in animal populations facilitates the colonisation of complex ecosystems. This suggests that intercropping systems are less vulnerable to insect population outbreaks than monoculture systems (Gold 1994). The current Rwandan policy of land consolidation and crop intensification, under which monoculture is favoured, may have facilitated the outbreak and high incidence of crop diseases and pests. Therefore, this policy should be reviewed, and other, new appropriate measures of disease and pest management should be adopted for achieving sustainable agriculture productions.

Different biological approaches have been reported for fall armyworm control. The success of any of these approaches depends on appropriate biological, ecological, and population studies of the involved species (Miller 1983; Murúa et al. 2009). A natural equilibrium governs the balance between plant pathogens and pests, and their natural predators such as birds, beneficial insects, entomopathogenic nematodes, and larvae of predators. Based on this this principle, the outbreaks of crop pests suggest that the populations of natural predators have decreased. This may be due to the application of the very toxic pesticides to kill crop pests. In this regard, it is advised to avoid the use of harmful pesticides or carrying out practices that would inadvertently destroy the natural predators of pests. Birds are especially fond of the moths, and they will pull larvae out from grasses and plants. Therefore, in case of armyworm infestations, birds should be given a chance to pick off the pupae that are exposed after turning the soil and prior putting it to bed (Veley 1902). Ground beetles (Carabidae), rove beetles (Staphylinidae), ants (Formicidae), and spiders (Araneae) are well known armyworm predators. When these generalist predators were selectively removed from the field, armyworm damage to corn plants was significantly greater than in the control, where predator populations were unaltered (Clark et al. 1999). This revealed the importance of natural predators in the control of plant pests. The beneficial insects, such as *Trichogramma* wasps, lacewing and ladybugs, have the potential to insert their eggs inside the pest body, killing them before they enter the plant-eating larval stage. The use of these parasitoids has shown potentials for controlling armyworm infestation (Clark et al. 1999; Shimat 2006). Entomopathogenic nematodes are killers of armyworm eggs and pupae found in the soil, and can feed on more than 200 pests. The release of these nematodes into soil has been recommended when there was an infestation or where conditions occur that might encourage the development of armyworms (Molina-Ochoa et al. 1999).

The use of live strains of *Bacillus thuringiensis* (Bt) has been shown experimentally to reduce the abundance of fall armyworm larvae in corn. However, its intensive application and success depend on having the product on the foliage when the larvae first appear to feed. Natural strains of *B. thuringiensis* tend not to be very potent, but the use of their genes in genetically modified crops improves their performance (All et al. 1996; Buntin et al. 2001). Several transgenic maize hybrids, such as Bt11 (Novartis Seeds) and MON810 (Monsanto Co.), which express the

insecticidal proteins Cry1Ab from *B. thuringiensis* in vegetative and reproductive structures, have been developed to control European corn borer (Buntin et al. 2001). These transgenic hybrids also revealed the potential to reduce losses due to fall armyworm (Buntin et al. 2001). Thus, cultivars expressing the Cry1F toxin against insect defoliators are currently widely commercialised in the western hemisphere (Goergen et al. 2016). Transgenic maize, containing genes encoding delta-endotoxins from *B. thuringiensis* subsp. *kurstaki*, have been commercialised in the USA and Brazil. Vegetative insecticidal proteins (VIP) have been isolated from *B. thuringiensis* during the vegetative phase of growth that show a wide spectrum of activities against *Spodoptera* spp. (Estruch et al. 1996). In a confined field trial in Kasese by NARO, it was observed that the only maize variety that was resistant to the fall armyworm was Bt maize, which is a genetically modified maize variety harbouring *B. thuringiensis* genes. However, this variety is not available on the market because of the absence of biotechnology and biosafety regulations (Lutaaya 2017). Although these toxins appear to control *Spodoptera* spp., the development of pest resistance is another concern (Moar et al. 1995). For example, Omoto et al. (2016) have reported that the efficacy of Bt maize expressing the Cry1Ab protein in controlling fall armyworm was reduced in Brazil. The release of transgenic Bt-maize in tropical Africa is not as straightforward, due to economic, logistic and socio-cultural considerations (Goergen et al. 2016). Moreover, the increase in the number of reports on the development of fall armyworm resistance to Bt toxins reveals the need to develop alternative control approaches, such as the use of endophytic entomopathogenic fungi, nucleopolyhedro viruses, insect biological control agents, and entomopathogenic nematodes (Storer et al. 2010; Goergen et al. 2016).

The use of resistant crop genotypes is a reliable approach to apply to control pests and diseases. In the study of foliar resistance to fall armyworm in corn germplasm lines that confer resistance to root-and ear-feeding insects, it was shown that there is the possibility of developing foliage, root, and ear-feeding insect-resistant germplasm covering multiple corn growth stages (Ni et al. 2011). According to Wiseman et al. (1980), a breeding programme to develop maize lines that are resistant to fall armyworm has been implemented. Within this project, inbred lines with resistance to fall armyworm have been developed and released. The plants that revealed the most resistance were self-pollinated and were evaluated in successive generations. In this programme, the Antigua Gpo. 2 genotype was used as a source of resistance to fall armyworm. The study aimed at identifying the quantitative trait loci that confer resistance to leaf-feeding damage by fall armyworm and southwestern corn borer. Data revealed that the resistance to fall armyworm and southwestern corn borer involves many of the same QTL, and candidate genes for insect resistance include the *glossy15* candidate locus on chromosome nine (Brooks et al. 2004).

In the screening of 20 maize genotypes, three genotypes that were derived from tropical maize germplasm, originated from Uruguay, Cuba, and Thailand, were identified as the best fall armyworm resistant lines, using the leaf injury ratings and predator survey data. These findings suggested that tropical germplasm is an important source of resistance genes to the fall armyworm (Ni et al. 2014). Genotypes

revealed variations for diseases and pest resistances. For example, Cruz et al. (1999) found that sweetcorn is much more susceptible to fall armyworm larval infestations than normal yellow or white endosperm and high-quality protein maize (Cruz et al. 1999). Efforts to increase the levels of resistance in maize to leaf feeding were initiated by the USDA-Agriculture Research Service team in Mississippi, in the mid'60s. This programme was expanded to include research on resistance to fall armyworm, *S. frugiperda*. Inbred germplasm lines with resistance to corn borer and fall armyworm have been developed and released (Williams and Davis 1989). This showed that plant breeders should consider this trait in the selection process for the development of new cereal crop varieties that are the main target of armyworms. Fall armyworm resistance breeding programmes have developed for field crop varieties with improved resistant traits. One resistance mechanism that appears to be operating in maize is increased leaf toughness, with a thicker epidermis (Mihm et al. 1988; Davis et al. 1995). All these data suggest the possibility of breeding maize to incorporate fall armyworm resistance in Rwanda.

In addition to their role in tracking and predicting fall armyworm infestation, pheromone traps are also used to control fall armyworms because the attraction of males by the traps disrupts the mating process of this pest. Fall armyworm pheromone traps have the potential to suppress moth populations, leading to reductions in eggs laid and in resultant larvae (Kinyua 2017). Malo et al. (2013) found that the pheromone Z9–14:TFMK acted as a pheromone antagonist under field conditions and caused a significant reduction of the electro-antennogram pheromone responses in *S. frugiperda*. It thus behaved as a pheromone antagonist in the field.

This finding suggests that this pheromone analogue may be a good candidate to consider as a mating disrupting technique in future strategies to control *S. frugiperda*. The perception of pheromones by insects is facilitated by olfactory receptor cells that are localised in long sensilla (trichodea) of the male antennae. This pheromone is catabolised by key enzymes such as antennal esterase (Hansson 1995). Therefore, the use of inhibitors of these enzymes in male olfactory tissues, like trifluoromethyl ketones, has shown the positive effects in insect pest control (Prestwich et al. 1986; Plettner and Gries 2010; Malo et al. 2013). Therefore, the efficient development of applicable pheromone traps to control fall armyworm requires an understanding of its olfactory system.

In cases of infestation, the handpicking and elimination of larvae and caterpillars that are feeding on the undersides of leaves and on new growth crop has been advised, prior insecticide spraying (Beseh 2017). A Push–Pull system has been developed by the International Centre of Insect Physiology and Ecology (ICIPE) in Kenya, and is effective in protecting maize from dangerous stem borers and the parasitic witchweed *Striga* (Cook et al. 2007; Tadele 2017). In this system, maize is intercropped with a forage legume called *Desmodium uncinatum* (Jacq.) D.C., whereas Napier grass (*Pennisetum purpureum* Schumach.) is planted around the field. While *Desmodium* produces a smell that drives stem borer adults away ("push"), it also produces a chemical that suppresses *Striga* from attaching to maize roots. The Napier grass instead attracts stem borer adults towards it ("pull"). The adult insects lay their eggs on the Napier grass, and when the eggs hatch, the grass

produces a sticky substance that kills the larvae and young stem borers. The system is also useful in reducing the amount of pesticide application (Cook et al. 2007; Tadele 2017). With the current outbreak of this new pest, fall armyworm in east and Central Africa, this approach has to be explored.

12.3.1 Management of Armyworm in Rwanda

When there is an outbreak of such a pest, the most serious problem is the shortage of appropriate pesticides, safety equipment, and means of transport. Gary (1994) reported that less than 10% of the pesticide applicators had respirators or other safety equipment during the outbreak of African armyworm in Ethiopia. The outbreak in Rwanda of armyworm in the earlier season B (March, 2017) raised the attention of various institutions to the threat of an extension of famine conditions that had been caused by the severe drought conditions that occurred in the growing season A in 2017 (September 2016–January 2017). Government institutions, such as the Rwanda Agriculture and Animal Resources Board (RAB), the Ministry of Agriculture and Animal Resources, and the Ministry of Defence, focused intensively on insecticide spraying and hand-picking of fall armyworms. In addition to these approaches, small-scale farmers tried other approaches, such as the application of mixtures of ash and hot pepper, and the use of cattle urine.

Community works were organised for hand-picking caterpillars of the fall armyworm. This approach can be efficient on a small plot, but the efficacy of this approach is complicated in the situation in Rwanda, where fields are scattered, with a dominance of an intercropping system of maize and beans. According to farmers and extension officers, various pesticides, such as Roket 44/EC, Pyrethrum 5 EW and Pyrethrum EWC, were sprayed to combat the fall armyworm. However, the efficacy of the pesticides was found to vary. At the recommended rate (2 ml/l of water), Pyrethrum 5 EW and Pyrethrum EWC did not provide significant effects in eliminating caterpillars of fall armyworm. The shortage of other pesticide brands and the lack of availability of these pyrethrenoids in Rwandan stock led to increases of the recommended dose, up to 8 ml/litre of water. This dose revealed appreciable toxic effects on fall armyworm, although the accompanying environmental contamination and other unexpected effects were not documented. Until now, Roket 44/EC has been the best pesticide preferred by farmers for controlling fall armyworm due to its quick and efficient killing effects on this pest. This pesticide has profenofos 40% and cypermethrin 4% EC as active ingredients. These two active ingredients have different modes of action; profenofos acts as an acetylcholine esterase inhibitor, while cypermethrin acts as a sodium channel modulator. Roket 44/EC is a non-systemic insecticide, having a contact and stomach action. It is effective against several insect pests, of both chewing and sucking types. Its application depends on the occurrence of insect pests, and application intervals of 10 to 15 days, at a rate of 2 ml/l, were suggested.

12.3.2 Implications of Management Approaches of Fall Armyworm in Rwanda

The outbreak of fall armyworm in Rwanda has negative effects on crop production. It is known that the occurrence of noticeable symptoms of the pest or disease damages in crop plantations means that the yield potential has been affected, in spite of measures taken to control the pest or the disease. Based on yield reductions of 15–73% reported by Malga (2017) and on an area of 16,000 ha of infected maize plantations reported by the Minister of Agriculture and Animal Resources, it is expected that estimated yield losses of between 7500 and 35,000 tons will be suffered, due to fall armyworm. Different approaches have been applied to control the fall armyworm in Rwanda. The Rwanda Agriculture and Animal Resources Board (RAB) conducted a follow-up exercise to eradicate this outbreak. Different pesticides, such as Pyrethrum 5EW, Pyrethrum EWC+, and Roket 44/EC, were sprayed in different doses in the various districts where the pest was identified. This model of spraying different pesticides, without controls, by farmers trying to rescue their maize plantations, could lead to the development of insecticide resistance by the pest. This outbreak of fall armyworm in Rwanda leads to wide spraying of insecticides, which will also cause harmful effects to beneficial insects, such as bees and other natural enemies of crop pests.

Other control approaches, such as hand-picking and the application of pepper and ash, have been applied to control the outbreak of fall armyworm in Rwanda. In the localities where fall armyworm was noticed, community works were organised for hand-picking caterpillars of fall armyworms, which were buried prior insecticide spraying. This approach revealed fruitful results, but its application on large plantations is complicated. Farmers on small maize plots reported that the application of the mixture of ash and pepper provided successful results in controlling the fall armyworm. Although this approach has been commonly used by rural Rwandan farmers to control post-harvest losses of grains, its precise application and recommended doses need further investigation.

The climate and vegetation of Rwanda are favourable for the fall armyworm. In Rwanda, there are no severe winters to reduce the fall armyworm, as there are in its endemic areas of America. The climate of Rwanda is characterised by an alternation of rainy and sunny seasons, with average temperatures varying between 15 and 25 °C. These climatic conditions are appropriate for the proliferation of fall armyworm. According to Flanders et al. (2011), more than 60 plant species have been reported to be hosts of the fall armyworm, including forage grasses, maize, millet, sorghum, rice, wheat, sugar cane, alfalfa, cotton, soybeans, and others vegetable crops. Some of these plants species are found in Rwanda. Therefore, the presence of a favourable climate and many host plant species for the fall armyworm, complicate its eradication in Rwanda.

12.4 Conclusion

The fall armyworm is a serious pest of plant species in the *Poaceae* family, including major food crops such as corn, sorghum, rice and wheat, and diverse pasture grasses. The life cycle of the pest passes through four main stages, from moth, egg, larvae, to pupae. The development from an egg to a fully grown larva takes from 14 to 28 days. In one growing season, three and more generations of fall armyworms can be produced. In regions with insignificant winters, fall armyworms will stagnate as eggs and pupae beneath the soil, while in warm climates, they will remain active. Caterpillars of fall armyworm harm crops and grasses by chewing plant tissues. Observations of this type of damage have revealed the gravity of its outbreak in African countries. Outbreaks of fall armyworm have been reported in Africa from January 2016. Previously, other armyworms (African Armyworm – *S. exempta*), different from the fall armyworms, were reported in 1994, 1996 and 1999 in the East African countries of Tanzania, Kenya and Ethiopia. The endless outbreak of African armyworms in the same locality leads to a prediction of outbreaks of the new coming pest, fall armyworms. The management of fall armyworm by different approaches, based on insecticide spraying, host-plant resistance, and biological control, has been suggested. The application of these approaches in the environmental conditions of Rwanda requires a specific exploration. The fall armyworm outbreak in Rwanda has led to the widespread spraying of insecticides. However, the effects of this spraying on other non target living organisms, such as bees and other natural enemies of crop pests, and on environmental pollution, have not been documented. The presence of a favourable climate and of many species of plants host for fall armyworm could complicate its eradication in Rwanda.

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References

Abrahams, P., Beale, T., Cock, M., Corniani, N., Day, R., Godwin, J., et al. (2017, April). *Fall armyworm status impacts and control options in Africa: Preliminary Evidence Note*. CABI.

Adams, I. P., Harju, V. A., Hodges, T., Hany, U., Skelton, A., Rai, S., et al. (2014). First report of maize lethal necrosis disease in Rwanda. *New Disease Reports, 29*, 22. https://doi.org/10.5197/j.2044-0588.2014.029.022.

Ali, A., Luttrell, R., Pitre, H., & Davis, F. (1989). Distribution of fall armyworm (*Lepidoptera: Noctuidae*) egg masses on cotton. *Environmental Entomology, 18*, 881–885.

All, J., Stancil, J., Johnson, T., & Gouger, R. (1996). Controlling fall armyworm infestations in whorl stage corn with genetically modified *Bacillus thuringiensis* formulations. *The Florida Entomologist, 79*, 311–317.

Altieri, M. A., Francis, C. A., Van Schoonhoven, A., & Doll, J. D. (1978). A review of insect prevalence in maize (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) polycultural systems. *Field Crops Research, 1*, 33–49.

Barfield, C., Stimac, J., & Keller, M. (1980). 1980 Fall armyworm symposium: State-of the art for predicting damaging infestations of fall armyworm. *The Florida Entomologist, 63*, 364–375.

Beseh, P. (2017). *Fall armyworm on maize. Pest management decision guide: Green and yellow list*. Plantwise, Ghana.

Bohmfalk, G. T., Frisbie, R. E., Sterling,W. L., Metzer, R. B., & Knutson, A. E. (2011). *Identification, biology and sampling of cotton insects*. http://www.soilcropandmore.info/crops/CottonInformation/insect/B-933/b-933.htm. Last modified: January 12, 2011 by Billy E. Warrick.

Borton, J. W. (1999). *Armyworms infestations in Ethiopia*. Addis Ababa: UNDP-EU.

Brooks, T. D., Martha, W. C., Williams, W. P., & Buckley, P. M. (2004). Quantitative trait loci conferring resistance to fall armyworm and southwestern corn borer leaf feeding damage. *Crop Science, 45*, 2430–2434.

Buntin, G. D., Lee, R. D., Wilson, D. M., & McPherson, R. M. (2001). Evaluation of yieldgard transgenic resistance for control of fall armyworm and corn earworm (*Lepidoptera*: *Noctuidae*) on corn. *The Florida Entomologist, 4*, 37–42.

Capinera, J. L. (2014). *Fall armyworm,* Spodoptera frugiperda *(J.E. Smith) (Insecta: Lepidoptera: Noctuidae)*. Gainesville: Entomology and Nematology Department, UF/IFAS Extension. http://entomology.ifas.ufl.edu/creatures

Clark, M. S., Luna, J. M., Stone, N. D., & Youngman, R. R. (1999). Generalist predator consumption of armyworm (*Lepidoptera: Noctuidae*) and effect of predator removal on damage in no-till corn. *Environmental Entomology, 23*, 617–622.

Cook, S. M., Khan, Z. R., & Pickett, J. A. (2007). The use of push-pull strategies in integrated pest management. *Annual Review of Entomology, 52*, 375–400.

CropLife. (2017, February 14). *Managing the fall armyworms (faw) outbreak in South Africa*. *A Cropfile South Africa perspective*. Ver. 2.

Cruz, I., Figueiredo, M., Oliveira, A. C., & Vasconcelos, C. A. (1999). Damage of *Spodoptera frugiperda* (Smith) in different maize genotypes cultivated in soil under three levels of aluminium saturation. *International Journal of Pest Management, 45*, 293–296.

Cruz, I., de Lourdes Corrêa Figueiredo, M., da Silva, R. B., da Silva, I. F., deSouza Paula, C., & Foster, J. E. (2012). Using sex pheromone traps in the decision making process for pesticide application against fall armyworm (*Spodoptera frugiperda* [Smith] [*Lepidoptera: Noctuidae*]) larvae in maize. *International Journal of Pest Management, 58*, 83–90.

Davis, F. M., Baker, G. T., & Williams, W. P. (1995). Anatomical characteristics of maize resistant to leaf feeding by southwestern corn borer (*Lepidoptera: Pyralidae*) and fall armyworm (*Lepidoptera: Noctuidae*). *Journal of Agricultural Entomology, 12*, 55–65.

EPPO. (2017). *Reporting service*. Paris: European and Mediterranean Plant Protection Organization.

Estruch, J. J., Warren, G. W., Mullins, M. A., Nye, G. J., Craig, J. A., & Koziel, M. G. (1996). Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proceedings of the National Academy of Sciences, 93*, 5389–5394.

EUROPHYT. (2017). *European union notification system for plant health interceptions* (Number of interceptions with harmful organisms, Vol. 160). https://ec.europa.eu/food/sites/food/files/plant/docs/ph_biosec_europhyt-interceptions-2017-05.pdf

FAO. (2003). *Trade reforms and food security: Conceptualizing the linkages*. Rome: Food and Agriculture Organization.

FAO. (2017). *Southern and Eastern African countries agree on urgent actions to tackle armyworm and other crop pests and animal diseases*. Regional Office for Africa: FAO.

Flanders, K. L., Cobb, P. P., & Ball, D. M. (2011). *Management of fall armyworm in pastures and hayfields*. The Alabama Cooperative Extension System, Alabama A&M and Auburn Universities.

Gary, J. (1994). *Environmental impact assessment of African armyworm control in Ethiopia: An amendment to supplemental environmental assessment of locust/grasshopper control in Ethiopia. African Emergency Locust and Grasshopper Assistance*. Addis Ababa: USAID.

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., & Tamò, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS One, 11*, e0165632. https://doi.org/10.1371/journal.pone.0165632.

Gold, C. (1994). The effects of cropping systems on cassava whiteflies in Colombia: Implications for control of African cassava mosaic virus disease. *African Crop Science Journal, 2*, 423–436.

Goldman, A. (1996). Pest and disease hazards and sustainability in African agriculture. *Experimental Agriculture, 32*, 199–211.

Halima, A. (2017). *Uganda confirms outbreak of fall armyworm in crops*. Kampala, Uganda. http://aa.com.tr/en/africa/

Hansson, B. (1995). Olfaction in lepidoptera. *Experientia, 51*, 1003–1027.

Johnson, S. J. (1987). Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the Western Hemisphere. *International Journal of Tropical Insect Science, 8*, 543–549.

Kinyua, Z. M. (2017). *Interim recommendations for management of fall armyworm in Kenya*. Nairobi: Crop Health Sub-Unit, KALRO.

Koehler, P., & Short, D. (1979). Scientific notes: Fall armyworm in Florida pasturegrass: 1977. *The Florida Entomologist, 62*, 154–155.

Kruger, K. (2017). *Why it's hard to control the fall armyworm in southern Africa*. University of Pretoria. http://theconversation.com/why-its-hard-to-control-the-fall-armyworm-in-southern-africa-72890

Lutaaya, H. (2017). Ugandan maize trial proves successful in controlling the armyworm but farmers cannot yet access the variety due to regulatory roadblocks. *The Sunrise*, March 17, 2017. http://www.sunrise.ug/news/201703/experts-on-pesticide-resistant-caterpillars.html

Malg, I. (2017). *General information note on fall armyworm Spodoptera frugiperda J. E. Smith: A very harmful and polyphagous pest to watch!* AGRHYMET Regional Centre/CILSS.

Mallet, J. (1989). The evolution of insecticide resistance: Have the insects won? *Trees, 4*, 336–340.

Malo, E., Rojas, J., Gago, R., & Guerrero, A. (2013). Inhibition of the responses to sex pheromone of the fall armyworm, *Spodoptera frugiperda*. *Journal of Insect Science*, *13*. Available online: http://www.insectscience.org/13.134

Marenco, R. J., Foster, R. E., & Sanchez, C. A. (1992). Sweet corn response to fall armyworm (*Lepidoptera: Noctuidae*) damage during vegetative growth. *Journal of Economic Entomology, 85*, 1285–1292.

Mihm, J., Smith, M., & Deutsch, J. (1988). Development of open pollinated varieties, non-conventional hybrids and inbred lines of tropical maize with resistance to fall armyworm, *Spodoptera frugiperda* (*Lepidoptera: Noctuidae*), at CIMMYT. *The Florida Entomologist, 5*, 262–268.

Miller, J. (1983). Ecological relationships among parasites and the practice of biological control. *Environmental Entomology, 12*, 620–624.

Moar, W. J., Pusztai-Carey, M., Van Faassen, H., Bosch, D., Frutos, R., Rang, C., et al. (1995). Development of *Bacillus thuringiensis* CryIC Resistance by *Spodoptera exigua* (Hubner) (*Lepidoptera: Noctuidae*). *Applied and Environmental Microbiology, 61*, 2086–2092.

Molina-Ochoa, J., Lezama-Gutierrez, R., Hamm, J. J., Wiseman, B. R., & Lopez-Edwards, M. (1999). Integrated control of fall armyworm (*Lepidoptera: Noctuidae*) using resistant plants and entomopathogenic nematodes (*Rhabditida: Steinernematidae*). *The Florida Entomologist, 82*, 263–271.

Mugabo, S. F. (2017). *Statement on cabinet decisions of 05/04/2017*. Republic of Rwanda. http://gov.rw/newsdetails2/

Murúa, G., & Virla, E. (2004). Population parameters of *Spodoptera frugiperda* (Smith)(Lep.: Noctuidae) fed on corn and two predominant grasses in Tucuman (Argentina). *Acta Zoologica Mexicana, 20*, 199–210.

Murúa, M., Molina-Ochoa, J., & Fidalgo, P. (2009). Natural distribution of parasitoids of larvae of the fall armyworm, *Spodoptera frugiperda*, in Argentina. *Journal of Insect Science (Online), 9*, 1–17.

Nambi, K. I. (2008). Farmer's knowledge: Armyworms invade Kigali. *Newtimes*, May 23, 2008. www.newtimes.co.rw/section/Printer/2008-05-23/3832/

Nduwumuremyi, A., Melis, R., Shanahan, P., & Asiimwe, T. (2016). Participatory appraisal of preferred traits, production constraints and postharvest challenges for cassava farmers in Rwanda. *Food Security, 8*, 375–388. https://doi.org/10.1007/s12571-016-0556-z.

Ni, X., Chen, Y., Hibbard, B. E., Wilson, J. P., Williams, W. P., Buntin, G. D., et al. (2011). Foliar resistance to fall armyworm in corn germplasm lines that confer resistance to root and ear-feeding insects. *The Florida Entomologist, 94*, 971–981.

Ni, X., Xu, W., Blanco, M. H., & Williams, W. P. (2014). Evaluation of fall armyworm resistance in maize germplasm lines using visual leaf injury rating and predator survey. *Insect Science, 21*, 541–555.

Nkuba, J., Tinzaara, W., Night, G., Niko, N., Jogo, W., Ndyetabula, I., et al. (2015). Adverse impact of Banana Xanthomonas Wilt on farmers' livelihoods in Eastern and Central Africa. *African Journal of Plant Science, 9*, 279–286. https://doi.org/10.5897/AJPS2015.1292.

Noko, L. (2013). *Armyworm infestation in Lesotho: FAO in emergency*. Lesotho: FAO.

Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science, 144*, 31–43.

Ogolla, E., Makonde, L., & Rachel, N. (2017). *Fall army worm outbreak, a blow to prospects of recovery for southern Africa*. FAO Regional Office for Africa. http://www.fao.org/africa/news/detail-news/en/c/469532/

Omoto, C., Bernardi, O., Salmeron, E., Sorgatto, R. J., Dourado, P. M., & al, C. A. (2016). Field evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. *Pest Management Science, 72*, 1727–1736.

Pair, S. D., Raulston, J. R., Sparks, A. N., Westbrook, J. K., & Douce, G. K. (1986). Fall armyworm distribution and population dynamics in the southeastern states. *The Florida Entomologist, 69*, 468–487.

Pashley, D. (1996). Host associated genetic differentiation in fall armyworm (*Lepidoptera: Noctuidae*) a sibling species complex. *Annals of the Entomological Society of America, 79*, 898–904.

Plettner, E., & Gries, R. (2010). Agonists and antagonists of antennal responses of gypsy moth (*Lymantria dispar*) to the pheromone (+)-disparlure and other odorants. *Journal of Agricultural and Food Chemistry, 58*, 3708–3719.

Prestwich, G. D., Vogt, R. G., & Riddiford, L. M. (1986). Binding and hydrolysis of radiolabeled pheromone and several analogs by male-specific antennal proteins of the moth *Antheraea polyphemus*. *Journal of Chemical Ecology, 12*, 323–333.

Prowell, D., McMichael, M., & Silvain, J. (2004). Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (*Lepidoptera: Noctuidae*). *Annals of the Entomological Society of America, 97*, 1034–1044.

Randall, P. G., & Joey, W. (2016). *Insect pests of sweet corn*. Horticulture Extension, Clemson University. http://www.clemson.edu/extension/hgic

Reynolds, T. W., Waddington, S. R., Anderson, C. L., Chew, A., True, Z., & Cullen, A. (2015). Environmental impacts and constraints associated with the production of major food crops in Sub-Saharan Africa and South Asia. *Food Security, 7*, 795–822.

Shimat, V. J. (2006). *The potential role of heteropteran predators – Geocoris punctipes (Say), Geocoris uliginosus (Say) (Geocoridae) and Orius insidiosus (Say) (Anthocoridae) in warm – Season turfgrass*. MSc. Thesis, University of Georgia.

Sparks, A. N. (1979). A review of the biology of the fall armyworm. *The Florida Entomologist, 62*, 282–287.

Storer, N., Babcock, J., Schlenz, M., Meade, T., Thompson, G., Bing, J., & Huckaba, R. (2010). Discovery and characterization of field resistance to Bt maize: Spodoptera frugiperda (*Lepidoptera: Noctuidae*) in Puerto Rico. *Journal of Economic Entomology, 103*, 1031–1038.

Tadele, Z. (2017). Raising crop productivity in Africa through intensification. *Agronomy, 7*, 1–30. https://doi.org/10.3390/agronomy7010022. www.mdpi.com/journal/agronomy

Veley, L. J. (1902). Birds attacking butterflies and moths. *Nature, 65*, 392–392.

WB. (2017). *World development indicators 2017*. Washington, DC: World Bank.

Williams, W. P., & Davis, F. M. (1989). *Breeding for resistance in maize to southwestern corn borer and fall armyworm.* Mississippi: USDA, Agricultural Research Service, Crop Science Research Laboratory.

Wiseman, B., Davis, F., & Campbell, J. (1980). 1980 Fall armyworm symposium: Mechanical infestation device used in fall armyworm plant resistance programs. *The Florida Entomologist, 63*, 425–432.

Chapter 13
Farmers' Perceptions and Preferences on Pesticide Use in the Management of Fall Armyworm in Rwanda

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Abstract The fall armyworm (FAW) is one of the most severe pests faced by maize growers in Rwanda. Due to FAW outbreak that affected maize fields in all districts of the country, farmers have sprayed more than eight types of pesticides to control the pest, since its first detection in February 2017. The objective of this study was to evaluate farmers' perceptions and their preference on pesticides to use in the FAW management in Rwanda. The findings show that the status of maize fields was good (for 51.6% of interviewed farmers) after pesticide application. Among the pesticides used, Rocket 44 EC was the most preferred (52.4%) and was considered stronger than other pesticides (56.8%). According to respondents' views, the reason behind the first choice of Rocket 44 EC was its high effectiveness, as compared with other insecticides. However, some farmers preferred to use Cypermethrin (7.2%). Regarding the effectiveness of Pyrethrum 5 EW, 5.6% of farmers found it to be 'excellent', while 30.8% found its effectiveness to be 'poor'. Therefore, only 22.4% of interviewed farmers 'highly recommended' pyrethrum, while 31.2% of them would 'recommend' another pesticide to other farmers. According to the findings, FAW was reduced in numbers due to pesticide application, and most of the interviewed farmers considered Rocket 44 EC as their first choice. The results of this study will give effective guidance on the management of FAW in future agricultural seasons.

Keywords Effectiveness · Maize · Cypermethrin · Rocket 44 EC

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13.1 Introduction

Fall armyworm (*Spodoptera frugiperda*, FAW), is an insect pest that feeds on the leaves and stems of more than 80 crop species, thereby causing significant damages to cultivated cereals of economic importance, such as maize, rice, sorghum, as well as to legumes, vegetable crops and cotton (FAO 2017).

It is estimated that the overall costs of losses of maize, sorghum, rice and sugarcane in Africa are approximately 13,383 million USD. For maize, for example, the yield loss is 13.5 million tonnes, equivalent to 3.058 million USD (Abrahams et al. 2017).

When a FAW outbreak is detected for the first time, immediate measures (recommendations) should be observed. They include: (i) the introduction of awareness-raising campaigns on FAW symptoms, early detection and control; (ii) the national preparation and communication of a list of recommended, regulated pesticides and biopesticides, and their appropriate application methods (CABI 2017).

FAW control requires an integrated pest management approach (CABI 2017). Monitoring for fall armyworm populations can be helpful in assessing population spread and density changes. Biological control, using species of parasitoids that attack the fall armyworm, is another alternative. However, FAW control has usually been accomplished with insecticide application (Hardke et al. 2015). The development of host-plant resistance (Knodel et al. 1995) and the use of sexual pheromone traps are other useful options used to control FAW. Furthermore, also transgenic hybrids have reduced the damage caused by the pest (Michelotto et al. 2017).

The presence of FAW in Rwanda was first reported in February 2017, in the Mushishito marshland, located in the Sectors of Uwinkingi and Kibilizi, in Nyamagabe District (Ministry in Charge of Cabinet Affairs 2017). FAW in Rwanda was also noticed by Ken Wilson (Lancaster University) in early March of 2017 (Abrahams et al. 2017). By the 24th of April 2017, the pest was reported in all districts of the country. In order to control this outbreak, MINAGRI in collaboration with MINALOC, MINADEF, the Rwanda national police, Provinces, Districts, and the population and stakeholders started and participated in a Fall Armyworm control campaign called upon all members of the population and stakeholders to fight the pest (Ministry in Charge of Cabinet Affairs 2017).

Although the above-mentioned government efforts were used towards FAW management, little was known about new outbreaks. Among the many knowledge gaps, was information about maize farmers' perceptions on pesticides use in the management of FAW, which was then unknown.

Therefore, this study has aimed at investigating farmers' perceptions and preferences on pesticides used against FAW in Rwanda.

13.2 Materials and Methods

13.2.1 Study Area

The study was carried out by means of a survey carried out in four districts of the Southern Province – Nyanza, Muhanga, Kamonyi and Nyaruguru Districts (see Fig. 13.1). The reason behind the choice of these districts was that they were most affected during the time of the survey. All districts are located in mid-altitude parts of Rwanda.

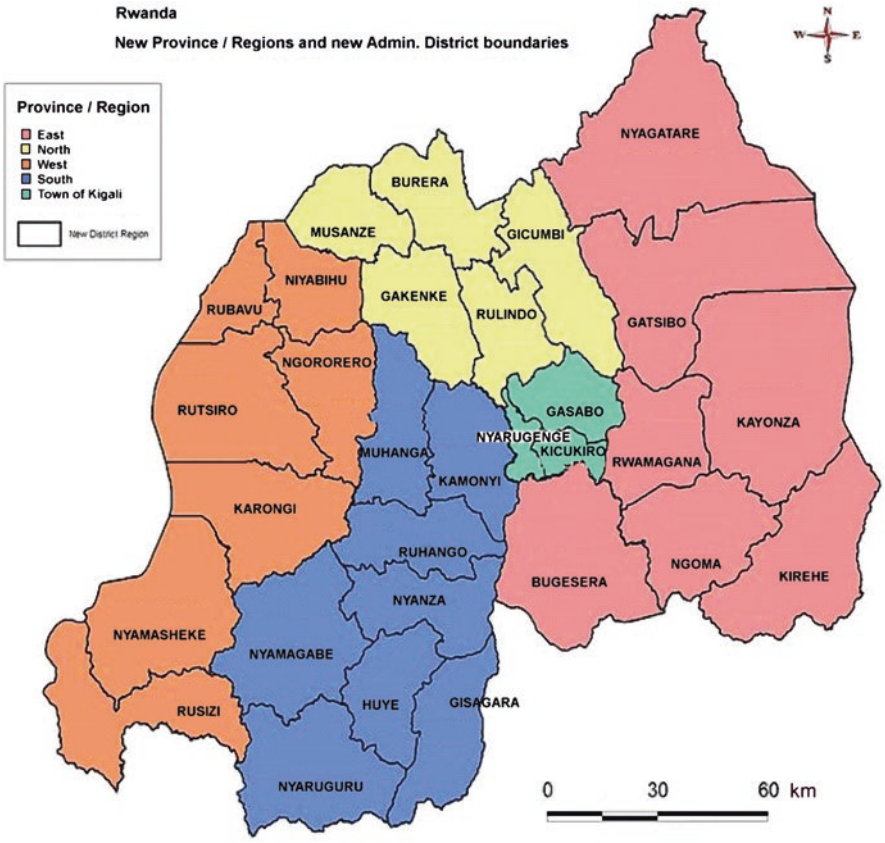

Fig. 13.1 Map of Rwanda showing the study area

Table 13.1 Farmers surveyed per district

District	Male farmers	Female farmers	Total
Kamonyi	18	12	30
Muhanga	35	28	63
Nyanza	21	70	91
Nyaruguru	48	18	66
Total	122	128	250

13.2.2 Sampling Procedures

The sampling unit was the farm household. A total of 250 respondents (including 122 males and 128 females) were randomly sampled in nine villages across the four districts (Table 13.1). The heads of villages were contacted, and they provided a long list of maize producers in their respective villages. From that list, a random selection was used to reach the household head respondents.

13.2.3 Data Collection and Analysis

This study was conducted in mid-May 2017, using a semi-structured questionnaire that targeted maize growers. The collected data from the four districts were entered in Excel sheets and analysed using the SPSS package. Tables and figures were generated to summarise the obtained data.

13.2.4 Information About Some Pesticides Used by Farmers against Fall Armyworm

13.2.4.1 Rocket 44 EC

Rocket 44 EC (Profenofos 40% + Cypermethrin 4% EC) is a broad-spectrum insecticide. It is composed of Profenofos a.i. (40% w/w), Cypermethrin a.i. (4% w/w), Castor oil polyglycol ether, 36/40 grade (9.75% w/w), Dodecyl benzene sulfonic acid, calcium salt (5.25% w/w), Soybean oil epoxydised (1.00% w/w) and Solvent C-IX to make 100.00% w/w.

Rocket 44 EC is manufactured by PI Industries Ltd., 237, GIDC, Panoli, Ankleshwar, Dist.: Bharuch (Gujarat) – India.

13.2.4.2 Pyrethrum 5 EW

Pyrethrum 5 EW is a contact insecticide for use outdoor and protected crops against chewing and sucking pests, including aphids, caterpillars, whitefly, red spider mite, capsids, mirids, cocoa borer and antestia (*Antestiopsis* sp.) bug. Being an advanced oil in water formulation, this organic insecticide contains 5% (W/V) pyrethrins. Pyrethrum 5 EW is manufactured by AgroPy Ltd., based in Musanze, Rwanda.

13.2.4.3 Lambdabex

Lambdabex, of which the active ingredient is lambda-cyhalothrin 2.5% EC, is a synthetic pyrethroid, non-systemic agricultural insecticide, being an acaricide with contact and stomach action, with repellent properties. It has some restriction actions on mites and has an ovicide action on Lepidoptera. Lambda-cyhalothrin 2.5% EC gives a rapid knock-down and has long residual activity, with a long persistence of around 1 month, even in hot conditions. Lamda-cyhalothrin 2.5% EC controls a wide range of agricultural pests, including aphids, Colorado beetles and butterfly larvae. It may be applied on rice, sorghum, maize, wheat, millet, cotton, cowpea, soybean, groundnuts, ornamentals, potato, vegetables and others. It is manufactured by Nanjing Red Sun Co. Ltd., Yaxi Town, Gaochun County, Nanjing, Jiangsu, China.

13.3 Results and Discussion

This study was related to the status of maize fields with regard to FAW control, the frequency of pesticide application, the most preferred pesticides, the recommendations for pyrethrum (by farmer to farmer), the ranking of pyrethrum effectiveness, and the comparison of pyrethrum with other insecticides used during the crop season 2017 B.

13.3.1 Fall Armyworm Status

The status of maize fields, in general, was considered as "good" (51.6%) following pesticide application. Only 2.8% of fields were highly affected. The number of sites that were slightly affected by FAW totalised about 40.4%, showing that, at the time of the survey, there was still a need to continue applying pesticides to fight against FAW caterpillars (Table 13.2).

Although the surveyed districts were the most affected by FAW at the time of survey, all districts of Rwanda were reported to be under attack by the pest in March 2017 (RAB 2017). This implies that further investigations are needed in all districts to fully assess the status of FAW in Rwanda.

13.3.2 Frequency of Pesticide Applications

The number of times that pesticides had been applied varied from 1 to 8 across the districts surveyed (Fig. 13.2). Generally, most of farmers (28.4%) applied the pesticides 3 times per season, while only 18% applied it 5 times. Very few farmers (2.8%) had applied pesticides 8 times.

13.3.3 Most Preferred Pesticide by Farmers

In terms of FAW control at the field level, Rocket 44 EC was the first preferred pesticide (52.4%), followed by Pyrethrum (32%). Only a few farmers (2.8%) preferred the Lambdabex pesticide, as detailed in Table 13.3.

Data Table 13.3 clearly show that, out of all the pesticides, Rocket 44 EC and Pyrethrum were chosen by farmers (in different proportions) in all the surveyed

Table 13.2 Status of fall armyworm

District	Good	Highly affected	Slightly affected	Very good	Total
Kamonyi	24	0	0	6	30
Muhanga	28	0	28	7	63
Nyanza	35	7	49	0	91
Nyaruguru	42	0	24	0	66
Total	129	7	101	13	250
%	51.6	2.8	40.4	5.2	100.0

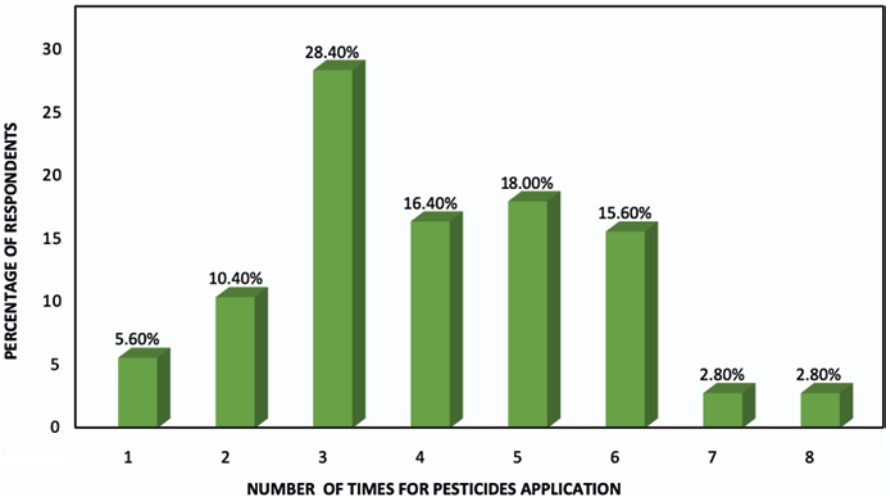

Fig. 13.2 Frequency of pesticide application

Table 13.3 Most preferred pesticide

District	Cypermethrin	Lambda Bex	Pyrethrum	Rocket 44% EC	Unknown	Total
Kamonyi	0	0	6	24	0	30
Muhanga	0	7	42	14	0	63
Nyanza	0	0	14	63	14	91
Nyaruguru	18	0	18	30	0	66
Total	18	7	80	131	14	250
%	7.2	2.8	32.0	52.4	5.6	100.0

Table 13.4 Recommendations of pyrethrum by a farmer to another farmer

District	High	Medium	NA	Would recommend another pesticide	Total
Kamonyi	0	6	0	24	30
Muhanga	42	7	7	7	63
Nyanza	14	21	21	35	91
Nyaruguru	0	54	0	12	66
Total	56	88	28	78	250
%	22.40	35.20	11.20	31.20	100.0

districts. Out of 131 farmers who considered Rocket 44 EC as their first choice, 63 (most of them) were from the Nyanza District, while most farmers who opted for Pyrethrum 5 EW were from the Muhanga District. Cypermethrin and Lambdabex were only chosen by farmers from Nyaruguru (18) and Muhanga (7), respectively.

13.3.4 Farmer to Farmer Recommendations for Pesticide Use

Farmers were further asked if they would recommend the use of Pyrethrum 5 EW to fellow farmers, and to rank the effectiveness of pyrethrum, compared with other pesticides. It was observed that only 22.4% of them 'highly recommended' Pyrethrum 5 EW, while 31.2% would 'recommend' another pesticide to other farmers (Table 13.4).

Whereas a great number of farmers (42) who recommended Pyrethrum 5 EW were from Muhanga district, most of those who would recommend another pesticide were from Nyanza (35) and Kamonyi (24) Districts.

13.3.5 Ranking Effectiveness of Pesticides

Regarding the effectiveness of Pyrethrum 5 EW, 5.6% found it 'excellent', 16.8% saw it as 'very good', 35.6% said it was 'good', and 30.8% found its effectiveness 'poor'.

Table 13.5 Pyrethrum compared with other insecticides

District	NA	Pyrethrum 'better'	'Weak' compared to Rocket 44 EC	Total
Kamonyi	0	6	24	30
Muhanga	7	42	14	63
Nyanza	21	14	56	91
Nyaruguru	0	18	48	66
Total	28	80	142	250
%	11.2	32.0	56.8	100.0

13.3.6 Comparison of Pyrethrum with Other Insecticides Used in FAW Control

During this study, respondents were asked to compare pyrethrum with other pesticides used to control FAW. Whereas 32% of them found Pyrethrum 5 EW 'better' than other insecticides, 56.8% found it 'weak', when compared with Rocket 44 EC (Table 13.5).

The results in Table 13.5 indicate that the majority of farmers (56.8%) consider Rocket 44 EC more effective than Pyrethrum 5 EW. This was confirmed by 56, 48, 24 and 14 farmers from Nyanza, Nyaruguru, Kamonyi, and Muhanga, respectively.

13.3.7 Discussion

This study aimed at evaluating farmers' perceptions and preferences on the pesticides used in the FAW management in Rwanda. Data collected revealed that the status of maize fields was generally considered 'good' after the application of pesticides. Although the surveyed districts were the most affected by FAW at the time of survey, all districts of Rwanda were reported to be under attack by the pest, in March 2017 (RAB 2017). This means that the potential for FAW to cause damage occurs everywhere in Rwanda, with significant potential economic losses, across the country. Further investigations are then needed in all districts to fully assess the status of FAW in Rwanda. However, farmers are advised to regularly monitor their fields (Abrahams et al. 2017) and report any occurrence of FAW caterpillars in their crops.

From the study results it was revealed that most of farmers (28.4%) applied pesticides 3 times per season, while only 2.8% applied them 7 times and more. This inconsistency in terms of frequencies of pesticide applications is probably attributable to a lack of knowledge and/or lack of money to purchase enough pesticides.

Consequently, this situation may lead to the insurgence of pesticide resistance in FAW populations, and consequent inadequacy of pesticides in controlling the pest. Farmers, in any case, had been already advised to spray at least once a week to kill FAW caterpillars.

Regarding the choice of pesticides, the data clearly indicate that most of farmers preferred Rocket 44 EC. The common reason behind this preference was that Roket 44 EC was preferred, being the most effective among the pesticides used. This is probably attributable to the fact that it is a combination of two active ingredients with different modes of action, i.e. profenofos 40% (systemic) and cypermethrin 4% EC (contact). Salato et al. (2017) recommended using chemicals with different modes of action to control FAW. Rocket 44 EC was also the most effective because it killed more caterpillars than the other insecticides used. This result converges well with the results reported by Kajuga et al. (2017), who found Rocket to be more efficient (it caused high percentage mortality, i.e. 62% of FAW caterpillars) than the other insecticides tested in the laboratory and fields. Therefore, due to its effectiveness, Roket 44 EC should be taken into account in future FAW management decisions.

As concerns the Pyrethrum 5 EW recommendations from farmer to farmer, only 22.4% of farmers 'highly recommended' it, while 31.2% would 'recommend' another pesticide. From this result, it is seen that farmers especially from Muhanga and Nyanza Districts, would recommend the use of pyrethrum to control FAW, as they found it to be efficient in their maize fields. According to the information given by farmers, it was realised that the effectiveness of pyrethrum could be linked to the dose (10 ml/l of water), which was far greater than that of other insecticides used (e.g. Rocket: 44 EC at 1.5 ml/l of water). However, other farmers (31.2%) recommended another pesticide against FAW. This suggests that pyrethrum was not effective for them. It is worth noting that FAW may develop resistance to Pyrethrum 5 EW, as reported from the Americas (Abrahams et al. 2017).

In regard to Pyrethrum 5 EW appreciation by farmers, 5.6% found it 'excellent', 16.8% 'very good', and 35.6% 'good', while 30.8% found it 'poorly effective'. The above results indicate that a few farmers would still purchase and use Pyrethrum 5 EW, but others would never use it. Pyrethrum 5 EW, being a contact mode of action, may not kill FAW caterpillars, especially older ones, because these tend to hide in the whorls (Abrahams et al. 2017). Therefore, farmers need to monitor their fields to detect young larvae and spray before they mature.

When Pyrethrum 5 EW was compared with other pesticides, only 32% of respondents found it better, while 56.8% found it weak, arguing that Rocket 44 EC was the best for them. This indicates that most farmers are convinced that Rocket 44 EC is more effective than Pyrethrum 5 EW. This confirms the results of Kajuga et al. (2017) in the laboratory and field.

Finally, the results of this study form a good basis for further studies and provide useful information for planning FAW management strategies in Rwanda.

13.4 Conclusion

FAW infestation is one of the most severe pests of maize in Rwanda. Data from this study showed that most farmers considered the status of maize fields as generally 'good' after pesticide applications. However, farmers are advised to regularly monitor their fields to detect young larvae and spray before they mature. Most of the farmers (28.4%) applied the pesticides 3 times, which is not enough to fight fall armyworm caterpillars, requiring further applications. Concerning pesticide choice, Rocket 44 EC was the most preferred pesticide, because of its high effectiveness, as compared with other insecticides used. Hence, Rocket 44 EC should be considered in further FAW management decisions.

Conflict of Interest This statement is to certify that all authors listed on the title page have contributed significantly to this work. They have read and approved the final manuscript submitted. They warrant that this research has not received prior publication and is not under consideration for publication elsewhere.

References

Abrahams, P., Beale, T., Cock, M., Corniani, N., Day, R., & Godwin, J., et al. (2017). *Fall armyworm status. Impacts and control options in Africa*. Preliminary Evidence Note, April 2017.

CABI. (2017). *Fall armyworm: Impacts and implications in Africa*. Evidence note (summary version), September 2017.

FAO. (2017). *Advisory note on fall armyworm (FAW) in Africa*. Rome: FAO.

Hardke, J. T., Lorenz, G. M., & Leonard, B. R. (2015). Fall armyworm (Lepidoptera: Noctuidae) ecology in Southeastern cotton. *Journal of Integrated Pest Management, 6*, 10. https://doi.org/10.1093/jipm/pmv009.

Kajuga, N. J., Bizimana, J. P., Waweru, W. B., Habinshuti, J. P., & Gasana, A. P. (2017). *Preliminary report on pesticide test to control fall army worm in southern zone*. Rwanda.

Knodel, J. J., Petzoldt, C. H., & Hoffmann, M. P. (1995). *Pheromone traps–effective tools for monitoring lepidopterous insect pests of sweet corn*. Department of Entomology: Cornell University.

Michelotto, M. D., Neto, J. C., Pirotta, M. Z., Duarte, A. P., Soares de Feitas, R., & Finoto, E. L. (2017). Efficacy of transgenic maize insecticide treatment to control fall armyworm in late-season maize in São Paulo state, Brazil. *Ciencia e Agrotecnologia, 41*(2), 128–138.

Ministry in Charge of Cabinet Affairs. (2017). *Statement on Cabinet Decisions of 26/04/2017*. Kigali, Rwanda.

RAB. (2017). *Fall Army Worm Campaign*. http://rab.gov.rw/

Salato, Z., Crozier, J., & Efa, N. (2017). *Fall armyworm (FAW) on maize*. Pest management decision guide: Green and yellow list. Ethiopia: Plantwise.

Chapter 14
Chronological Review of Fruit Fly Research and Management Practices in Sudan

Mohammed E. E. Mahmoud, Abdelgadir M. Abdellah, Mohamedazim I. Basher, Samira A. Mohamed, and Sunday Ekesi

Abstract Fruit flies are the main constraints that limit horticultural production in Sudan. More than 39 fruit fly species have been sampled by the Insect Museum of the Agricultural Research Corporation since the early nineteenth century. Studies from 2008 to 2017 have reported 19 species of economic importance. The situation regarding fruit flies worsened after the invasion of Sudan by the alien invasive species, *Bactrocera dorsalis*, in 2005 and by *B. zonata* in 2012. A competitive displacement was noticed between *B. dorsalis* and the species of the genus *Ceratitis* MacLeay, later also reported between *B. zonata* and *B. dorsalis* on mango and guava, mainly. Several host plants were reported to attract various species of fruit flies. Species of genera *Bactrocera* Macquart, *Ceratitis* MacLeay, and *Dacus* Fabricius responded positively to food bait attractants, mainly Torula and Mazoferm. More than ten local attractants are comparable with standard attractants and are capable of attracting both sexes of different fruit flies species. Male annihilation was the only governmental control option applied, countrywide, to control *B. dorsalis* for 12 years, and lately *B. zonata*, although resistance of the latter species to Malathion (an insecticide applied with Methyl eugenol) has been recently reported. The integration of different control measures, including cultural practices, the use of food-based attractants, the utilisation of biocontrol agents, and the application of post-harvest techniques, are suggested for application in an eco-friendly management approach.

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14.1 Introduction

14.1.1 Importance of Horticultural Sector in Sudan

Sudan is a large country, measuring an estimated 1,765,048 Km^2, with a population of 41,511,526 in 2018 (World Bank 2018). Its arable land is estimated to amount to 83,333.3 million hectares (only 20% of total area) (Mahgoub 2014) . The areas utilised in agriculture are distributed over nine states and are divided into five distinct ecological zones: desert, semi-desert, woodland savannah, flood region, and montane vegetation. Fertile soils, with diverse sources of water – rivers, springs and rains – allow the country to produce various crops. The agricultural sector in Sudan is the most important source of income and livelihoods for 60–80% of the population, and represents 31% of the national income (Elgali 2010; Abdelkareem 2003). The areas used in horticultural production, ca 104,166 hectares, provide 12% of the national agricultural income (Idris 2006; Mahgoub 2014).

Mango, *Mangifera indica* (family Anacardiaceae), is the main fruit in Sudan, and is produced along the banks of the rivers and tributaries in South Kordofan, Khartoum, Kassala, River Nile and Western Darfur States. The area cultivated for mango in Sudan was estimated to be about 2,814,000 ha in 2004, with more than 57 cultivars. The most important exported cultivars are Zibda, Alphons, Malgoba, Hindi, Sinaria, Shendi, Taimoor, Nailam, Mabroka, Dibsha Abu Samaka, and lately, Tommy Atkins. Generally, 60% of grape fruit (*Citrus* × *paradisi*) and 40% of orange (*C. sinensis*), both belonging to the family Rutaceae, are produced mainly in the Kassala, River Nile and Northern States. Orange is also produced in Western Darfur, while banana, *Musa* sp., family *Musaceae*, is produced in Kassala and Blue Nile States, in percentages of 35 and 25%, respectively (Elbashir and Imam 2010).

Guava (*Psidium guajava*, family Myrtaceae), is produced all around the country, in gardens and houses, and in Sudan institutions, all year round (Ali et al. 2014).

Date fruits (*Phoenix dactylifera*, family Arecaceae), are produced in the Northern and River Nile States, of which 75% are dry and 25% are soft dates. The production of vegetables has been estimated to amount to 1.9 million tons, on 186,000 ha (Elbashir and Imam 2010).

The estimated productions of main crops, during the period from 2006 to 2009, are shown in Fig. 14.1, and the average amounts of exported fruits are presented in Fig. 14.2.

The main areas of mango production in Sudan extend along the banks of the River Nile in the Khartoum, River Nile and Northern States. Mango is also grown on a small scale along the banks of the Blue Nile and White Nile Rivers in central Sudan. In Kassala State, fruit trees are grown on the banks of the Gash River and in some parts of South Kordofan, on the banks of the Khor Abuhabil, while another cultivated species of mango is found in Darfur State (UNEP 2005).

Elyas (2008) reported that the fragmentation of lands, lack of financing, low-yield varieties, absence of quality control measures, high cost of transportation, and the incidence of pests and diseases, are the main constraints that hinder horticultural production in Sudan.

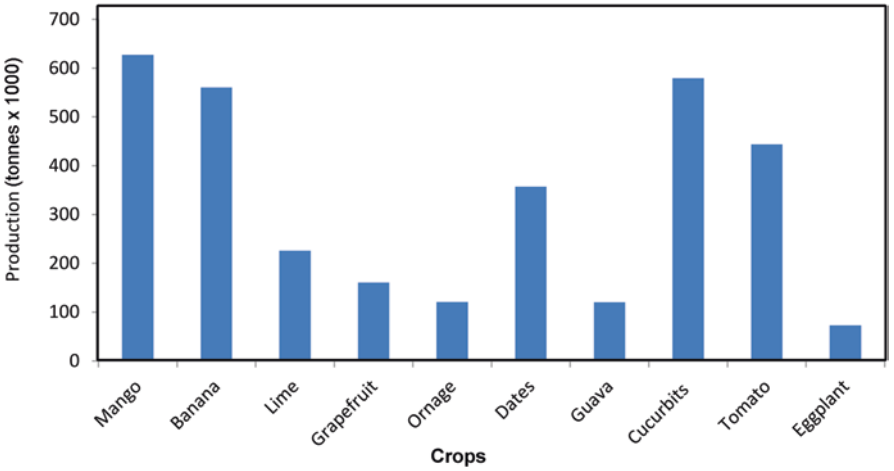

Fig. 14.1 Mean production/ton of certain horticultural crops in Sudan, 2006–2009. (Source: Ministry of Agriculture, Department of Horticulture, Sudan)

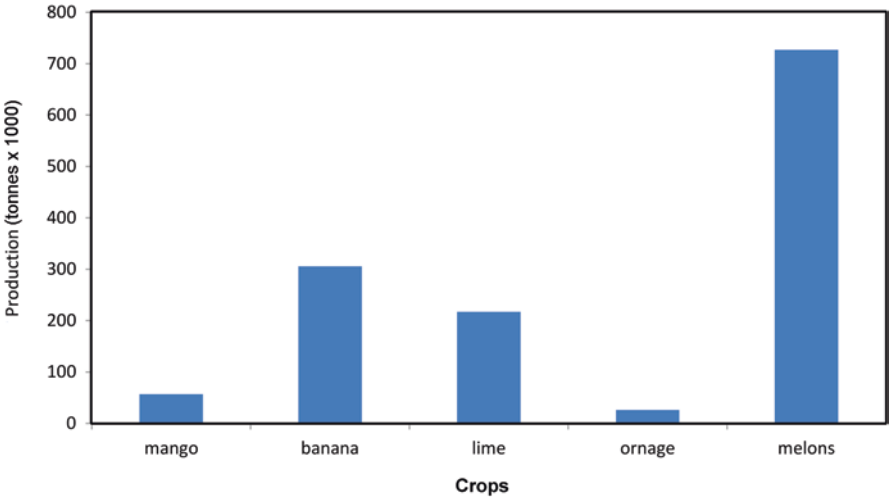

Fig. 14.2 Average amount of Sudan exported fruits (2009). (Source: Ministry of Agriculture, Department of Horticulture, Sudan)

Among pests and diseases, fruit flies are reported as the main threat, causing severe losses to fruit production, which exceed 80% of guava and 30–50% of the Abu Samaka mango variety, from 2005 to 2008 (PPD 2008; Gesmallah et al. 2014). According to El Tahir and Taha Yousif (2004), in addition to the problems already faced by the indigenous fly species in Sudan (*Ceratitis capitata*, *C. cosyra*, *Dacus vertebratus* and *D. ciliatus*), the country is threatened by invasions of exotic species

from across its borders with several neighbouring countries, and by its weak interception and quarantine procedures.

14.1.2 Fruit Fly Species Compositions in Sudan

Fruit flies have been reported in Sudan since the early nineteenth century. Specimens of more than 39 Tephritid fruit flies have been preserved and documented at the Insect Taxonomy Unit of the Agricultural Research Corporation, Wad Medani, Sudan. Of this number, the information for 19 species has been well documented, including their place of collection and host plants.

Venkatarman and El Khidir (1965) and Ali (1967) were the first to report the presence of the Mediterranean fruit fly, *C. capitata*, in Sudan. Schmutterer (1969) reported *C. quinaria* as major pest of grapefruit and *Dacus vertebratus* as a pest of watermelon. *Ceratitis cosyra* has been considered as the predominant species from 1990 to 2008 in mango production areas (Arop 1990; Ahmed 2001; Elhewairs 2003; Mohamed 2003; Abdellah and Mohammed 2010).

Beije et al. (1996) reported that *Zeugodacus cucurbitae*, *D. ciliatus*, *D. vertebratus*, *D. longistylus* on watermelon and melon from Kassala and Gash, with 92% relative abundance for *D. ciliatus*. They also reported Baluchistan fruit fly *Carpomya (Myiopardalis) pardalina* that was reared on musk melon. In addition to the species that attack watermelon, *C. capitata*, *C. quinaria* and *C. cosyra* were the main fruit flies infesting guava and mango in the same study area, causing 11–33% infestation levels during the study period (Beiji 1997).

In 2005, the country witnessed the invasion of numbers of *B. dorsalis*, which were captured in McPhail traps by Abdellah at Shendi, River Nile State, in a collaborative study between the Agricultural Research Corporation (ARC), the Sudan and the International Centre for Insect Physiology and Ecology (ICIPE). This pest changed the situation regarding fruit flies in Sudan and led to an upgrade regarding fruit flies being added to the list of notorious national pests in Sudan in 2008. The same species, except the *Carpomya* (*Myiopardalis) pardalina* Baluchistan fruit fly, were recently reported by Mahmoud et al. (2012a, c) in Kassala, Khartoum, South Kordofan and Gedarif States. They have also been recorded by Fadlelmula et al. (2014) in Blue Nile State, by Gesmallah and Abdellah (2011) in Sennar state, and by Abdel Magid et al. (2012) and Suliman et al. (2014) in River Nile State. Surveys carried out in 2008 in Kassala and South Kordofan, using male attractants, revealed that *C. cosyra* was not in the group of fruit flies found in Kassala State, and that *C. capitata* was not present in South Kordofan State (Mahmoud et al. 2012b, c; Mahmoud 2017).

In 2011, Gesmallah and Abdellah reported the occurrence of *D. punctatifrons* in Sennar State, and recently, the same species was reported from River Nile State (Mahmoud et al. 2015, unpublished).

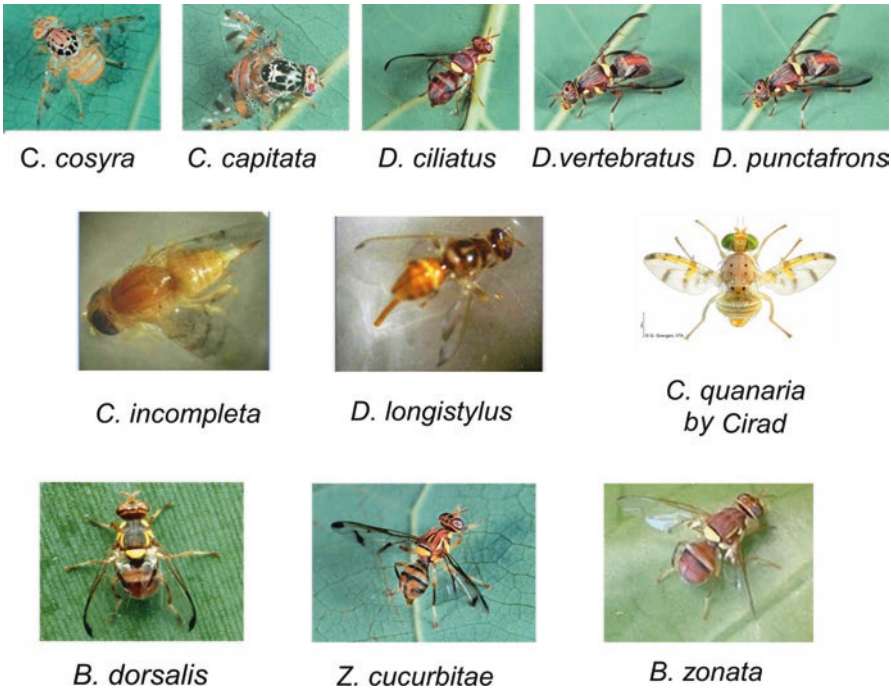

Fig. 14.3 Fruit flies of Sudan

Peach fruit fly, *B. zonata*, was first reported in Gezira and Sennar States by Salah et al. (2012), and later it was recorded in the Northern, River Nile, Khartoum, Gedarif, White Nile, North Kordofan and South Kordofan States (Mahmoud et al. 2015, unpublished). The occurrence of the peach fruit fly in the south and east of Sudan is threatening both Ethiopia and Kenya, as well as other African countries. Figure 14.3 and Table 14.1 show the most important fruit flies found in Sudan.

14.1.3 Fingerprints of Fruit Flies from Sudan

Sequences of genomic DNA of *B. dorsalis*, *C. cosyra*, *C. capitata*, *C. quinaria*, *Zeugodacus cucurbitae*, *D. ciliatus*, *D. vertebratus* and *D. longistylus* were determined using Cytochrome Oxidase (COI) 1, applying 2 sets of primers. The sequences of certain species of fruit flies from Sudan were compared with those for *B. dorsalis* and *C. capitata* deposited in the International Gene bank database (Mahmoud et al. 2012a).

Table 14.1 Tephritids deposited and documented at the Insect Museum Unit of the Agricultural Research Corporation, Wad Medani, Sudan

Genus	Species and authority
Carpomyia	*incompleta* Beck.
Ceratitis	*capitata* Wied.
Pardalaspis	*melanaspis* Bezzi
Elaphromyia	*adatha* Walker
Acanthiophilus	*helianthi* Rossi
Paroxyna	*sororcula* Wied.
Paroxyna	*ignobilis* Loew.
Paroxyna	*praetexta* Lw.
Craspedoxantha	*marginalis* Wd.
Dacus	*vertebratus* Bezzi
Dacus	*ciliatus* Lw. = *brevistylus* Bezzi
Coelotrypes	*vittatus* Bezzi
Dacus	*longistylus* Wied.
Dacus	*marginalis* Bezzi
Dacus	*vertebratus* Bezzi *var*. *marginalis* Bezzi
Dacus	*frontalis* Becker
Myiopardalis	*pardalina* big.
Platensia	*diaphasis* big.
Pterandrus	*anonae* Grah.
Spheniscomyia	*sexmaculata* Macq.
Bactrocera (*Dacus*)	*zonata* Saunders
Bactrocera (*Dacus*).	*oleae* Rossi
Carpomyia (*Pardalaspis*)	*quinaria* Bezzi
Carpophthoromyia	*superba* Bezzi
Celidodacus	*obnubilus* Ksm.
Cheliyophora	*magnicps* Bezzi
Ceratitis (Pardalaspis)	*bipustulata* Bezzi
Euribia.	*tristrigata* Bezzi
Rhacoclaena.	*pulchella* Bezzi
Rhabdochae.	*taneavei* Bezzi
Tephrella.	*cyclopica* Bezzi
Trypanea	*amoena* Frauenfeld
Trypanea	*auger* Frauenfeld
Trypanea	*kingi* Bezzi
Trypanea.	*eluta* Meigen
Leptoxyda	*longistylus* Wd.

CO1F and CO1R (first set of primers) gave around 1300 bps length and 425 kDa of DNA weight, and the lengths of DNA of each species were as follows: 1328, 1334, 1303, 1336, 1328, 1323, and 1332 bp for *C. quinaria*, *C. capitata*, *C. incompleta*, *C. cosyra D. longistylus*, *B. invadens* 1, *B. invadens* 2, *B. invadens* 3, and

D. vertebratus, respectively, while the kDa weights were 428.5; 430.4; 420.1; 430.5; 428.6; 426.7; and 429.4; respectively.

Samples of *B. dorsalis* (1-2-3-4-5), *Z. cucurbitae* and *D. ciliatus* were successfully sequenced using UEA7 and UEA10 primers (2nd set). These species gave around 222.7, 223.1, 223.2, 222.4, 223.5, 222.8, and 221.2 kDa of DNA weight, and 696, 697, 697, 695, 698, 695, and 692 bp length, respectively (Mahmoud et al. 2012c).

14.1.4 Biology of Fruit Flies

In laboratory conditions, at 25 °C and 60% relative humidity, the rearing of *B. zonata* on fruits of guava and mango and an artificial diet resulted in 18.6, 18.7, and 23.6 days, respectively (Mahmoud et al. 2016), while the life cycles of *B. dorsalis*, *C. capitata* and *C. cosyra* were 18.4, 22.3, and 23.3 days, respectively, when reared only on mango fruits (Abdel Magid et al. 2012).

14.1.5 Host Range of Fruit Flies in Sudan

Mango and guava are the main host plants for *C. capitata*, *C. cosyra* and *C. quinaria*, *B. dorsalis*, and *B. zonata*. Grape fruit, orange, mandarin, banana, lemon, annona, papaya, cantaloupe, Brazilia (*Terminalia braziliensis*), and wild strawberry (*Fragaria vesca*) were reported as hosts for *B. dorsalis* (Fadlelmula et al. 2014). Cucumber, water melon, musk melon and wild strawberry were found infested by *D. ciliatus*, *D. vertebratus* and *Z. Cucurbitae*, while Sidir (*Ziziphus spina-christi*) is infested by *Carpomya incompleta*. Indian date, *Z. jujube*, is infested by *B. dorsalis* and *Z. cucurbitae*. Usher, (*Calotropis procera*), is attacked by *D. longistylus* (Abdellah 2010; Mahmoud 2011).

Since its detection in Sudan, *B. zonata* has been reported to attack fruits of mango, guava, grape fruit and orange, while it also lives on ivy fruit (*Coccina grandis*) and the Indian date (Mahmoud et al. 2016). On other hand, *B. zonata* is capable of attacking sweet lemon, banana, cucumber, date palm, tomato, eggplant and sweet pepper, as indicated in 'choice' and 'no choice' tests (Mahmoud et al. 2016; Mahmoud 2017).

14.1.6 Seasonal Abundance

B. dorsalis has become the most abundant species in Sudan since 2005, and has displaced *C. cosyra*, *C. capitata* from mango and guava fruits all around the country (Fadlelmula et al. 2014; Abdel Magid et al. 2012; Abdalla et al. 2014; Gesmallah and Abdellah 2011; Mahmoud et al. 2012a; Mahmoud et al. 2015).

In 2008, *B. dorsalis* was reported as the dominant species, during all the year, and all over the country. Its highest populations were reported during September to December in Khartoum, and from October to November in Kassala, while in South Kordofan, it crested two times, June and August in autumn, and November and December in winter, due to high production of fruits at low temperatures and high relative humidity (Mahmoud et al. 2015).

The highest population of *C. cosyra* was recorded in May, September, October and November in Khartoum State, while the population of *C. capitata* flared up from August to November. In South Kordofan, the population of *Z. cucurbitae* peaked during the period from January to March, which represents the period of maturity of cucurbits.

According to Mahmoud et al. (2015), *B. dorsalis* was the most dominant species all around the country, where it had displaced *C. cosyra* and *C. capitata* in the Kassala, Gezira, White Nile, Blue Nile, South Kordofan, and Sennar States.

In South Kordofan, only *C. quinaria* (0.1%) was found to coexist with *B. dorsalis* (99.9%) on mango and guava (Mahmoud et al. 2012a).

In Khartoum State, few numbers of *C. capitata*, *C. cosyra* and *C. quinaria* (0.01%) were reared from fruits of guava. In the upper parts of Northern State, only *C. cosyra* (0.001%), together with numerous numbers of *B. zonata,* was reared from mango and orange.

Bactrocera zonata peaked two times in Gezira States during 2014 and 2015, coinciding with the fruiting season for mango trees, which bear fruit twice a year in Sudan (Mahmoud et al. 2016).

14.1.7 Competitive Displacement Between Fruit Fly Species in Sudan

During the invasion of *B. dorsalis* in 2005, the populations of *C. capitata*, *C. cosyra* and *C. quinaria* decreased drastically to very low levels, and *B. dorsalis* became dominant in various states. In 2010, the populations of *C. capitata* and *C. cosyra* started to increase again because the heavy mass-trapping operations of the invasive species conducted by Plant Protection Directorate had resulted in reducing the numbers of *B. dorsalis*. In 2012, *B. zonata* invaded some states and started to displace *B. dorsalis* and other *Ceratitis* species from guava and mango trees in several orchards in Khartoum and Gezira States, and has become the main insect pest.

The dominance of *B. zonata* is attributed to the high reproductive characteristics of the species, its ability to infest more than 50% of plant species, and its ability to develop resistance to the extermination action of several insecticides, as reported in Asia, and especially to malathion (Nadeem et al. 2014; Radwan 2012).

14.1.8 Response of Fruit Flies to Food Bait Attractants

Ceratitis capitata, B. dorsalis, C. cosyra, C. quinaria, and *Z. cucurbitae* have responded positively to Nulure, *Torula* yeast, AFFI, GF-120, and Mazoferm (Mahmoud et al. 2012a). The highest number (fruit flies/trap/day) was attained by *Torula* yeast, while the least was attained by AFFI.

Despite the potency of Nulure and Mazoferm for trapping fruit flies, it was found that they also serve as good media for the growth of a layer of fungi that hindered the emission of volatiles and thus reduced their efficiency as baits. Both males and females of the above-mentioned species were attracted to human urine (Mahmoud et al. 2012b), urea, and water extracts of guava, mango, cucumber and apple. Tests with ready-made juice solutions proved the effectiveness of the solutions in attracting the five most important species: *B. zonata* responded to *Torula* yeast, Mazoferm and GF-120, and also positively to water extracts of sorghum, millet and maize (Basher et al. 2017).

The use of Mazoferm in combination with Tracer in traps reduced the population and infestation levels of *B. zonata* in guava orchards to very low levels (2 flies/trap/day), when compared with untreated traps (25 flies/trap/day) (Mahmoud 2017).

14.2 Control Practices in Sudan

14.2.1 Cultural Control

Abdel Magid et al. (2014) have reported that cultural practices carried out at mango orchards, including hoeing, flooding, cleaning and early harvesting before fruit ripening, reduced the infestation levels of fruit flies by more than 70% on mango fruits. In another study, (Mahmoud 2011) it was proved that the removal of infested, fallen fruits reduces the infestation percentages from 92.3% to 7.3%.

Figure 14.4 shows the efforts taken by the Plant Protection Directorate in controlling fruit flies in Sudan, especially *B. dorsalis*, over three consecutive years (2012, to 2014).

Control operations of fruit flies conducted by Plant Protection Directorate incorporated pheromone traps and local food baits (yeast + sugar + pesticides) since 2008, the year of recommendation of using methyl eugenol on mass trapping of fruit flies. Data for years 2012, 2013 and 2014 revealed that the treated area was 14,900.9, 13,000, 9796.7 ha respectively. The distributed traps were 68,514, 35,550 and 36,064 for methyl eugenol and the number of distributed food attractant traps were 13,336, 8800 and 32,370, for the above-mentioned years, respectively (Fig. 14.4).

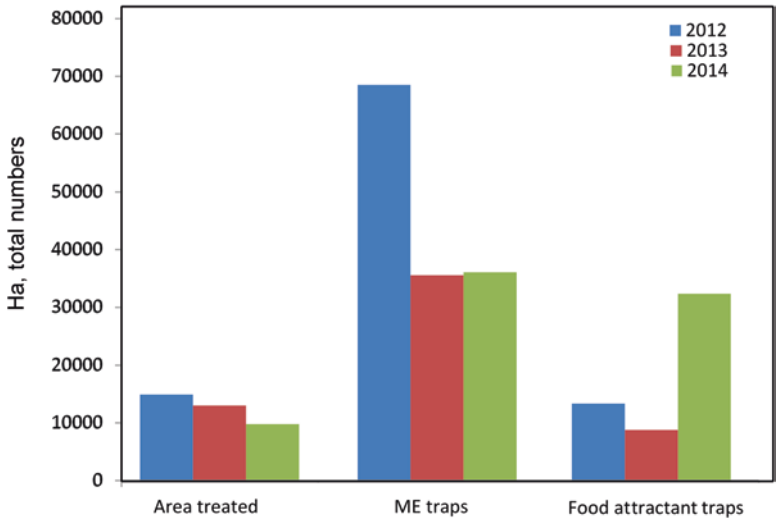

Fig. 14.4 Number of distributed pheromone and local food traps and area/ha in 2012, 2013 and 2014

14.3 Aspects of Biological Control

14.3.1 Parasitoids

Three parasitoids have been reported to attack *B. zonata* reared from guava and mango fruits: *Tetrastichus giffardianus* (Hymenoptera: Eulophidae), *Aganaspis* sp. (Figitidae: Eucoilinae) and *Psyttalia* sp. (Braconidae: Opiinae). *Tetrastichus giffardianus* has been reported as the most efficient parasitoid that naturally controls *B. zonata*, where it is found all year round, with parasitism percentages ranging from 10 to 60% (Mahmoud 2017) (Fig. 14.5).

14.3.2 Entomopathogens

Applications of insect parasitic fungi *Beauveria bassiana* (at 6.5×10^{10} conidia/ml) and *Metarhizium anisopliae* (at 4.3×10^{10} conidia/ml) caused 62.6 and 72.8% mortality to the larval stages of *Z. cucurbitae* and *D. vertebratus*, respectively. They also caused 65.7 and 51.9% mortality to the pupal stage of both species, respectively Musa (2014). Abdellah et al. (2017) stated that adult emergence from the pupae of *B. dorsalis* was inhibited by the tested concentrations of *M. anisopliae*. The inhibition increased with increases in concentration. The LC_{50} and LC_{90} values for *M. anisopliae* were 10×10^6 and 18×10^6 conidia/ml, respectively. The mentioned fungus can be incorporated in fruit fly management strategies.

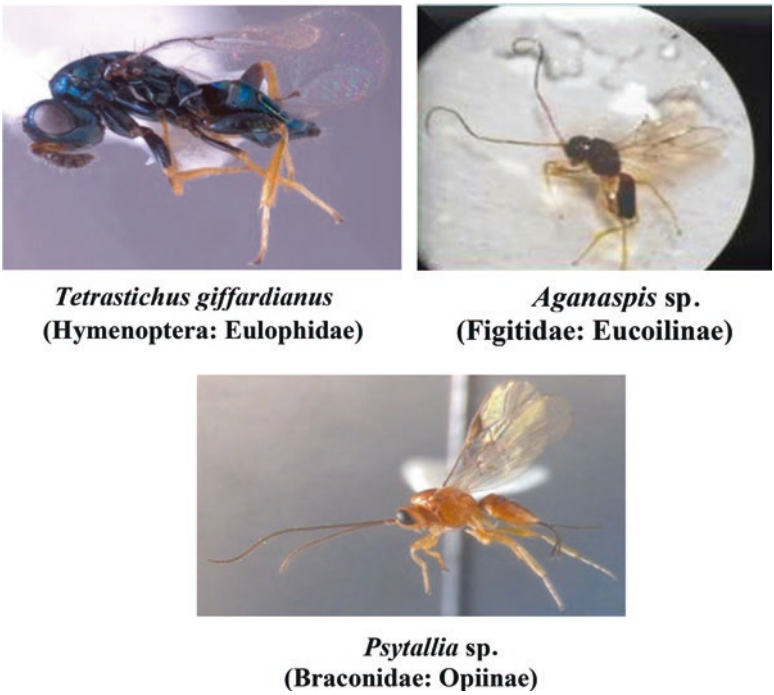

Fig. 14.5 Parasitoids of *Bactrocera zonata*

14.3.3 Botanical Extracts

Neem Azal 15 ppm was found very effective against *Z. cucurbitae* and *D. vertebratus*. Various concentrations of ethanolic extracts of neem and basil caused high mortality to adult flies of *B. dorsalis*, when applied topically and as a food bait (Hussein 2015).

14.4 Chemical Control

Chemical control is practiced by farmers in all states, with different groups of insecticides used, including malathion, cypermethrin and deltamethrin. Suliman et al. (2014) reported that three doses of deltamethrin – 1, 1.5 and 0.25 L/feddan – gave high reductions in populations of *B. dorsalis* when applied using fogging machines.

Gesmallah et al. (2014) reported that chlorpyrifos, imidacloprid and neem seed powder extracts are very effective when applied against the third larval stage of *B. dorsalis* by inhibiting the emergence of adults, when applied on sand under laboratory condition.

14.4.1 Post-Harvest Treatment

Various studies have been conducted to assess the role of heat treatment in controlling the immature stages of fruit flies on fruits (Bashir 2008). The results were promising, and according to that finding, the private sector initiated the Sudanese Centre for Sterilization of Horticultural Exports in 2013. The Centre uses advanced technology that applies vapour heat treatments, according to international standards and regulations.

14.4.2 Role of Extension Services in Dissemination of Knowledge on Fruit Fly Control

Sidahmed et al. (2014), in his assessment of farmers' knowledge of fruit flies and their management, reported that only 12% of interviewed farmers had high experience of fruit flies, 52% had received extension services and information from plant protection officials, and 43% used methyl eugenol to control fruit flies. However, only 17% of respondents applied the correct dose.

14.4.3 Capacity Buildings, Trainings and Public Awareness

Several Training of Trainers programmes (TOT), for staff of Plant Protection Directorate, universities teaching staff, and extension and plant quarantine officers, have been instituted under the auspices of the Plant Protection Directorate and Agricultural Research Corporation. In 2006, the International Centre of Insect Physiology and Ecology held one TOT at the Agricultural Research Corporation, Wad Medani. More than 15 PhD and 25 MSc degree holders from several Sudanese universities discussed various topics that dealt with basic studies and applied control.

Public awareness has been raised through various radio and television programmes, at national and state levels, that give information about the pest and the prospects for the integrated management of fruit flies, as well as the safe use of insecticides.

The awareness of fruit flies and their management reached a peak after the invasion of the alien exotic species had significantly affected the produce and exports, and these species had been placed in the list of phytosanitary dangerous organisms. Awareness was also raised through the proceedings of a national conference on the situation concerning fruit flies and their management, that was held in 2008 in Khartoum.

14.5 Future Studies

To enhance knowledge on fruit flies and promote ecologically sound management options, further studies are most especially required in the:

- Evaluation of the effectiveness of local plant materials as food-based attractants for the mass trapping of fruit flies.
- Evaluation of local diet for mass rearing.
- Evaluation of various materials as repellents to oviposition.
- Determination of the host marking pheromones and assessment of their roles as deterrents to oviposition.
- Evaluation of the phenomenon of competitive displacement between species, the factors governing this phenomenon, and the fate of displaced species.
- Examination of the effects of cross-mating between *B. zonata* and *B. dorsalis* which occurred under laboratory condition, the determination of its side effects on the aggressiveness of the progenies, and the determination of the genetic makeup of the progenies.
- Breaking down of the resistance of *B. zonata* to the malathion used in male annihilation technique.
- Incorporation of indigenous entomopathogens in control programmes.
- Evaluation of the efficacy of indigenous and exotic parasitoids in controlling Tephritid fruit flies.

14.6 Conclusion

The main problem in controlling fruit flies is given by the reliance on one control option, neglecting the role of other options. This dependence is attributed to many factors, such as economic costs, technical difficulties, availability, and certain impracticalities, for various reasons.

The use of one control option might lead to the resurgence of neglected insect pests and promoted them to the class of primary pests, which has actually happened in Sudan. Before the invasion of the country by *B. dorsalis*, the main fruit flies that infested mango and guava were *C. cosyra*, *C. capitata* and *C. quinaria*. Because of the high fecundity and rapid invasion of *B. dorsalis* into different crops and territories, encouraged by the attraction of different host plants, suitable temperature and relative humidity, the alien invasive pest in Sudan has displaced species of genus *Ceratitis* from mango and guava. The population of *Ceratitis* species started diminishing to very few numbers in 2008–2009 (Mahmoud et al. 2015). During that time, fruit flies were declared as national pests and a control campaign was instituted by the Plant Protection Directorate, that recommended the adoption of a lure and kill system using methyl eugenol with malathion in cotton wicks, to control *B. dorsalis*.

The campaign continued for 12 years, and resulted in excellent mass trapping of the species, but also gave the opportunity to the population of *Ceratitis* spp. to build up again. This occurred because it had been left without control, while the Plant Protection Directorate had been concentrated completely on using one method to control only one targeted species. In 2012, *B. zonata* invaded Sudan and started to displace all other species. By 2017 it had displaced *B. dorsalis*, *C. capitata* and *C. cosyra* from fruits of guava in two states. Then, a huge concern developed when it was noted that *B. zonata* generates resistance to malathion, not being killed by this pesticide any more.

As an agricultural country needs to export its production to other countries, it is imperative that measures be taken in Sudan to (i) adopt good practices that reduce reliance on pesticides, thereby achieving the goals of Good Agricultural Practices (GAP), and (ii) reduce the risk of safety hazards in food, as required by the Hazard Analysis and Critical Control Point (*HACCP*) regulations. Moreover, there is a need to adopt effective integrated pest management plans, including cultural, physical, biological options to meet the growing demands of global markets.

References

Abdalla, S. A. I., Mohamed, S. A., Mahmoud, M. E. E., Sabiel, S. A. I., Ali, S., & Ali, A. (2014). Monitoring of Tephritidae of fruit trees and their level of infestation in South Kordofan State, Sudan. *International Journal of Agriculture Innovations and Research 2*, 687–693.

Abdel Magid, F. M. A., Ali, E. E., & Mahmoud, M. E. E. (2012). Life cycle study of *Bactrocera invadens*, *Ceratitis cosyra* and *Ceratitis capitata* (Tephritidae: Diptera) and assessment of their damage to mango, guava and citrus in Shendi Area, Sudan (2012). *Persian Gulf Crop Protection, 1*, 22–27.

Abdel Magid, F. M. A., Mahmoud, M. E. E., Eltigani, E. A., & Amani, M. K. (2014). Cultural practices: The hidden role on management of Tephritid fruit flies (Diptera). In *3rd conference on pests Management in Sudan*, 3–4 February 2014. Sudan: ARC.

Abdelkareem, M. I. (2003). *Report of core assessment mission in Sudan*. Khartoum: United Nations Industrial Development Organization.

Abdellah, A. M., & Mohammed, S. A. (2010). Monitoring and host range of tephritid fruit flies in the River Nile and Gezira states, Sudan. *Sudan Journal of Agricultural Research, 15*, 123–130.

Abdellah, A. M., Hassan, A. E. M., Eisa, A. A., Adam, Y. S., & Dafallah, A. B. (2017). The efficacy of entomopathogenic fungus, *Metarhizium anisopliae* Sorokin (Sudanese strain) on puparia of *Bactrocera dorsalis*. In *22nd meeting and conference of the African Association of Insect Scientists*, 23–26 October 2017. ARC, Wad Medani, Sudan.

Ahmed, E. E. (2001). *Studies on mango fruit fly, Ceratitis cosyra in Central Sudan*. M.Sc. Thesis, National Institute of Promotion of Horticultural Exports, University of Gezira, Sudan.

Ali, D. O. M, Ahmed, A. R., & Babikir, E. B. (2014, August 31). Suitability of Local Sudanese Guava (Psidium guajava L.) Cultivars for concentrates production. *Journal of Agri-Food and Applied Sciences, 2*(8), 225–229. Available online at jaas.blue-ap.org ©2014 JAAS Journal. E-ISSN: 2311-6730.

Ali. E. E. (1967). *Studies on some Trypetidae of economic importance in the Sudan*. M.Sc. Thesis, Faculty of Agriculture, University of Khartoum, Sudan.

Arop, L. D. (1990). *Studies on the behavior, host range, seasonal abundance and chemical control of the medfly, Ceratitis capitata (Weidmann) in Sudan*. M.Sc. Thesis, University of Khartoum.

Bashir, Y. G. E. (2008). *Studies on ecology and control of the mango fruit fly, Ceratitis cosyra (W) (Diptera) on mango in North Kordofan State, Sudan*. Ph.D. Thesis, University of Gezira, Sudan.

Basher, M. I., Abdellah, A. G. M., & Mahmoud, M. E. E. (2017). Field attraction of Tephritid fruit flies to aqueous extracts of maize, sorghum and millet. In *22nd meeting and conference of the African Association of Insect Scientists*, 23–26 October 2017. ARC, Wad Medani, Sudan.

Beiji, C. M. (1997). *Report on field trip to Kassala and Gash Delta*. FAO IPM Project GCP/SUD/025/NET, ARC, Wad Medani, Sudan.

Beije, C. M., Bakheit, S. B., Ehassan, A., & Mohamed, H. O. (1996). *Preliminary observations on fruit fly in the Gash delta and Gezira in integrated pest management in vegetables, wheat and cotton in the Sudan - A participatory approach*. FAO IPM Project GCP/SUD/025/NET, ARC, Wad Medani, Sudan: ICIPE, Science Press.

El Tahir, I. M., & Taha Yousif, M. (2004). Indigenous melons (*Cucumis melo* L.) in Sudan: a review of their genetic resources and prospects for use as sources of disease and insect resistance. *Plant Genetic Resources Newsletter, 138*, 36–42.

Elbashir, H. A., & Imam, A. M. (2010). Status report on fruits and vegetables production and processing industry in Sudan. In *AARDO Workshop on technology on reducing post-harvest losses and maintain quality of fruit and vegetables* (pp. 186–179).

Elgali, M. B. (2010). Development of the agricultural crops trade sector of Sudan under the increasing world food prices. In *AAAE third conference/AEASA 48th conference*, Cape Town.

Elhewaris, O. M. (2003). *Studies on the biology and ecology of Ceratitis cosyra (Diptera: Tephritidae) on mango fruits in Sinnar State*. Sudan. M.Sc. Thesis, Faculty of Agriculture, University of Sinnar, Sudan.

Elyas, A. (2008). *The problem of exported crops in Sudan*. General Department of Standards Metrology, Sudanese standards and Metrology Organization, Ministry of Ministries.

Fadlelmula, A., Ali, A., & Breima, E. (2014). Geographical distribution, host range and seasonal abundance of fruit fly species (Diptera: Tephritidae) in the Blue Nile state, Sudan. In *3rd conference on Pests Management in Sudan*, 3–4 February 2014. ARC, Sudan.

Gesmallah, A. E., & Abdellah, A. M. (2011). Species complex of fruit flies at Abu Naama area and identification of *Dacus punctafrons*. *Gezira Journal of Agricultural Science, 9*.

Gesmallah, A. E. B., Hassan, N., & Assad, Y. A. (2014). Identification, damage, host range, seasonal abundance and chemical control of mango fruit fly species (Diptera: Tephritidae) in Sennar State, Sudan. In *3rd conference on Pests Management in Sudan*, 3–4 February, 2014. ARC, Sudan.

Hussein, W. A.. (2015). *The effect of Bee glue powder and Ethanolic extracts of Neem and Basil on mortality of the Asian Fruit Fly (Bactrocerainvadens, Drew and White) (Diptera: Tephritidae)*. M.Sc. thesis, College of Agricultural Studies, Sudan University of Science and Technology.

Idris, B. (2006). *Horticultural export, technical report*. Faculty of Agriculture: University of Khartoum.

Mahgoub, F. (2014). Current status of agriculture and future challenge in Sudan. *Current African Issues, 57*. ISSN 0280-2171. ISBN 978-91-7106-748-7.

Mahmoud, M. E. E. (2011). Population dynamics, mass trapping and molecular characterization of major fruit flies (Diptera: Tephritidae) in Khartoum, Kassala and South Kordofan States (Sudan). Ph.D. Thesis, University of Khartoum, Sudan.

Mahmoud, M. E. E. (2017). Tephritid fruit flies in Sudan: Species composition, distribution, abundance, host plants and prospects of management. In *Book of abstract of 22nd meeting and conference of the African Association of Insect Scientists*, 23 – 26 October 2017. Wad Medani: ARC.

Mahmoud, M. E. E., Kambal, M. A. O., & Abukashwa, S. M. A. (2012a). Prospects of using protein hydrolastes for trapping and monitoring major fruit flies (Tephritidae: Diptera) in Sudan. *Persian Gulf Crop Protection Journal, 1*, 6–14.

Mahmoud, M. E. E, Kambal, M. A. O., & Abukashawa, S. M. A. (2012b). Potential use of human urine as attractant to Tephritid fruit flies in Sudan. *Persian Gulf Crops Protection Journal, 1*, 1–6.

Mahmoud, M. E. E., Binti, A. N. A., Abukashwa, S. M. A., & Kambal, M. A. O. (2012c). DNA fingerprints of major fruit flies (Tephritidae: Diptera) of Sudan. In 86th meeting of the National Pests and Diseases Committee. ARC, Sudan.

Mahmoud, M. E. E, Kambal, M. A. O., Abukashawa, S. M. A., Mohamed, S. A., & Ekesi, S. (2015). Seasonal abundance of major fruit flies Diptera: Tephritidae in Khartoum, Kassala and South Kordofan States, Sudan. *Sudan Journal of Agricultural Reasearch, 26*, 79–90.

Mahmoud, M. E., Mohamed, S. A., Khamis, F. M., & Ekesi, S. (2016). Spatial and temporal distribution, relative abundance and host range of Bactrocera zonata in Sudan. In *3rd International Symposium of TEAM (Tephritid Workers of Europe, Africa and the Middle East) in Stellenbosch (South Africa)*.

Mohamed, S. A. (2003). *Biology, host and host plant relationships of two Psyttalia species (Hymenoptera, Braconidae), parasitoids of fruit flies (Diptera: Tephritidae) in Kenya*. Sudan: University of Gezira.

Musa, I. O. (2014). Control of cucurbits fruit fly using entomopathogenic fungi, *Beauvaria bassiana* (Bals), *Metarhizium anisopliae* (Met) and the botanical insecticide Neem Azal. In *3rd conference on Pests Management in Sudan*, 3–4 February 2014. ARC, Sudan.

Nadeem, M. K., Ahmed, S., Nadeem, S., Ishfaq, M., & Fiaz, M. (2014). Assessment of insecticides resistance in field population of *Bactrocera zonata* (saunders) (Diptera: Tephritidae). *Journal of Animal and Plant Sciences, 24*, 172–178.

Plant Protection Directorate (PPD). (2008). *Ministry of Agriculture, Sudan*. Annual Report.

Radwan, E. M. M. (2012). Malathion resistance and acetylcholinesterase enzyme changes in field population of the peach fruit fly, *Bactrocera zonata* (Saunders). *Journal of American Sciences, 8*.

Salah, F. E. E., Abdelgader, H., & De Villiers, M. (2012). The occurrence of thepeach fruit fly, Bactrocera zonata (Saunders) (Tephritidae) in Sudan. In *TEAM 2nd international meeting: Biological invasions of tephritidae tvephritidae: Ecological and economic impacts* (pp. 3–6). Greece: Kolymbari, Crete.

Schmutterer, H. (1969). *Pests of crops in Northeast and Central Africa*. Gustav Fischer Verlag. Stuttgart: Portland-USA Universitats druckerei Mainz GmbH.

Sidahmed, O. A., Abdalla, I., Allagabo, A. H., & Taha, A. K. (2014). Farmer knowledge about fruit flies problem and their control in guava and mango orchards in three states in Sudan. In *3rd conference on Pests Management in Sudan*, 3–4 February 2014. ARC, Sudan.

Suliman, A. I., Bashir, N. H., & Assad, Y. O. (2014). *Bactrocera invadens* (Drew, Tsuruta and White) (Diptera: Tephritidae), identification, population incidence and control in Wad Medani locality, Gezira State, The Sudan. In *3rd conference on Pests Management in Sudan*, 3–4 February, 2014. ARC, Sudan.

UNEP. (2005). *United and Action Plan – Sudan (Part II, English version), Nations Environment Programme*. Convention on Biological Diversity (CBD), Strategy, pp 16–17. http://www.cbd.int/countries/?country=sd

Venkatraman, T. V., & El Khidir, E. I. (1965). *Outbreak and new records (Sudan)*. FAO Plant Protection Bullettin, p. 22.

World Bank. (2018). https://data.worldbank.org/country/sudan

Chapter 15
Integrated Pest Management for Control the Date Palm Green Pit Scale Insect *Palmapsis phoenicis* Ra (Homoptera: Asterolecaniidae) in Sudan

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Abstract Date palm (*Phoenix dactylifera* L.) is an economic and food security crop in Sudan. Estimated annual date production from 8 million date palm trees is about 431,000 mt, which is far below the country's potential. Sudan has been famous in the world for its production of dry dates. Six good local commercial cultivars are available, and research is coming up with better compositions of cultivars by local selection and foreign introduction from tissue laboratories. Sudan is still free from the devastating red palm weevil and the destructive bayoud caused by *Fusarium oxysporum* f.sp. *albedinis.* The green date palm scale insect is an exotic pest, which appeared in the Golid area (1974). In other palm groves of the world it is considered of minor or no importance, but it developed in Sudan in a very explosive way. The total number of infested trees is 1,200,000. In the past, and due to a lack of indigenous knowledge of appropriate control measures to control the insect, the control efforts adopted in Sudan, basd on foliar applications of contact insecticides and mineral oils, were not successful (1991–1992). Hence, the level of infestation has steadily increased. Following intensive research, an Integrated Pest Management (IPM) approach began by adopting the use of cultural practices or sanitary measures, which were supplemented with chemical controls, using systemic neonicotinoid insecticides (thiamethoxam and imidacloprid), such as Confidor 200 SL and Actara 25 WG, and augmented by utilising the impact of natural enemies of the pest, together with plant quarantine legislation. A comprehensive programme by the Plant Protection Directorate (PPD) has been conducted in infested areas. The sustainable biological control of the green pit scale is vitally important. Results of surveys have revealed that many natural enemies associated with the insect have been recorded, with the Nitidulidae beetle predators found in all surveyed areas. The beetle *Cypocephalus dudichi* L., the lady bird *Pharoscymnus numidicus*, and *Chrysoperla* sp., with the parasitoid *Metaphycus* sp. are mostly found in association with green pit scale insect.

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Keywords Date palm · Green pit scale insect · Systemic insecticides · Biological control

15.1 Introduction

The date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops, providing a primary source of food and a product for commerce, in the great desert areas from Western North Africa to India, and many other subtropical areas. The tree is drought and salt tolerant, and its tasty fruits have high nutritional value and good storage properties. Date fruits constitute the most important agricultural crop in the area and provide highly nutritious food as well as a primary source of income to the majority of the inhabitants. The date palm tree is cultivated in the Northern Sudan along the banks of the Nile, for about 900 kilometres. The total number of trees is about 8 million. According to FAO (2005), the mean annual production of dates is 328.2 metric tons. This ranks Sudan as the seventh largest producer of dates among Arab countries.

However, the date palm industry is facing many serious problems, related to low yields, lack of appropriate packing and presentation, as well as limited processing of date products. The low yields in most countries, including Sudan, are due to soil salinity, poor fertility, insect pests and diseases, lack of maintenance and care due to increasing cost of labour and to shortage of personnel trained in improved cultural practices. As a result of the high cost of production and low prices of the produce, farmers tend to neglect or even abandon their orchards. Although commonly known insect pests like the red weevil, and diseases like bayoud, have not been reported yet in Sudan, the yield of the date palm is affected by many biotic factors among which insects are the most important.

In Sudan, the green date palm pit scale insect *Palmapsis phoenicis* Bodenheimer (*Asterolecanium phoenicis* Rao), is considered the key pest. This genus, a native of central Asia (Iran), was not known in Sudan before 1989, when it was firstly reported by Ali (1989) in El Golid area, as a result of the introduction of some offshoots from Saudi Arabia in 1974. Later, the pest crossed the natural barrier of the Baja desert to invade Elgaba Scheme (150 km south of Dongola, 400 km north of Khartoum) and has become a real threat to date palm cultivation in Northern Sudan. The infested area in El Golid, Elgaba and Old Dongola comprises about 5000 hectares, extending over 60 and 50 kilometres along the west and east banks of the River Nile, respectively. The newly reported infestation in Artigasha Island, Burgag Scheme and Orbi in Dongola area, Abuhamad in the River Nile State and Khartoum State, provides evidence that the pest may continue to spread.

15.2 The Date Green Pit Scale Insect, *Palmapsis phoenicis*

The green scale of the date palm was originally described in the genus *Asterolecanium* (Asterolecaniidae: pit scale insects) in 1922 by Ramachandra Rao, but was designated as type-species of the genus *Palmapsis* by Bodenheimer (1944). *Palmapsis phoenicis* can be a serious pest for date palms, causing yellowing and dieback of the leaf pinnate, scarring and reduction in value of the fruits.

15.2.1 Signs and Symptoms of Damage

The insect attacks the leaflets, leaf rachis and fruits, thus preventing respiration and photosynthesis (Fig. 15.1). It feeds on the leaflets and fruits of the date palms, covering the date clusters and shoots, causing chlorosis, degeneration of leaves, and malformation of fruits before maturity, leading to losses in production ranging from 30 to 50 kg per tree (Ali and El-Nasr 1992). The losses may range between 85 and 90% according to the infestation rate, variety infested, and management conditions (Ahmed 2001, 2004).

Palmapsis phoenicis, like all pit scales, does not produce honeydew. A significant increase in the dry weight/fresh weight ratio was observed in the infested trees. Total and reducing sugars are significantly higher in infected leaflets than in healthy ones. Phenolic compounds increased significantly in the trees infested by green scale insects. Elemental contents of total N, Ca, K, Cu, Mn and Cd were different in diseased and healthy leaflets, but P, Mg, Zn, Fe, Se and B contents were unchanged (Al-Whaibi 1997).

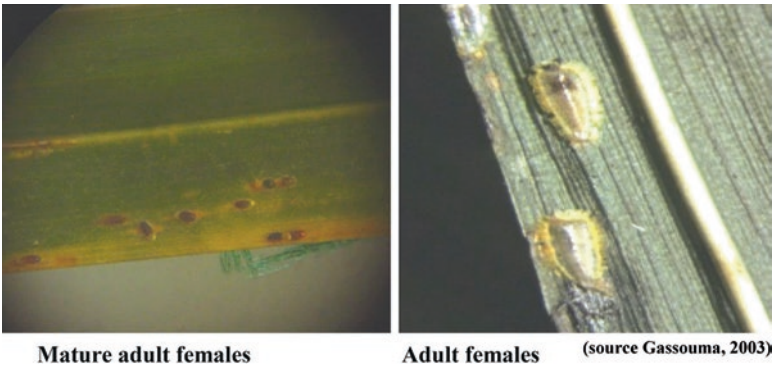

Fig. 15.1 The date palm green pit scale *Palmapsis phoenicis* Ra (Homoptera: Asterolecaniidae)

15.2.2 Classification

The classification of the date palm pit scale insect was in a state of confusion prior to the revision of the genus *Asterolecanium* by Green (1922) as *Asterolecanium phoenicis*. However, as Mani (1982) reported, Russell (1941) had identified the species as belonging to the genus *Asterolecanium* (family Asterolecaniidae), which is characterised with paired wax glands, arranged in rows.

The species was also reported by Bodenheimer (1944) to belong to the genus *Palmapsis*, but he clearly stated the closeness of *Palmapsis* to the *Asterolecanium* group of Russell (1941). Morrison and Morrison (1966) and Bendov and Harpaz (1985) agreed with the findings of Russell (1941). However, Gharib (1974) reported both *Palmapsis phoenicis* Rao, and *Asterolecanium phoenicis* Rao, as synonyms under the family Asterolecaniidae.

Gassouma (2003) reported that the green scale of date palm was originally described in the genus *Asterolecanium* (Asterolecaniidae: pit scale insects). It was designated the type-species of the genus *Palmapsis*by Bodenheimer (1944).

15.2.3 Morphology

Armoured scales belong to the family Diaspididae. They are spherical in shape, less than 3.1 mm in diameter, and have a plate-like cover, that usually can be removed from the scale body. They are called "armoured scales" because the cover is quite dense and provides a certain degree of protection from pesticides and parasites. The hatching armoured scales settle down, lose their legs, and form a hard cover that is usually separated from their body. Armoured scales do not excrete honeydew. Examples of armoured scales include the green date palm pit scale, the California red scale, the greedy scale, the oyster shell scale, and the San Jose scale.

Females of soft scales, family Coccidae, may be smooth or cottony, with a diameter of 6.2 mm or less. They are usually larger and more rounded and convex than armoured scales. Their surface is the actual body wall of the insect and cannot be removed. Most immature soft scales retain their barely visible legs and antennae after settling and are able to move, although slowly. Soft scales produce large quantities of honeydew, which is a modified plant sap that drips from their bodies. Examples of soft scales are the black scale, the brown soft scale, and the European fruit Lecanium.

The date palm pit scale, *Asterolecanium phoenicis* Rao (Asterolecaniidae), has been first described by Rao (1921), and then reviewed by Rao and Dutt (1922), Green (1922), Russel (1941), Kehat and Amitai (1967), Ali (1989), and others. The first instar nymph is ellipsoid, flattened, 0.4–0.6 mm long, 0.16–0.27 mm broad, bright yellow to greenish, and the dorsal surface carries a thin, transparent wax plate with long and thin, whitish filaments on each body ring. The margin has 28 pairs of elongate-shaped filaments, and the posterior part has two long filaments. The head

is furnished with a pair of reddish-brown ocelli and a pair of antennae. The abdomen carries three pairs of well-developed legs (Kehat and Amitai 1967).

Microscopically, the margin has two anterior 10 μm long setae and 28 (8-shaped) marginal pores in a single row. A row of 6–9 pairs of 8-shaped pores, about half the size of the marginal ones, appears on the dorsal surface, with two very small disk-shaped pores, close to second and third minute 8-shaped pores in the median area. The antennae on the ventral surface are 6-jointed. The basal joint with one seta, the first with two, the fourth and the fifth each with one, while the sixth has three long, two stout, and two fairly stout setae. A quinquelocular pore was found lateral to each spiracle on the ventral surface. The legs carry a number of setae, the femur one and the tarsus five.

The second instar nymph was described microscopically by Kehat and Amitai (1967) as elongated, ellipsoid, flattened, 0.55–0.9 mm long, 0.29–0.44 mm broad, and greenish-yellow to dark yellow. Its dorsal surface is covered with a wide transparent wax and carries long thin filaments on each body ring. There are 155–156 pairs of whitish filaments on the margin, and 28 pairs of elongate S-shaped filaments on the sub-margin. The posterior margin has two long filaments. Microscopically, the margin has 8-shaped pores, numbering 155–156, 10 μm long. The dorsal surface has 18–26 small 8-shaped pores, with tubular ducts in scattered groups. The ventral surface carries one-segmented antenna with two blunt and two minute setae. There is a pair of short setae anterior to the anal opening, and 30–32 disk-shaped pores, in the sub-marginal area.

The adult female has a pale yellowish green scale (Fig. 15.1). The body of the insect shows a reddish-brown median patch. The scale is about 1.25–1.5 mm long and 0.75 mm wide, convex in shape and posteriorly narrow, with a long marginal fringe, except on the fifth posterior part (Green 1922). The scale is elongated and wide at the anterior third, tapering towards both ends. It is wider and rounded posteriorly and convex ventrally, with whitish marginal filaments and semi-circular slit for larval exit (Russell 1941).

The adult female insect is slipper-shaped, with a narrow and rounded posterior (Fig. 15.1). Antennae are rudimentary, with two small, relatively stout setae. Mouthparts are rather large and conspicuous. The size of the adult female scale was described by Green (1922) and Russell (1941) as 1–1.5 mm long and 0.75 mm wide, and 1.4–1.75 mm long and 0.65–0.85 mm wide, respectively. Kehat and Amitai (1967) described the adult female microscopically and showed that the dorsal surface is convex, with constrictions near the posterior end. It is pale yellowish green posteriorly and reddish brown to dark violet interiorly. The margin carries 156–166 filaments, resembling those of the second instar nymph, while the sub-margin carries rows of filaments resembling those of the first instar and second instar nymph.

The microscopic description of the adult female by Kehat and Amitai (1967) showed the margin with 8-shaped pores, numbering 155–156, about 10 μm wide, in a single row. On the dorsal surface, there are many 8-shaped pores, about half the size of the marginal pores. The antennae on the ventral surface are segmented and very short, with two blunt and two minute setae. The female body is furnished with a disk-shaped pore on each side of the mouthparts, while two parallel rows of 4

disk-shaped pores occur midway between the mouthparts and the anal opening. Twenty-four short setae are found around the genital opening, 25–35 quinquelocular pores extend from spiracles to the body margin, with a row of 10 min setae in the sub marginal area and 50 disk-shaped pores in an irregular row, terminating parallel to the marginal, 8-shaped pores. The apex of the abdomen has two 60 μm long and two minute setae. The exuvial can be seen beside the posterior portion of the body. The young female resembles the second instar nymph in shape and can only be distinguished by size and by the space between the posterior wax filaments.

15.2.4 Ecology, Biology and Population Density

The green date palm pit scale produces three generations annually in Iran, with two short generations in spring and summer, and one long generation in autumn and winter (Gharib 1974). In Palestine, it produces two short-lived summer generations and a protracted autumn–winter generation (Kehat and Amitai 1967; Avidov and Harpaz 1969), while in Sudan it produces three generations, with one long winter and two short summer generations (Ali 1989). In Iran, the insect over-winters as immature females and pairing takes place in May (Gharib 1974). In Palestine, the development is slow during the winter months, thus the scale population remains as second instars (Kehat and Amitai 1967).

The female developmental cycle lasts around 3 months in spring and summer, and 5–6 months in autumn and winter. The males, which constitute a quarter of the total population in the palms, take around 2 months to develop (see life cycle below). Male flights can be observed in the May–October period (Gharib 1974). The phenological study carried out by Kehat and Amitai (1967) in the Betshean area in Palestine revealed that all stages, except for the male, occur in the area throughout the year. In this area, the autumn–winter generation starts in November with the appearance of great number of first instar nymphs and continues for 7–8 months, through winter til early summer. In autumn, the population of the first instar nymphs reaches its peak early and transforms to second instar nymphs. The scale population remains in this stage in winter. With the rise of temperatures in early spring, these nymphs change to adults (Kehat and Amitai 1967).

At the beginning of summer, the numbers of females in the population increase following the transformation of second instar nymphs into young females. These females begin, in a short time, to give rise to the second generation. The nymphs of this generation attack the fruits soon after hatching. The beginning of the third and last generation is marked by the appearance of great numbers of first instar nymphs at the beginning of September, ending by the beginning of November. The nymphs of this generation attack the late fruits as they begin to ripen. The adults of the third generation that appear in October give rise to the winter generation, that appears in October, which gives rise to winter nymphs (Kehat and Amitai 1967).

In damp groves with a relative humidity of over 50–60%, the scale *Parlatoria blanchardii* (Targ.) was found together with *Asterolecanium* (*Palmapsis*) (Gharib

1974). A study of the wind effect (Ali 1989) revealed that the wind in the area plays a major role in the spreading of the pit scale crawlers and their congregations on the leaflets on the tree sides. The wind direction also explains the north–south spreading patterns of the pit scale insect observed in the Northern State, Sudan.

15.2.5 Geographical Distribution

Palmapsis phoenicis (A. phoenicis Rao) was not reported in the list prepared by Buxton (1920) on the scale insects of date palms in Iraq. However, Green (1922) received a group of Coccidae collected by entomologists from Iraq, which included *P. phoenicis*, that was reported as a new pest species on date palms. Iraq is reported as being the origin of this pest, that occurs from Basra to Bagdad (Bodenheimer 1943). The pest was reported in Iran in the Mohammara and Khuzestan regions (Bodenheimer 1944). Ezz and Abu El Ezz (1961) examined coccid slides brought from El Hassa in Saudi Arabia, which revealed the existence of the pest in Saudi Arabia before 1950, in contrast to the statement of pest existence in the Al-Karag area in 1954. Ezz (1973) reported the existence of the pest in the Mallawy and Barragil areas in Egypt during a survey carried out in 1969, and stated it had entered this country with date palms originally imported from Iraq and Saudi Arabia in 1929. This was considered the first record of the species in Egypt. In Palestine, the scale occurs in the Bet Shean and Jordan valleys, as well as in the Arava area of the Negev Desert (Kehat and Amitai 1967). Gharib (1974) reported the scale in the districts of Fars, Khuzestan and Baluchistan in Iran. Al-Azawi (1985) reported the species in Qatar on Kornish date palm and in Um Salal Mohamed.

Dowson (1982) reported *A. phoenicis* on date palms in Iraq, Iran, Palastine, and Saudi Arabia. The first report of its presence in Sudan dates back to 1989 (Ali 1989). A recent epidemic spread of date palm scale has been reported from Northern Sudan by ARC Sudan, March 2003 (http://www.arcsudan.org/recommendations.htm), and Ahmed (2007).

15.2.6 Host Plants and Economic Importance

Avidov and Harpaz (1969) stated that date palm is the sole host of *Asterolecanium phoenicis* Rao. Green (1922), Bodenheimer (1943), Büttiker and Krupp (1984), reported that the pit scale infests the date palm stalks, leaves, leaflets and fruits. It causes chlorotic stains that appear on the leaves as a consequence of heavy feeding. The leaves subsequently turn yellow and finally degenerate completely. The insects feed on the leaflets and fruits covering the date clusters and shoots (Fig. 15.2) and interfere with natural physiological processes such as respiration and photosynthesis (Gharib 1974).

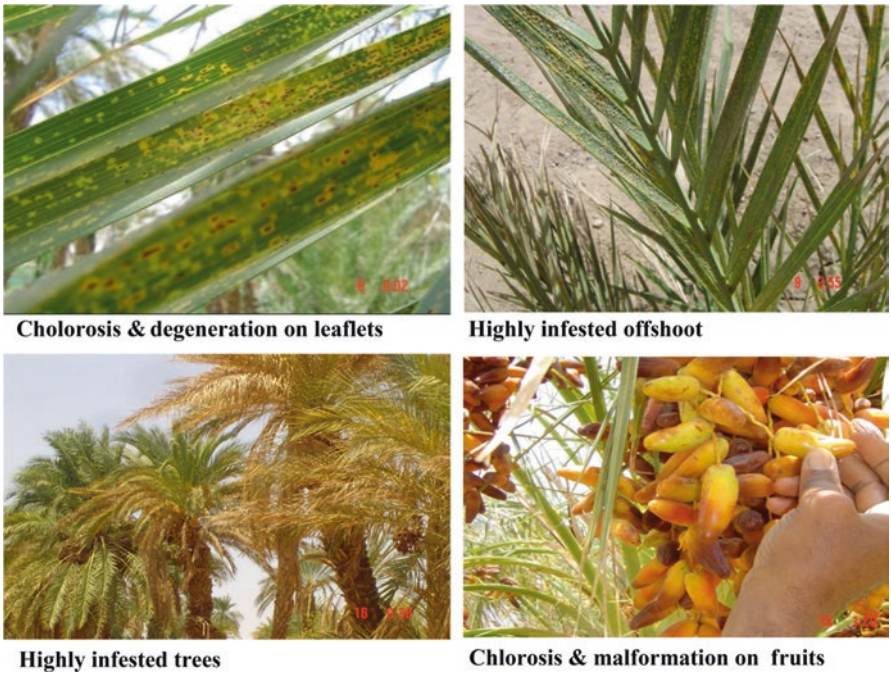

Fig. 15.2 Damage due to the date palm green pit scale on leaves and fruit"

The pest normally congregates along the veins on the pinnate, mainly on the under surface (Avidov and Harpaz 1969). The damage to fruits consists of fouling, which greatly reduces quality and sometimes renders the fruit unfit for marketing (Kehat and Amitai 1967). The damage is also reflected in fruit disfigurement and discoloration, to the point of culling, or reduction in its economic value (Kehat 1967; Kehat et al. 1964). Severe attacks by this insect likely delay the development of the tree, leading to stunting, and may cause its death (Kehat 1967; Kehat and Amitai 1967). The date palm, *Phoenix dactylifera*, is the only host for this pest.

15.2.7 Life Cycle

Information is available on the life history of *P. phoenicis* Green (*A. phoenicis* Rao) on date palm and on the damage that it causes in Iran, especially in the districts of Fars, Khuzestan and Baluchistan (Gharib 1974). It feeds on the leaflets and fruits, covering the date clusters and shoots, preventing respiration and photosynthesis. It has two short generations in spring and summer, and one long one in autumn and winter. The scale over-winters as an immature female and pairing occurs in the following May. The female development cycle lasts 85–95 days in spring and summer and 150–180 days in autumn and winter. The males take only 50–60 days to develop,

and male flights were observed in May–October. Males form only 25% of the total *Palmapsis* population in the palms.

In Sudan, the life cycle has been studied by Ali (1989), who observed that the developmental period of the female is longer in winter (80.5 days) than in summer (57.4 days). The durations of the female first nymphal instars were 50 and 45 days in winter and summer, respectively, while the duration of the female second nymphal instars were 27.5 and 4.8 days in winter and summer, respectively. Kehet and Amitai (1967) reported that the population of green pit scale in Palestine remained in the second nymphal instar in winter, leading to a slower population development. Ali (1989) reported that the developmental period of the males was shorter in summer (46 days) than in winter (66 days).

Most armoured scales have several generations per year, while most soft scales (brown soft scales are an important exception) often have only a single generation. Eggs of both types of scales are usually hidden under the adult female. They hatch into tiny, usually yellow, crawlers with legs. Crawlers walk over the plant surface, are blown by winds to other trees, or are inadvertently moved by people or birds. Armoured scales settle down permanently after spending a day or two in the crawler stage. They then moult, and begin to form their characteristic covers. Soft scales move around for a while longer, but also eventually settle at permanent feeding sites, while half-grown individuals of some soft scale species move once again in fall from leaves to wood, to overwinter. Adult female scales are immobile and have a characteristic scale cover. Adult male scales are tiny winged insects that superficially resemble parasitic wasps. They are rarely seen, do not feed, and live only a few hours. Females of many soft scale species can reproduce without mating.

15.3 Management of the Pest

15.3.1 Cultural Control

Plants need to be provided with good growing conditions and proper cultural care, such as appropriate irrigation, and pruning-off of heavily infested twigs and branches, to become more resistant to the scale damage (Ahmed 2003). Also, they should be provided with good growing conditions and proper cultural care, especially appropriate irrigation, to become more resistant to the scale damage.

15.3.2 Chemical Control

Chemical control trials against date palm pit scale have been conducted in Palestine (Kehat et al. 1964) and Iran (Gharib 1974), where the pest has caused severe damages to date palms. Other species of genus *Asterolecanium* have also been reported

to be controlled by chemical insecticides in different countries (James 1932; Morril and Otanes 1947; Shread 1960; Kehat et al. 1964, 1974; Gharib 1974; El-Kifil et al. 1980; Felippe et al. 2005).

Kehat et al. (1964) carried out eight trials in Bet Shean valley in 1959–1960. Eighteen different chemical compounds, including fumigants, sulphurs, chlorinated hydrocarbons and organic phosphates, were used either alone or mixed with mineral oils, and their effects on the different stages of the scale insect were tested. Results showed that the majority of chemicals were effective, especially against the nymphal stages. Hoever, they did not prevent the reappearance of the first instars and the renewal of the scale population.

Kehat et al. (1964) stated that the effective combinations against date palm pit scale, in descending order, were: 2% Malathion with oil, (special formulation which contains 712 kg white medium oil, 88 kg technical Malathion, 200 kg water and emulgator); 0.2% Diazinon 40% WP + 2% oil; 0.5% Gusathion 20% EC + 2% oil; and 0.5–1% Malathion 25% WP + 2% oil, which was the least effective of the above-mentionned combinations.

Gharib (1974) reported that the most effective treatment was provided by Malathion 57% EC at 200 g a.i./100 L of water, mixed with two L of Volck oil (a petroleum-based insecticide), which gave 94.2% mortality. It was followed by Dimethoate 40% EC, Diazinon 25% W. and Azinophos-methyl (Gusathion) 20% EC, all at 200 g a.i./100 L of water, in combinations with 2 litres of Volck oil. This combination gave between 84.2% and 90.4% mortality.

Kehat et al. (1964) reported that the best time for chemical control in Palestine was during the autumn and winter, when nymphs are more easily controlled than females. At the time of mass appearance of first nymphal instars during June and July, summer spraying may be necessary to prevent excessive settling of crawlers on the fruits. Kehat and Amitai (1967) reported the best time for control is from November to March, when the relatively susceptible nymphs constitute a large part of the population, coinciding with the absence of flowers and fruits that might be damaged by spraying. Gharib (1974) indicated the best times for chemical control in Iran to be between May–June, November and December, when 75% of the nymphs had left the parent scale.

Chemical control practices have been reported against other species of the genus *Asterolecanium*. Shread (1960) tried granulated systemic insecticides against the scale *Asterolecanium* spp. Infested holly plants were treated with Phorate 2%, Phosdrin 60%, Systam and Disyston, used as soil drenches in 4-inch pots. Phorate and Phosdrin controlled the insect, but Disyston and Dystam were inefficient. El Kifil et al. (1980) tested organophosphorus compounds, such as Malathion, Roger, Parathion and mineral oil, against the fig scale *A. pustulans* Ckll, in Egypt. Better results were obtained when the organophosphorus compounds were added to oil than when the oil was used alone. Other chemical control trials were carried out against other species of the pit scale, such as *A. coffeae* Newst. in Kenya (James 1932), *A. bambusae* Boisd. (Morril and Otanes 1947), and *A. variolosum* Ratz., *A. minus* Lindinger and *A. oercicola* Bouche, on oak trees in California (Pritchard and Robert 1950).

In Sudan, chemical control attempts have been carried out in the field only against the date white scale, *Parlatoria blanchardi* Targ. (Siddig 1975). Petroleum oil at 2%, Dimethoate 40% EC at 0.1%, Malathion 57% at 0.2%, and methyl parathion 48% EC at 0.1% were all tested, alone or in combinations with petroleum oil, in a large-scale trial in Bauga area. The above chemicals, in addition to fenitrothion in oil (Sumifene + oil) 5% and carbaryl 85% WP, were used in small-scale trials. The results of the small and large trials showed that the petroleum oil, mixed with Dimethoate or Malathion, and methyl parathion alone caused considerable reductions in the populations of the pest. The mixture of petroleum oil and Dimethoate, and of petroleum oil and Malathion, were recommended for control of date palm white scale *Parlatoria blanchardi* Targ., in Sudan.

In Sudan, no control trials have been carried out against the date palm pit scale. Small- and semi-large-scale trials were conducted by Ali (1989), using contact and systemic insecticides and Albolenium oil (white oil, alone or in a mixture with insecticides) in the El Golid area against the date palm pit scale. The results proved that all the tested chemicals reduced the numbers of the green scale pest, and that they were significantly different from the control throughout the test period, except the Albolenium oil. However, no recommendation was reported. The chemical campaign arranged by the Plant Protection Directorate, during 1990–1992, used foliar spraying (aerial and ground) of the recommended chemicals for the white scale and many other compounds, but failed to control the pest and the insect spread from the target area to other places. A successful control was achieved by Ahmed (2003) and Ahmed (2005, 2007), when soil application and trunk injection of neonictonid insecticides was recommended, such as imidacloprid, in different commercial formulations.

According to past chemical control measures undertaken in Iran, the best times for chemical control were May–June, November and December, when 75% of nymphs had left the parent scale. The most effective treatment was 57% Malathion at 200 g/100 L, which gave 94.2% mortality, followed by 40% Dimethoate (Roxion), 25% Diazinon and 20% Azinphos-methyl (Gusathion), also at 200 g, and emulsifiable Volck oil at 2 litres/100 litres. All of these gave 84.290.4% kill rates (Gharib 1974). Soil application of imidacloprid provided good control, with the least environmental impact (Ahmed 2003).

An oil emulsion spray in spring and another in autumn kills the crawlers of the white scale insect and prevents the development of severe infestations. Infestations on small- to medium-sized trees can be adequately managed with one annual application, over several consecutive years. Insecticidal oil should be diluted to a solution of 1.5–2% (1.5 to 2 parts oil to 100 parts water).

Mixtures of oil and organophosphate insecticides applied in spring to kill hatching crawlers are not recommended because of the environmental hazards associated with applying these insecticides to large plants, especially in residential areas, the difficulty of getting adequate coverage with available application equipment, and the availability of safer, more effective alternatives (Ahmed 2007). Applications of Albolenium oil (Ali 1989) and chlorpyrifos (Ahmed 2007) were not effective against the adults of the green pit scale, under Northern State conditions.

15.3.3 Biological Control

Scales are usually controlled by natural enemies, including many species of small, dark, lady beetles, *Hyperaspis species*, which are tiny, shiny, black lady beetles with several red, orange, or yellow spots on the back. *Rhyzobius lophanthaes* is a lady beetle with a reddish head and underside, and a greyish back, densely covered with tiny hairs. The twice stabbed lady beetle, *Chilocorus orbus*, is shiny black with two red spots on its back, and a reddish colour underneath. The larvae of certain predaceous lady beetles can be found under the female of soft scales feeding on scale eggs and crawlers.

Many parasitic wasps are important natural enemies of scales, such as *Aphytis*, *Coccophagus*, *Encarsia* and *Metaphycus*. The parasitoids activity can be estimated by checking scale covers for the round exit holes made by emerging adults. Covers of armoured scales can be removed to examine immature parasitoids beneath. Growing flowering plants near scale-infested trees and shrubs will help to augment natural enemies (Zaid and Arias 1999).

None of the natural enemies associated with the scale, *A. phoenicis*, were reported before 1967, even in catalogues of parasites and predators (Thompson 1950; Thompson and Simmonds 1964). Kehat (1968) reported that females of *A. phoenicis* were highly resistant to attack by the coccinellid predator, *Pharoscymnus numidicus* Pic., especially larvae and adults, apparently due to their hard scale coverings, whereas nymphs were readily devoured. Yinon (1969) reported that the Asterolecaniids, the soft scales, and the mealy bugs, in addition to the armoured scales, constitute the host range of the Coccinellid predator *Chilocorus bipustulatus* L.

The intensity of the *A. phoenicis* predation was measured as only one-third of that on armoured scale insects (Yinon 1969). The presence of the parasitoid *Habrolepis dalmanni* (*Encyrtidae*) resulted in the virtual elimination of golden oak scale *Asterolecanium variolosum*, a closely related species, in New Zealand. The coccinellid *Chilocorus cacti* has been successfully introduced to control *Asterolecanium bambusae* (bamboo scale) and *Asterolecanium pustulans* (oleander pit scale) in other countries.

15.4 The Situation of the Green Date Palm Pit Scale Insect in Sudan

Palmapsis phoenicis was recorded on date palms in Sudan for the first time during 1986–1987 (Ali and El-Nasr 1992). The pest was first reported in the El Golid area, as a result of the illegal introduction of some offshoots from Saudi Arabia in 1974. Later, it crossed the natural barrier of the Baja desert to invade Elgaba Scheme (150 km south of Dongola, 400 km north of Khartoum). In 1996, it became a real threat to date palm cultivation in Northern Sudan (Ahmed et al. 2002). The infested

areas in El Golid, Elgaba and Old Dongola cover about 5000 hectares, extending over 60 and 50 kilometres along the west and east banks of the River Nile, respectively. The newly reported infestation in Artigasha Island, Orbi and El Burgig Scheme in the Dongola area and Abuhamad in the River Nile State (23,000 infested palm trees) is evidence that the pest is still rapidly spreading. The total number of infested trees is about 1 million (Ahmed 2005).

In the past, the control efforts were not successful due to lack of indigenous knowledge of appropriate control measures. Hence, the level of infestation steadily increased. The insect attacks the leaflets, leaf rachis and fruits. It causes chlorosis, degeneration of the leaves, and malformation of fruits before maturity, leading to losses in production ranging from 30–50 kg to 5 kg per tree (Ali and El Nasr 1992). The losses may range between 85% and 90%, according to the infestation rate, variety infested, and management conditions (Ahmed 2001, 2004).

Studies have been conducted on the biology and population development of the pest, its seasonal abundance, susceptibility of date palm varieties, losses and control methods (Ali 1989). An eradication programme was implemented, based on pruning, local quarantine and aerial insecticide application. The insecticides applied, at spray volumes of 100 L of water, were Diazinone 60 EC (340 ml), Roger 32% EC (225 ml), and Folimate 80% (200 ml), each including 2 litres of 80% Albolenium oil. A 96.4% control rate was achieved, thus lowering the infestation within the targeted area drastically to 3.6%, and nearly ended in apparently curtailing its infiltration outside the infested area Ali and Tibin (1992). However, the infestation flared back to more than 50% in less than one year. Eradication of the insect was doomed to failure. Attempts to locate a biological control agent failed, when a Coccinellid beetle was introduced (Ali 1989). Results from research conducted at Dongola Research Station indicate that this pest can be controlled using an IPM package that includes cultural, chemical and biological control measures. The systemic insecticide used for this purpose was imidacloprid (Ahmed et al. 2002).

15.4.1 Varietal Susceptibility

A study was conducted by Ahmed (2001) in Elgaba Scheme, Northern State, Sudan, to evaluate the susceptibility of four date palm varieties (Barakawi, Gondeilla, Moshrig/Waddlagi, and Jaw) to green pit scale insects. The results indicated that the order of susceptibility among the cultivars was highest in Gondeilla, Barakawi, and Jaw, and that cultivar Moshrig/Waddlagi was the least susceptible. A similar result was obtained by Ali (1989) in the El Golid area, where the varietal susceptibility studies showed that Moshrig and Gondeilla varieties were the most susceptible. Barakawi was moderately susceptible, while Jaw and Tamoda were the least susceptible, according to average number of scales per leaflet.

15.4.2 Damages

In Sudan the insect attacks the leaflets, leaf rachis and fruits. It causes chlorosis, degeneration of leaves, and malformation of fruits before maturity, leading to losses in production ranging from 5 to 30–50 kg per tree (Ali and El-Nasr 1992). The losses may range between 85% and 90%, according to infestation rate, variety infested, and management conditions (Ahmed et al. 2002; Ahmed 2004).

15.4.3 Control Efforts

In the past, and due to lack of indigenous knowledge of appropriate control measures to adopt to control the date palm green pit scale in Sudan, the treatment control efforts using foliar applications of contact insecticides and mineral oils were not successful (1991–1992). Hence, the level of infestation steadily increased.

15.4.4 IPM

Following intensive research, an Integrated Pest Management (IPM) method was developed to include cultural practices or sanitary measures, supplemented with chemical control using systemic neonicotinoids (thiamethoxam or imidacloprid, such as Confidor 200 SL and Actara 25 WG), strengthened by the impacts of natural enemies, together with implementing plant quarantine legislations. A comprehensive programme by Plant Protection Directorate (PPD) has been implemented in infested areas.

A partial budget analysis indicated the profitability of the package recommended by ARC for using systemic neonicotinoids (imidacloprid or thiamethoxam), as indicated by the marginal rate of returns of 364% for imidacloprid (Ahmed 2005). A socioeconomic study indicated that the insect is currently threatening the stability of farmers and has increased their vulnerability to serious shocks.

15.4.5 Sustainable Control

Sustainable biological control of the green pit scale is very important. Results of the survey revealed that many natural enemies associated with insect have been recorded: the nitidulid beetle predators found in all surveyed areas were *Cypocephalus dudichi* L., ladybird *Pharoscymnus numidicus*, *Chrysoperla* sp., and the parasitoid *Metaphycus* sp. These were found in association with green pit scale insect, and the percentage of parasitism was 16% in some areas (Dafalla and Ahmed 2010).

15.5 Conclusion

- Date palms are of major socioeconomic and social importance in the Northern State.
- Date palm is still grown in Sudan by conventional methods without attention to irrigation, fertilization, or other cultivation practices.
- Date palm production and plantations have considerably deteriorated in the last years as a result of biotic and abiotic stress, among which the green pit scale is the most important.
- Soil application and trunk injection of imidacloprid (Confidor 200SL, Rinfidor20%SL Comodor 20%, Zathron 20% SLand Sinfidor 20%SL) and thiamethoxam (Actara 25WG) were highly effective in controlling the green pit scale insect.
- They proved to be very effective as a protective measure against new infestation.
- The two methods of application do not require any expensive machinery or labour for application. They can be safely applied.
- Trunk injection truly effective and reliable method for controlling the green pit scale insect, with minimal environmental impact.
- The two methods of application are highly economical and safe for the user and appear to be safe for the beneficial insects.
- Date palms were treated with different insecticides using the two methods, developed normally during four seasons and no phytotoxicity has been noticed in the treated trees.
- The tested insecticides checked termites and many other pests, but did not affect
- Cultivation of the Wadlagi variety is recommended in the infested areas.
- Further studies are needed to study the susceptibility of introduced soft and semi-soft date palm varieties to green scale infestation.
- The current surveys indicated that there is sign of natural enemies (predators and parasites) of this pest.
- The effort of research in Dongola Research Station (ARC) succeeded in isolating an endoparasite, identified as *Metaphycus sp.*
- Further studies are needed to confirm the efficacy and mass rearing of the natural enemies.

References

Ahmed, M. A. (2001). *Susceptibility of some date palm varieties to green pit scale insect infestation.* ARC, Palm Research Program Annual Report 2000/2001.

Ahmed, M. A. (2003). Evaluation of the efficacy of imidacloprid (Confidor 200SL) against the green date palm pit scale insect *(Asterolecanium phoenicis* Rao.) (Homoptera: Asterolecaniidae). In *Proceedings of the international date palm conference, 16–19 September 2003*. Faculty of Agricultural Sciences and Veterinary, El Gassim, King Saud University, K.S.A.

Ahmed, M. A. (2004). The efficacy of Confidor 200 SL against the green date palm pit scale insect *(Asterolecanium phoenicis* Rao.) (Homoptera: Asterolecaniidae). In *Proceedings of second National Pest Management conference in the Sudan, 6–9 December, 2004*. Faculty of Agricultural Sciences, University of Gazira, Sudan.

Ahmed, M. A. (2005). Recommendation of four systemic insecticides for control of the green date palm pit scale insect (*Asterolecanium phoenicis* Rao.) (Homoptera: Asterolecaniidae), using two method of application. In *Proceedings of 67th meeting of the Pests and Diseases Committee*. ARC Conference Hall, Wad Medani, Sudan, June 2002.

Ahmed, M. A. (2007). *The efficacy of chlorpyrifos 480 EC as a contact insecticide against the green date palm pit scale insect (Asterolecanium phoenicis Rao.) (Palmapsis phoenicis) (Homoptera: Asterolecaniidae) in Northern Sudan*. Progress Report, submitted to Plant Protection Research Centre Agricultural Research Corporation ARC, Sudan. November 2007.

Ahmed, M. A., Oman, A. M., & Makawi, H. M. (2002). Recommendation of Confidor 200 SL (imidacloprid) for control of the green date palm pit scale insect *(Asterolecanium Phoenicis* Rao.) (Homoptera: Asterolecaniidae). In *Proceedings of 67th meeting of the Pests and Diseases Committee*, ARC Conference Hall, Wad Medani, Sudan, June 2002.

Al-Azawi, A. F. (1985). A survey of insect pest of date palm in Qatar. *Date Palm Journal, 4*, 247–266.

Ali, A. A. (1989). *Studies on Astrolecanium phoenicis (Rao). A date palm scale insect in El Golid area*. MSc. Thesis Faculty of Agriculture, UK.

Ali, A. A., & El-Nasr, Y. E. S. (1992). New record of green pit scale insect, *Asterolecanium phoenicis* Rao, on date palm in the Sudan. *FAO Plant Protection Bulletin, 40*, 115.

Ali, A. A., & Tibin, A. B. (1992). *Chemical control trials against termites and green pit scale insect in Dongola Province*. Pest and Disease Committee Annual Meeting September 1992. ARC HQ, Wad Medani, Gezira Sudan.

Al-Whaibi, M. H. (1997). Some metabolic changes of chlorotic and green leaflets of date palm tree. *Journal of King Saud University, Science, 9*(1), 1–9.

Avidove, Z., & Harpaz, I. (1969). *Plant pests of Israel* (pp. 456–157). Jerusalem: Israel University Press (Abstract).

Ben-Dov, I., & Harpaz, I. (1985). An annotated list of Taxa of Coccoidae (Homoptera) described by F.S. Bodenheimer., correction and addition. *Israel Journal of Entomology, 19*, 23–36.

Bodenheimer, F. S. (1943). A first survey of the Coccoidea of Iraq. Ministry of Economics, Directorate. *Journal of Agriculture. Iraq Bullettin, 28*, 25.

Bodenheimer, F. S. (1944). Note of Coccoidea of Iraq with description of new species (Hemiptera – Homoptera) Asterolecanidae. *Bulletin de la Société Fouad 1st Entomologist. 18*, 85–100.

Büttiker, W., & Krupp, F. (1984). Fauna of Saudi Arabia, insects of Saudi Arabia, Homoptera, Meteor. *Environmental Protection Administration Fauna Public.* No. 6.

Buxton, P. A. (1920). Insect pest of dates and date palm in Mesopotamia and else where. *Bulletin of Entomological Research, 11*, 287–303.

Daffalla, E. Y., & Ahmed, H. M. (2010). *Metaphycus* sp. (Hymenoptera:Encyrtidae), a parasitoid of the green pit scale insect on date palm in the Northern State, Sudan: A new record. In *The 82nd meeting of the Pests and Diseases committee*. ARC, WadMadni, June 2010.

Dowson, V. H. W. (1982). *Date production and protection with special reference to North Africa and the Near East*. FAO Technical Bullettin No. 35, p. 294.

El – Kifi, A. H., Salama, H. S., & Handy, M. K. (1980). Chemical control of scale insect infesting fig tree in Egypt. *Review of Applied Entomology, 68*, 55.

Ezz, I. A. (1973). *Asterolecanium phoenicis* (Homoptera-Coccoidea), a date palm pest recorded for the first time in Egypt. *Agricultural Research Review, 51*, 47.

Ezz, A. I., & El Ezz, A. A. (1961). *Asterolecanium phoenices* Rao. Infesting date palm in Saudi Arabia. *Bullettin of the Entomological Soceity of Egypt, XLV*(45), 407.

FAO. (2005). Agro-statistics database. http://faostat.fao.org/site/340/default.aspx

Felippe, M. R., Garbim, L. F., Coelho, J. H. C., Ximenes, N. L., Sanchez, A. L., & Yamamoto, P. T. (2005). Chemical control of orthezia in citrus. *Laranja, 26*, 251–264.

Gassouma, S. S. (2003). *Palmaspis phoenicis*. http://ecoport.org/ep. 9.9.2003.
Gharib, A. (1974). *Palmaspi sphoenicis* Rao. *Asterolecanium phoencis* Rao (Homoptera: Asterolecanidae). *Entomologie et Phytopathologie Appliquees, 36.*
Green, E. E. (1922). On a small collection of Coccoidae from Mesopotamia with description of a new species. *Bulletin of Entomological Research, 8*, 469–470.
James, H. C. (1932). The control of Asterolecanium (The Fringed scale of coffee). *Bulletin of the Department of Agriculture, Kenya, 23*, 4.
Kehat, M. (1967). Survey and distribution of ladybeetles (Coleoptera: Coccinellidae) on date palm trees in occupied Palestine. *Entomophaga, 12*, 119–125.
Kehat, M. (1968). The feeding behaviour of *Pharoscymnus numidicus* (Coccinellidae), predator of the date palm scale *Parlatoria blancharidi* Targ. *Entomologia Experimentalis et Applicata, 11*, 30–42.
Kehat, M., & Amitai, S. (1967). The morphology and phenology of the scale *Asterolecanium phoenicis* Rao on date palm in Beat Shean valley. *International Journal of Agricultural Research, 17*, 85–93.
Kehat, M., Swiriski, E., & Bankier, E. (1964). Trials in the control of the scale *Asterolecanium phoenicis* Rao on date palms. *International Journal of Agricultural Research, 14*, 19–26.
Kehat, M., Swiriski, E., Blumberg, D., & Greenberg, S. (1974). Integrated control of date palm pests in Israel. *Phytoparasitica, 2*, P141–P149.
Mani, M. S. (1982). *General entomology* (3rd ed.). New Delhi: Oxford and IBH Publication.
Morril, A. W., & Otanes, F. Q. (1947). DDT emulsions to control mealy bugsand scales. *Journal of Economic Entomology, 40*, 599.
Morrision, H., & Morrision, E. (1966). *An annotated list of generic names of the scale insects (Homoptera: Coccidea)*. USDA Miscellaneous Publicaitons N. 1015.
Pritchard, A. E., & Ropert, E. (1950). Biology and control of *Asterolecanium* scale on Oaks in California. *Journal of Economic Entomology, 43*, 494–497.
Rao, Y. R. (1921). *A preliminary list of insect pests of Iraq*. Department of Agriculture, Iraq, Memair No. 7. Time press Bombay.
Rao, Y. R., & Dutt, A. (1922). *The pest of the date palm in the Iraq*. Department of Agriculture, Iraq, Memair No. 6, Time press, Bombay.
Russel, L. M. (1941). *A classification of the scale insect genus Asterolecanium*. USDA Miscellaneous Publications No. 424. Washington, pp. 154–155.
Shread, J. C. (1960). Systemic insecticides for control of scales, leaf miners and lacebug. *Journal of Economic Entomology, 53*(3), 406–408.
Siddig, S. A. (1975). Field control of the scale insect *Parlatoria blancharidi* Targ. (Diaspididae) infesting date palm in the Sudan. *Journal of Horticulture, 50*, 13–19.
Thompson, W. R. (1950). *A catalogue of the parasites and predators of insect pests. Sec.1: Parasite Host catalogue Part 3; Parasites of Homoptera* (2nd ed.). The Commonwealth Bureau of Biological Control, Canada.
Thompson, W. R., & Simmonds, F. J. (1964). *A catalogue of the parasites and predators of insect pests Sec. 3: Predator Host catalogue*. The Commonwealth Bureau of Biological Control, Canada.
Yinon, A. (1969). Food consumption of the armoured scale lady beetle *Chilocorus bipustulatus* L. *Entomologia Experimentalis et Applicata, 12*, 139–146.
Zaid, A., & Arias-Jimenez, E. J. (1999). *Date palm cultivation*. FAO Plant Production and Protection Paper No. 156, 285 pp.

Chapter 16
Use of Para-pheromone Methyl Eugenol for Suppression of the Mango Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) in Southern Ethiopia

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Abstract Mango growers in Southern Ethiopia are faced with the severe challenge of controlling mango fruit fly, *Bactrocera dorsalis*. Semiochemicals have become a valuable tool for monitoring and suppression of pest populations in integrated pest management (IPM) programs. Since pheromone traps are relatively easy to use, cost-effective, species-specific and environmentally friendly tools for IPM programs, we evaluated the performance of methyl eugenol (ME) vs. *B. dorsalis* in Gamo-Gofa zone, in Southern Ethiopia. A 3-month (February–April 2018) trial on *B. dorsalis* population reduction was implemented in six intervention sites of Arba minch zuria district, and three control sites of Mierab Abaya district. Results showed that continuous application of ME was effective in reducing the fruit fly population. Fruit fly captures and fruit infestation in six intervention sites were significantly lower during the 3 months than those recorded in the three control sites. Therefore, the strategy of including mango fruit fly suppression techniques using pheromone and/or parapheromone lures such as ME (related to mating behaviour) in IPM approach, is recommended.

Keywords Mango · Mating disruption · Fruit fly · Horticulture

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16.1 Introduction

Mango is one of the most important fruit crop produced and exported in Ethiopia. In the recent years, its production showed a steady increase in the planted acreage. Like other perennial fruit crops, mango production is plagued with many insect pests. In Africa, of the 1.9 million tons of mangoes produced annually, about 40% is lost due to fruit flies, where infestation rates vary among countries and seasons, ranging from 5 to 100% (Lux 1999; Goergen 2011).

Across Southern and Eastern parts of Ethiopia, *Ceratitis* spp. (*C. capitata* and *C. fasciventris*) and *Bactrocera* spp. (mostly *B. dorsalis*), are the dominant species, with a great economic importance across the country (Ferdu et al. 2007; Dawit et al. 2015). However, the most important fruit flies on which efforts of pest management are concentrated is the mango or Oriental fruit fly, *B. dorsalis* Hendel (Diptera: Tephritidae).

In the recent past, Ekesi et al. (2009, 2010) reported that *B. dorsalis* competes with other endemic fruit fly species such as *C. cosyra* Walker, *C. capitata* Wiedemann and *C. ditissima* (Munro) causing species displacement. It is estimated that where plantations are not managed, there could be total fruit loss, although losses less than 30% can be salvaged if control measures are applied. *Bactrocera dorsalis* was first detected in Kenya in 2003 (Lux et al. 2003; Drew et al. 2005; Ekesi and Billah 2009) and in Ghana in 2005 (Billah et al. 2006). It is a serious pest, requiring the adoption of area-wide control measures to suppress the populations below their economic threshold.

Several control measures against fruit flies have been practised including, among others, chemical control. Some of the most widely used control measures which pose little or no harm to the environment is the use of traps and semiochemicals such as sex pheromones, lures and baits (Cunningham et al. 1978; Agunloye 1987; McQuate et al. 2005). These are generally used for masse trapping, spot spray and in "attract and kill" approaches. The success of the mass trapping strategy depends on the efficiency of traps and lures (Cohen and Yuval 2000). Trap designs, including assorted colours and shapes, can also influence efficacy in fruit fly catches (Epsky et al. 1995; Vargas et al. 1997).

Traps baited with sex pheromones attract males of the same species for mating. They have become a valuable tool for monitoring and suppression of fruit fly pest populations in survey and integrated pest management (IPM) programs. Many insect sex pheromones can now be chemically synthesized for use in pest monitoring and mating disruption. Since pheromone traps are relatively easy to use, cheap, species-specific, and environmentally benign, they make ideal tools for IPM programs.

For tephritid fruit fly suppression, methyl eugenol (ME) and cue-lure are highly attractive kairomone lures to *B. dorsalis* and the melon fly, *B. cucurbitae* (Coquillett), respectively.

The effectiveness and performance in the field of ME-based baits against mango fruit fly in Ethiopia have not yet been documented in the literature, though other studies have suggested its effectiveness, elsewhere.

The International Centre of Insect Physiology and Ecology (ICIPE-Ethiopia) initiated an area-wide fruit fly suppression program through pheromone traps in Gamo-Gofa zone of Southern Regional State of Ethiopia, since 2017. Therefore, the purpose of this study was to demonstrate and validate the performance of solid male lure ME formulated with killer insecticide for suppression of mango fruit flyin mango fields, as alternatives to current control systems based on organophosphate insecticides.

16.2 Materials and Methods

16.2.1 Description of Study Sites for Intervention and Control

The study was conducted in Arba Minch Zuria and Mierab Abaya Districts of Gamo-Gofa zone, in the Southern Regional State of Ethiopia. The two districts are located at a grid reference between (5° 50.46′ N and 37° 27.72′ E) and (6° 27.32′ N and 37° 44.64′ E), respectively. Six villages (Ankober, Wajifo, Kolashele, Elgo, Chelba and Lante) in Arba Minch Zuria District were selected for intervention, while three villages (Ugayehu, Molie and Kolamulato) in Mierab, Abaya District, were selected for control purposes. The GPS coordinates, temperature and rainfall of all nine locations are shown in Table 16.1. These two Districts and nine villages are known for major mango production in the zone. Majority of the inhabitants of these areas are involved in subsistence agriculture cultivating mango, banana, apple, avocado, papaya and guava.

Table 16.1 GPS position, altitude, temperature and rain fall of study sites

Location	GPS Position	Altitude (mamsl)	Temperature (°C)	Rainfall (mm)
Ankober	6° 14.581′ N, 37° 44.680′ E	1209	19–32	595.5
Wajifo	6° 27.320′ N, 37°44.643′ E	1220	18–34	610
Kolashele	5° 52.919′ N, 37° 29.740′ E	1162	17.6–31.6	588.4
Elgo	5° 50.469′ N, 37° 27.721′ E	1122	20.6–35.6	588.4
Chelba	6° 6.496′ N, 37° 35.074′ E	1203	17.6–31.6	590.4
Lante	6° 7.834′ N, 37° 38.165′ E	1190	17.6–32.6	588.4
Ugayehu	6° 15.417′ N, 37° 45.663′ E	1218	19–34	610
Molie	6° 16.25″ N, 37° 46.367′ E	1215	19–34	603
Kolamulato	6° 27.942′ N, 37° 45.081′ E	1202	18–34	600

Fig. 16.1 Lynfield bucket traps

16.2.2 Treatments for Insect Population Suppression

Fruit fly baits are generally short distance attractants. For this reason, we chose whole orchards for para-pheromone ME attract and kill applications. The attract and kill concept consists in eliminating males from the vicinity, for mating disruption.

The following sites: Ankober, Wajifo, Kolashele, Elgo, Chelba and Lante were treated with the commonly used polymeric plug of solid para-pheromone ME formulated with insecticide malathion, to attract and kill males *B. dorsalis* at monthly intervals, throughout the fruit development period. The treatment interval selected was in line with the manufacturer's recommendation (4–6 weeks), based on research with codling moth (Charmillot et al. 2000). Control sites within a 20–30 km radius (Ugayehu, Molie and Kolamulato) were used as untreated control orchards.

Fruit fly populations were monitored using yellow Lynfield traps, a bucket type trap composed of a cylindrical plastic container with four equidistant holes on the upper third (Fig. 16.1). The lid of the trap contains a hook to which a ME dispenser could be fitted. Intervention traps were maintained on the field for 3 months (February–April 2018). Between 50 and 100 traps were placed in each intervention site, depending on the farm size, at the distance of 50 m apart, hanged at a height of 1–2 m from the ground.

The control villages were at farmers practice. 8 ME baited monitoring traps were deployed in each control site at the distance of 80 m apart. They stayed for 24 h per month, and caught flies were collected at a 1-week interval during the 3 months, and counted.

16.2.3 Insect Population Monitoring

All traps were placed at the rate of ten traps per hectare and placed at un-shaded area to sunlight on the windward side of a field, so that the pheromone should be blown into the field. Traps were coded and numbered. They were managed by individual

farmers who were responsible for the traps safety and service. The traps were serviced at weekly interval and caught flies were collected weekly at each service time and visit, counted and data recorded for further analyses.

The caught flies per trap per day (FTD) were also calculated to facilitate comparison across the different intervention and controlled localities.

The formula used to calculate FTD was:

$$\text{FTD} = F / T \times D$$

whereby F = total number of flies; T = number of serviced traps; D = average number of days the traps were exposed in the field.

16.2.4 Assessment of Fruit Infestation

Throughout the study period the fruit fly infestation was assessed by monthly fruit collections from mango orchards. Every month, 5 to 10 kg fruits were sampled from both intervention and control sites. Fruits were weighed and then assessed visually for rotting and presence of maggots. Afterwards, the fruits were sorted into "Infested" and "Non-Infested" categories, counted and weighed again separately. The clean-looking mango fruits were kept in containers for a week then dissected for the presence of fruit fly maggots. The proportions of damaged fruits were added in to "Infested" category to recalculate the level of infestations. Percent infested fruits was determined as ratio of number of infested fruits per total number of collected fruits. The experiment was repeated three times, one month apart.

16.2.5 Fruit Fly Impact Assessment and Statistical Analyses

The impact of the fruit fly on mango at zone level was assessed at the yield and economic levels. Key informant interviews (KIIs) were used to assess the outcome and impact of fruit fly on mango production

16.2.6 Data Analysis

For normalization, data on fruit fly captures and proportion of fruit infestation were log- transformed to (Log10) and angular (arcsine $\sqrt{}$proportion), respectively, for statistical comparisons. Untransformed means are presented in both figures and tables. Analysis of variance was used to determine differences among study sites using SAS Statistical Program. Once a significant difference was detected, data were subjected to post hoc analysis for means separation using LSD test, ($P = 0.05$).

Comparison of fruit fly population levels between intervention and control sites (fruit fly suppression test) was made using t-tests (Sokal and Rohlf 1981) of log-transformed trap catch results by Statistical Package for Social Science (SPSS) software. Comparison of percentage fruit infestation by fruit fly between intervention and control sites was made using paired t-tests of angular transformed proportion damaged data.

16.3 Results

16.3.1 Fruit Fly Populations

The attract-and-kill technique significantly reduced the population of *B. dorsalis*, in mango orchards. Average trap catch data in intervention and control sites of each orchard and trap servicing month are presented in Fig. 16.2. Average trap catch in the intervention sites was lower than in the control section in all months, with catch significantly lower in March and April (Fig. 16.2). Trap catch per day in the control sites were more than 20-fold higher, during the whole period of study (Fig. 16.2).

A continuous decrease in fruit fly population was observed over the 3 months under intervention trial sites (Fig. 16.3). At three of the intervention sites (Lante, Elgo, and Chelba) the *B. dorsalis* populations were the lowest throughout the study time. In general, from the time of the first month until the end of the trial, oriental fruit fly trap catch was not higher than 20 flies/trap/day in any intervention sites except in Ankober which was higher than 45 flies/trap/day (Fig. 16.3). In the first month, trap catch in all intervention sites, except Wajifo and Chelba, was higher

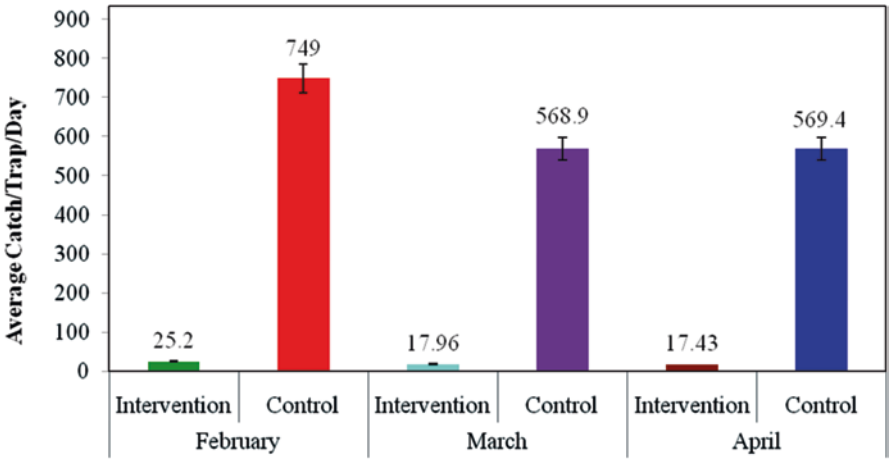

Fig. 16.2 Average catch (flies/trap/day) of *Bactrocera dorsalis* in baited traps with para-pheromone methyl eugenol blend, in intervention versus control sites, at each trap service month

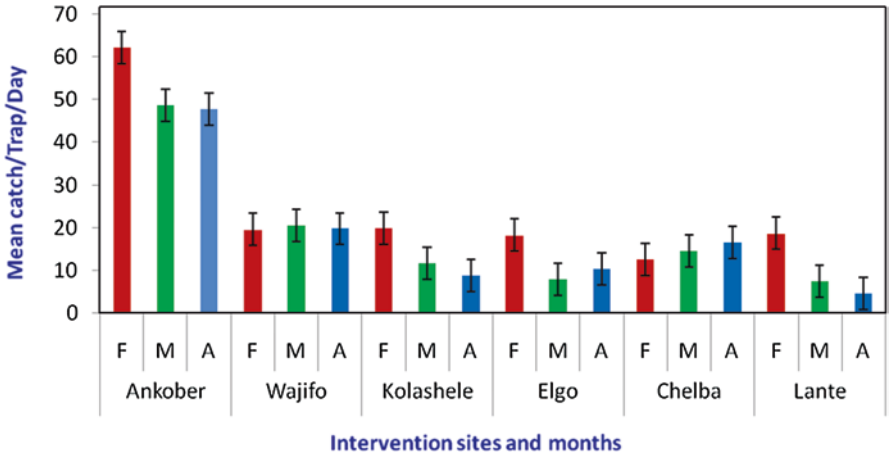

Fig. 16.3 Mean total mango *B. dorsalis* trap catches (flies/trap/day ± SEM) in intervention sites of six villages, at each trap service month

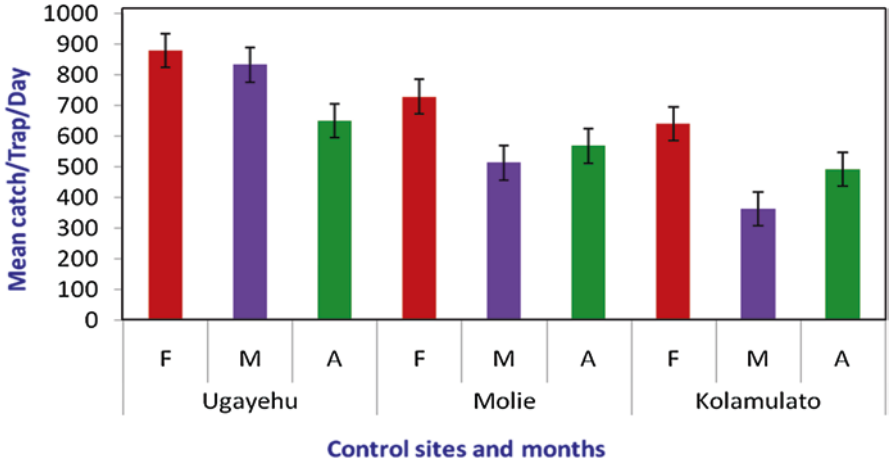

Fig. 16.4 Total mango *B. dorsalis* trap catches (flies/trap/day, ± SEM), in control sites of three villages, at each trap service month

than in the second and third months, although trap catches at second and third months in all intervention sites were not statistically different. The oriental fruit fly populations in the control sites (Ugayehu, Molie and Kolamulato), collected within 24 h of the 3 months were considerably higher throughout trapping periods (Fig. 16.4). Highest fly catches per trap per day were recorded at Ugayehu in February and March (879 and 832 flies, respectively). The lowest catches (362 and 492 flies/trap/day) were observed at Kolamulato in March and April, respectively (Fig. 16.4).

Table 16.2 Mean catches of *Bactrocera dorsalis* males (flies/trap/day ± SEM), from February to April 2018 at Arbaminch and Mierab Abaya districts, Gamo-Gofa zone

Locations	Fruit fly moth catch/trap∗
Ankober	52.84 ± 4.69 c
Wajifo	19.95 ± 3.18 c
Kolashele	13.44 ± 3.35 c
Elgo	12.12 ± 3.15 c
Chelba	14.57 ± 1.12 c
Lante	8.25 ± 5.26 c
Ugayehu (control)	786.67 ± 70.16 a
Molie (control)	602.63 ± 64.63 b
Kolamulato (control)	498.00 ± 80.31 b
Mean	223.16
LSD (5%)	113.18
CV (%)	29.29

∗Means followed by the same latter in a column are not significantly different ($P \leq 0.05$)

Average trap catches in both intervention and control locations of each site are shown in Table 16.2. For all intervention and control orchards considered together, there was significant difference in mango fruit fly trap catch between treated and control locations. However, there was no significant difference in trap catch in intervention sites while a significant difference was observed among control sites. At three of the control sites (Ugayehu, Molie, and Kolamulato) mango fruit fly trap catches were significantly higher. The mango fruit fly populations in the Ugayehu site (786.67 ± 70.16), however, were considerably highest and statistically significant than in the other remaining sites (Table 16.2). However, significantly lower flies were caught in the intervention sites. Numerically the lowest trap catches were recorded from Lante (8.25 ± 5.26), but the difference was not statistically significant from the other intervention sites ($P < 0.05$, Table 16.2).

16.3.2 Fruit Fly Infestations

Fruit infestation rates decreased over time in both sites. However, a significant difference was observed in the intervention compared to the control sites (Fig. 16.5). Average fruit fly infestation in the mango fruits collected from intervention sites at each of the treatment interval ranged from 11% to 13%, while the infestation in control sites ranged between 24 and 68.4% (Fig. 16.5). Fruit fly infestation obtained from control site was fivefold higher than infestation obtained from intervention site (Fig. 16.5). Overall there were no significant differences in infestation rates among intervention sites, at any of the collection times, while a significant difference in infestations was observed among fruit collection times. The highest infestation rates in any site with the intervention (13%) and control (68.4%), were found in the first

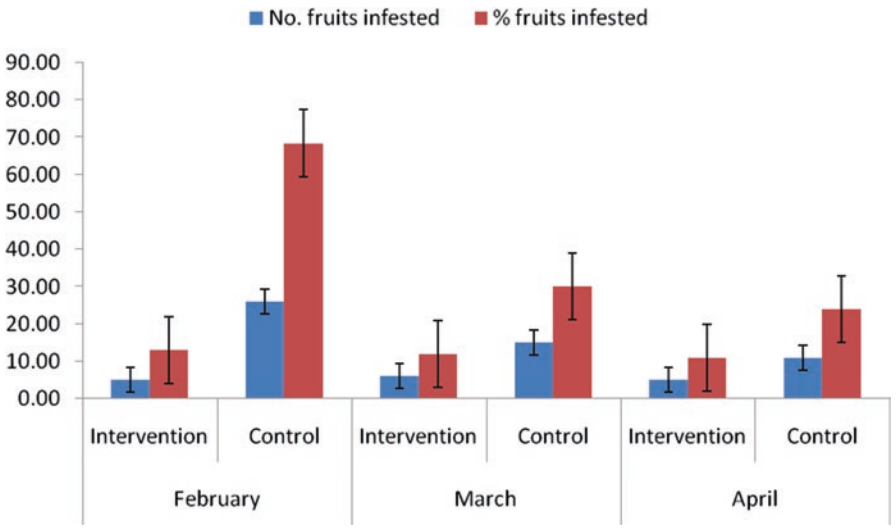

Fig. 16.5 Average (± SEM) number and percentages of fruit fly infestation in intervention versus control sites, at each trap service month

Table 16.3 The extent of mango fruit infestation by fruit flies in different locations of Gamo-Gofa zone, Southern Ethiopia

Location	Number of fruit sampled	Fruits infested (%)∗
Lanate	119	24.23 ± 7.54 c
Chano mille	125	29.85 ± 2.17 c
Kolla shelle	110	27.09 ± 7.97 c
Ugayehu	119	60.91 ± 7.15 a
Molie	125	50.15 ± 8.03 b
Kolamulato	110	43.24 ± 9.38 b
Mean		39.24
LSD (5%)		9.63
CV (%)		13.49

∗Means followed by the same latter in a column are not significantly different ($P \leq 0.05$)

fruit collection month. This month was also had the highest fruit fly population numbers, based on trap catches.

In general, mango infestation levels varied among separate locations of Arbaminch and Mierab Abaya Districts of Gamo-Gofa zone. The average infestation ranged from 24% to 60% in the study locations (Table 16.3 and Fig. 16.6). Significantly higher mango fruit infestations were recorded in Ugayehu, Molie and Kolamulato with respective mean infestations of 60.91%, 50.15% and 43.24% at $P < 0.05$ (Table 16.3). The lowest level of infestation (24.23%) was observed in Lanate (Table 16.3).

Fig. 16.6 Comparison between mango quality at intervention and control sites

Table 16.4 The extent of mango losses by fruit fly in Gamo-Gofa zone during 2017

Parameters	Extent of loss
Yield loss (Qt)	199,620
Yield loss (% of total)	30
Monetary loss (Birr)	159,696,000

NB: Total mango production area in the zone = 2218 ha; mango productivity for 2017 = 300 qt/ha; total mango produced in the zone during 2017 = 665 400 qt; farmgate price of mango in 2017 = 8.00 ETB/kg

16.3.3 Yield and Economic Loss of Mango by Fruit Fly

Table 16.4 summarizes the extent of mango fruit losses due to fruit fly in Gamo-Gofa zone, both in quantity and economic terms. Based on secondary data obtained from the zone and the District Bureau of Agriculture during the year of 2017, damage due to the fruit fly amounted to a yield loss of 199 620 qt (equivalent to 30% of the total mango produced, 665 400 qt) and around 159.7 million Birr (Table 16.4).

16.4 Discussion

Mango fruit flies cause considerable economic damage in the Gamo-Gofa zone of the Southern Region of Ethiopia. We applied mass trapping technique which was one of the most effective strategies for fruit fly management (Aluja 1999; McQuate et al. 2005). Mass trapping consists of the use of traps and baits that release specific

volatile substances that attract insects to the trap, in which fruit flies are captured and killed (El-Sayed et al. 2009). However, for some fruit fly species, the use of mass trapping as a control tool depends on the availability of an effective and cheap attractant (Villalobos et al. 2017). If the attractant is not specific, it might lead to failure (Suckling et al. 2016).

Male fruit flies are usually attracted by parapheromones (IAEA 2003). In contrast, lures for attracting female fruit flies into traps are based primarily on food or host lures (Dominiak and Nicol 2010). Our study showed that the use of ME has the potential to minimize population of *B. dorsalis*, and the related impact (Navarro-Llopis et al. 2008). Inclusion in monitoring networks of food-based lures that capture both females and males is useful. ME is a powerful attractant to male *B. dorsalis* (Kafu et al. 2012). In combination with malathion it attracted and killed the male mango fruit flies as such rates that mating was disrupted, effectively reducing the population density. Previous work on the evaluation of ME dispensers on males of *Dacus zonatus* under field conditions showed that it effectively attracted high numbers of males, with a potential for monitoring and control of this pest (Qureshi et al. 1992). Similarly, research with treatments containing ME and malathion (EC50) evaluated nutritional attractants including protein hydrolysate, palm extract, sugar, water and dishwashing liquids and ripe mango, under field conditions. The results showed that mango fruit fly populations were attracted more to the protein hydrolysate than to other treatments (Agarwal and Kumar 1999; Khosravi et al. 2018). It seems that the simultaneous use of hydrolyzed protein in bucket traps along with ME and malathion destroyed significant part of the pest population, disrupting mating and significantly reducing damages. These results confirm our findings.

In general, our results confirmed that the capturing rate of fruit flies showed similar dynamics. In both intervention and control sites and within three consecutive months catched fruit flies were lower in the second and third months than in the first one, even if the population in the control sites were considerably higher, compared to the intervention sites. This decline could be due to regular actions of treating in the first month. Based on results, the highest rate of adult insect capture was related to the treatment of ME and malathion, used during previous years of test implementation. However, in some locations since mango had been cultivated at the desired location mixed with avocado, banana, coffee and citrus tress (that are one of the favourite hosts of the mango fruit flies), the remaining population could have survived on these hosts re-starting the populations during the mango seasons. For this reason, it is advisable to avoid mixed planting of mango with other tropical fruits, to prevent damage by polyphagous fruit flies.

The ME in the traps worked quite well in attracting male flies, when serviced weekly to increase the pest control ngconfirming previous findings (Samuel et al. 2016). Our results also agree with Asquith and Kido (1994) and Howarth and Howarth (2000) as placing the lure and toxicant at 1–2 m above the ground in the uncovered canopy was more effective in controlling the fly populations. Our data further suggest that attractants placed closer than 50 m apart will interfere with each other and would be less cost-efficient.

Results showed a significant reduction in fruit infestation in the intervention compared to the control sitesas ME led to a reduction in losses by attracting male flies and impairing mating.

Infestation levels vary among seasons, countries, regions, agro-ecological areas and cultivars (Vayssières et al. 2009), however fruit flies are still a severe limiting factor for crops on a continental scale. Lux et al. (2003) reported that, of the 1.9 million tons of mangoes annually produced in Africa, about 40% was lost due to fruit flies.

In addition to effectively controlling *B. dorsalis* and fruit damage in mango orchards, the attract-and-kill bait stations caused less harm to non-target insects than conventional insecticides. In conventional control, which depends mainly on the application of broad-spectrum insecticides, non-target insects are exposed to acute and long-term toxic effects of insecticides, both directly via contact and/or indirectly, via ingestion of contaminated preys (Bostanian et al. 2009). In attract-and kill systems, the use of insecticides (the killing agent) is limited to the treated devices. Consequently, attract-and-kill bait stations have minimal lethal and sub-lethal effects on non-target insects and other invertebrates. The use of a pheromone should benefit further by increasing the bait station selectivity, maximizing the number of flies attracted and the dispensers lifespan (Hafsi et al. 2015).

16.5 Conclusions

Studies on fruit flies continue to increase and provide useful knowledge for researchers working in the areas of monitoring and control tactics. So far, there has been an emphasis on chemical control research, especially the use of organophosphates. However, the continued use of insecticides is increasingly limited, making it necessary to evaluate other control strategies for inclusion in fruit fly management. The use of a mass-trapping method using ME reduced mango fruit flies effectively. Therefore, it can then be a candidate technique to replace aerial treatments with synthetic insecticides, applied to suppress this pest. Even if the number of potential trapping targets by this para-pheromone lure is smaller, as only males are strongly attracted, its impact on consequent progeny reduction by mating disruption is very high. Our study demonstrates that an attract-and-kill method using solid ME lure formulated with an insecticide was effective for suppression of the *B. dorsalis* populations in mango orchards. These results indicate that attract-and-kill methods using ME represent a suitable alternative to conventional insecticide sprays for the control of *B. dorsalis*. Therefore, this product can be used in conjunction with other environment-friendly, area-wide IPM programmes, such as sanitation and protein bait sprays, for management of mango fruit fly. This study provides fact-based information and evidence to end-users and policymakers, to facilitate the application of this bait at local markets to control mango fruit flies in Ethiopia.

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References

Agarwal, M. L., & Kumar, P. (1999). Effect of weather parameters on population of peach fruit fly, *Bactrocera zona*ta (Saunders). *Entomon, 24*, 81–84.

Agunloye, O. J. (1987). Trapping and chemical control of *Ceratitis capitata* (Wied) (Diptera, Tephritidae) on sweet orange (*Citrus sinensis*) in Nigeria. *Journal of Horticultural Science, 62*, 269–271.

Aluja, M. (1999). Fruit fly (Diptera: Tephritidae) research in Latin America: myths, realities and dreams. *Anais da Sociedade Entomológica do Brasil, 28*, 565–594.

Asquith, A., & Kido, M. (1994). Native Hawaiian insects attracted to the semiochemical methyl eugenol, used for male annihilation of the oriental fruit fly (Diptera: Tephritidae). *Environmental Entomology, 23*, 1397–1408.

Billah, M. K., Wilson, D. D., Cobblah, M. A., Lux, S. A., & Tumfo, J. A. (2006). Detection and preliminary survey of the invasive fruit fly, (Diptera: Tephritidae) in Ghana. *Journal of the Ghana Science Association, 2*, 38–14.

Bostanian, N. J., Thistlewood, H. A., Hardman, J. M., Laurin, M. C., & Racette, G. (2009). Effect of seven new orchard pesticides on *Galendromus occidentalis* in laboratory studies. *Pest Management Science, 65*, 635–639.

Chang, V. C. S. (1995). Trapping *Cryptophlebia illepida* and *C. ombrodelta* (Lepidoptera: Tortricidae) in macadamia in Hawaii. *International Journal of Pest Management, 41*, 104–108.

Charmillot, P. J., Hofer, D., & Pasquier, D. (2000). Attract and kill: a new method for control of the codling moth *Cydia pomonella*. *Entomologia Experimentalis et Applicata, 94*, 211–216.

Cohen, H., & Yuval, B. (2000). Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera: Tephritidae) damage on different host species in Israel. *J. Econ. Entamol., 93*, 721–725.

Cunningham, R. T., Nakagawa, S., Suda, D. Y., & Urago, T. (1978). Tephritid (Diptera Tephritidae) fruit-fly trapping liquid food baits in high and low rainfall climates. *Journal of Economic Entomology, 71*, 762–763.

Dawit, G., Ferdu, A., & Yibrha, B. (2015). Species composition of fruit flies (Dipteral: Tephritidae) and extent of damage on mango fruit in eastern Ethiopia. *International Journal of Innovation and Scientific Research, 19*, 95–102.

Dias, N. P., Zotti, M. J., Montoya, P., Carvalho, I. R., & Nava, D. E. (2018). Fruit fly management research: A systematic review of monitoring and control tactics in the world. *Crop Protection, 112*, 187–200.

Dominiak, B. C., & Nicol, H. I. (2010). Field performance of Lynfield and McPhail traps for monitoring male and female sterile *Bactrocera tryoni* (Froggatt) and wild *Dacus newmani* (Perkins). *Pest Management Science, 66*, 741–744.

Drew, R. A. I., Tsuruta, K., & White, I. M. (2005). A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *African Entomology, 13*, 149–154.

Ekesi, S. (2010). *Development and implementation of a sustainable IPM program for major mango pests and opportunity for improving market and processing information in sub-Saharan Africa. Progress Report*. Eschborn: Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH.

Ekesi, S., & Billah, M. K. (2009). *A field guide to the management of economically important tephritid fruit flies in Africa* (2nd ed.). Nairobi: ICIPE Science Press.

Ekesi, S., Billah, M. K., Nderitu, P. W., Lux, S. A., & Rwomushana, I. (2010). Evidence for competitive displacement of *Ceratitis cosyra* by the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) on mango and mechanisms contributing to the displacement. *Vitamins and Hormones, 83*, 575–595.

Eliopoulos, P. A. (2007). Evaluation of commercial traps of various designs for capturing the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae). *International Journal of Pest Management, 53*, 245–252.

El-Sayed, A. M., Suckling, D. M., Byers, J. A., Jang, E. B., & Wearing, C. H. (2009). Potential of 'lure and kill' in long-term pest management and eradication of invasive species. *Journal of Economic Entomology, 102*, 815–835.

Enkerlin, W., Lopez, L., & Celedonio, H. (1996). Increased accuracy in discrimination between captured wild unmarked and released dye-marked adults in fruit fly (Diptera: Tephritidae) sterile released programs. *Journal of Economic Entomology, 89*, 946–949.

Epsky, N. D., Heath, R. R., Guzman, A., & Meyer, W. L. (1995). Visual cue and chemical cue interactions in a dry trap with food-based synthetic attractant for *Ceratitis capitata* and *Anastrepha ludens* (Diptera: Tephritidae). *Environmental Entomology, 24*, 1387–1395.

Ferdu, A., Mohammed, D., Defabachew, B., & Bezawork, M. (2007). Review of entomological research on fruit crops in Ethiopia. Fruit fly species composition and extent of damage on mango at UAAIE, 74–75.

Goergen, G. (2011). *Bactrocera invadens* (Diptera: Tephritidae), a new invasive fruit fly pest for the Afrotropical region: host plant range and distribution in West and Central Africa. *Environmental Entomology, 40*, 844–854.

Hafsi, A., Abbes, K., Harbi, A., Duyck, P. F., & Chermiti, B. (2015). Attract-and-kill systems efficiency against *Ceratitis capitata* (Diptera: Tephritidae) and effects on non-target insects in peach orchards. *Journal of Applied Entomology, 140*, 28–36. https://doi.org/10.1111/jen.12259.

Howarth, V. M. C., & Howarth, F. G. (2000). Attractiveness of Methyl Eugenol–baited traps to oriental fruit fly (Diptera: Tephritidae): Effects of dosage, placement, and color. *Proceedings of the Hawaiian Entomological Society, 34*, 167–178.

IAEA. (2003). *Trapping guidelines for area-wide fruit fly programmes*. Vienna: International Atomic Energy Agency.

Kafu, A. A., Hassan, N., Badi, A. A., & Zanati, A. (2012). Evaluation of the efficacy of zonatrac system (lure and kill) to control Peach fruit fly *Bactrocera zonata* by application of male annihilation technique. *Tunisian Journal of Plant Protection, 7*, 127.

Khosravi, M., Sahebzadeh, N., Kolyaie, R., & Mokhtari, A. (2018). Field evaluation of controlling methods of mango fruit flies *Bactrocera zonata* (Diptera: Tephritidae) in the southern part of Iran. *Trakia Journal of Sciences, 1*, 62–69.

Krupke, C. H., Roitberg, B. D., & ad Judd G. J. R. (2002). Field and laboratory responses of male codling moth (Lepidoptera: Tortricidae) to a pheromone-based attract-and-kill strategy. *Environmental Entomology, 31*, 189–197.

Lux, S. A. (1999). *African fruit fly initiative, Pan-African initiative to promote productivity and trade of fruits and vegetables through management of African fruit flies* (28 pp). Nairobi: International Centre for Insect Physiology and Ecology.

Lux, S. A., Copeland, R. S., White, I. M., Manrakhan, A., & Billah, M. K. (2003). A new invasive fruit fly species from the *Bactrocera dorsalis* (Hendel) group detected in East Africa. *Insect Science and Its Application, 23*, 355–361.

McQuate, G. T., & Follett, P. A. (2006). Use of attractants to suppress oriental fruit fly and *Cryptophlebia* spp. in Litchi. *Proceedings of the Hawaiian Entomological Society, 38*, 27–40.

McQuate, G. T., Sylva, C. D., & Jang, E. B. (2005). Mediterranean fruit fly (Dipt., Tephritidae) suppression in persimmon through bait sprays in adjacent coffee plantings. *Journal of Applied Entomology, 129*, 110–117.

Navarro-Llopis, V., Alfaro, F., Domínguez, J., Sanchís, J., & Primo, J. (2008). Evaluation of traps and lures for mass trapping of Mediterranean fruit fly in citrus groves. *Journal of Economic Entomology, 101*, 126–131.

Qureshi, Z. A., Siddiqui, Q. H., & Hussain, T. (1992). Field evaluation of various dispensers for methyl eugenol, an attractant of *Dacus zonatus* (Saund.) (Dipt., Tephritidae). *Journal of Applied Entomology, 113*, 365–367.

Samuel, A. B., Selorm, O., George, Y., & Maxwell, B. (2016). Evaluation of two trap types on the capture of fruit flies (Diptera: Tephritidae) in the Assin North Municipality, Ghana. *Agricultural Science Research Journal, 6*, 235–240.

Sokal, R. R., & Rohlf, F. J. (1981). *Biometry*. New York: W. H. Freeman and Company.

Suckling, D. M., Kean, J. M., Stringer, L. D., Cáceres-Barrios, C., Hendrichs, J., Reyes-Flores, J., & Dominiak, B. C. (2016). Eradication of tephritid fruit fly pest populations: outcomes and prospects. *Pest Management Science, 72*, 456–465.

Vargas, R. I., Walsh, W. A., Kanehira, D., Jang, E. B., & Armstrong, J. W. (1997). Demography of four Hawaiian fruit flies (Diptera: Tephritidae) reared at five constant temperatures. *Annals of the Entomological Society of America, 90*, 162–168.

Vayssières, J. F., Korie, S., & Ayegnon, D. (2009). Correlation of fruit fly (Diptera Tephritidae) infestation of major mango cultivars in Borgou (Benin) with abiotic and biotic factors and assessment of damage. *Crop Protection, 28*, 477–488.

Villalobos, J., Flores, S., Liedo, P., & Malo, E. A. (2017). Mass trapping is as effective as ground bait sprays for the control of *Anastrepha* (Diptera: Tephritidae) fruit flies in mango orchards. *Pest Management Science, 73*, 2105–2110.

Chapter 17
Sudan Thematic Implementation Plan for the Management of Invasive Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier)

Mahdi Abdelrhman Ahmed

Abstract Date palm (*Phoenix dactylifera L.*) is believed to be cultivated in northern Sudan and upper Nubia since 3200 BC. Dry date cultivars might have originated in Southern Egypt and Northern Sudan. Date palm is an economic and food security crop in Sudan. Estimated annual date production from 8 million date palm trees is about 431,000 mt, which is far below the country's potential, that has been famous in the world for the production of dry dates. Six suitable local commercial cultivars are available, and research yielded a better composition of cultivars by local selection and foreign introduction from tissue culture laboratories. Sudan is still free from-devastating red palm weevil (RPW) and the destructive bayoud caused by *Fusarium oxysporum* f. sp. *albedensis.* RPW, *Rhynchophorus ferrugineus* (Olivier), is a category-1 pest on date palms in Middle-East countries. It is one of the biggest threats to global agriculture of these days. These little crimson pests eviscerate coconut, date and oil palms. They are native to South Asia. Over the last three decades, the pest spread to more than 60 countries from the Caribbean to Southern Europe. For farmers across East Asia, North Africa, Europe and the Middle East, the red palm weevil evokes serious anxiety. These pests, which attack 40 different species of palms, have caused economic losses in the millions of dollars annually, worldwide. One female weevil can lay up to 300 eggs – hiding them inside holes and cavities in the trunk of a palm. Once they hatch, the larvae burrow deep inside the palms, munching their way through the tree, and destroying it from the inside. Sudan is expecting that RPW would eventually come. The thematic implementation plan is the major component of a strategy to be adopted for a country that is not infested with RPW. It involves (1) Quarantine, (2) Monitoring/Surveillance and (3) Training/Capacity Building. Here are discussed some points that could be adopted to strengthen the components mentioned above should an infestation occur in Sudan. In this case, the strategy should aim at containing the spread and eradicating the pest.

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Keywords Sudan · Date palm · Red palm weevil · Strategy adopted

17.1 Introduction

Date palm (*Phoenix dactylifera*) L. is believed to be cultivated in northern Sudan and upper Nubia since 3200 BC. Dry date cultivars might have originated in Southern Egypt and Northern Sudan. It is considered as a key, economic and food security crop in Sudan. Estimated annual production of dates from 8 million date palm trees reaches about 431,000 mt (FAO 2010), which is far below the country's potential. Sudan has been famous in the world for the production of dry dates. Six suitable local commercial cultivars are available, and research is developing a better composition of cultivars by local selection and foreign s from tissue culture laboratories (Khairi 2015).

However, the date palm industry is facing many serious problems, related to low yields, lack of appropriate packing and presentation as well as limited processing of date products. The low yields in most countries, including Sudan, are due to soil salinity, poor fertility, insect pests and diseases, lack of maintenance and care due to the increasing cost of labour and to the shortage of personnel trained in improved cultural practices. As a result of the high cost of production and low prices of the produce, farmers tend to neglect or even abandon their orchards. Although the commonly known, insect pests i.e. the devastating red weevil and the destructive disease known as bayoud (caused by *Fusarium oxysporum* f. sp. *albedensis*), have not been reported yet in Sudan (Ahmed et al. 2013; Faleiro 2017; El Hassan 2006), the date palm crop is affected by many biotic factors among which insects are the most important.

17.2 Red Palm Weevil (RPW)

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier), is a category-1 pest on date palms in all the Middle-East countries. It is one of the biggest threats to global agriculture in these days. The little crimson pests eviscerate coconut, date and oil palms. Over the last three decades, they spread from native South Asia to more than 60 countries, from the Caribbean to Southern Europe. For farmers across East Asia, North Africa, Europe and the Middle East, the red palm weevil represents a severe threat. RPW, which attacks 40 different species of palms, have caused economic losses in the millions of dollars annually, worldwide

Rhynchophorus ferrugineus is a lethal pest of the date palm tree. Its area of speciation corresponds to South and South-East Asia, where it has been a key pest of coconut. RPW is now reported from nearly 50 countries in all the continents from 40 palm species, worldwide (Wattanapongsiri 1966; Faleiro 2006; Giblin-Davis

et al. 2013; http://www.savealgarvepalms.com/en/weevil-facts/host-palm-trees). The global spread of RPW has been rapid during the last three decades, primarily through infested planting material. FAO has designated RPW as a category-1 pest of the date palm. Early detection of infested palms is the key to its successful control in countries where it occurs. In the case of Sudan, enforcing strict external and internal quarantine regimes is vital to prevent the entry of the pest (Table 17.1).

There is a good reason for all the panic. Citing Mark Hoddle, an entomologist at the University of California, Riverside: "*Red palm weevils are notoriously difficult to detect – until it is too late. One female weevil can lay up to 300 eggs – hiding them inside holes and cavities in the trunk of a palm. Once they hatch, the larvae burrow deep inside the palms, munching their way through the tree, and destroying it from the inside*".

17.3 The Thematic Implementation Plan

A thematic implementation plan is the major component of a strategy to be adopted for a country that is not yet infested by RPW. It revolves around the following actions: (1) Quarantine, (2) Monitoring/Surveillance and (3) Training/Capacity Building. Here are some points that could be adopted to strengthen the components as mentioned above, to be adopted in case an infestation occurs in Sudan. In this situation, the strategy should mostly be aimed at containing the spread and eradicating the pest.

17.3.1 Quarantine

- Registration of nurseries/importers: The Ministry of Agriculture/National Plant Protection Organization (NPPO) should keep a register for all palm nurseries/importers (growers, nurseries, dealers, etc.) and establish a database on importation and destination of palms.
- No palm tree should leave nurseries without a **movement certificate**, issued by NPPO.
- Prohibition of importation of palm trees from a particular site/origin (infested area/countries).
- Palm trees originating from authorized nurseries should only be imported, from non-infested countries.
- In case of the date palm, only date palms obtained by *in vitro* propagation could be imported in test-tubes, from officially certified nurseries.
- Offshoots produced within the country should be permitted to be transported under the close supervision of NPPO, preferably after treatment (dipping of the bole of offshoots in 0.004% Fipronil for 30 min). A simple, superficial spray does not help.

Table 17.1 Thematic implementation plan for the management of invasive Red Palm Weevil[a]

Theme	Recommended actions	Implementing agencies
Awareness, Training and Education	(i) Develop and implement a public awareness programs about that invasive pest and its impact on biodiversity and livelihood of the local communities.	Min. of Agric., Min. of Educ., NGOs, ARC, Universities
	(ii) Encourage media organizations and extension workers to participate in the dissemination of information about the impact of this invasive insect.	
	(iii) Support education institutions to incorporate issues of RPW, identification, prevention, eradication and management into their curricula.	
	(iv) Develop a database of RPW, identification guides and make the information accessible to Stakeholders.	
	(v) Qualify and train taxonomy specialists in insects.	
Policies	(i) Strengthen quarantine measures and border control to ensure that intentional plant introductions are subject to appropriate authorization.	Min. of Agric.
	(ii) Develop risk assessment and management programs and guidelines for newly introduced species.	
	(iii) Develop and implement effective response procedures for the prevention of new potentially invasive species.	
	(iv) Encourage and support the involvement of all stakeholders in alien invasive species management program.	
	(v) Develop invasive species management plans that emphasize prevention of introductions, control and eradication of invasive species.	
	(vi) Develop effective systems and tools for monitoring and evaluation of invasive species.	
Legislation	(i) Harmonize state and sectoral rules and regulations relevant to invasive species and formulate policies and legislation for the control of introductions, movement and management of date palm.	Min. of Agric.
	(ii) Enforcing the international regulation for RPW.	
Conservation	(i) Identify RPW problems and recommend management actions.	ARC
	(ii) Develop appropriate methods to monitor, prevent and stop the spread of invasive RPW.	
	(iii) Assess the movement of RPW and develop maps of the distribution of the invasive pest.	
	(iv) Formulate and implement result-oriented research on the characterization of invasive RPW; vulnerability of ecosystems, social and economic impact; prevention, control, eradication and management methods.	
	(v) Promote research on the use of traditional knowledge in the development and implementation of measures to manage RPW.	

(continued)

Table 17.1 (continued)

Theme	Recommended actions	Implementing agencies
Sustainable Use	(i) Strengthen existing institutions to coordinate research, management and eradication of invasive RPW.	Min. of Sc., ARC, Universities.
	(ii) Produce an inventory of RPW data and evaluate their economic, social and environmental impacts.	

[a]Summary of the thematic plan (with some modification) as reported in the **National Biodiversity Strategy and Action Plan 2015–2020**. Issued by Higher Council for Environment and Natural Resources (HCENR) of Ministry of Environment, Natural Resources and Physical Development, The Republic of Sudan, June 2015

- In case of tissue culture palms produced within Sudan, localize geographically (GIS) the mother tree, label it (each mother plant must be marked with the year, sampling area and serial number), under the supervision of NPPO.
- Movement of offshoots from one oasis to another across the country should not be permitted, to localize infestation in case of an outbreak.
- Nurseries should be inspected at least three times a year.
- Import of offshoots should be banned from infested countries.
- Develop regulation manuals with clear requirements for import, movement and nursery certification.
- Support the establishment of tissue culture laboratories for the production and supply of RPW-free planting material.
- Train Plant Quarantine Staff and other law enforcement authorities

17.3.2 Detection

- Create awareness among farmers and other stakeholders about the seriousness of the RPW issue.
- Develop a protocol for visual inspection in a simple and easy way to understand, in the languages of the farmer and other support staff.
- Urgent need to develop a quick and reliable, cost-effective, and easy to handle early detection device for RPW. These may include and rely on: remote sensing, acoustics, thermal imaging, chemical signatures, laser-induced breakdown spectroscopy, near-infrared spectroscopy, X-ray, biological and physiological stress indicators, sniffer dogs etc.

17.3.3 Measures for Surveillance, Monitoring and RPW Control

1. Regular inspection and monitoring
2. Population disruption (pheromone trapping)

3. Plant quarantine
4. Extension programs
5. RPW-IPM

In addition to regular visual inspection of date palms in the susceptible age group (<20 years), it is recommended to monitor the incidence of adult weevils during their peak activity from March to May and again from September to November. This could be achieved by setting food baited pheromone traps in plantations along roads, at a distance of 1 km between two monitor traps.

17.3.4 Awareness

1. Strengthen extension programs, activities, knowledge sharing mechanisms, communications, and farmers' organizations.
2. Establish defined coordination mechanisms with NGO's, private sector, and cooperatives to make the program more effective.
3. Introduce a participatory approach (Farmers Field School) for farmers and farm workers to empower them with knowledge and field practices.
4. Use of social media to expedite the transmission of information
5. Strengthen cooperation among institutions at the National level and initiate programs of cooperation at the Regional and International levels.

17.3.5 Capacity Building

Training and capacity building of all stakeholders (Farmers, Agriculture officers, Quarantine officials, NGOs, Cooperatives, Farmer Groups etc.) on the RPW eradication strategy (should the pest be detected) should be carried out/intensified.

Farmer participation and involvement in the RPW control programs is crucial for successful control. The advantage of involving the farmers and other stakeholders in the control program is considerable as they are present in the farm and can assist in detecting infested palms in the early stage of the attack. This action that constitutes the key to control and eradicate the pest.

Pilot projects to experiment and demonstrate the feasibility to involve farmers/stakeholders should be initiated in all the major date palm oasis of Sudan.

17.4 RPW-IPM Programs

1. Mass trapping to be taken up by lead/trained farmers.
2. Introduce attract and kill strategy in mass trapping programs.

3. Evaluate the dry trap using electro-magnetic technology.
4. Carry out a risk assessment of the area adopting visual observation and pheromone traps.
5. Develop good agronomic practices that limit the RPW attack.
6. Preventive measures should be practiced, including sanitation, wounds treatment, removal of neglected orchards, pheromone trapping, and insecticide applications via spray and injection.
7. Explore potential indigenous strains of entomopathogenic nematodes and fungi and develop an efficient delivery system.
8. Develop RPW-IPM programs and ensure farmers/stakeholder participation.

17.5 Data Management

1. Develop a GIS and spatial database to be used operationally by countries.
2. Managing mass trapping through the GIS with RFID (barcoding) of traps.
3. Use remote sensing imagery to geo-referenced palm trees in countries to be used as a primary base map in the GIS.
4. Develop a user-friendly mobile application for reporting, data collection and transmission.

17.6 Should an Infestation Occur – What Is to Be Done?

In this case, the goal should be to contain the spread and eradicate the pest. To achieve this:

- Remove (eradicate) all infested palms. Cut the infested portion of the palm into small bits (20 cm long) and drench with insecticide *in-situ*.
- Do not move the infested palm for eradication to another site.
- Establish a 10 km radius buffer zone.
- Intensify phytosanitary/quarantine regulations.
- Inspect all palms in the buffer zone at bi-monthly intervals.
- Mass intervention with traps in the area (1 km radius from the infested palm) at 1 trap/ha.
- Deploy attract and kill interventions, if infestation is severe, i.e. 3 weevils/trap/week.
- Prohibit movement of all palms from the buffer zone.
- Intensify training on RPW-IPM.
- Encourage farmers' participation in the RPW-IPM program, especially with regard to the detection of infested palms.
- The country is to be declared pest (RPW)-free if no new infestation/weevil is detected for three years.

17.7 Challenges in Red Palm Weevil Mentoring

1. Early detection difficulties of RPW infestation.
2. Farming system.
3. Lack of adequate human and financial resources
4. Lack of active involvement/ training of farmers.

References

Ahmed, M. A., Gubara, S., Sanhuri, S., Gebrel, K., & Siddig, I. (2013). The efficacy of two systemic insecticides against the green pit scale insect (*Palmapsis phoenicis* Bodenheimer (Green)) (Homoptera: Asterolecaniidae) infesting date palm in Northern Sudan. In: Proceedings of 72nd meeting of the Pests and Diseases Committee, ARTC, Wad Madani, Sudan.

El Hassan, E. M. (2006). Date palm diseases in Merawi and Debba areas. In *The Northern State, Sudan* (Crop Protection Research Center Annual Report, 2006/2007). Agricultural Research Corporation (ARC), Wad Madani.

Faleiro, J. R. (2006). A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. *International Journal of Tropical Insect Science, 26*, 135–154.

Faleiro, J. R. (2017). Report of red palm weevil mission to Sudan. On behalf of 'Khalifa international award for date palm and agricultural innovation'. First international Sudanese date palm conference, December 2017, Khartoum, Sudan.

FAO. (2010). *Agro-Statistics Database*. http://faostat.fao.org/site/340/default.aspx.

Giblin-Davis, R. M., Faleiro, J. R., Jacas, J. A., Peña, J. E., & Vidyasagar, P. S. P. V. (2013). Coleoptera: Biology and management of the red palm weevil, *Rhynchophorus ferrugineus*. In J. E. Peña (Ed.), *Potential invasive pests of agricultural crop species* (pp. 1–34). UK: CABI Wallingford.

Khairi, M. M. A. (2015). Date palm status and prospective in Sudan. In J. M. Al-Khayri (Ed.), *Date palm genetic resources and utilization, Vol. 1: Africa and the Americas*. Dordrecht: Springer. https://doi.org/10.1007/978-91-017-9694-1-5.

Wattanapongsiri, A. (1966). A revision of the genera *Rhynchophorus* and *Dynamis* (Coleoptera: Curculionidae). Bangkok: Department of Agriculture Science Bulletin 1, 328 pp.

Chapter 18
Management of Pests of Quarantine Importance in Ghana's Export Vegetables (Chili, Eggplant and Ridged Gourds)

K. O. Fening, M. K. Billah, and C. M. K. Nankinga

Abstract The Ghana vegetable exports experienced several interceptions from 2012 to 2015, largely due to the presence of harmful organisms, specifically quarantine pests (thrips, whiteflies, fruit flies and false codling moth) in the consignments destined for export to the European Union. Therefore, Ghana was placed under a ban for some vegetables, such as chili pepper, eggplant, aubergine, and gourds. This situation lasted from 2015 until December 2016, and was further extended to December 2017. Consequently, the Ministry of Food and Agriculture (MoFA) set up an Exports Taskforce to help reverse the ban. In spite of the progress made, well-coordinated scientific research was weak in these efforts. Here we discuss the outcome of the scientific interventions undertaken between 2016 and 2017, to ensure that these pests of quarantine importance were effectively managed at the farm level. These interventions also endeavoured us to equip the Plant Quarantine Inspectors (PQI) of the National Plant Protection Organisation (NPPO), known as Plant Protection and Regulatory Services Directorate (PPRSD) of MoFA, with updated knowledge on the sampling, detection and identification of these pests to ensure pest-free export produce. A quick survey was undertaken to understand the

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crop protection strategies employed by farmers (farmer practices), and the gaps identified were then taken into consideration in the development of a scientific protocol that was tested in the exporter farms. The protocols comprised the testing of six products belonging to different classes of insecticides (synthetic, botanical, microbial and physically acting), use of sticky traps, food baits and lures for the target pests, and the promotion of good farm hygiene. The action was targeted at three selected crops: chili, ridged gourd and eggplant, in six selected out-grower farms of exporters, in three different agro-ecological zones. From the quick-scan survey, poor farm hygiene (mainly heaps of rotten fruits left lying near the farm) was the main source of re-infestation for false codling moths and fruit flies, onto newly established crop fields. Species-specific lures for mass trapping of false codling moths and fruit flies offered an effective control of these pests, with fruits harvested and incubated being generally pest free. Aqueous neem kernel extract (ANKE), Maltodextrin 282 g/L, *Bacillus thuringiensis* (32,000 IU/mg), and Acetamiprid 16 g/L + Indoxacarb 30 g/L were among the most effective insecticides for the target pests. The outcome of the trial was used as baseline information, to be used in addition to best crop protection practices, for developing an Integrated Pest Management (IPM) strategy known as a roadmap for pest reduction on the target export vegetables. This roadmap was further implemented through demonstration trials in over ten exporter out-grower farms, to help upscale the technology and disseminate the information to the greater numbers of exporters and their outgrowers, field staff of PPRSD, MoFA Agriculture Extension Agents, and other relevant stakeholders. The training of the PQI at the point of exit ensured that produce sent through the exit points are pest free. The positive steps undertaken ultimately influenced the decision of the EU Commission to lift the ban on the export of the selected vegetables, from 1st January 2018.

Keywords Quarantine pests · Harmful organisms · Export vegetables · Pesticides · Field trials · Insect pests

18.1 Introduction

The Ghana exports of vegetables experienced several interceptions from 2012 to 2015, largely due to the presence of harmful organisms, specifically quarantine pests, in the exported consignments to the European Union (GhanaVeg Sector Reports 2016, 2017). These pests comprise: *Thrips palmi* Karny (Thysanoptera: Thripidae) whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae); fruit flies, *Bactrocera, Ceratitis, Dacus* and *Zeugodacus* spp. (Diptera: Tephritidae), and the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). Therefore, Ghana was placed under a ban for some vegetables, mainly *Capsicum* sp. (chili pepper), *Solanum* spp. (eggplant and aubergine) and gourds

such as luffa, bitter and bottle gourds, (*Luffa*, *Momordica*, *Lagenaria* spp., respectively). This situation lasted from 2015 until December 2016, and was further extended till December 2017 (GhanaVeg Sector Reports 2016, 2017). Consequently, the Ministry of Food and Agriculture (MoFA) set up an Exports Taskforce to help reverse the situation. In spite of the progress made, well-coordinated scientific research was weak in these efforts (GhanaVeg Sector Reports 2017).

International trade also requires strict adherence to Sanitary and Phytosanitary (SPS) regulations stipulated by the National Plant Protection Organisation (NPPO) of the exporting or importing country. This is done in order to satisfy the awareness and demand by consumers worldwide for wholesome and safe vegetables, devoid of pests and pesticides residues, and to ensure that these crops are produced using methods that preserve the integrity of the environment for posterity.

This article, therefore, seeks to discuss the outcome of the well-coordinated scientific interventions that were undertaken between 2016 and 2017 to ensure that pests of quarantine importance were effectively managed at the farm level. These interventions also endeavoured to equip the Plant Quarantine Inspectors (PQI) of the NPPO of Ghana, known as Plant Protection and Regulatory Services Directorate (PPRSD) of MoFA, with updated knowledge on the sampling, detection and identification of these pests, to ensure that exported produce is pest-free. These positive steps undertaken ultimately influenced the decision of the EU Commission to lift the ban on the export of the selected vegetables, from 1st January 2018.

18.2 Materials and Methods

18.2.1 Site Selection and Trial Locations

A quick survey of prospective farmer fields was undertaken from 26th May to 1st June, 2016. Eighteen farms belonging to out-growers of the vegetable exporters were inspected in three agro-ecological zones in the Eastern, Central, Volta and the Greater-Accra regions, to gain an understanding on their farm-management practices. Six of these farms (AB farms at Adeiso, Param Farms at Akatsi, Joekopan farms at Begoro, Joekopan farms at Torgorme, Shrigan farms at Begoro, and Vegpro at Torgorme) were selected for the trials. The farm selection was mainly based on the fact that the production sites were a hotspot or an area with high pest pressure (Fening et al. 2016).

18.2.2 Land Preparation, Field Layout, Treatments and Trap Placements

The crops used for the trials included chili pepper, eggplant (garden eggs) and ridged gourd (turia or luffa). The land was de-stumped, cleared of weeds, ploughed, and harrowed to obtain a uniform field. A nursery was established for sowing pepper and

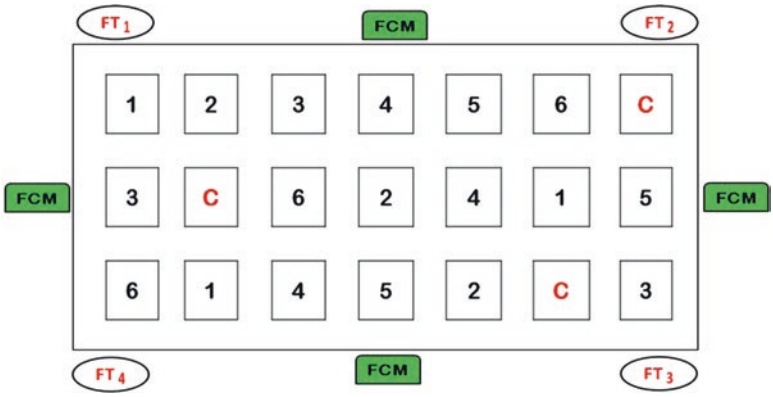

Fig. 18.1 Field layout for each crop and positioning of traps for monitoring fruit flies and false codling moth (FCM). FCM delta sticky trap with lure was absent in turia plots and present in pepper and garden egg plots. C = control plot (only food bait and yellow sticky traps applied for in-plot monitoring, without any insecticide treatment)

eggplant seeds, protecting the young seedlings with a mosquito-proof net. The seedlings were transplanted to the main field at 4 and 6 weeks old, for eggplant and pepper, respectively. Turia was planted direct at a spacing of 150 cm × 150 cm. The spacing for pepper was 60 cm × 60 cm, and 90 cm × 90 cm for eggplant. Each plot size was 5 m × 5 m, with 4 m interplot alleys, for the three crops. The experiment was laid in a Randomised Complete Block Design (RCBD), with three replications (Fig. 18.1). Trap placement for FCM and fruit flies (lures) is also shown in Fig. 18.1.

The six insecticides treatments (T1–T6) were applied, and the related information are summarised in Table 18.1 below.

The insecticides were applied using a knapsack. Separate knapsacks were used for the synthetic and natural insecticides. Spray applications were done in the late afternoon or evening in order to avoid peaks of high UV radiation.

18.2.3 Fruit Fly Pheromone Traps

The four fruit fly lures used were:

FT_1 = Methyl Eugenol (ME)
FT_2 = Terpinyl Acetate (TA)
FT_3 = Trimedlure (TML)
FT_4 = CueLure (CUE)

To prevent flies from learning the positions of the fruit fly attractants, the trap locations were changed on a weekly basis after every inspection and collection, in a rotational fashion, until the end of the trial (Billah et al. 2013; Billah and Wilson 2016). Data from these traps were used to determine the general fruit fly population densities in the field (IAEA 2003). The trap arrangement (food baits and sticky traps) within a plot for each crop is shown in Fig. 18.2.

Table 18.1 Insecticide treatment information

Treatment and commercial name of insecticide	Active ingredient (s)	Grouping	Target Pests	Application rate (g or ml/15 L of water)
1. Eradicoat T GH®	Maltodextrin 282 g/L	Physically acting by blocking spiracles of insect	Thrips, whiteflies, fruit flies, False codling moth	150 ml
2. Protocol®	Lambda cyhalothrin 15 g/L + Acetamiprid 20 g/L	Synthetic	Thrips, whiteflies, fruit flies, False codling moth	40 ml
3. ANKE	Azadirachtin	Botanical –plant origin	Thrips, whiteflies, fruit flies, False codling moth	750 g
4. Cydim Super EC®	Dimethoate (400 g/L) + Cypermethrin (36 g/L)	Synthetic	Thrips, whiteflies, fruit flies, False codling moth	35 ml
5. Viper 46 EC®	Acetamiprid 16 g/L + Indoxacarb 30 g/L	Synthetic	Thrips, whiteflies, fruit flies, False codling moth	40–50 ml
6. Ecopel®	*Bacillus thuringiensis* (32,000 IU/mg)	Microbial	False codling moth	20–25 g

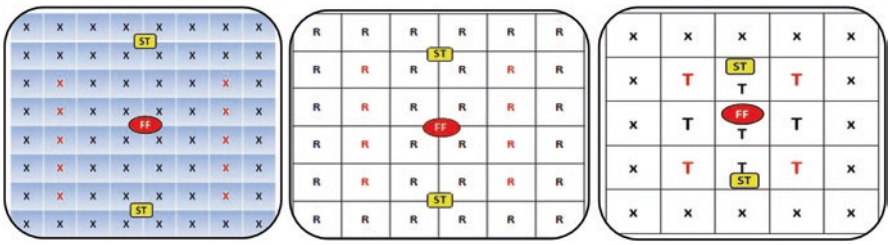

Fig. 18.2 Set up of traps for chili pepper, garden eggs and turia. FF = fruit fly trap (food bait); ST = sticky trap. X, R or T in red colour selected plants for data collection

18.2.4 Sampling and Data Collection

Chili fruits, eggplant, and turia were collected on each sampling date, on at least 10 tagged sample plants (except turia where 4 plants were selected) to assess for false codling moth and fruit fly infestations, on each farm (Fening et al. 2016). Harvesting of the samples was done on 0, 14, 28, 42, 56, 70, and 82 days after pesticide application. The collected fruits were counted, weighed and incubated in the University of Ghana Entomology laboratory, and the numbers of emerged larvae/pupa/adults were recorded.

Details of trap data were used to calculate relative pest densities (i.e. no. of pests/no. of traps/no. of days of trap exposure, F/T/D) in the area (IAEA 2003) to determine pest status, prevalence and requirements of Fruit Fly-Areas of Low Pest Prevalence (FF-ALPP). This index allows comparison across different localities and over different exposure periods, irrespective of the number of traps used. Ten flowers were randomly sampled weekly at each plot and placed into 70% alcohol in vials, and the number of thrips counted using a hand lens. In addition, sticky traps placed in the centre of each plot were used to monitor within-plot thrips populations. Counts of whiteflies were undertaken early morning, at 6–8 am, when the flies were inactive. The five topmost expanded leaves were inspected from five plants in the middle rows of each plot (Fening et al. 2014). Sticky trap catches were also recorded. Good agronomic practices (GAP) were followed, such as supplementary irrigation and fertiliser application.

18.2.5 Data Analysis

Count data for insect catches from traps were square-root transformed and subjected to ANOVA. Data on weekly counts of insects were also subjected to ANOVA. Significant differences in means were separated using an SNK test ($P \leq 0.05$). All analyses were carried out using SAS (SAS Inc. 2008).

18.3 Results and Discussion

18.3.1 Highlights from Scientific Trials for Managing Export Vegetable Pests in the Field

Ridged Gourd (Turia/luffa)

1. No single fruit fly was collected in the food bait traps that were deployed in-plot.
2. No fruit fly was recorded in the fruit incubation process.
3. All fruit flies recorded were trapped in the pheromone traps deployed at the periphery of the crop field.

The fruit flies species identified from the trap catches included *Bactrocera dorsalis*, *Ceratitis anonae*, *Dacus bivittatus*, *D. punctatifrons*, *D. vertebratus* and *Zeugodacus cucurbitae.* These species of fruit flies have been intercepted several times on produce from Ghana being exported to the EU (EUROPHYT 2016). The fruit flies species, *B. dorsalis* and *Z. cucurbitae*, are included in the European and Mediterranean Plant Protection Organization (EPPO/OEPP) A1 list as quarantine pests that are absent from the EPPO region. They are regarded as organisms of serious phytosanitary concern, and must be regulated accordingly, as they pose a very high phytosanitary risk (EPPO 2017).

Eggplant

Both fruit flies and FCM were recorded on this crop. The species of fruit flies recorded included *D. bivittatus, D. vertebratus*, *Z. cucurbitae* and a single specimen of *B. dorsalis*. Although FCM was recorded, their numbers were low. The weekly fruit fly catches are shown in Table 18.2 and Fig. 18.3. The initial high fruit fly population at the beginning of the trial was gradually brought to low levels, with time. These ranged from 0.1 to 22.0 flies per trap per day, with an average of 9.1 flies per trap per day across the different zones. Although the relative fly density levels were high and flies were collected in traps from all the turia growing farms, no single individual fruit fly emerged from the more than 400 individual turia fruits (weighing over 100 kg) that were sampled from the trial fields for incubation. This could be largely attributed to the new "two-level" trapping layout, where four different fruit fly pheromone traps were deployed at the periphery of each plot (and rotated on a weekly basis), and the placement of additional fruit fly food bait traps in individual plots (GhanaVeg Sector reports 2017).

The most active traps were the CueLure and Methyl Eugenol (ME) traps, which attracted most of the *Dacus*, *Zeugodacus* and *Bactrocera* species. These species are known to be specifically attracted to crops of the family Cucurbitaceae and a wide range of other crops.

18.3.2 FCM Results from Chili and Garden Egg Fields

The emergence of FCM from incubated chili fruits and garden eggs from Begoro is shown in Table 18.3. From the data, three of the insecticide treatments – Neem, Viper and Protocol – were able to give a 100% protection to the chili fruits, while Cydim Super, Ecopel and Eradicoat offered 71.2, 85.8 and 85.8% protection, respectively, to the chili plots (relative to the control plots) (Table 18.3).

With respect to the garden eggs, only Neem and Viper offered 100% protection, while Cydim Super, Ecopel, Eradicoat and Protocol gave 74.7, 90.5, 91.4 and 96.0 % protection, respectively, to the plots (relative to the control plots). It is interesting to note that the Neem kernel extract provided total protection against FCM in both chili and garden egg fields. Here, the levels of protection ranged from 71.2 to 100% for all the six products. Hitherto, the farmers had used some of these same products

Table 18.2 Weekly fruit fly catches for different fruit fly lures

Trap	13.12.16	21.12.16	30.12.16	6.01.17	13.01.17	20.01.17	26.01.17	03.02.17	Total	No. Traps	Trap Days	Density F/T/D
ME	209	127	137	63	157	70	25	13	801	1	56	14.3
TA	0	0	0	0	0	0	0	0	0	1	56	0.0
TML	0	0	3	0	0	0	0	0	3	1	56	0.1
CUE	354	143	156	247	96	102	88	46	1232	1	56	22.0
Total	563	270	294	310	253	172	113	59	2036	4	56	9.1

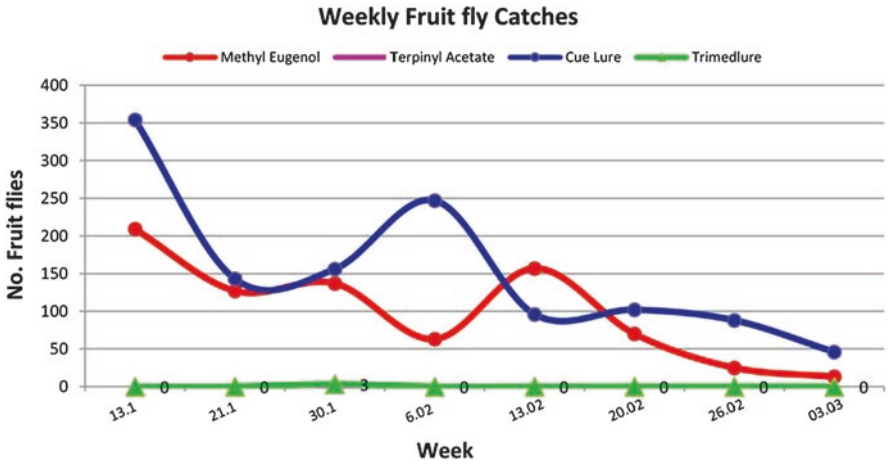

Fig. 18.3 Weekly fruit fly catches for different fruit fly lures

Table 18.3 Emergence of false codling moths from incubation of fruits from chili and garden egg fields in Begoro

Treatment (T)	No. fruits	No. Puparia	Pupa/fruit	Diff. (C−T)	% Protection (Rel. to control)
Begoro – chili					
Neem	30	0	0.00	–	**100**
Cydim	30	2	0.067	0.166	**71.2**
Ecopel	30	1	0.033	0.200	**85.8**
Eradicoat	30	1	0.033	0.200	**85.8**
Viper	30	0	0.00	–	**100**
Protocol	30	0	0.00	–	**100**
Control©	30	7	0.233	–	–
Begoro – garden eggs					
Neem	60	0	0.00	–	**100**
Cydim	60	8	0.133	0.392	**74.7**
Ecopel	60	3	0.050	0.475	**90.5**
Eradicoat	44	2	0.045	0.480	**91.4**
Viper	40	0	0.00	–	**100**
Protocol	47	1	0.021	0.504	**96.0**
Control©	40	21	0.525	–	–

before, but with little success. The plausible reason for the success now could be the consistent and systematic procedure applied when using the products. They include the "when and how" they were applied with the appropriate applicator, coupled with the good planting distances used for the different crops, as well as the good agronomic practices used during the trials. Farmers always plant as many seeds or seedlings as possible in a fixed plot of land, in order to harvest as many fruits as possible. These results indicate that, with the proper scientific basis, good

Table 18.4 Relative density based on weekly trap catches of FCM from two trial fields from Begoro, for garden eggs and chili

	Garden eggs				Chili			
Date	No. adults	No. traps	Days		No. adults	No. traps	Days	
26.12.16	13	4	7		9	4	7	
04.01.17	18	4	14		13	4	14	
11.01.17	23	4	21		11	4	21	
18.01.17	43	4	28		19	4	28	
25.01.17	40	4	35		15	4	35	
01.02.17	15	4	42		8	4	42	
08.02.17	9	4	49		6	4	49	
15.02.17	3	4	56		2	4	56	
F/T/D	164	4	56	0.732	83	4	56	0.371

agronomic practices, and appropriate application of treatments, high quality fruits and vegetables can be produced for both the local and export markets.

The relative density of FCM adults based on trap catches on chili and garden eggs at Begoro is shown below (Table 18.4 and Fig. 18.4). The general population of FCM for the two fields were calculated as 0.732 and 0.371 moths per trap per day, respectively, for the garden egg and chili fields (Table 18.4). This means it will take approximately one-and-a-half days and three days for a single trap to collect FCM adult moths from the vicinity of the garden eggs and chili fields, respectively. No wonder the total numbers of adult moths collected during the 8-week period were 164 (for the garden egg farm) and 83 (for the chili farm). These indices are used to categorise crop production areas into areas of low, medium or high pest prevalent, and are important in the import/export trade of fruits and vegetables.

A visual observation of the trap catches from the two fields show a gradual increase at the onset of the traps to peak periods around the fourth week after trap set (corresponding to the period of fruit bearing by the plants) (Table 18.4 and Fig. 18.4). Thereafter, the numbers begin to decline to levels below the initial numbers. This shows that the traps played a significant role in the observed levels of damage to the fruits, thus collecting high numbers of insects (Fig. 18.4), without which the damage would have been higher.

18.3.3 Monitoring of Whiteflies, Bemisia tabaci

The application of neem, Eradicoat and Ecopel resulted in low numbers of the whiteflies in the other treatment plots on garden egg at Vegpro farms at Torgorme (Fig. 18.5). Generally, the initial numbers of whiteflies were high, but they were reduced significantly as the weeks progressed.

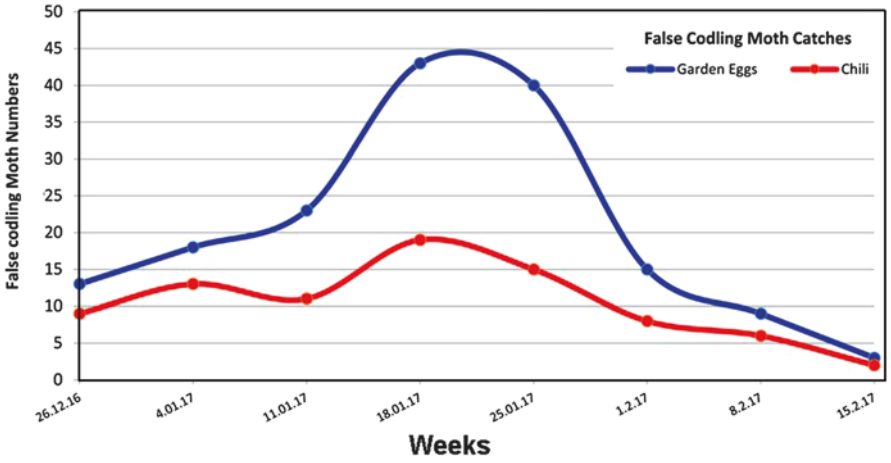

Fig. 18.4 Plot of weekly FCM trap catches from garden eggs and chili fields from Begoro

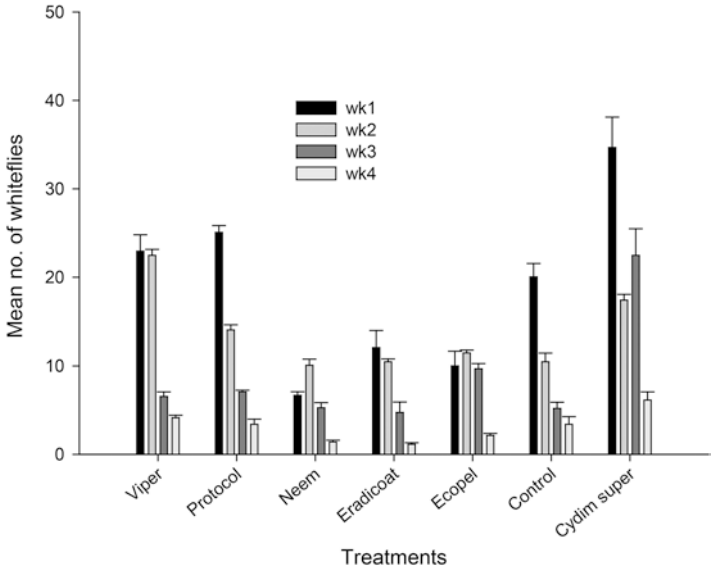

Fig. 18.5 Mean number of whiteflies, *Bemisia tabaci*, on garden egg at Vegpro, Torgorme in Ghana

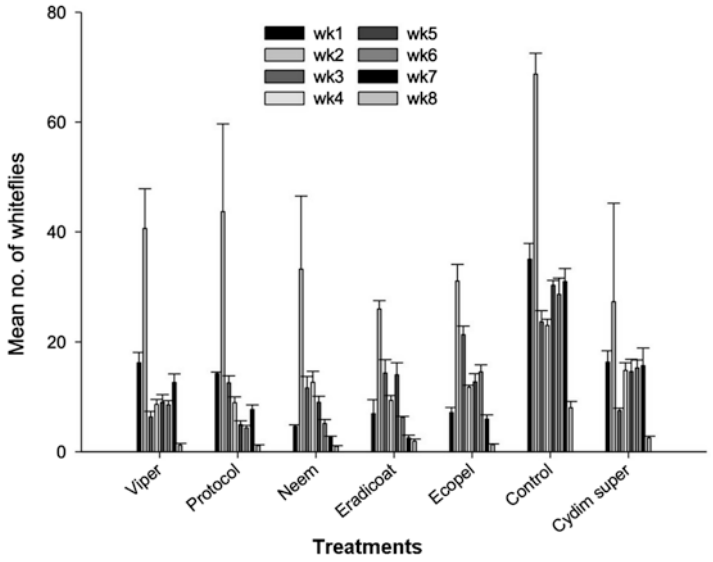

Fig. 18.6 Mean number of whiteflies, *B. tabaci* on garden egg at Begoro

Generally, at Begoro the initial numbers of whiteflies were high, and reduced significantly as the weeks progressed (Fig. 18.6). The treated plots had lower numbers of whiteflies than the control plots. Whitefly populations on chilies were lower than on garden eggs (Fig. 18.7) at Begoro.

Viper, Eradicoat and Cydim Super were more effective on thrips, followed by Neem and Ecopel, with the control plot being the least affected (Fig. 18.8).

The mean weights of harvested chilies and garden eggs were significant for the different treatments (Table 18.5), and similar in the case of Turia (Table 18.6 and Fig. 18.9). Irrespective of the area of the cultivation of crop, 2 or 3 of the selected treatment products worked well, or they all performed creditably, as indicated on turia across all zones (Table 18.6). Although the observed differences in the weights of the turia fruits were not statistically significant under the different treatments, the total yield was considered satisfactory by farmers.

18.3.4 Roadmap for Reducing the Incidence of Pests in Ghana Export Vegetables

A roadmap is a plan or strategy intended to achieve a particular goal. In this regard, the roadmap for reducing the incidence of the targeted quarantine pests in Ghana export vegetables to the EU comprised a compilation of guidelines based on IPM strategies. Main goal is to ensure that the incidences of each of these pests associated with export vegetables are reduced drastically to meet the strict EU phytosanitary

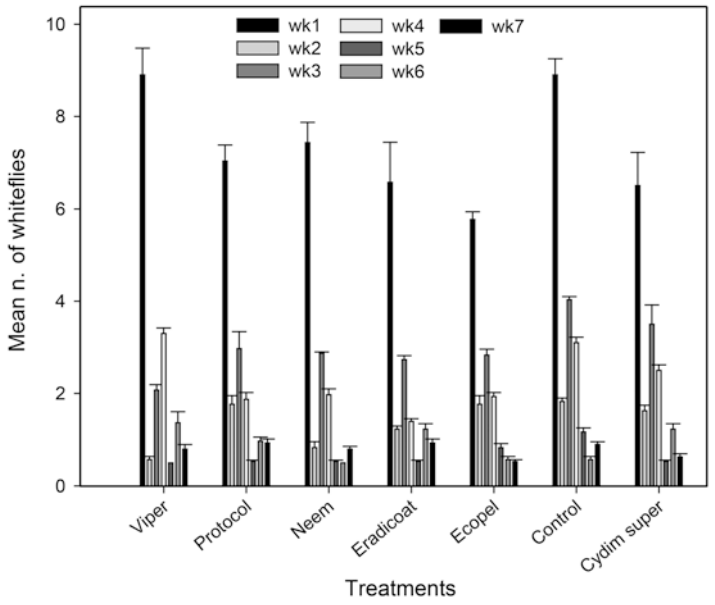

Fig. 18.7 Mean number of whiteflies, *B. tabaci* on pepper at Begoro

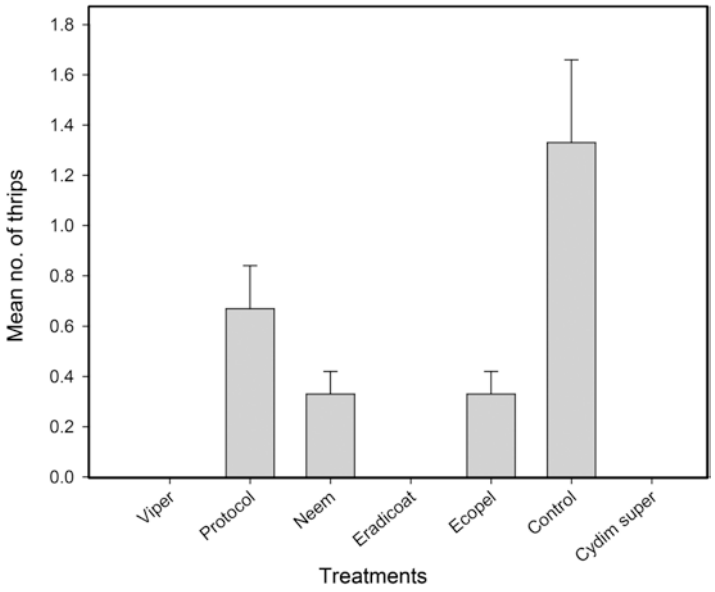

Fig. 18.8 Mean number of thrips on garden egg at Vegpro farms at Torgorme

Table 18.5 Mean weight (g) of Chili and garden eggs fruits from the treated field at Begoro

Treatment	Chili	Garden eggs
Neem	21.56 ± 1.43 b	310.67 ± 23.34 a
Eradicoat	26.60 ± 2.18 ab	112.50 ± 26.42 b
Protocol	33.89 ± 2.88 a	215.00 ± 37.58 ab
Ecopel	23.51 ± 0.34 b	195.00 ± 30.55 ab
Cydim	20.31 ± 2.86 b	250.00 ± 36.52 ab
Viper	29.01 ± 1.85 ab	215.00 ± 37.58 ab
Control	25.14 ± 2.46 ab	218.75 ± 70.25 ab
F	4.49	3.04
df	6, 13	6, 30
P	0.0112	0.0191

Means within a column followed by the same letter do not differ significantly from each other (P > 0.05; SAS, PROC GLM, SNK)

Table 18.6 Mean weight (g) of turia fruits from trial fields

Treatment	AB farms	Joekopan farms	Param farms	Comparison across all farms
Neem	346.67 ± 58.33 a	457.22 ± 77.55 a	375.00 ± 76.38 a	418.67 ± 49.91 a
Eradicoat	330.00 ± 52.04 a	368.89 ± 45.31 a	416.67 ± 72.65 a	370.67 ± 31.40 a
Protocol	521.67 ± 129.37 a	389.44 ± 64.12 a	475.00 ± 175.00 a	433.00 ± 54.60 a
Ecopel	288.33 ± 58.33 a	410.00 ± 55.55 a	475.00 ± 300.00 a	393.21 ± 50.90 a
Cydim Sup.	380.00 ± 38.19 a	463.89 ± 80.38 a	675.00 ± 128.29 a	489.33 ± 62.17 a
Viper	380.00 ± 90.14 a	406.11 ± 67.18 a	400.00 ± 52.04 a	399.67 ± 43.18 a
Control	405.0 ± 123.32 a	492.78 ± 76.29 a	550.00 ± 94.65 a	486.67 ± 53.29 a
F	0.75	0.44	0.70	0.86
df	6, 14	6, 56	6, 13	6, 94
P	0.6214	0.8483	0.6559	0.5271

Means within a column followed by the same letter do not differ significantly from each other (P > 0.05; SAS, PROC GLM, SNK)

regulations. The roadmap strategy adopts the IPM principles of putting in place **preventive measures**, including **monitoring** of the status of each of the four target pests (thrips, fruit flies, whiteflies and false codling moth), and eventually **acting** (by undertaking control measures, most likely in an integrated manner) based on an informed decision (outcome of the monitoring).

The approach for pest reduction must constitute a combination of IPM tactics, mainly employing good cultural control practices (planting of healthy seeds, improving soil health and soil fertility, regular weeding, destruction of harvested

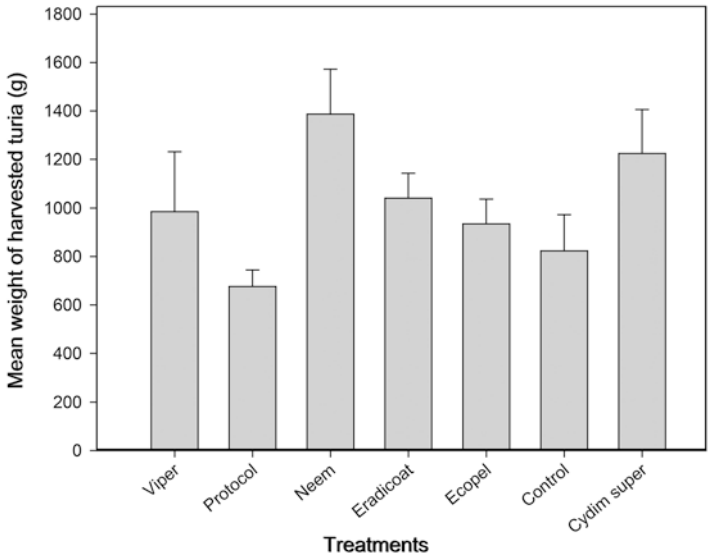

Fig. 18.9 Mean weight of three harvested turia (gourd) per plot at Joekopan farm, Torgorme

crop residues, crop rotation, intercropping, and prompt harvesting), biological control (promoting the use of natural enemies of pest, parasitoids and predators), planting of resistant varieties of crops if available, physical or mechanical control, monitoring (mass trapping of insect pests using sticky and pheromone traps), and chemical control (as the last resort). The use of less persistent and selectively acting insecticides is preferred to the use of persistent and broad-spectrum insecticides. Insecticides must be used judiciously and must be applied during the late afternoon for enhanced action. Application of the insecticides, especially the synthetic pyrethroids (e.g. Lambda cyhalothrin) and the botanicals (neem) during the hot afternoon, will be ineffective because long periods of exposure to the sun (UV light) readily breaks down the active ingredient, thereby reducing their efficacy. The misuse of synthetic insecticides (repeated and much higher dosing than recommended) may result in the pests developing early resistance to them, thereby making them ineffective and also leading to pesticide residues in the produce.

Thus, a conscious effort must be made in regulating the use of pesticides in the field to ensure the safety of the applicator (farmer), the produce (vegetable), and the environment. Farmers must wear Personal Protective Equipment (PPE) when applying insecticides. The trials have demonstrated that most of the insecticides used have the potential to manage insect pests in the field. In order to minimise the possibility of the pests becoming resistant to them, there will be the need to alternate the use of these different classes of synthetic insecticides (Viper, Cydim Super, Protocol and Ecopel) with the plant-based aqueous neem-seed extract, and the physically-acting Eradicoat. The synthetic insecticides must be applied bi-weekly, with at least 14 days pre-harvest interval. Neem and Eradicoat can be applied weekly and they have no pre-harvest interval, but it is advised that produce be har-

vested 3 days after their application. Thus, the use of the ANKE (azadirachtin) has a great potential in vegetable production, to ensure that the produce is safe and free of insect pests.

In addition, the use of sticky traps and species-specific lures for the monitoring and mass trapping of the target insect pests was an integral part of the interventions developed (roadmap) to reduce their incidence in the field. Now, the farmers, exporters, and the Agriculture Extension Agents and PPRSD field staff (regulators) are all familiar with the enormous potential of this method for controlling pests in the field, and have adopted its use in vegetable production.

Finally, the Plant Quarantine Inspectors (PQI) of PPRSD, which manages the two main ports of exit (Kotaka International Airport and Tema Harbour), were trained in the effective sampling and thorough inspection of consignments to detect any hidden pests, their proper identification, and decision making. Thus, the number of notifications on the interceptions of harmful organisms (quarantine pests) by EU Members States and Switzerland in consignments exported from Ghana has progressively reduced drastically from 280 in 2015, to 19 in 2016, and 1 in 2017 (source EUROPHYT). This positive outcome has been achieved through the collective effort of all the stakeholders in the vegetable sub-sector. It is no wonder, then, that the EU decided to lift the ban on the export of the selected vegetables from Ghana to the EU, from 1st January 2018.

18.4 Conclusion

Based on the promising outcome of the scientific trials and demonstrations on the farms, and adopting the best practices and principles in IPM, specific recommendations were made to manage each of the target pests in the field. However, the details are not presented in this article. Those details have been used to develop pictorial posters showing the different stages and characteristic damage of each of the target pests, together with the required information (in bullets points) needed for their management, based on the IPM principles to **prevent, monitor** and **act** (control or manage). The PPRSD and the Extension Division of MoFA are at the forefront in disseminating this information to all vegetable exporters and their out-growers, and all relevant stakeholders in Ghana, to ensure the production of wholesome vegetables that are devoid of pests and chemical residues, for the local and international markets.

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References

Billah, M. K., Braimah, K., Ofosu-Budu, G., Nkansah, G. O., Agboka, M., Monney, E., Bleppony, H., Garveh, E., Avar, V., Djannie, A. N. D. A., & Attatsi, R. (2013). Fruit fly management in Ghana. *Export Marketing and Quality Awareness Project (EMQAP) of MoFA* (p. 100). Accra Ghana. ISBN: 978-9988-8562-4-3.

Billah, M. K., & Wilson, D. D. (2016). Integrated management of fruit flies: Case studies in Ghana. In S. Ekesi, S. A. Mohamed, & M. De Meyer (Eds.), *Fruit fly Research and development in Africa – Towards a Sustainable Management Strategy to Improve Horticulture* (pp. 601–627).

EPPO. (2017). EPPO Pest Lists: purposes and procedures. European and Mediterranean Plant Protection Organization (last updated 2017/02). https://www.eppo.int/QUARANTINE/quarantine.htm. Accessed on 7 Jan 2018.

EUROPHYT. (2016). European Phytosanitary notification and rapid alert system dealing with Interceptions for plant health reasons of consignments of plants and plant products imported into the EU or being traded within the EU itself. https://ec.europa.eu/food/sites/food/files/plant/docs/ph_biosec_europhyt-interceptions. Accessed on 7 Jan 2018.

Fening, K. O., Lamptey, J. N. L., Mochiah, M. B., Amoabeng, B. W., Adama, I., Manu-Aduening, J. A., et al. (2014). Assessing arthropod pests and disease occurrence in cassava (*Manihot esculenta* Crantz) and cowpea (*Vigna unguiculata* L. Walp) intercropping system in the Ashanti region, Ghana. *Ghana*. *Journal of Agricultural Science, 47*, 95–110.

Fening, K. O., Billah, M. K., & Nankinga, C. M. K. (2016). *Protocol for evaluating the efficacy of six products against the False Codling moth, Fruit flies, Thrips and Whiteflies on Chilies, Garden egg and Ridged Gourd in Ghana*. Accra: GhanaVeg., 35 pp.

GhanaVeg Sector Reports. (2016). *GhanaVeg vegetable business opportunities in Ghana* (Gonzalez, Y. S., Dijkxhoorn, Y., Koomen, I., van der Maden, E., Herms, S., Joosten, F. et al., Eds.). Accra: Embassy of the Kingdom of the Netherlands.

GhanaVeg Sector Reports. (2017). *Roadmap for pest reduction in Ghana's export vegetable sector* (Fening K. O., Billah M. K. & Nankinga, C. M. K. Eds.). Accra: GhanaVeg., 28 pp.

IAEA. (2003). International Atomic Energy Agency *Trapping Guidelines for area-wide fly programmes*. Vienna: IAEA, 47 pp.

Statistical Analyses Software (SAS) Institute Inc. (2008). *SAS/STAT1 User's Guide, Version 9.2*. Cary, NC, USA.

Chapter 19
The Role of Plantwise in Improving Detection and Action on Pest Situations

L. Migiro and W. Otieno

Abstract Agriculture is a vital sector for many countries in Africa. It is an important source of employment and constitutes the primary source of export earnings. Among the constraints to agricultural production is damage caused by pests. The detection of new pests, or of a new host for an existing pest, or of the spread of an existing pest to a new location, has significant implications in crop production and trade. Agricultural advisory models, such as the CABI-led Plantwise programme, enable early detection of pests, thereby providing an opportunity for prompt action to be taken by responsible plant protection authorities. These circumstances also present opportunities for researchers to develop methods for pest management. Plantwise supports agricultural extension systems provided by governments in developing countries to assist smallholder farmers with access to advice and information needed to sustainably manage plant health problems. It works through networks of plant clinics run by specially trained extension officers, called 'plant doctors'. Plant clinics are reinforced by the Plantwise Knowledge Bank, an online and off-line interactive resource with information on pest diagnosis, management and distribution. Plantwise works through partnerships with relevant actors in the areas of extension, research, regulation and input supply. In the plant clinic model, a farmer brings a sample of affected plant and a plant doctor diagnoses the problem and gives advice. During this interaction, information on the farmer, plant health problem, and the recommendation given is recorded and is fed into a database. Plant clinics have shown potential to enable early detection of pests, monitoring outbreaks and introductions, as well as changes in their status, based on the experiences with some of the emerging pests such as *Tuta absoluta*, *Spodoptera frugiperda*, maize lethal necrosis disease, and Napier stunt disease. The Plant clinic database is therefore useful for general surveillance and as a source of information for Pest Risk Analysis.

Keywords Extension · ICT · Invasive species · Pest management · Pest risk analysis · Plant clinic · Plant health problems · Plantwise

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19.1 Introduction

Many rural households in developing countries rely on small-scale farming for food and income. However, production is constrained by both biotic and abiotic factors, such as pests and diseases, degraded soils deficient in nutrients and drought. Estimates of crop losses associated with pests and diseases alone range between 20 and 40% of the yield (Oerke 2006).

The CABI-led Plantwise programme is a global initiative that aims to help smallholder farmers to reduce losses of what they grow due to plant health problems. Currently, the programme runs in 33 countries around the world; 12 in Africa (Kenya, Uganda, Ethiopia, Tanzania, Rwanda, Malawi, Mozambique, Zambia, Democratic Republic of Congo, Burkina Faso, Ghana, and Sierra Leone), 11 in Asia (Bangladesh, India, Nepal, Pakistan, Sri Lanka, Cambodia, Myanmar, Thailand, Vietnam, China, and Afghanistan), and 10 in the Americas (Brazil, Peru, Barbados, Grenada, Jamaica, Trinidad and Tobago, Bolivia, Costa Rica, Honduras, and Nicaragua). The programme has three components: plant clinics, knowledge bank, and monitoring and evaluation. Networks of locally owned plant clinics, which are based on a similar approach to human health clinics (Bentley et al. 2009), have been established in all these countries where they serve as the first interface between the farmer and the extension officer, referred to in this case as the 'plant doctor'. Plant doctors are Government extension personnel who undergo further training by CABI on how to diagnose plant health problems using visual symptoms and give advice as recommendations, based on national and international best practice standards to farmers. At the plant clinic, farmers bring in "sick" plants and a plant doctor diagnoses the problem and gives a recommendation on management, based on IPM principles. Data regarding the farmer, the problem presented at the clinic, and the recommendation given are collected and entered into either a paper-based prescription form or onto a tablet computer, which details are then fed into a database called Plantwise Online Management Systems (POMS), which is the access controlled part of the Plantwise Knowledge Bank.

The Plantwise Knowledge Bank- https://www.plantwise.org/KnowledgeBank/home.aspx- is an interactive website that is available both online and offline. The offline knowledge bank is available through the Plantwise factsheet library app. For both Android and iOS, and through the Plantwise Factsheet Library, with key information from the Plantwise Knowledge Bank being stored on a USB stick. The Knowledge Bank contains information on pest diagnosis, pest management, and distribution data for more than 2500 key pests, with an individual portal tailored for each Plantwise country. It contains a simple, image-based pest diagnosis tool that helps plant doctors and other users to recognise pests that may be new to them. Targeted management advice is provided in linked factsheets created through Plantwise activities, CABI's own scientific resources, or from a wide range of partner organisations, such as the Consultative Group on International Agricultural Research (CGIAR) e.g. the International Centre for Tropical Agriculture (CIAT), the International Rice Research Institute (IRRI) and other organizations such as the

US Department of Agriculture (USDA) and the UK Food and Environment Research Agency (FERA). Interactive maps, which show where pests occur globally or nationally, are overlaid with other datasets, such as climate zones, to aid prediction and modelling, thus giving users free and open-access plant health information. Users of Knowledge Bank can also access plant health news and information as pest alerts (Fig. 19.1).

Country homepage is viewed by selecting a respective country. To further enhance a plant doctor's skills for pest diagnosis through game play, Plantwise, in collaboration with Bondi Labs (Australia), developed the PestSmart Diagnostic Simulator game that is now available through Google Play Store.

Since data in POMS contain private as well as trade-sensitive information, it is access restricted by national partners that are also the key users and must provide

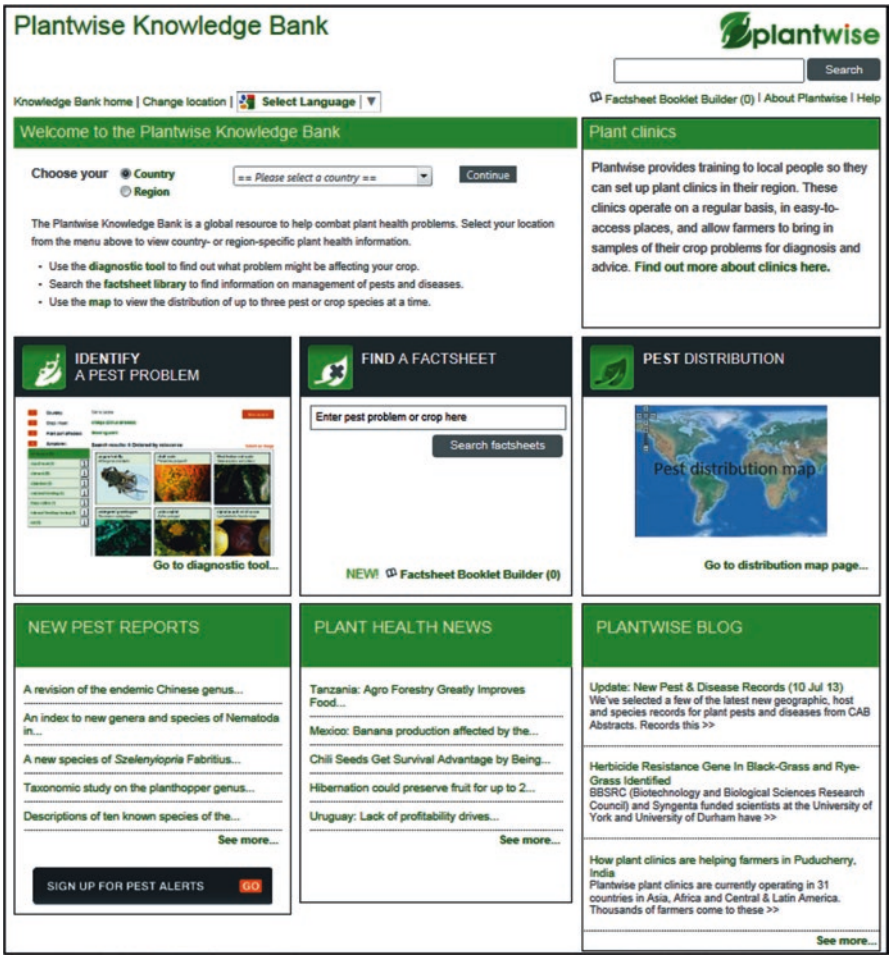

Fig. 19.1 Plantwise knowledge bank homepage

authorization of access and use. The increasing use of ICTs, coupled with continuous developments in data management processes has made the data more accessible and useful for partners. These developments comprise of automated processes for data loading (using data collection apps), semi-automated tools for harmonising data, basic analytics and enhanced options by which the clean data can be easily downloaded and analysed offline (using the offline data analysis spreadsheet or simply using inbuilt data analytics for basic analyses on, for example, crops by gender, recommended management practices and pests brought to the clinics). There is evidence that the data is used by Plantwise partners for purposes such as identifying training and research needs, choice of priority topics for mass extension campaigns and plant heath rallies, developing extension material, and monitoring pest spread and plant doctor performance.

Although plant clinics are primarily part of a rural agricultural advisory service that delivers information to farmers, they have been demonstrated to also have a value in creating a framework for vigilance on pests. They have also contributed to the identification and monitoring of the spread of pests new to a country.

The management of invasive species, through i.e. prevention, eradication, containment, resource protection and long-term management, will depend on the phase of invasion (Harvey and Mazzotti 2018). To prevent the introduction of invasive and key quarantine pests at points of entry for instance, the Plantwise programme in partnership with respective National Plant Protection Organisations, is involved in activities to build capacity of staff of regulatory agencies and other actors in plant health, e.g. in Kenya and Zambia, in skills for identification and monitoring.

Where an introduction has already taken place, early detection and rapid response is critical. Plant clinics have aided in the identification of newly introduced invasive species in a number of countries such as the banana skipper (*Erionota thrax*) in Sri Lanka in 2015, which resulted in a rapid response by the Ministry of Agriculture, thereby averting economic damage. Bio-economic modelling of the potential cost of this outbreak of banana skipper showed that without early detection through plant clinics, crop losses could have been as high as 13 metric tonnes per ha, with a market value of approximately 20 million USD. It can be concluded that the potential losses caused, if a new pest outbreak is not quickly detected and controlled, will result in direct negative effect on yields and farmer livelihoods. The pest seems to have been eradicated since there have not been any further reports of its occurrence in the country. In Rwanda, plant clinics supported the National Plant Protection Organisation to identify the papaya mealybug as a new pest problem.

Once an invasive pest becomes established in an area, efforts shift to containment of the increase of the core population and to prevention of its spread to new areas. Plant clinics have been at the fore front in not only identification but also monitoring the spread and creating awareness through the design and dissemination of targeted information on key invasive pests, further complemented by plant health rallies and mass extension campaigns. Examples include Maize Lethal Necrosis Disease (MLND) in Rwanda in 2013, tomato leaf miner (*T. absoluta*) in Uganda in 2015, and the fall armyworm (*S. frugiperda*) in Ghana in 2016. Plant clinics also helped in confirming the presence of the pest in Ghana and Zambia.

Some of the response action following detection of new pests have included provision and setting up of monitoring traps to monitor e.g. *T. absoluta* in several eastern and southern African countries including Kenya, Rwanda, Uganda, DRC, Malawi and Zambia (Fig. 19.2), and working with partners to develop country-specific information material such as posters and flyers, for use in managing the problem and raising awareness about the economic importance of the pest.

The first report of *S. frugiperda* in Africa was from Ghana, and the pest was also reported through a plant clinic in 2016. By 2017, the pest had been reported in several other African countries (Fig. 19.3). Several organizations (CABI, ICIPE, CIMMYT, FAO, the World Bank, DFID, USAID, and national and regional plant protection agencies) are currently involved in the development of a management strategy for containing this polyphagous pest.

Over the years, Plantwise has introduced the use of ICT tools in its activities, especially the use of tablet computers at plant clinics that have now replaced the use of paper prescription forms. Over 1000 plant doctors have been trained in the use of tablets, and are running over 500 e-plant clinics in 21 Plantwise countries. This has greatly improved efficiency in data management and transformed communication among plant doctors, farmers, and other stakeholders within the plant health system. With this has come the use of apps, e.g. WhatsApp, Telegram, Facebook, Line and WeChat by plant doctors that are increasingly enabling rapid sharing of images and information on plants' health, resulting in near real-time support on diagnosis and delivery of management advice from peers and other experts within the networks. There are at least 25 Plantwise countries with one or more such digital support groups, with over 6000 photos having been shared since 2015 through this channel. This helped trigger quick action on emerging pest problems in some of the countries

Fig. 19.2 Distribution of the tomato leaf miner, *Tuta absoluta* as at 2018. (Source: CABI invasive species compendium, https://www.cabi.org/ISC/tuta)

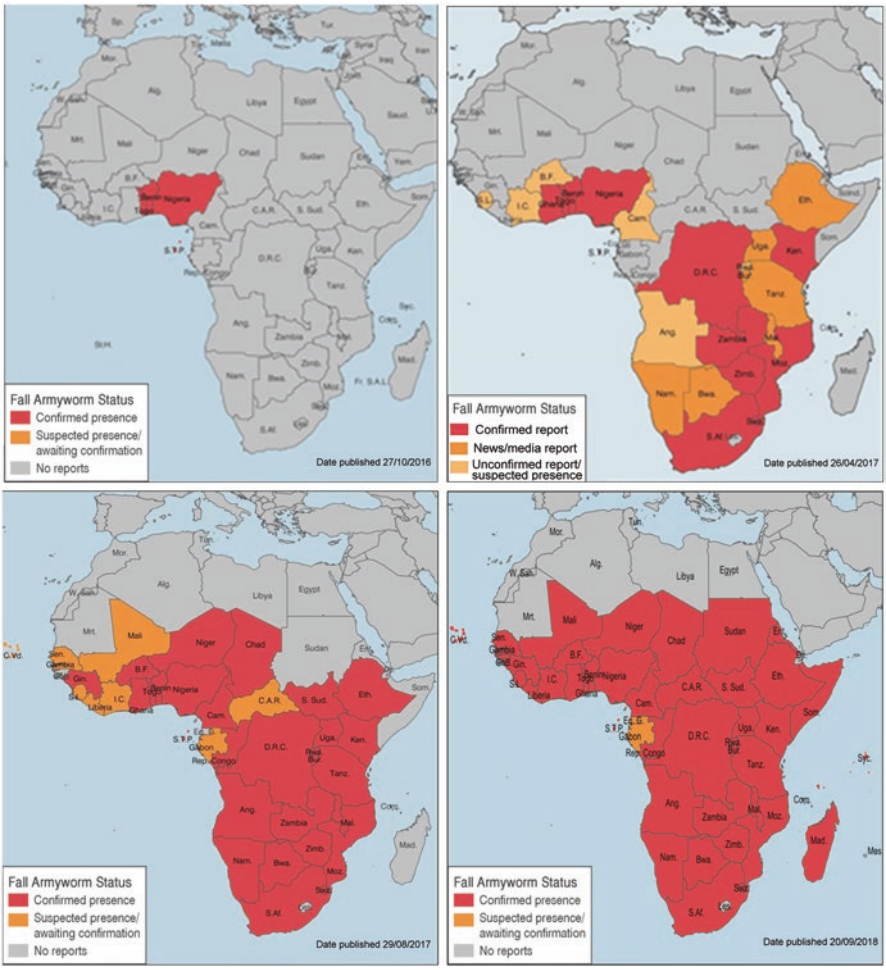

Fig. 19.3 Spread of fall armyworm across several African countries as at 2018. (Source: CABI)

e.g. bacterial blight of rice in Ghana in 2015 (which was later traced back to a contaminated seed lot) and banana skipper in Sri Lanka in 2015, resulting in prompt action from the governments. Recently, images of *S. frugiperda* have been shared across these networks and this helped track its spread. In Zambia, plant doctors used distinct images on tomato shared through a Telegram network to seek support in identification of suspected cases of *T. absoluta*. Linking local diagnostic experts with other expert groups was coordinated by CABI to confirm that identifications through these networks are correct. In some of the cases, it is the linkage between plant clinics and national plant protection organizations that helped to bring problems to the attention of the national authorities and to seek expert confirmatory identifications.

Once an invasive pest becomes widespread and abundant, long-term management strategies become essential. An optimal management effort is one that minimizes the sum of both the management and impact costs (Yokomizo et al. 2009), hence the need for organizations like CABI, ICIPE and IITA to continue partnering with national and other international organizations to ensure implementation of innovative, ecologically sustainable management practices for responsible production as part of the contribution to sustainable development goals, to reduce hunger and poverty particularly among smallholder farmers in Africa.

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References

Bentley, J. W., Boa, E., Danielsen, S., Franco, P., Antezana, O., Villarroel, B., et al. (2009). Plant health clinics in Bolivia 2000—2009: Operations and preliminary results. *Food Security, 1*, 371–386.

Harvey, R., & Mazzotti, F. (2018). *The invasion curve: A tool for understanding invasive species management in South Florida*. Gainesville: University of Florida, Institute of Food and Agriculture Safety.

Oerke, E. C. (2006). Crop losses to pests. *Journal of Agricultural Science, 144*, 31–43.

Yokomizo, H., Possingham, H. P., Thomas, M. B., & Buckley, Y. M. (2009). Managing the impact of invasive species: The value of knowing the density-impact curve. *Ecological Applications, 19*, 376–386.

Chapter 20
Semiochemical-Baited Autodissemination Device for Managing BFT on Cowpea

D. K. Mfuti, S. Subramanian, S. Niassy, H. du Plessis, S. Ekesi, and N. K. Maniania

Abstract Various autodissemination devices, baited with Lurem-TR and methyl anthranilate, were compared for their effective control of Bean flower thrips (BFT) on cowpea crops. The BFT density in treatment plots with semiochemical-baited autodissemination devices was significantly different during the two evaluation seasons ($P < 0.001$). In the first season, plots treated with the fungus-free device recorded the lowest BFT density (12.1 ± 1.0 thrips), while those treated with the autodissemination baited with Lurem-TR recorded the highest BFT density (19.1 ± 1.5 thrips), which was significantly different from plots treated with the device baited with methyl anthranilate (16.4 ± 1.3 thrips). The same scenario was observed in the second season, corresponding to high infestation season, where the autodissemination devices baited with Lurem-TR and methyl anthranilate and the fungus-free device recorded 59.9 ± 3.5, 48.4 ± 3.5 and 27.6 ± 4.3 thrips, respectively. In all autoinoculation devices, at least 45% of *M. anisopliae* conidia remained viable 12–15 days post-exposure. No significant difference in *M. anisopliae* conidial persistence and acquisition was found between the two contamination devices baited with both semiochemicals. This study has demonstrated that methyl anthranilate could be used in autodissemination, as well as Lurem-TR, to control BFT.

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Keywords Lurem-TR · Methyl anthranilate · Entomopathogenic fungus · Autodissemination · Thrips · Lure and infect

20.1 Introduction

Cowpea, *Vigna unguiculata* L. (Walp.), is among the most important grain legumes in Kenya, providing protein to consumers. However, its production is insufficient to meet demand, particularly for dry grains (Mergeai et al. 2001). The leaves, green pods and dry grains are consumed. The crop is grown under low-input conditions and grain yields are low, averaging less than 500 kg ha^{-1} (Adipala et al. 1999). The annual production of cowpea in Kenya is declining, despite increases in areas planted (Belmain et al. 2013; Kiprotich et al. 2015). This is attributed to many factors, the most important of which comprise heavy biotic pressures from insects and other pests (Abate and Ampofo 1996; Jackai and Daoust 1986; Rachie 1985). The Bean flower thrips (BFT), *Megaluthrips sjostedti* (Trybom), is the dominant thrips species on cowpea, causing severe damage to the crop. This specie can be found throughout tropical Africa (Fritsche and Tamo 2000). To control *M. sjostedti* outbreaks, growers are often forced to apply chemical treatments intensively (Gachu et al. 2012). The increase in insecticide applications has created detrimental effects in the environment, causing residual effect, adverse effects on no target insects, and development of insect resistance (Jensen 2004).

Reducing the quantities of insecticides applied in the environment is a major objective that drives research for the discovery of new behaviour-modifying chemicals (semiochemicals) and for investigation of their potential in pest management and eradication of invasive species (Davidson et al. 2007; El-Sayed et al. 2009; Teulon et al. 2007). Therefore, less environmentally contaminating methods have been developed that involve biological control with the use of parasitoids, predators and entomopathogenic fungi (EPF) (Ekesi and Maniania 2007; Loomans and Lenteren 1995; Maniania 2002; Niassy et al. 2012b; Teulon et al. 2014).

In the latter case, the fungus can be applied alone through inundation methods (Jaronski 2010) or combined with an attractant in a trap (Dimbi et al. 2003; Mfuti et al. 2016a; Migiro et al. 2010; Niassy et al. 2012a). The combination of semiochemicals with a killing agent such as entomopathogens is termed “lure and kill” or “lure and infect”. Lure and kill typically uses semiochemicals and biopesticticides in a concentrated area at the lure source to provide pest control (Mfuti et al. 2016a, 2017; Niassy et al. 2012a).

The use of a “lure and kill” strategy, employing an autoinoculation device or spot spray applications, could reduce the quantity of inoculums and related costs, and sustain fungal persistence in the field (Maniania 2002; Mfuti et al. 2016a, 2017; Nana et al. 2014; Niassy et al. 2012a). However, the success of this technology depends on the compatibility of the attractants with the entomopathogens. In this

regard, Niassy et al. (2012a) showed the incompatibility between the commercial thrips attractant, Lurem-TR, and the EPF *Metarhizium anisopliae* isolate 69. Thus, from that incompatibility, Mfuti et al. (2016a) investigated the best way to combine the two agents by separating them spatially. A distance of 0–5 cm away from the conidial source was found to optimise thrips attraction and conidial acquisition. Moreover, Mfuti et al. (2016b) screened methyl anthranilate among alternative thrips attractants to be combined with *M. anisopliae*, in an autoinoculation device.

Therefore, the aim of this study was to evaluate the potential of semiochemicals Lurem-TR spatially separated from EPF and methyl anthranilate, in direct exposure with EPF, in an autodissemination system for the management of BFT on cowpea.

20.2 Materials and Methods

20.2.1 Study Site

Field experiments were conducted from June to December 2014, in Western Kenya at the Mbita Thomas Odhiambo Campus (0° 26′ 06.19″ S, 34° 12′ 53.13″ E; 1137 m above sea level). Mbita is a field station of the International Centre of Insect Physiology and Ecology (ICIPE). This station is situated on the eastern shores of Lake Victoria. The station measures ca. 24.5 ha in extent. The vegetation type around the station comprises mainly savannah grassland with mixed combretum and acacia trees to the north, and papyrus along the shores of the lake.

The experiments were conducted in two seasons: the first one during the long rainy season, corresponding with the low infestation season (LIS) of *Megaluthrips sjostedti.* Cowpea was planted on 6/6/2014 and the experiment ran from July to August 2014. The second season was the short rainy season, corresponding with the high infestation season (HIS). Cowpea was planted on 10/8/2014 and the experiment ran from October to December 2014.

20.2.2 Experimental Crop

The field was divided into four blocks, each with three equal experimental plots measuring 8 × 10 m. Blocks were separated at 5 m intervals, while within the blocks the plots were separated at 2 m intervals. Cowpea, *V. unguiculata* variety Ken-Kunde 1, was planted in 80 m^2 plots with an intra-row spacing of 50 cm and inter-row spacing of 20 cm. An irrigation plan was scheduled once a week, and weeding was conducted every 2 weeks at the beginning, and was spaced later, depending on the abundance of weeds. No fertilisers, organic matter, or synthetic chemical insecticides were applied during the experimental period.

20.2.3 Mass Production of the Fungus

Conidia of *M. anisopliae* isolate ICIPE 69 were mass-produced on a long rice substrate in Milner bags (60 cm long by 35 cm wide). Rice was autoclaved for 1 h at 121 °C and inoculated with a 3-day-old culture of blastospores (Jenkins and Goettel 1997). The rice containing fungal spores was then allowed to dry for 5 days at room temperature. Conidia were harvested by sifting the substrate through a 295-μm mesh sieve and stored at 4–6 °C until use. Conidial viability was determined by spread-plating 0.1 ml of the suspension (3×10^6 conidia ml^{-1}) on Sabouraud Dextrose Agar (SDA) plates. Sterile microscope cover slips were placed on each plate. Plates were then incubated at 24–28 °C, 12:12 L:D of photoperiod, and examined after 16–20 h. Percentage germination was determined by counting the number of germ tubes formed among 100 random conidia for each plate at 400×, under a light microscope (Goettel and Inglis 1997).

20.2.4 Autodissemination Device (ADD)

The ADD used in the current study is an improved device, as compared to the previous one described by Migiro et al. (2010) and Niassy et al. (2012a) (Figs. 20.1 and 20.2). It has been refined following the findings of Mfuti et al. (2016a), in which the conidia were separated from the semiochemical and placed inside a small container

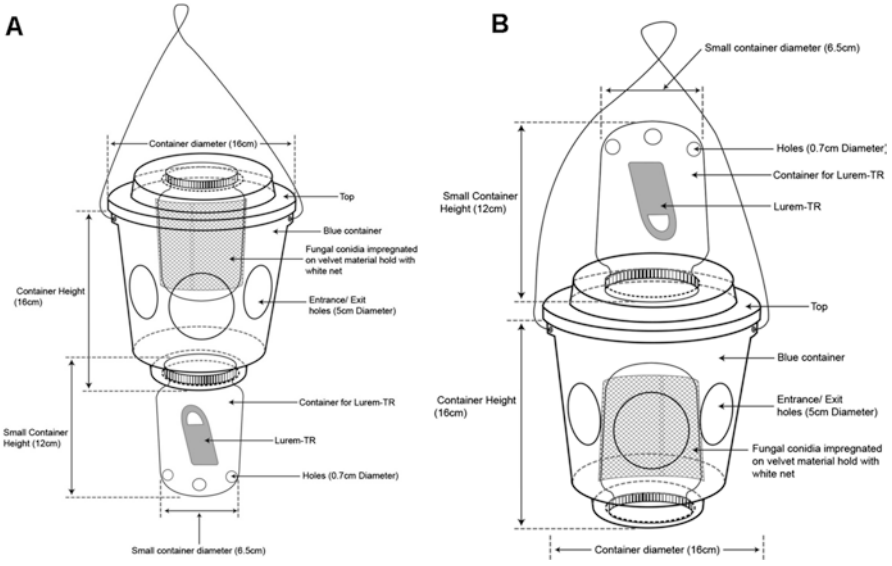

Fig. 20.1 Autodissemination device (ADD) made for spatial separation of semiochemical and entomopathogenic fungi with conidia container placed above (**a**) or below (**b**)

Fig. 20.2 An autodissemination device (ADD) baited with Lurem-TR placed in cowpea field for bean flower thrips management

(6.5 cm diameter × 12 cm high) fixed just below (Fig. 20.1a) or above (Fig. 20.1b). The small container is perforated with six holes (0.7 cm diam.) to allow volatiles to be released. The entire container forming the trap is blue in colour. This blue trap (16 cm diameter × 16 cm high) is also perforated with six entry/exit holes (5 cm diam.) in the right middle of the bottle, at alternate positions. A velvet cloth (8 × 8.5 cm) and a white netting (3.5 × 11 cm) are wrapped around a smaller inner cylindrical bottle (6.5 cm diameter × 6 cm high) that is then hung in the trap. Approximately 2–3 g of dry conidia are spread evenly on the velvet cloth of the autoinoculation device. The white netting is then wrapped around the velvet cloth containing the spores and tightened with two office pins. The device was then hung at canopy level (35 cm).

20.2.5 Semiochemicals

Two semiochemicals, Lurem-TR and methyl anthranilate, were used in the ADD. Lurem-TR, a commercial semiochemical with methyl isonicotinate as active ingredient, had been previously reported to be effective in monitoring thrips populations (Davidson et al. 2007; Muvea et al. 2014). It was obtained from Pherobank (Wageningen, The Netherlands).

Methyl anthranilate was obtained from Sigma-Aldrich Chemicals GmbH, Germany. Its active ingredient is ester anthranilic. It is reported to be attractive to *Thrips coloratus* and *T. hawaiiensis* (Imai et al. 2001; Murai et al. 2000). Recently, Mfuti et al. (2016b) have found it to be attractive to *Megalurothrips sjostedti*.

20.2.6 *Effect of Semiochemical-Baited ADD on the Attraction of* M. sjostedti

The attraction of *M. sjostedti* to the semiochemical-baited autodissemination was evaluated every 3 days to determine the number of individuals visiting the device. To assess *M. sjostedti* attraction, 5 cowpea plants were randomly sampled around each treatment. The number of thrips was recorded, using a whole plant tapping technique which consists of tapping plants on a white barber tray (25 × 45 cm) during which the tray is held underneath the selected plant, while the plant is tapped gently (5 taps) using the palm of a hand (Pearsall and Myers 2000). The tray was cleaned between sampling plants.

Treatments in the experiment consist of the use of ADDs baited with Lurem-TR, spatially separated with *M. anisopliae* (T1), the use of ADDs baited with methyl anthranilate (T2), and the use of a fungus-free device as control (T3). Treatments were laid out in a complete randomised block design with four blocks as replicates. Within the replicate, autodissemination placement was separated at distances of 12 m, to avoid interference between treatments.

20.2.7 *Effect of Semiochemical-Baited ADDs on* M. anisopliae *Conidial Persistence*

The persistence of conidia of *M. anisopliae* was evaluated for a period of 2 weeks after the onset of the experiment. It was conducted for the same interval of time during the flowering and podding stages.

Three-day intervals were used to sample conidial spores. A moist cotton bud was used to collect conidial samples The end of the cotton bud was cut, suspended in 10-ml 0.05% (w/v) Triton X-100 and vortexed for 1 min to dislodge the conidia. A sample of 100 µl was spread-plated on SDA plates and incubated for 16 h at 25 ± 2 °C and L12: D12 photoperiod. Germination of conidia was determined as described above.

For this experiment, treatments consisted of the use of autodissemination with *M. anisopliae* baited with Lurem-TR in spatial separation (T1), the use of autodissemination baited with methyl anthranilate (T2), and a fungus-free device as control. The experiment was replicated four times.

20.2.8 *Effect of Semiochemical-Baited ADDs on Conidial Acquisition*

To assess the amount of conidia acquired by single *M. sjostedti* from each treatment, five cowpea plants were sampled around the autoinoculation device (2 m radius), and 20 insects were collected in separate glass containers (10-ml) using an aspirator.

Containers were labelled and stored in a refrigerator for immobilisation. Insects were transferred individually into 2-ml cryogenic tubes containing 1 ml of sterile 0.05% Triton X-100. The tube was then vortexed for 2–3 min to dislodge conidia from the insect, and the concentration of conidia was determined using a Neubauer haemocytometer.

For this experiment, the two semiochemical-baited ADDs were compared in terms of their conidial acquisition.

20.2.9 Statistical Analyses

Data on Bean flower thrips densities were checked for normality and homogeneity of variance using Shapiro–Wilk and Bartlett tests, before being subjected to repeated measures of ANOVA analysis. Multiple comparisons of means were made with a Tukey HSD test.

Prior to the t-test analysis, data on *M. anisopliae* conidial persistence were arcsine transformed. Similarly, *M. anisopliae* conidial persistence count and data on conidial acquisition acquired by a single M. sjostedti were subjected to t-test analysis, to compare the effect of the two semiochemical-baited ADDs on *M. sjostedti* acquisition. All data analyses were performed using R statistical software (Version 3.1.3, 2015) (R Core Development Team 2014).

20.3 Results

20.3.1 Effect of Semiochemical-Baited ADDs on M. sjostedti *Density*

The BFT density in plots treated with semiochemical-baited ADDs was significantly different during the two evaluation seasons, as compared with the control device (Season I: $F_{2, 86} = 14.6$; $P < 0.001$; Season II: $F_{2, 86} = 19.9$; $P < 0.001$). In the first season, plots treated with the fungus-free device recorded the lowest BFT density, while plots treated with the ADD baited with Lurem-TR recorded the highest BFT numbers. The same scenario was observed in the second season (Table 20.1).

Table 20.1 Bean flower thrips density during the two experimental seasons

	Season I	Season II
Treatments	BFT density ± SE	BFT density ± SE
Free ADD (control)	12.1 ± 1.0c	27.6 ± 4.3c
ADD + Lurem-TR	19.1 ± 1.5a	59.9 ± 3.5a
ADD + MA	16.4 ± 1.3b	48.4 ± 3.5b
	$F_{2,86} = 14.6$; $P < 0.001$	$F_{2,86} = 19.9$; $P < 0.001$

In the first season, a significant difference was observed during stages ($F_{1, 86} = 81.9$; $P < 0.001$) and days ($F_{3, 86} = 2.7$; $P = 0.5$) of evaluation, while it was not observed in the second season. Greater numbers of BFT were recorded during the flowering than the podding stage. Considering the two cropping stages, it was observed that a high number of BFT was recorded in day 3 of evaluation. However, this was not significantly different, as compared with days 6 and 9.

20.3.2 *Effect of Semiochemical-Baited ADDs on* M. anisopliae *Conidial Persistence*

There was a significant difference on *M. anisopliae* conidial persistence among treatments during the two experimental seasons (season I: $F_{2, 141} = 35.9$; $P < 0.001$; season II: $F_{2, 141} = 280.2$; $P < 0.01$). Conidial persistence was higher in the fungus-free device (control) than in the semiochemical-baited ADDs, over the two experimental seasons. However, no significant difference in *M. anisopliae* conidial persistence was found between the two contamination devices, baited with both semiochemicals. The persistence trend was similar among treatments, within the stages of the two seasons (Table 20.2). However, except during the flowering stage of the second season, the control and ADD baited with Lurem-TR treatments were significantly different, as compared with the ADD baited methyl anthranilate (Table 20.3).

Table 20.2 Conidial persistence during the two different experimental seasons

	Season I	Season II
Treatments	% Germination ± SE	% Germination ± SE
ADD without attractant (control)	64.0 ± 3.6a	63.8 ± 8.0a
ADD + LUREM	53.3 ± 4.8b	56.6 ± 10.1b
ADD + MA	52.1 ± 4.8b	54.3 ± 10.2b
	$F_{2,141} = 35.9$; $P < 0.001$	$F_{2,141} = 280.2$; $P < 0.01$

Table 20.3 Conidial persistence during different stages of the two experimental seasons

	Crop stage			
	Season I		Season II	
Treatments	Flowering stage	Podding stage	Flowering stage	Podding stage
ADD without attractant (control)	65.2 ± 3.1a	64.5 ± 3.1a	64.1 ± 17.3a	64.5 ± 4.5a
ADD + LUREM	57.2 ± 4.1b	50.8 ± 4.2b	57.2 ± 22.3a	54.1 ± 5.0b
ADD + MA	55.0 ± 4.1b	51.1 ± 4.1b	51.6 ± 20.2b	55.0 ± 5.1b
	$F_{2,70} = 60.0$; $P < 0.001$	$F_{2,62} = 60.2$; $P < 0.001$	$F_{2,70} = 146.2$; $P = 0.2$	$F_{2,62} = 60.3$; $P < 0.001$

Significant differences in *M. anisopliae* conidial persistence were found over time during the two seasons (season I: $F_{4, 141} = 17.2$, $P < 0.001$; season II: $F_{4, 141} = 237.6$; $P < 0.001$). During the flowering and podding stages of the first season, significant differences were found between the semiochemical-baited ADDs and the control. The persistence in the control device remained higher in all sampling days. However, in the second season, no significant differences were found between the semiochemical-baited autodissemination devices during sampling days of the flowering stage, while in the podding stage, the fungal persistence trend was similar to that of first season stages, with significant differences among treatments (Fig. 20.3).

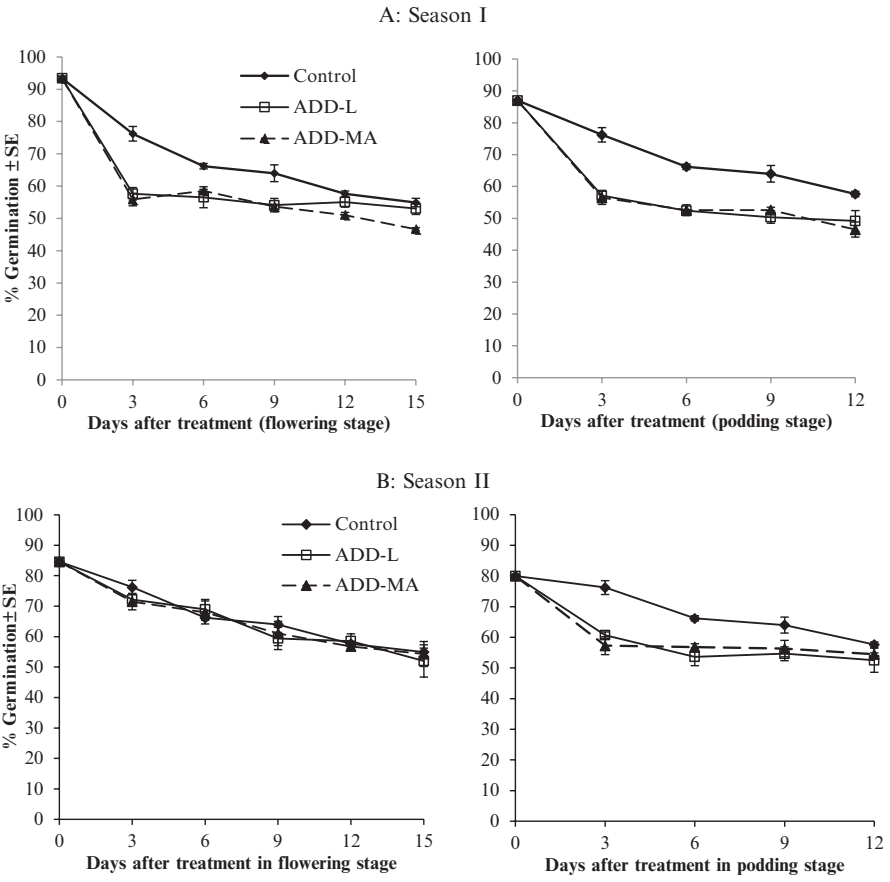

Fig. 20.3 Effect of semiochemical-baited autoinoculation devices on conidial persistence of *Metarhizium anisopliae* during the flowering and podding stages of cowpea, over seasons I (**a**) and II (**b**)

20.3.3 *Effect of Semiochemical-Baited ADDs on Conidial Acquisition by* M. sjostedti

There were no significant differences in conidia acquired by a single *M. sjostedti* between semiochemical-baited ADD treatments in both experimental seasons (season I: t = −0.6, df = 53.9, P = 0.6; season II: t = −1.2, df = 48.3, P = 0.2). Within the first season, a similar trend was observed over the crop stages. However, during the second season, significant differences were found between treatments during the podding stage (t-test: t = −2.2, df = 17.8, P = 0.04) (Table 20.4). *Megalurothrips sjostedti* acquired more conidia in the ADD baited with methyl anthranilate than in the one baited with Lurem-TR. No significant differences were detected during the flowering stage (t-test: t = 0, df = 28.8, P = 1) (Table 20.4).

Table 20.4 Over all conidial acquisition during different stages of the two experimental seasons

Season	Crop stage	Days after treatment	Number of conidia/individual thrips	
			ADD[a] + Lurem-TR (10^3 conidia/individual)	ADD + MA (10^3 conidia/individual)
Season I	Flowering	6	4.0 ± 0.2	1.0 ± 0.1
		9	5.0 ± 0.2	8.0 ± 0.3
		12	10.0 ± 0.5	4.0 ± 0.2
		15	6.0 ± 0.3	8.0 ± 0.3
		F = 0.3; df = 1, 21; P = 0.6		
	Podding	6	3.0 ± 0.2	10.0 ± 0.4
		9	3.0 ± 0.3	8.0 ± 0.3
		12	1.0 ± 0.1	1.0 ± 0.1
		F = 3.3; df = 1, 15; P = 0.1		
Season II	Flowering	6	6.0 ± 0.5	6.0 ± 0.6
		9	1.0 ± 0.1	1.0 ± 0.1
		12	1.0 ± 0.1	0.0 ± 0.0
		15	0.0 ± 0.0	1.0 ± 0.1
		F = 0.0; df = 1, 21; P = 1.0		
	Podding	6	0.0 ± 0.0	5.0 ± 0.2
		9	1.0 ± 0.1	8.0 ± 0.3
		12	5.0 ± 0.2	6.0 ± 0.4
		F = 4.4; df = 1, 15; P = 0.05		

[a] Autodissemination device

Means (±SE) followed by the same small letters are not significantly different by the Tukey HSD test

20.4 Discussion

Generally, entomopathogens are applied through inundative techniques. However, it was demonstrated that the inundative application of fungus presents some limitations, such as the short persistence of spores due to UV light, exposure to droughts, and drainage by rain (Jaronski 2010). The use of the ADD was conceived to address the shortcomings of inundative applications of EPF, as described above (Dimbi et al. 2003; Mfuti et al. 2016a; Niassy et al. 2012a). The advantage of the ADD is that it sustains fungal persistence, as the conidial spores are well protected against climate influences.

To enhance the efficacy of the strategy, the ADD was combined with a thrips attractant. In the case where Lurem-TR was used, the two items were spatially separated, following the findings of Mfuti et al. (2016a), to maximise the compatibility between the fungus and the attractant. In fact, it has been reported previously that the attractant Lurem-TR presents antifungal activities when placed in direct contact with conidia of the EPF, in an ADD (Niassy et al. 2012a). However, in the device baited with methyl anthranilate, the conidia were placed in direct exposure to the attractant, following research result of Mfuti et al. (2016b).

The management of thrips using a semiochemical-baited ADD system was experimented with by Niassy et al. (2012a) and Mfuti et al. (2016a) to assess the control of populations of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and *M. sjostedti*, respectively. Niassy et al. (2012a), for instance, reported a significant reduction of thrips density per plant in a contamination device baited with a semiochemical, as compared with one without semiochemical and a control (fungus-free) device. In the current study, a significant reduction in BFT was obtained in the ADD baited with both attractants (Lurem-TR and methyl anthranilate), as compared to the control device, where no attractant was added. However, when comparing the two semiochemical-baited ADDs, it was observed that the autodissemination baited with Lurem-TR recorded the highest BFT density, as compared with the one with methyl anthranilate. This finding could be explained by the fact that the separation made between Lurem-TR and EPF in their compatibility could play a significant role in luring the BFT to the contamination source, for their effective control (Mfuti et al. 2016a).

The conidial viability of *M. anisopliae* decreased over time in all treatments. However, in all ADDs, at least 45% of *M. anisopliae* conidia remained viable for 12–15 days post-exposure. However, Ekesi et al. (2001) reported persistence of about 3–4 days of this fungal isolate on cowpea and Daoust and Pereira (1986) reported persistence of 1–2 days on cowpea leaves, for *M. anisopliae* and *B. bassiana.* The radiation in the ultraviolet region is considered as the major factor affecting the survival of conidia on plant foliage (Smits et al. 1996). In the present study, conidia were not directly exposed to solar radiation, as they were applied within the

ADDs. This may, therefore, explain the observed persistence of >45% for 12–15 days post-exposure. In a field cage studies, Niassy et al. (2012a) reported that conidial viability was not affected in the autoinoculation device without a semiochemical, 7 days post-treatment, but in the Lurem-TR-baited autoinoculation device, conidial viability decreased from 80% to 6% at 2- and 7-days post-inoculation, respectively. The difference between the two studies is that conidia were directly exposed to Lurem-TR (Niassy et al. 2012a), while they were spatially separated in the present study, thereby enhancing compatibility (Mfuti et al. 2016a). Maniania (2002) reported that conidia of *M. anisopliae* could retain up to 60% of their viability after a 31-day exposure, in a contamination device.

The mean number of conidia acquired by a single thrips in both semiochemical-baited devices varied between 2 and 10×10^3 conidia, and was lower than what was reported by Niassy et al. (2012a) for *F. occidentalis*. The study by Niassy et al. (2012a) was, however, conducted in experimental cages where multiple infections were possible, as compared with the present study under field conditions. These results have shown that methyl anthranilate is as effective as Lurem-TR, in terms of conidial acquisition.

This study demonstrated that methyl anthranilate could be used in autodissemination, as well as Lurem-TR, to control BFT. However, methyl anthranilate has some advantages in being used in direct exposure to *M. anisopliae*. BFT tended to collect more conidial spores in the ADD baited with methyl anthranilate, than in the autodissemination device baited with Lurem-TR.

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References

Abate, T., & Ampofo, J. K. (1996). Insect pests of beans in Africa: Their ecology and management. *Annual Review of Entomology, 41*, 45–73.

Adipala, E., Omongo, C. A., Sabiti, A., Obuo, J. E., Edema, R., Bua, B., et al. (1999). Pests and diseases on cowpea in Uganda: Experiences from a diagnostic survey. *African Crop Science Journal, 7*, 465–478.

Belmain, S. R., Haggar, J., Holt, J., & Stevenson, P. C. (2013). Managing legume pests in sub-Saharan Africa. Challenges and prospects for improving food security and nutrition through agro-ecological intensification. Natural Resources Institute, University of Greenwich, UK, 34.

Daoust, R. A., & Pereira, R. M. (1986). Stability of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on beetle-attracting tubers and cowpea foliage in Brazil. *Environmental Entomology, 15*, 1237–1243.

Davidson, M. M., Butler, R. C., Winkler, S., & Teulon, D. A. J. (2007). Pyridine compounds increase trap capture of *Frankliniella occidentalis* (Pergande) in a covered crop. *New Zealand Plant Protection, 60*, 56–60.

Dimbi, S., Maniania, N. K., Lux, S. A., Ekesi, S., & Mueke, J. M. (2003). Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). *Mycopathologia, 156*, 375–382.

Ekesi, S., & Maniania, N. K. (2007). *Use of entomopathogenic fungi in biological pest management*. Kerala: Research Signpost.

Ekesi, S., Maniania, N. K., Ampong-Nyarko, K., & Akpa, A. D. (2001). Importance of timing of application of the entomopathogenic fungus, *Metarhizium anisopliae* for the control of legume flower thrips, *Megalurothrips sjostedti* and its persistence in cowpea. *Archives of Phytopathology and Plant Protection, 33*, 431–445.

El-Sayed, A. M., Mitchell, V. J., McLaren, G. F., Manning, L. M., Bunn, B., & Suckling, D. M. (2009). Attraction of New Zealand flower thrips, *Thrips obscuratus*, to cis-jasmone, a volatile identified from Japanese honey suckle flowers. *Journal of Chemical Ecology, 35*, 656–663.

Fritsche, M. E., & Tamo, M. (2000). Influence of thrips species on the life history and behaviour of *Orius albidipennis*. *Entomologia Experimentalis et Applicata, 96*, 111–118.

Gachu, S. M., Muthomi, J. W., Narla, R. D., Nderitu, J. H., Olubayo, F. M., & Wagacha, J. M. (2012). Management of thrips (*Thrips tabaci*) in bulb onion by use of vegetable intercrops. *International Journal of AgriScience, 2*, 393–402.

Goettel, M. S., & Inglis, G. D. (1997). Fungi: Hyphomycetes. In L. Lacey (Ed.), *Manual techniques in insect pathology* (pp. 213–214). San Diego/London: Academic.

Imai, T., Maekawa, M., & Murai, T. (2001). Attractiveness of methyl anthranilate and its related compounds to the flower thrips, *Thrips hawaiiensis* (Morgan), *T. coloratus* Schmutz, *T. flavus* Schrank and *Megalurothrips distalis* (Karny) (Thysanoptera: Thripidae). *Applied Entomology and Zoology, 36*, 475–478.

Jackai, L. E. N., & Daoust, R. A. (1986). Insect pests of cowpeas. *Annual Review of Entomology, 31*, 95–119.

Jaronski, S. T. (2010). Ecological factors in the inundative use of fungal entomopathogens. *BioControl, 55*, 159–185.

Jenkins, N. E., Goettel, M. S. (1997). Methods for mass production of microbial control agents of grasshoppers and locusts. In M. S. Goettel & D. L. Johnson (Eds.), *Microbial control of grasshoppers and locusts* The memoirs of the Entomology Society of Canada, pp. 37–48.

Jensen, S. E. (2004). Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. *Integrated Pest Management Reviews, 5*, 131–146.

Kiprotich, M. J., Mamati, E., & Bikketi, E. (2015). Effect of climate change on cowpea production in Mwania watershed: A case of Machakos County. *International Journal of Educational Research, 3*, 287–298.

Loomans A. J. M., & van Lenteren J.C. (1995). Biological control of thrips pests: A review on thrips parasitoids. Wageningen Agricultural University Papers, 95, 92–201.

Maniania, N. K. (2002). A low-cost contamination device for infecting adult tsetse flies, *Glossina spp*., with the entomopathogenic fungus *Metarhizium anisopliae* in the field. *Biocontrol Science and Technology, 12*, 59–66.

Mergeai, G., Kimani, P., Mwang'ombe, A., Olubayo, F., Smith, C., Audi, P., & Le Roi, A. (2001). Survey of pigeonpea production systems, utilization and marketing in semi-arid lands of Kenya. *Biotechnologie, Agronomie, Société et Environnement, 5*, 145–153.

Mfuti, D. K., Subramanian, S., van Tol, R. W. H. M., Wiegers, G. L., De Kogel, W. J., Niassy, S., et al. (2016a). Spatial separation of semiochemical Lurem-TR and entomopathogenic fungi to enhance their compatibility and infectivity in an autoinoculation system for thrips management. *Pest Management Science, 72*, 131–139.

Mfuti, D. K., Subramanian, S., Niassy, S., Salifu, D., du Plessis, H., Ekesi, S., & Maniania, N. K. (2016b). Screening for attractants compatible with *Metarhizium anisopliae* for use in thrips management. *African Journal of Biotechnology, 15*, 714–721.

Mfuti, D. K., Niassy, S., Subramanian, S., du Plessis, H., Ekesi, S., & Maniania, N. K. (2017). Lure and infect strategy for application of entomopathogenic fungus for the control of bean flower thrips in cowpea. *BioControl, 107*, 70–76.

Migiro, L. N., Maniania, N. K., Chabi-Olaye, A., & Vandenberg, J. (2010). Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) isolates to the adult pea leafminer (Diptera: Agromyzidae) and prospects of an autoinoculation device for infection in the field. *Environmental Entomology, 39*, 468–475.

Murai, T., Imai, T., & Maekawa, M. (2000). Methyl anthranilate as an attractant for two thrips species and the thrips parasitoid *Ceranisus menes*. *Journal of Chemical Ecology, 26*, 2557–2565.

Muvea, A. M., Waiganjo, M. M., Kutima, H. L., Osiemo, Z., Nyasani, J. O., & Subramanian, S. (2014). Attraction of pest thrips (Thysanoptera: Thripidae) infesting French beans to coloured sticky traps with Lurem-TR and its utility for monitoring thrips populations. *International Journal of Tropical Insect Science, 34*, 197–206.

Nana, P., Nchu, F., Ekesi, S., Boga, H. I., Kamtchouing, P., & Maniania, N. K. (2014). Efficacy of spot-spray application of *Metarhizium anisopliae* formulated in emulsifiable extract of *Calpurnia aurea* in attracting and infecting adult *Rhipicephalus appendiculatus* ticks in semifield experiments. *Journal of Pest Science, 88*, 613–619. https://doi.org/10.1007/s10340-014-0637-8.

Niassy, S., Maniania, N. K., Subramanian, S., Gitonga, L. M., & Ekesi, S. (2012a). Performance of a semiochemical-baited autoinoculation device treated with *Metarhizium anisopliae* for control of *Frankliniella occidentalis* on French bean in field cages. *Entomologia Experimentalis et Applicata, 142*, 97–103.

Niassy, S., Maniania, N. K., Subramanian, S., Gitonga, L. M., Mburu, D. M., Masiga, D., & Ekesi, S. (2012b). Selection of promising fungal biological control agent of the western flower thrips *Frankliniella occidentalis* (Pergande). *Letters in Applied Microbiology, 54*, 487–493.

Pearsall, I. A., & Myers, J. H. (2000). Population dynamics of western flower thrips (Thysanoptera: Thripidae) in British Columbia. *Journal of Economic Entomology, 93*, 264–275.

R Core Development Team. (2014). *A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Rachie, K. O. (1985). Introduction. In R. H. Singh & K. O. Rachie (Eds.), *Cowpea research, production and utilization* (pp. XXI–XXVIII). Chichester: Wiley.

Smits, N., Fargues, J., Rougier, M., Goujet, R., & Itier, B. (1996). Effect of temperature and solar radiation interactions on the survival of quiescent conidia of the entomopathogenic fungus *Paecilomyces fumusoroseus* (Wize) Brown and Smith. *Mycopathologia, 135*, 163–170.

Teulon, D. A. J., Davidson, M. M., Ducan, I. H., Dale, E. J., Cllum, D. F., Lesley, L., Vanessa, C. G., & Nigel, B. P. (2007). 4-Pyridyl carbonyl and related compounds as thrips lures: Effectiveness for onion thrips and New Zealand flower thrips in field experiments. *Journal of Agricultural and Food Chemistry, 55*, 6198–6205.

Teulon, D. A. J., Castañé, C., Nielsen, M.-C., El-Sayed, A. M., Davidson, M. M., Gardner-Gee, R., et al. (2014). Evaluation of new volatile compounds as lures for western flower thrips and onion thrips in New Zealand and Spain. *Journal of the New Zealand Plant Protection Society, 67*, 175–183.

Chapter 21
Influence of Predatory Mites, *Phytoseiulus longipes* Evans. on the Within-Plant Diurnal Migration and Distribution of the Red Spider Mite, *Tetranychus evansi*, Baker and Pritchard on African Nightshade, *Solanum scabrum*

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Abstract Understanding the within-plant diurnal migration and distribution of the Red spider mite, *Tetranychus evansi* Baker and Pritchard, in the presence of the predatory mite *Phytoseiulus longipes* Evans, on African nightshade, *S. scabrum*, is critical in developing an Integrated Pest Management (IPM) strategy for the pest. The *T. evansi* density, day and night cycles, and presence of *P. longipes*, on the within-plant migration and distribution of *T. evansi* on African nightshade were hence investigated. The results indicated that *T. evansi* does not exhibit a circadian migratory movement pattern on *S. scabrum* at lower densities (50 and 100 mites). However, *T. evansi* was observed to have a density-dependent collective displace-

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ment and distribution to the top of the plant, as densities increased (300 and 600 mites). The presence of *P. longipes* on the plant enhanced the within-plant migration of *T. evansi*, even at low densities (50 and 100 mites). However, there was no apparent pattern of migration and movement that was observed within the plant. The increased within-plant movement of *T. evansi* in presence of the predatory mites and collective displacement of *T. evansi* at higher densities indicate a potential for developing an IPM strategy using the acaricide treated net, which is discussed further.

Keywords Density dependence · Distribution mite · Predation · Within-plant migration

21.1 Introduction

African Nightshade, *Solanum scabrum* (Solanaceae), is an important African indigenous vegetable (AIV). With increased demand and promotion of AIVs as alternative sources of nutrients in East and West Africa, there has been increased, continuous cultivation of *S. scabrum* in Kenya (Farm concern report, 2015), eventually resulting in increased pests' incidence and damage to the crop. African nightshade is alleged to be tolerant to pest and diseases (Maundu et al. 1999), although just like other vegetables, it is susceptible to pests such as Bean aphids (*Aphis fabae*) (Chweya 1999), and spider mites (Murungi et al. 2010). Heavy infestation and damage resulting in webbing on leaves have been noted on the plants, which are due to the recently introduced invasive pest, the red spider mite, *Tetranychus evansi* Baker and Pritchard. During a survey conducted by the ICIPE-AIV Department, most farmers interviewed reported that they abandoned nightshade cultivation due to heavy infestation and damage by the mite, that led to huge losses (Unpublished ICIPE AIV survey data and pers. comm., 2014, 2015; Murungi et al. 2011). *Tetranychus evansi* is known to be a serious invasive pest of solanaceae crops (Smith Meyer 1996; Migeon et al. 2009). In Africa, it was first recorded in Zimbabwe in

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1979 (Blair 1983) and in 2001 in Kenya (Bugeme 2008) on tomato plants. It was also found infesting African nightshade in Kenya in the year 2009, during a country-wide survey of both Kenya and Tanzania (Toroitich 2006). *Tetranychus evansi* has a high reproductive performance on *S. scabrum* (Murungi 2011), being highly destructive, with a very rapid population growth, short development time, high birth rate, a long adult survival (Hussy and Parr 1963; Boykin and Campbell 1984), and a wide temperature range (Bonato 1999). It has, therefore, become a major pest of horticultural crops, mainly Solanaceae, causing up to 90% yield losses (Sibanda et al. 2000; Knapp et al. 2003). Control of *T. evansi* in Africa is mainly achieved through frequent application of synthetic acaricides (Smith Meyer 1996; Saunyama and Knapp 2003; Machini 2005; Toroitich et al. 2014), but with dire consequences, due to their excessive and inappropriate use.

The broad leaves of the *S. scabrum* pose greater challenges in spider-mite scouting and pesticide application, as mite populations build up under the leaves, thus being covered from harsh environmental conditions. The protected *T. evansi* thus increase their survival rates, and population build-up goes unnoticed. *Tetranychus evansi* infestation lowers production and quality of the marketable leaves, that are utilised for food.

Unfortunately, most farmers only recognise a *T. evansi* attack when the infestation is high and webbing is evident on top leaves. By the time that mites are detected on the apex of the plant webbing, they have already entered their dispersal phase and populations are very high, with lower and middle leaves destroyed by feeding marks. It therefore becomes difficult to initiate an effective control strategy for the mites using acaricide sprays or biological control agents such as the *T. evansi* predator, the effective predator mite *Phytoseiulus longipes* Evans (Kungu et al. 2019).

Spider mites exhibit the behaviour of congregating at the apices of the upper leaflets when the mite population builds up, and plants becomes heavily infested (Clotuche et al. 2011).

According to Hussey and Parr, *T. urticae*, a species closely related to *T. evansi*, forms a 'ball' by crowding together upon one another in an agitating mass. There is an obvious general movement of spider mites upwards to penultimate leaves, as nearly all movement of the mites, walking from leaf to leaf, is upwards. Clotuche et al. (2011) noted that as *T. urticae* migrate, they leave trails of silk thread. Research conducted on the dispersal, migration and recruitment behaviour of spider mites showed that within-plant dispersal and distribution of mites may vary widely, in different spider mite and host plant species (Sabelis 1985; Azandémè-Hounmalon et al. 2014). In high populations, *T. urticae* exhibits within-plant movements in conditions of overcrowding and food depletion, forming silk balls at the apex of their over-exploited host plants (Sabelis 1985; Pralavorio et al. 1989). This is characterised by phases of diurnal size increase and nocturnal decrease, which is likely to be related to a circadian rhythm (day and night cycles), during which *T. urticae* populations move up the plant in the early afternoon, and migrate to its bottom during the night (Pralavorio et al. 1989; Clotuche et al. 2011).

In spider mites, these movements within the plant could form patterns, although wind dispersal has been proposed as one mean of long-distance dissemination,

especially when spider mite colonies are overcrowded, or the host plant is no longer suitable (Boykin and Campbell 1984). Nevertheless, infestation appears even on wind-protected crops over time, suggesting other factors and forms of dispersal. The suddenness of spider mite population build-ups on *S. scabrum* makes it difficult to control the pest, as it appears to migrate along the plant, unnoticed by farmers.

To manage the pest populations and build-up on *S. scabrum* plants, farmers rely heavily on the use of highly toxic pesticides that are sprayed directly on the leaves, but with little or no success. Most of the pesticides used are listed as candidates for persistent organic pollutants (POPs) by UNEP. Pesticide sprays also lead to high pesticide residues on the vegetables, causing potential human and environmental health hazards. In addition, mites have developed resistance to most classes of pesticides, leading to their decreased efficacy. There is, hence, a need to develop alternative control strategies that would lower the costs of production.

Recently, *P. longipes* was found during exploration studies in Uruguaiana, Rio Grande do Sul State, southern Brazil (Furtado et al. 2006; Silva et al. 2008). Tests conducted under laboratory and screenhouse conditions indicated the potential of *P. longipes* as a successful control agent of *T. evansi* (da Silva et al. 2008; Furtado et al. 2007). The predatory mite was therefore imported into Kenya for the control of *T. evansi*. However, it could also be eliminated by the excessive use of acaricides. There is, therefore, an urgent need for the development of other control measures that are friendly to the environment, through studying and understanding the ecology and behaviour of the pest, *T. evansi*.

On the other hand, the cultivation of nightshades and other traditional vegetables has been promoted in recent years by development agencies and governments, which shows likelihood of severe outbreaks (Murungi et al. 2010). An intensive survey conducted by ICIPE in Kenya and Tanzania in 2014 (unpublished data) revealed that farmers were abandoning the cultivation of *S. scabrum* in Kenya, due to heavy infestation with spider mites or resulting in excessive use of dangerous synthetic acaricides to control *T. evansi*. This management approach had minimum success, and excessive use of synthetic pesticides is not recommended in most cases, since the leaves are eaten (Murungi et al. 2010). An innovative acaricide-treated net was introduced in IPM programmes to control spider mites, as a viable alternative solution to the problems arising from excessive use of synthetic pesticides. The technique involves covering the infested plants with an acaricide-treated net that traps and kills mites that are crawling on the plants. It has been effective in the control of *Polyphagotarsonemus latus* (Banks) and *T. urticae* (Martin et al. 2010). However, it was reported to be unsuccessful against *T. evansi* (Martin pers. comm.), contrary to *T. urticae* (Deletre et al. 2014). Placing an acaricide-treated net on top of infested plant patches of these leaf vegetables could be safer and less harmful. However, to be more effective, it would be of paramount importance to gain a deeper understanding of the within-plant distribution and movement of *T. evansi* along the *S. scabrum* plants. There is currently not much available information, and not many studies carried out, on the migration and distribution of *T. evansi* within *S. scabrum* plants. This type of knowledge would benefit IPM

strategies in developing other *T. evansi* control methods. On the other hand, with the introduction of *P. longipes* in the system, less is known of how within-plant migration and dispersal is affected by the presence of the predator, despite predators have been reported to induce movement in prey (Pallini et al. 1999; Meng et al. 2012). Studies on how *P. longipes* would affect within-plant migration and dispersal of *T. evansi* on *S. scabrum* are limited or non-existent.

It appears that gaining an understanding of the within-plant migration behaviour and distribution of the Red spider mite *T. evansi*, on the *S. scabrum* in presence of the predatory mite, *P. longipes*, holds the key for the development of effective and safe IPM strategies.

21.2 Materials and Methods

21.2.1 Plant Materials

The seeds for the African Nightshade *S. scabrum*, var. SS 52, were obtained from the World Vegetable Centre (AVRDC), Arusha, Tanzania. The seedlings were raised in the greenhouse in seeding trays for 4 weeks on media containing three parts of red soil, two parts of manure, and one part of sand. Four weeks after germination, the seedlings were transplanted in black polythene bags (15 × 30 cm) filled with a mixture of topsoil, sand and manure at a ratio of 3:2:1. Two weeks after transplanting, the plants were watered daily and each pot was nourished with 3 g calcium ammonium nitrate (CAN, 26% N) from Agrovet (Nairobi, Kenya). Afterwards, these plants were used for the experiments, or for spider mite and predatory mite rearing.

All the experiments were carried out in the laboratory at the International Centre of Insect Physiology and Ecology (ICIPE). The temperatures of the laboratory ranged between 20 °C and 24 °C, with 53% relative humidity (RH).

21.2.2 Mites

Quantities of the spider mite used in this study, *T. evansi,* were obtained from a regularly regenerated colony maintained at ICIPE on potted African nightshade plants. The mass cultures of *T. evansi* were maintained at a temperature of 25 ± 1 °C, 50–70% RH, and a 12-h photoperiod.

Specimens of *P. longipes* were obtained from a colony maintained at ICIPE rearing rooms at 25 ± 1 °C, 60–70% RH, and a 12-h photoperiod, and reared on *T. evansi* on detached, infested leaves.

21.2.3 *Migration Study of* Tetranychus evansi *Alone*

Test plants were randomly selected from the greenhouse plants when they had at least seven, fully developed leaves, and were placed on the laboratory benches for inoculation of *T. evansi*. For each density of *T. evansi* – 50, 100, 300 and 600 mites per plant – random samples of seven plants each were used. Each plant was divided into three levels, the bottom, middle and top sections. Same-age young adult mites, previously isolated as deutonymphs, were released on the stem of each plant, at 1 cm above the soil level. The plants were kept aside for 3 days, and initial observations of mite movements commenced 6 h after introduction, which was taken to be day one, with a second observation on day 4, at 6-h intervals, starting from 2 pm to 8 pm, the following day.

21.2.4 *Migration Study of* Tetranychus evansi *in Presence of* Phytoseiulus longipes

In the same way, random sample of 15 plants were used for each density, 50 and 100 mites per plant. Four hours after the introduction of *T. evansi*, *P. longipes* of the same age were introduced onto the plant, in the ratio of one predator to five mites on the stem of each plant, 1 cm above the soil level.

21.2.5 *Data Collection*

In each case, diurnal migration (day and night cycle) at 6-h intervals over a period 30 h was evaluated. Six observations were recorded at 2 pm, 8 pm, 2 am, 8 am, 2 pm and 8 pm. The counting of *T. evansi* and *P. longipes* mites was done with the aid of a 20× hand lens at each plant level, from bottom, middle and top levels, including adjacent stems. The main lights were put off at night during the whole experimental period, and counting was aided by the use of a dimmed spot light.

21.2.6 *Data Analysis*

Experiment 1: *Tetranychus evansi* **Within Plant Migration at Varying Densities**
The mite counts collected at each time of the day, for each density level and at a given plant level, were analysed using a generalised linear mixed model with Poisson distribution and log link. The fixed effects were *T. evansi* density (fitted as a qualitative factor), plant level (qualitative factor: bottom, middle, top) and time of day (fitted as a qualitative factor, six levels) and their interactions. The plant was

considered as a random effect (Bolker et al. 2008). The model was implemented using the *glmer function* in the lme4 package (Bates et al. 2014) of R3.1.1 (R Core Team 2014). The analyses were carried out separately for data collected at day 1 and 4 days after *T. evansi* introduction.

Experiment 2: *Tetranychus evansi* **Within Plant Migration in Presence of Predator**

The mite counts collected at each time of the day, for each density level and at a given plant level, were first transformed using log10(x + 1) to normalise data and stabilise variance, prior to subjecting data to a linear mixed model. Because of problems with sparse data in some plant levels, the data on *T. evansi* alone and *T. evansi* in presence of predator were analysed separately for each density. The fixed effects were plant level (qualitative factor) and time of day (qualitative factor) and their interaction. The plant was considered as a random effect (Bolker et al. 2008). The model was implemented using the *lmer function* in the lme4 package (Bates et al. 2014) of R3.1.1 (R Core Team 2014). The analyses were carried out separately for data collected at day 1 and 4 days after mites introduction.

21.3 Results

21.3.1 Within-Plant Migration and Distribution of T. evansi *Alone Within* S. scabrum

The analysis of mite distributions on single plants on day one and on the fourth day after introduction, showed no significant interaction between mite density, plant level and time of day, and no significant interaction between mite density and time of day (Table 21.1), thus suggesting the tendency of mites to stay in the same position over the time of day.

However, the interaction of mite density and plant level was significant, meaning that the within-plant migration of *T. evansi* depends on the initial mite density introduced on the plants (Table 21.1). While marginal effects of density and plant level were significant, the marginal effect of time of day was not significant, indicating no mite movement over day and night. These results were similar for both data collected at days one and four after inoculation (Table 21.1).

At the low mite density of 100, more mites were recorded at the middle and bottom than the top of the plant, throughout the day and night. Since the mites were introduced on the stem below the bottom leaves, they migrated and settled at the bottom and middle leaves, with no further migration over time of day to the subsequent plant level (Fig. 21.1a, c).

Analyses of mite counts at the higher densities of 300 and 600 mites, respectively, showed that the interaction between plant level and time of day was, as in the overall analysis above, not significant, with Wald $\chi^2 = 2.03$, df = 10, p = 0.996 for density at 300, and Wald $\chi^2 = 0.24$, df = 5, p = 0.998 for density 600. The time of

Table 21.1 Analysis of mite counts on single potted *Solanum scabrum* plants sampled from different plant levels (bottom, middle, top) over a 6 h-day and night cycle

1 day after mites introduction on stem			
Factor	Wald χ^2	d.f	P
Mite density	111.5	2	<0.001
Plant level	15.7	2	<0.001
Time of day	1.3	5	0.934
Mite density ∗ plant level	26.0	4	<0.001
Mite density ∗time of day	0.6	10	0.999
Plant level ∗ time of day	1.8	10	0.998
Mite density ∗ plant level ∗ time of day	1.7	20	1.000
4 days after mites introduction on stem			
Mite density	130.1	2	<0.001
Plant level	30.4	2	<0.001
Time of day	1.7	5	0.886
Mite density ∗ plant level	39.7	4	<0.001
Mite density ∗time of day	1.3	10	0.994
Plant level ∗ time of day	12.7	10	0.242
Mite density ∗ plant level ∗ time of day	11.2	20	0.941

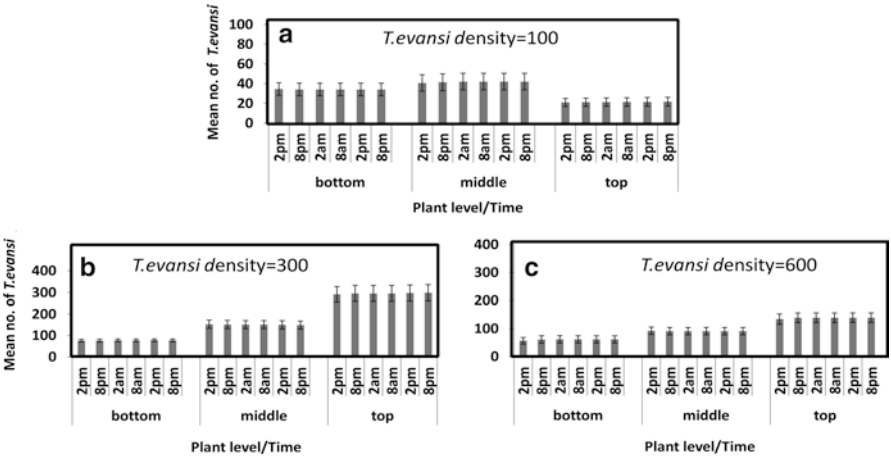

Fig. 21.1 Within-plant distribution of *Tetranychus evansi* at densities 100, 300, 600 (day 1 after inoculation)

day effect was also not significant. However, marginal effects of plant level were significant at both densities, with more mites at the top than middle and bottom at day 1 (Fig. 21.1b, c). Thus, mites at these higher densities collectively migrated to the top of the plant after being introduced at the bottom, and remained at top throughout the day and night recording time points (Figs. 21.1b, c and 21.2b, c).

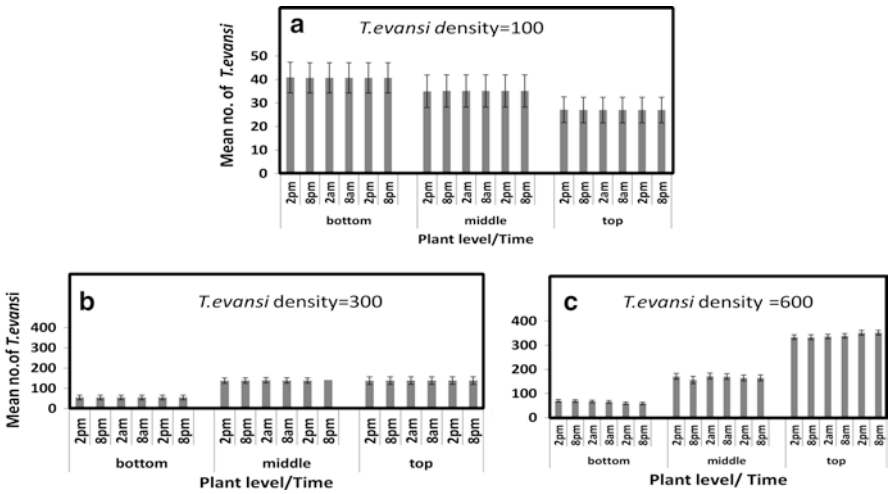

Fig. 21.2 Within-plant distribution of *T. evansi* at densities 100, 300, 600 (day 4 after inoculation)

Table 21.2 Analysis of mite counts on single potted *S. scabrum* plants sampled from different plant levels (bottom, middle, top) over a 6-h day and night cycle at density 50, in presence and absence of predator, *Phytoseiulus longipes*

1 day after mites introduction on stem						
	T. evansi with *P. longipes*					
Factor	Wald χ^2	d.f	P			
Plant level	65.3	2	<0.001			
Time of day	157.8	5	<0.001			
Plant level ∗ time of day	938.4	10	<0.001			
4 days after mites introduction on stem						
	T. evansi with predator			*T. evansi* alone		
Factor	Wald χ^2	d.f	P	Wald χ^2	d.f	P
Plant level	1023.2	2	<0.001	2964.6	2	<0.001
Time of day	96.2	5	<0.001	2	5	0.549
Plant level ∗ time of day	123.4	10	<0.001	13	10	0.224

21.3.2 Within Plant Distribution of T. evansi *in Presence of* P. longipes

At a low density, 50 mites per plant in presence of the predator, the interaction of plant level and time of day was highly significant for mites sampled at first day of introducing mites and predator, as well at the fourth day, suggesting that in presence of *P. longipes*, the abundance of mites at plant levels depended on the time of day. For plants with *T. evansi* alone, the interaction of plant level and time of day, and main effect of time of day were not significant, respectively (Table 21.2).

Table 21.3 Analysis of mite counts on single potted *S. scabrum* plants sampled from different plant levels (bottom, middle, top) over a 6 h-day and night cycle at density 100 in the presence and absence of predator, *P. longipes*

1 day after introduction of mites on stem of plant						
	T. evansi with predator					
Factor	Wald χ^2	d.f	P			
Plant level	38.1	2	<0.001			
Time of day	221.8	5	<0.001			
Plant level ∗ time of day	3608.6	10	<0.001			
4 days after introduction of mites on stem of plant						
	T. evansi with predator			*T. evansi* alone		
Factor	Wald χ^2	d.f	P	Wald χ^2	d.f	P
Plant level	4191.2	2	<0.001	36.8	2	<0.001
Time of day	588.9	5	<0.001	1.12	5	0.953
Plant level ∗ time of day	563.9	10	<0.001	61.5	10	<0.001

At density of 100 *T. evansi* per plant in presence of the predator, the interaction of plant level and time of day was also highly significant for mites sampled the first day of mites and predator introduction, as well than the fourth day, suggesting that the migration of mites at plant levels depended on the time of day. For plants with *T. evansi* alone, the interaction of plant level and time of day was significant, while the main effect of time of day was not significant (Table 21.3).

At 50 *T. evansi* per plant, the mites showed a pronounced diurnal movement within the plant, in the presence of the predator. They were found in higher numbers at the bottom of the plant at 2 pm, 8 pm and 2 am, and on its top in the next morning, (8 am), where they remained at 2 pm and 8 pm (Fig. 21.2a). This behaviour was similar to that shown at the higher mite density (100 specimens) (Figs. 21.2a and 21.3b).

Four days after introduction of *T. evansi* and predator, the more mites found at the top than those found at the same time, in day 1, at the middle and bottom levels. The mites were almost absent at the bottom level for both low (50) and higher density (100), in presence of the predator (Figs. 21.3a, b and 21.4a, b). Thus, the mites exhibited an avoidance response to *P. longipes.*

Analysis of the density and time of day effects in the predator treatment, 4 days after introduction of mites and predator, showed no significant interaction between density and time of day (Wald $\chi^2 = 1.37$, df = 5, p = 0.928). Thus, the effect of time of day was independent of the density level, suggesting that movement of *T. evansi* was indeed a result of the predator, and not of its own density.

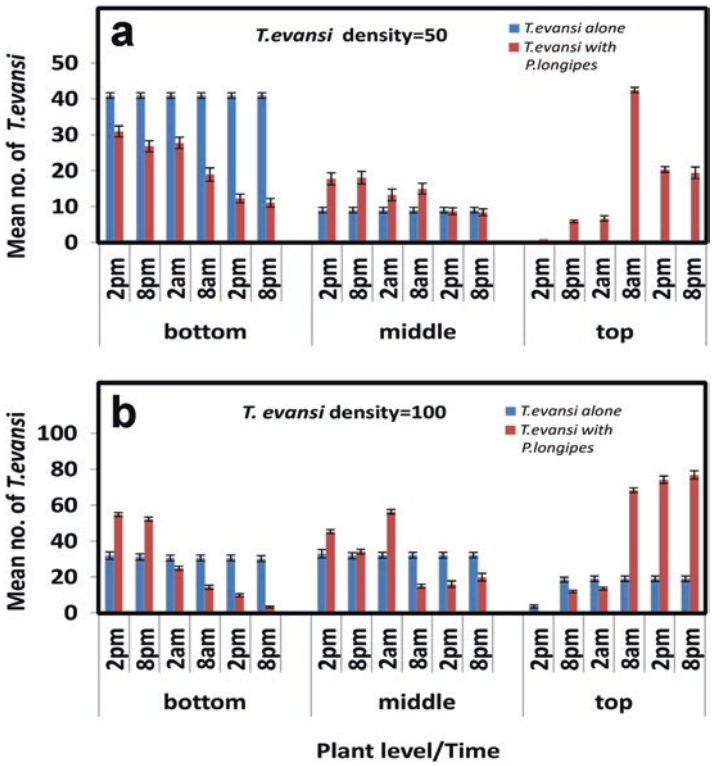

Fig. 21.3 Within-plant distribution of *T. evansi* alone and in presence of *Phytoseiulus longipes* at 50 and 100 specimens inoculation densities (day 1 after inoculation)

21.4 Discussion

We found that *T. evansi* did not possess a defined pattern of circadian migration within the *S. Scabrum* plant. The initial numbers of *T. evansi* recorded at each plant level did not change significantly at each time point, in 30 and in 72 h. Therefore, there was no definite pattern of migration within the time of the day/night. All mites remained in the same position that they had settled on after introduction onto the plant. However, we found a positive density-dependent, within-plant collective migration and distribution of *T. evansi*. At density 100, there was a significantly higher number of mites settling on the bottom leaves, 6 h after introduction, whereas at densities 300 and 600, a significantly higher number of mites moved furthest towards on-top leaves. The numbers of mites at each plant level, over the 30-h period of observations on day one and 4 days later, did not change significantly, although we had expected migration to occur as the mites had started to reproduce on the third day. This was an indication of no or minimum migration during the 4-day observation period. This could probably be due to the broad width of *S. scabrum* leaves, where feeding and multiplying mites could migrate horizontally first

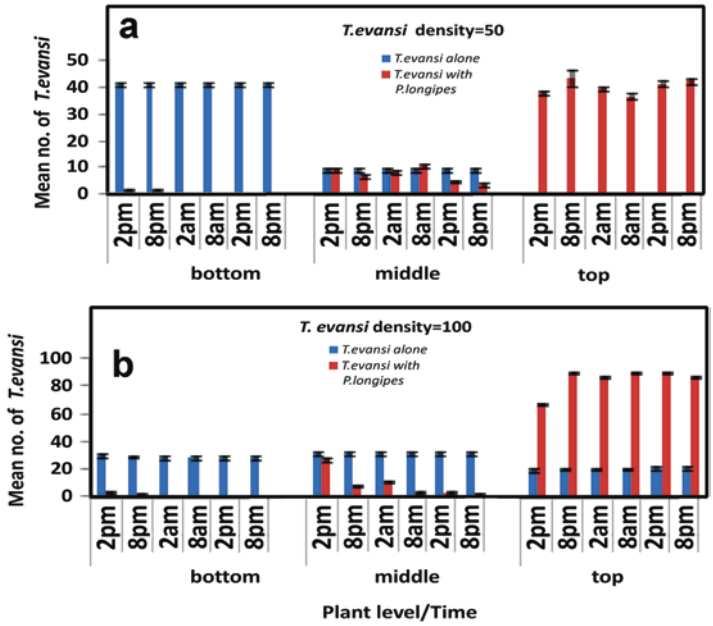

Fig. 21.4 Within-plant distribution of *T. evansi* alone and in presence of *P. longipes* at 50 and 100 specimens inoculation densities (day 4 after inoculation)

within the leaf, before they started to move up on the plant. This result suggests that *T. evansi*, being gregarious, would remain on the same patch spot that they first infested on arrival, especially in cases where it arrives on a plant from ballooning mite balls, which fall randomly off the plant and onto the leaves of, or ground around, clean plants. Furthermore, this might explain the occurrence of mite outbreaks where, in cases of low-density mite infestation, *T. evansi* individuals remain on the same leaves for a longer time, reproduce quickly, and damage all the lower leaves unnoticed, to infest suddenly the whole crop that becomes noticeably damaged. On the other hand, in cases of high-densities, the mites arrive on a patch together, probably from a ball of mites, collectively migrating further up the plant to infest the top-most leaves. In these circumstances, the mites get noticed earlier when the lower leaves and the whole crop is not heavily damaged, but would remain on the same leaves as long as these remain green to reproduce, causing damages and eventually dispersing to adjacent plants or neighbouring fields.

Hussy and Parr (1963) showed, using *T. urticae* placed on bean leaves, that the movements of ovipositing females were much more restricted, compared to pre-oviposition females. They remained on the same leaf 3 days after infestation. These authors further postulated that the movement of mites, walking from leaf to leaf, is mainly upwards, and that re-infestation of lower leaves occurs only when migrants drop from above or climb upwards from the soil. This upward migration is in anticipation to dispersal. On highly infested plants, the upper foliage is often more

severely damaged than lower foliage. In Tetranychid dispersal, it has been shown that factors that influence within-plant movement and distribution of mites vary between host plant, species, life cycle stage, sex, environmental factors, and the presence of predators in the system. Furthermore, the silk threads produced by spider mites facilitate further dispersal.

In *T. urticae*, collective displacement occurs in conditions of overcrowding and food depletion (Clotuche et al. 2011). Collective movements are ubiquitous in gregarious invertebrates (Clotuche et al. 2011). Hussey and Parr (1963) also postulated that the spider mite *T. urticae* spreads by migration of teneral females to oviposition sites, usually bean plants, and they noted that, during the short pre-oviposition period of about 24 h, teneral females migrated to fresh leaves higher up the host plant. The females migrated from the leaves on which they developed, even when leaves were slightly damage dirrespective of their infestation level. On the other hand, ovipositing females continued to feed and multiply on the initial leaves they had infested, causing leaf damage on the lower canopy. They remained in the same position and only abandoned the host when all the apical foliage was fully infested and chlorotic, with no migration from the host at an earlier stage. In our case, the experimental conditions and the fact that *T. evansi* were introduced at the bottom of the plant simultaneously, suggests that a high density could affect and contribute to the upward collective displacement and movement of *T. evansi* to infest the top leaves of *S. scabrum*. This collective movement could lead to sudden outbreaks of mites on previously uninfested crops. This could be due to the recruitment behaviour of spider mites, where the silk threads they produce recruit others to follow the trail. However, Astudillo Fernandez et al. (2012) tested the potential of silk trails left by the spider mites to elicit collective choices for *T. urticae*. He showed that the presence of a silk trail influences the mites, but not sufficiently to systematically provoke a collective choice. Further experiments need to be done, under full cropping cycle, multiplication on *S. scabrum*, and different plant and mite stages, to establish and confirm these results, while taking into account the distances that *T. evansi* would travel towards the apex of the plant, in various densities.

However, on introducing *P. longipes* onto plants previously inoculated with *T. evansi*, observations made 4 h later showed that varying numbers of *T. evansi* were recorded on the top, middle and bottom leaves at each 6-h interval, at densities 50 and 100. This indicates movement within the plant that is evidence of migration and dispersal in presence of *P. longipes*. In this regard, the study shows that the introduction of *P. longipes* to plants infested with *T. evansi* could be counterproductive, since *P. longipes* seemed to initiate upward movement and migration of *T. evansi*, which could lead to disturbance, and early or increased prey dispersal. This probably explains why, although the introduction of predatory mites reduces the population of spider mites, it does not sufficiently suppress their population. New infestations occur in other patches, prompting repeated releases of predatory mites to control the dispersing mites. The acaricide-treated net was effective in the control of *T. urtica*e (Martin et al. 2010), but ineffective in the control of *T. evansi.* This inefficiency could be explained by its gregarious nature. The decision of spider mites to migrate from a habitat is widely known to be affected by the quality of food

present in that habitat, and by the population density (Meng et al. 2012; Driessen and Bernstein 1999). Such knowledge could be of benefit in the management and control of this species, before the population builds up, inflicting high losses to the crop and other neighbouring fields. Spider mites in fact may avoid habitats with predators, and this could lead to premature dispersal (Kriesch and Dicke 1997) and other infestations elsewhere. To maintain balance between species, local extinctions, colonisation and persistence of the predatory mites in the system, it is of paramount importance to incorporate different control methods, studying the dispersal behaviour of both prey and predator (Zemek and Nachman 1998; Taylor 1990).

In conclusion, although early detection of *T. evansi* and subsequent introduction of *P. longipes* onto the plants are known to lower the mite population significantly (Furtado et al. 2006; Silva et al. 2008), data from this study show that *P. longipes* could induce possible migration within the plant and disturb its habitat, thereby inducing dispersal. Incorporating acaricide-treated nets in the management of *T. evansi* with *P. longipes* could help trap the dispersing mites, while the predators would feed on the eggs left behind by the dispersing females before catching the adults, after which the net could be removed. Moreover, since results suggest that *T. evansi* collectively migrate upwards towards the plant apex due to population pressure, migrating mites could be trapped by placing an acaricide-treated net on heavily infested plants. This would subsequently lower the mites' population, while at the same time preventing inter-plant infestations and aerial dispersal of mites from patches containing parental colonies. In the long run, this would prevent *T. evansi* outbreaks and delay the founding of new colonies on uninfested plants and help in conserving *P. longipes* in the environment, avoiding direct acaricide sprays. This approach may therefore contribute greatly in effective and successful integrated mite management strategies.

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References

Astudillo Fernandez, A., Hance, T., Clotuche, G. et al. (2012). Testing for collective choices in the two-spotted spider mite. *Experimental and Applied Acarology, 58*, 11–22. https://doi.org/10.1007/s10493-012-9558-5

Azandémè-Hounmalon, G. Y., Fellous, S., Kreiter, S., Fiaboe, K. K. M., Subramanian, S., Kungu, M., et al. (2014). Dispersal behavior of *Tetranychus evansi* and *T. urticae* on tomato at several spatial scales and densities: Implications for integrated pest management. *PLoS One, 9*, e95071. https://doi.org/10.1371/journal.pone.0095071.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7. http://CRAN.R-project.org/package=lme4

Blair, B. W. (1983). *Tetranychus evansi Baker and Pritchard (Acari: Tetranychidae): A new pest of tobacco in Zimbabwe*. Bergerac: CORESTA Phytopathology and Agronomy Study Group.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. (2008). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution, 24*, 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Bonato, O. (1999). The effect of temperature on life history parameters of *Tetranychus evansi* (Acari: Tetranychidae). *Experimental and Applied Acarology, 23*, 11–19. https://doi.org/10.1023/A:1006144610009

Boykin, L. S., & Campbell, W. V. (1984, February 1). Wind dispersal of the twospotted spider mite (Acari: Tetranychidae) in North Carolina peanut fields. *Environmental Entomology, 13*(1), 221–227. https://doi.org/10.1093/ee/13.1.221

Bugeme, D. M. (2008). *Potential of entomopathogenic fungi in the control of economically important spider mite species Tetranychus urticae Koch and Tetranychus evansi Baker and Pritchard*. Phd. Thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya.

Clotuche, G., Mailleux, A.-C., Astudillo Fernández, A., Deneubourg, J.-L., Detrain, C., & Hance, T. (2011). The formation of collective silk balls in the spider mite *Tetranychus urticae* Koch. *PLoS One, 6*, e18854. https://doi.org/10.1371/journal.pone.0018854.

da Silva, F. R., De Moraes, G. J., & Knapp, M. (2008) Distribution of *Tetranychus evansi* and its predator *Phytoseiulus longipes* (Acari: Tetranychidae, Phtoseiidae) in Southern Brazil. *Experimental and Applied Acarology, 45*, 137–145.

Dawson, P. (2001). The biodiversity of traditional leafy vegetables. Edited by J. A. Chweya and P. B. Eyzaguire. Rome: IPGRI (1999), pp. 182, no price quoted. ISBN 92-9043-404X. *Experimental Agriculture, 37*(2), 269–274. https://doi.org/10.1017/S0014479701212071

Deletre, E. M., Bonafo, R., & Martin, T. (2014). Evaluation of acaricide-treated string curtains for control of two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) on greenhouse roses and impact of the string curtain on the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). *Crop Protection, 60*, 34–43.

Driessen, G., & Bernstein, C. 1999. "Patch Departure Mechanisms and Optimal Host Exploitation in an Insect Parasitoid." *Journal of Animal Ecology 68*(3): 445–59. https://doi.org/10.1046/j.1365-2656.1999.00296.x.

Furtado, I. P., De Moraes, G. J., Kreiter, S., & Knapp, M. (2006). Search for effective natural enemies of *Tetranychus evansi* in south and southeast Brazil. *Experimental and Applied Acarology, 40*, 157–174.

Furtado, I. P., De Moraes, G. J., Kreiter, S., Tixier, M. S., & Knapp, M. (2007). Potential of a Brazilian population of the predatory mite, *Phytoseiulus longipes* as a biological control agent of *Tetranychus evansi* (Acari: Phytoseiidae, Tetranychidae). *Biological Control, 42*, 139–147.

Hussey, N. W., & Parr, W. J. (1963). Dispersal of glasshouse red spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Entomologia Experimentalis et Applicata, 6*, 207–214.

Knapp, M., Saunyama, I. G. M., Sarr, I., & De Moraes, G. J. (2003). *Tetranychus evansi* in Africa-Status, distribution, damage and control options. Abstract presented at the Institutional.

Kriesch, S., Dicke, M. (1997) Avoidance of predatory mites by the two-spotted spider mite *Tetranychus urticae*: The role of infochemicals. *Proceedings of the Section Experimental and Applied Entomology-Netherlands Entomological Society*, Amsterdam. M. J. Sommeijer, & P. J. Francke (Eds.), 8, 121–126.

Kungu, M., Deletre, E., Subramanian, S., Fiaboe, K. K., Gitonga, L., Lagat, Z. O., & Martin, T. (2019). A new mite IPM strategy: Predator avoidance behaviour resulting from the synergetic effects of predator release and acaricide-treated nets: New mite IPM strategy combining predator release and acaricide-treated nets. *Pest Management Science, 75*(4), 979–985. https://doi.org/10.1002/ps.5203.

Machini J. M. (2005). Study of the efficacy of Oberon 240 SC (Spiromesifen) and D-C-Tron plus on the red spider mite (*Tetranychus* spp.) on tomatoes (*Lycopersicon esculentum*) (Mill) and

their effect on predatory mite (*Phytoseiulus persimilis*) (Athias Henriot). Msc thesis, 2005, Nairobi University, Kenya.

Martin, T., Assogba-Komlan, F., Sidick, I., Ahle, V., & Chandre, F. (2010). An acaricide-treated net to control phytophagous mites. *Crop Protection, 29*, 470–475.

Maundu, P. M., Ngugi, G. W., & Kabuye, C. H. S. (1999). *Traditional food plants of Kenya* (270 pages). Kenya Resource Centre for Indigenous Knowledge, National Museums of Kenya.

Meng, R. X., Sabelis, M. W., & Janssen, A. (2012). Limited predator-induced dispersal in whiteflies. *PLoS One, 7*(9), e45487. https://doi.org/10.1371/journal.pone.0045487

Migeon, A., Ferragut, F., Escudero, A., Fiaboe, K. K. M., Knapp, M., de Moraes, G. J., Ueckermann, E., & Navajas, M. (2009). Modeling the potential distribution of the invasive tomato red spider mite, *Tetranychus evansi* (Acari: Tetranychidae). *Experimental and Applied Acarology, 48*, 199–212.

Murungi, L. K. (2011). Biological performance, response and population dynamics of *Tetranychus evansi* (Acari: Tetranychidae), as influenced by different African nightshade (*Solanum* sp.) species. PhD. Thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya.

Murungi, L. K., Nyende, A., Wesonga, J., Masinde, P., & Knapp, M. (2010). Effect of African nightshade species (Solanaceae) on developmental time and life table parameters of *Tetranychus evansi* (Acari: Tetranychidae). *Experimental & Applied Acarology, 52*, 19–27.

Murungi, L., Knapp, M., Salifu, D., Wesonga, J., Nyende, A., Masinde, P., & Torto, B. (2011). Differential effects of various African nightshade species on thefecundity and movement of *Tetranychus evansi* (Acari: Tetranychidae). *International Journal of Tropical Insect Science, 31*(4), 269–276.https://doi.org/10.1017/S1742758411000403

Pallini, A., Janssen, A., & Sabelis, M. (1999). Spider mites avoid plants with predators. *Experimental and Applied Acarology, 23*, 803–815. https://doi.org/10.1023/A:1006266232714

Pralavorio, M., Fournier, D., & Millot, P. (1989). Activité migratoire des tétranyques: mise en évidence d'un rythme. *Entomophaga, 34*, 129–134.

R Core Team. (2014). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. http://www.R-project.org/

Sabelis, M. W. (1985). In W. Helle & M. W. Sabelis (Eds.), *Predation on spider mites. Their biology, natural enemies and control* (pp. 73–83). Amsterdam: Elsevier.

Saunyama, I. G. M., & Knapp, M. (2003). The effects of pruning and trellising of Tomatoes (*Lcycoprscon esculentum* Mill.) on red spider mite (*Tetranychus evansi* Baker and Pritchard) incidence and crop yield in Zimbabwe. *African Crop Science Journal, 11*, 269–277.

Sibanda, T., Dobson, H. M., Cooper, J. F., Manyangaririwa, W., & Chiimba, W. (2000). Pest management challenges for smallholder vegetable farms in Zimbabwe. *Crop Protection, 19*, 807–815.

Silva, F. R., Moraes, G. J., & Knapp, M. (2008). Distribution of *Tetranychus evansi* and its predator *Phytoseiulus longipes* (Acari: Tetranychidae, Phytoseiidae) in southern Brazil. *Experimental & Applied Acarology, 45*, 137–145. https://doi.org/10.1007/s10493-008-9184-4

Smith Meyer, M. K. P. (1996). *Mite pests and their predators on cultivated plants in Southern Africa vegetables and berries* (Plant Production Research Institute, Handbook no 6). Pretoria: ARC-Plant Protection Research Institute. 90 pp.

Taylor, A. D. (1990). Metapopulations, dispersal, and predator-prey dynamics: An overview. *Ecology, 71*, 429–433.

Toroitich F. J. (2006). Effect of pesticides on the tobacco spider mite *Tetranychus evansi* Baker and Pritchard on tomatoes in Kenya. Msc thesis, University of Nairobi, Kenya.

Toroitich, F. J., Knapp, M., Nderitu, J. H., Olubayo, F. M., & Obonyo, M. (2014). Susceptibility of geographically isolated populations of the tomato red spider mite (*Tetranychus evansi* Baker & Pritchard) to commonly used acaricides on tomato crops in Kenya. *Journal of Entomological and Acarological Research, 46*, 18–23.

Zemek, R., & Nachman, G. (1998). Interactions in a trirophic acarine predator-prey metapopulation system: Effects of *Tetranychus urticae* on the dispersal rates of *Phytoseiulus persimilis* (Acarina: Tetranychidae, Phytoseiidae). *Experimental and Applied Acarology, 22*, 259–278. https://doi.org/10.1023/A:1006022500205

Chapter 22
Effects and Persistence of Endophytic *Beauveria bassiana* in Tomato Varieties on Mite Density *Tetranychus evansi* in the Screenhouse

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Abstract The tomato red spider mite (RSM), *Tetranychus evansi* Baker and Pritchard, is an important exotic pest in the production of tomatoes and other solanaceous plants in Africa. Isolates of *Beauveria bassiana* ICIPE 273, ICIPE 279, ICIPE 283, ICIPE 10, and ICIPE 35 were previously tested for their endophytic activity in tomato in a screenhouse assay. ICIPE 35 was able to colonise leaves, stems and roots of the tomato varieties Cal J, Kilele and Anna F1, whereas the other four isolates were undetectable in all plant parts of the varieties tested, thus confirming no colonisation. Colonisation was assessed after every two-week period by plating the various plant parts on selective media. Persistence was examined by comparing colonisation from week 2, 4 and 6, since there was no colonisation detected after this time, for all the tested tomato varieties. There were no significant differences in levels of colonisation in the various parts of tomato varieties, i.e. stem ($F = 1.7$, $DF = 2$, $P = 0.186$), roots ($F = 2.0$, $DF = 2$, $P = 0.127$), and leaves ($F = 0.28$, $DF = 2$, $P = 0.752$). The density of *T. evansi* was lower in endophyte-colonised plants than the controls. In conclusion, this study revealed that *B. bassiana* can

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colonise and persist in the tested tomato varieties for a period of 6 weeks in the screenhouse and reduce adult RSM populations.

Keywords Endophytes · *Solanum lycopersicum* · Fungal Entomopathogens · Spider mites

22.1 Introduction

Tomato, *Solanum lycopersicum* Mill. (Solanales: Solanaceae), is a major vegetable crop, worldwide (Rice et al. 1987). It can be grown in backyards for home consumption, as well as in open fields and controlled environments for commercial purposes (van Dam et al. 2005). Tomato production in Kenya has been on the decline due to environmental stresses, declining soil fertility, poor crop management, and low quality seeds (KHDP 2008). Pest and disease pressures have also increased, reducing yields (FAO 2003).

Sustainable methods are being considered to manage pest populations in tomato crops. Among them there is the use of entomopathogenic fungi (Chandler et al. 2000; Maniania et al. 2008). These fungi also play additional roles in nature, such as endophytes, antagonists of plant pathogens, and possibly even plant growth-promoting agents (Vega et al. 2009).

Isolates of *Beauveria bassiana* have been tested against *Tetranychus evansi* and found to be highly virulent, suggesting a potential for their utilisation in the management of this pest (Wekesa et al. 2005). However, lack of an effective delivery system for entomopathogenic fungi limit their wide application. Hence, the need to develop an economically feasible mass-production and delivery system.

Beauveria bassiana has been detected inside tomato seedlings following seed treatment with the fungus. The endophytic association promoted growth and provided protection to tomato seedlings from damping-off disease (Ownley et al. 2004, 2008). The effectiveness of *B. bassiana* as an endophyte is determined by its survival and establishment within tomato plants.

Although *B. bassiana* is known to have the ability to exist in plants as an endophyte, there is a need to study its persistence in tomato plant tissues, so that the fungus can offer long-term protection against the *T. evansi* in tomatoes in the screenhouses. This study, therefore, aimed at investigating the endophytic potential and persistence of ICIPE 35 isolate of *B. bassiana* in the Cal J, Kilele, and Anna tomato varieties in the screenhouse, study the effects of the fungus on RSM density, and assess the endophytic colonisation and persistence of *B. bassiana*.

22.2 Materials and Methods

22.2.1 Fungal Culture

From previous screening work done on five isolates of *B. bassiana*, ICIPE 273, ICIPE 279, ICIPE 283, ICIPE 10 and ICIPE 35, only ICIPE 35 was able to establish as an endophyte in tested tomato varieties. The isolates were obtained from the ICIPE Arthropod Germplasm Centre, (Nairobi, Kenya), and sub-cultured on Sabouraud Dextrose Agar (SDA) medium, amended with 0.05 g chloramphenicol antibiotics to minimise the bacterial contamination, and incubated for 3 weeks at 27 °C (Inglis et al. 1996). Conidia were gently scrapped from fungal cultures and suspended in 10 ml sterile distilled water in a 20-ml universal bottle containing 0.01% Tween-20 and glass beads. The suspension was vortexed for 5 min to produce a homogenous conidial distribution. From the stock solution, a concentration of 1×10^9 conidia ml^{-1} was prepared, since the standard concentration of 1×10^8 used in preliminary studies did not establish well in the tomato varieties. The viability of conidia was assessed prior to bioassays by spread-plating 0.1 mL of 3×10^6 conidia mL^{-1} onto 90-mm Petri dishes containing SDA (Goettel and Inglis 1997). The plates were incubated at 27 ± 2 °C and were examined after 16–20 h under a compound microscope (×40 magnifications). Conidia were considered as germinated when the germ tube was twice the diameter of the conidium. The experiment was replicated four times.

22.2.2 Tomato Plants

The tomato plants (*Solanum esculentum* var. Cal-J, Kilele and Anna) that were used in this study were raised in the screenhouse at ICIPE. Cal-J is highly susceptible to red spider mites, but is commonly grown by farmers, while Kilele and Anna are popular new hybrids among farmers.

22.2.3 Red Spider Mite Culture

Tetranychus evansi was obtained from a regularly regenerated colony reared on tomato variety Cal-J (ICIPE) at 25 ± 2 °C, 60–70% R.H., and a photoperiod of 12:12 h (L:D). The initial culture originated using mites collected from Mwea Irrigation Scheme, Kenya, in 2001.

22.2.4 Seed Inoculation and Colonization

Seeds of Cal-J and two hybrid cultivars (Kilele and Anna) were surface-sterilised in 70% ethanol for 1 min and then in 1.5% sodium hypochlorite for 3 min. They were then washed three times with sterile distilled water and blot dried on sterile paper towels to remove the excess water. The last rinse water was plated out to assess the effectiveness of the surface sterilisation procedure. Inoculation was carried out by soaking seeds in conidial suspension (10 ml) of *B. bassiana* isolate ICIPE 35 at the concentration of 1×10^9 conidia ml^{-1} for 2 h. The seeds were hand stirred at a 30-min intervals until they were uniformly coated (Powell et al. 2009). Control seeds were soaked in sterile distilled water containing 0.01% Tween-20 for 2 h. The seeds were then removed and sown in pots filled with sterilised soil. There were 8 individual pots for each tomato variety for ICIPE 35 and for their respective controls. The pots containing the seedlings were grown in the screen house at 28 ± 2 °C and 70–80% R.H. for 6 weeks. The endophytic colonisation by *B. bassiana* on tomato plant was examined every 2 weeks after inoculation through destructive sampling. Plants were carefully removed from the pots, washed with tap water, and surface-disinfected by submersing in 70% ethanol for 15 s, followed by 3 min in 1.85% sodium hypochlorite, and rinsed 3 times in sterile distilled water. To determine the effectiveness of the disinfecting process, the final rinse water was plated on SDA, as described above. Plants were dried on sterile paper towels, and tissues from leaves, shoots and roots were cut into 4 mm^2 sections with a sterile scalpel, and about 5 of them were placed on Petri dishes containing SDA medium. Four plates were cultured for each tissue (roots, shoots and leaves) and for each treatment, including the control. They were allowed to sporulate for 2 weeks in the laboratory, and then the *B. bassiana* was identified, based on its morphological characteristics. For each plant part, the percentage of colonisation was calculated as the number of sections exhibiting *B. bassiana* outgrowth per total number of sections (Fisher and Petrini 1987).

22.2.5 Data Analysis

The colonisation frequency (CF) was calculated as described by Fisher and Petrini (1987). The proportions of fungal colonisation per plant part and persistence, which were expressed as percentages, were arcsine square root transformed before being subjected to ANOVA using the procedure generalised linear models of SAS software. Whenever treatment effects were significant, then means were compared using SNK (Student-Newman-Keuls). All tests were performed at 5% level of significance. Population counts of mites for the greenhouse experiment were log transformed to normalise data and transformed values were subjected to ANOVA. A

Student's t-test was used to compare treatments and control. All data were analysed using the SAS statistical package ver. 8.

22.3 Results

22.3.1 Endophytic Colonisation of B. bassiana *on Tomato Varieties*

The *B. bassiana* isolate ICIPE 35 was able to colonise the roots, stems and leaves of all the tomato varieties in the screen house. However, there were no significant differences in levels of colonisation among parts of the three tomato varieties, i.e. stems (F = 1.7, DF = 2, p = 0.18), roots (F = 2.0, DF = 2, p = 0.12), and leaves (F = 0.28, DF = 2, p = 0.75) (Fig. 22.1). For example, in Anna, there was no significance difference in colonisation among the various plant parts, (leaves = 4.5%, stem = 5%, roots = 3.3%). Colonisation was slightly higher in the leaves of Cal J variety (5.8%), compared with the stem, (2.9%), and roots (3.3%). In Kilele, colonisation was highest in leaves (4.8%), followed by stem and roots (3.4 and 1%), respectively (Fig. 22.1). There was no colonisation of plant parts of the varieties tested as control.

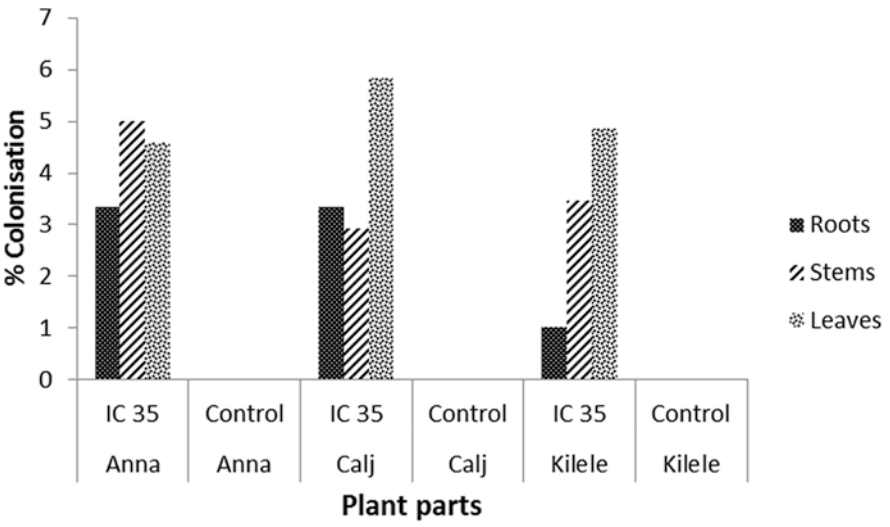

Fig. 22.1 Colonisation of different parts of tomato varieties by *Beauveria bassiana* isolate ICIPE 35

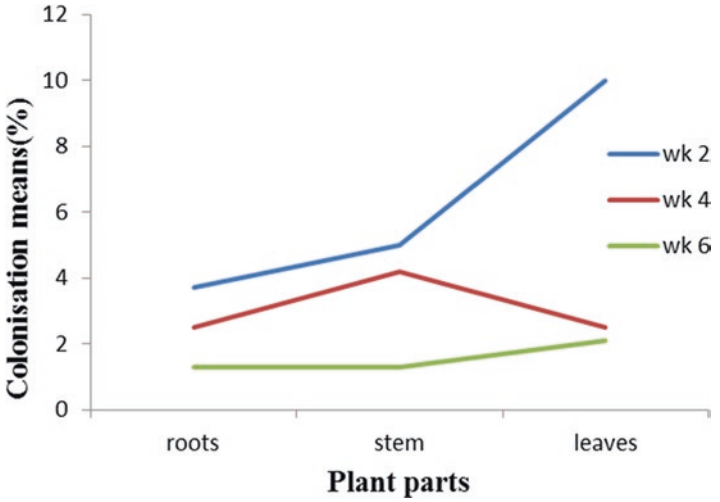

Fig. 22.2 Persistence of different parts of tomato varieties by *B. bassiana* isolate ICIPE 35, 6 weeks after inoculation in the screen house

There was no interaction between the variety and treatment for the various plant parts at 2, 4, 6, weeks after inoculation: roots (F = 2.3, df = 2, P = 0.10), stems (F = 1.8, df = 2, P = 0.17), and leaves (F = 0.34, df = 2, P = 0.71). Consequently, there was no interaction between the variety and the weeks: roots (F = 1.9, df = 4, P = 0.10), stems (F = 0.50, df = 4, P = 0.74), and leaves (F = 0.34, df = 4, P = 0.71). Although there was no interaction for the treatment and week for plant roots, (F = 1.7, df = 2, P = 0.18), there was a significant interaction for stems (F = 3.87, df = 2, P = 0.02) and leaves (F = 8.9, df = 2, P = 0.00). Despite the general decline in percentage colonisation over time, the rate of decline was not different among the various plant parts for all the tested varieties, i.e. stem (F = 1.7, df = 2, P = 0.18), roots (F = 2.0, df = 2, P = 0.12), and leaves (F = 0.28, df = 2, P = 0.75).

The percentage for colonisation was generally high for week 2, as compared with weeks 4 and 6, although there were no significant differences for plant roots for weeks 2, 4 and 6. For stems, week 6 was significantly different from weeks 2 and 4, which were not different, while for the leaves, week 2 was significantly different from weeks 4 and 6, which were not different (Fig. 22.2).

The correlation between the top leaf and low leaf has an r value of 0.619, which is more than 0.05, and is a strong value. For top leaf and total mite, the value is 0.978, which is also a strong value. The correlation between the top leaf and leaf area has a weak value of 0.399. Generally, from the results above, as the leaf area increases, the mite density decreases, and vice versa (Fig. 22.3). Tomato plants inoculated with ICIPE 35 had less RSM, compared with the controls.

		Correlations			
		topleaf	lowleaf	Tot_mite	l/area cm3
topleaf	Pearson Correlation	1	.619**	.978**	-.399**
	Sig. (2-tailed)		0	0	0
	N	181	158	181	181
lowleaf	Pearson Correlation	.619**	1	.748**	-.188*
	Sig. (2-tailed)	0		0	0.018
	N	158	158	158	158
Tot_mite	Pearson Correlation	.978**	.748**	1	-.393**
	Sig. (2-tailed)	0	0		0
	N	181	158	181	181
l/area cm3	Pearson Correlation	-.399**	-.188*	-.393**	1
	Sig. (2-tailed)	0	0.018	0	
	N	181	158	181	181
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

Fig. 22.3 Correlations for top leaf and low leaf for adult mite populations

22.4 Discussion

Beauveria bassiana isolate ICIPE 35 was able to colonise the root, stem and leaves of Cal-J, Kilele and Anna tomato varieties, in the screenhouse. The levels of colonisation also differed among the various plant parts, likely due to the external environment or the biological differences within the plant tissues (Bayman et al. 1997). These results confirm those by Muvea et al. (2014), who found differences in the level of colonisation of different plant parts by fungal isolates. Similar results were also reported on French beans and Faba beans (Akutse et al. 2013), and coffee (Posada et al. 2007).

The fact that the fungus was found in several plant parts indicates that it was able to spread within the plant. This is important, especially if there is vertical transmission of the endophytes through the seeds, meaning that subsequent generations may not require seed dressing (Schardl et al. 2004). Colonisation of plants by entomopathogens can take several pathways, which all depend on the fungi and the plant in question. However, systemic spread has been advocated by most authors (Bing and Lewis 1991). This supports previous studies that attributed passive movement of the fungal hyphae within the xylem and vascular tissues, eliciting a systemic reaction (Bing and Lewis 1992).

The colonisation in the Anna variety was slightly higher in the stem than in leaves, while in Kilele and Cal J the leaves experienced a higher degree of colonisation, compared with the other parts. It is possible that the leaves provide a suitable environment for the establishment and survival of *B. bassiana*, as compared with

the other plant parts in these two varieties (Fisher et al. 1992). Previous studies showed that most endophytic fungi are highly adapted to particular conditions present in a given plant organ (Carroll 1988; Fisher et al. 1992). This is likely due to micro-ecological and physiological conditions existing in the different tomato plant tissues, which confer varying survival degrees for *B. bassiana* (Bayman et al. 1997).

Although the various parts of the tomato varieties were colonised 2 weeks after inoculation, a decrease was noted by week 6, and by week 8, colonisation declined as it was not observed in any of the plant parts. The rate of decline was faster in leaves, followed by the stems, and lastly, the roots, which could probably be due to no multiplication taking place within the plant rhizosphere, or inhibition of *B. bassiana* germination or growth (Quesada-Moraga et al. 2006). Similar results were observed in cocoa plants, where *B. bassiana* became established as an endophyte, but did not persist beyond 2 months (Posada and Vega 2005). Decline in colonisation of the various plant parts may also be caused by the plant response to the endophytic fungus, by other fungi that occur naturally within the plant, and also by the expansion of its tissues and parts (Posada et al. 2007).

The delivery of *B. bassiana* as endophyte bypasses the limitations imposed by their direct use, especially foliar spraying, where they are affected by UV radiation, varying conditions of temperature, and humidity, that frequently reduce conidial viability (Vega et al. 2008). Once established in the plant system, these endophytes provide plant protection against pests, reduce operation costs because no repeated applications are needed, and guarantee efficacy because the fungus is protected against abiotic factors.

The plant-defence ability exhibited by *B. bassiana* by reducing mite populations in tomato requires a greater understanding of the mechanisms that suppress herbivory, so as not compromise human and animal safety, as was the case with Epichloe, which protects grasses against nematodes but is also toxic to vertebrates (Schardl et al. 2004).

This study demonstrated that *B. bassiana* isolate ICIPE 35 can colonise tomato plants and persist up to 6 weeks in a screenhouse from seed inoculation. It also reduced the adult population of RSM. This can be used to complement existing control measures for controlling RSM in tomato crops.

References

Akutse, K., Maniania, N., Fiaboe, K., Van den Berg, J., & Ekesi, S. (2013). Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Fungal Ecology, 6*, 293–301.

Bayman, P., Lebron, L. L., Tremblay, R. L., & Lodge, D. J. (1997). Variation in endophytic fungi from roots and leaves of Lepanthes (Orchidaceae). *The New Phytologist, 135*, 143–149.

Bing, L. A., & Lewis, L. C. (1991). Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. *Environmental Entomology, 20*, 1207–1211.

Bing, L. A., & Lewis, L. C. (1992). Endophytic *Beauveria bassiana* (Balsamo) Vuillemin in corn: The influence of the plant growth stage and *Ostrinia nubilalis* (Hübner). *Biocontrol Science and Technology, 2*, 39–47.

Carroll, G. (1988). Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. *Ecology, 69*, 2–9.

Chandler, D., Davidson, G., Pell, J., Ball, B., Shaw, K., & Sunderland, K. (2000). Fungal biocontrol of Acari. *Biocontrol Science and Technology, 10*, 357–384.

FAO. (2003). World Agriculture Information Centre Data base. http://www.fao.org/waicent/portal/statistics-en.asp

Fisher, P., & Petrini, O. (1987). Location of fungal endophytes in tissues of Suaeda fruticosa: A preliminary study. *Transactions of the British Mycological Society, 89*, 246–249.

Fisher, P., Petrini, O., & Scott, H. L. (1992). The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). *The New Phytologist, 122*, 299–305.

Goettel, M., & Inglis, G. (1997). Fungi: Hyphomycetes. In L. A. Lacey (Ed.), *Manual of techniques in insect pathology* (pp. 213–249). London: Academic.

Inglis, G. D., Johnson, D. L., & Goettel, M. S. (1996). An oil-bait bioassay method used to test the efficacy of *Beauveria bassiana* against grasshoppers. *Journal of Invertebrate Pathology, 67*, 312–315.

Kenya Horticultural Development Programme (KHDP). (2008). *Keeping small holders in Business*, p. 10.

Maniania, N. K., Bugeme, D. M., Wekesa, V. W., Delalibera, I., & Knapp, M. (2008). Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. *Experimental & Applied Acarology, 46*, 259–274.

Muvea, A. M., Meyhofer, R., Subramanian, S., Poehling, H. M., Ekesi, S., & Maniania, N. K. (2014). Colonization of onions by endophytic fungi and their impacts on the biology of Thrips tabaci. *PLoS One 9*(9), e108242.

Ownley, B. H., Pereira, R. M., Klingeman, W. E., Quigley, N. B., & Leckie, B. M. (2004). *Beauveria bassiana*, a dual purpose biocontrol organism, with activity against insect pests and plant pathogens. Information Systems Division, National Agricultural Library.

Ownley, B. H., Griffin, M. R., Klingeman, W. E., Gwinn, K. D., Moulton, J. K., & Pereira, R. M. (2008). *Beauveria bassiana*: Endophytic colonization and plant disease control. *Journal of Invertebrate Pathology, 98*, 267–270.

Posada, F., & Vega, F. E. (2005). Establishment of the fungal entomopathogen Beauveria bassiana (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*). *Mycologia, 97*, 1195–1200.

Posada, F., Aime, M. C., Peterson, S. W., Rehner, S. A., & Vega, F. E. (2007). Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). *Mycological Research, 111*, 748–757.

Powell, W. A., Klingeman, W. E., Ownley, B. H., & Gwinn, K. D. (2009). Evidence of endophytic *Beauveria bassiana* in seed-treated tomato plants acting as a systemic entomopathogen to larval *Helicoverpa zea* (Lepidoptera: Noctuidae). *Journal of Entomological, 44*, 391–396.

Quesada-Moraga, E., Landa, B., Muñoz-Ledesma, J., Jiménez-Diáz, R., & Santiago-Alvarez, C. (2006). Endophytic colonisation of opium poppy, *Papaver somniferum*, by an entomopathogenic *Beauveria bassiana* strain. *Mycopathologia, 161*, 323–329.

Rice, R. P., Rice, L. W., & Tindall, H. D. (1987). *Fruit and vegetable production in Africa*. Basingstoke: Macmillan Publishers.

Schardl, C. L., Leuchtmann, A., & Spiering, M. J. (2004). Symbioses of grasses with seedborne fungal endophytes. *Annual Review of Plant Biology, 55*, 315–340.

van Dam, B., Goffau, M. D., van Lidt de Jeude, J., & Naika, S. (2005). *Cultivation of tomato: production, processing and marketing*. Wageningen: Digigrafi.

Vega, F. E., Posada, F., Aime, M. C., Pava-Ripoll, M., Infante, F., & Rehner, S. A. (2008). Entomopathogenic fungal endophytes. *Biological Control, 46*, 72–82.

Vega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. A., Keller, S., et al. (2009). Fungal entomopathogens: New insights on their ecology. *Fungal Ecology, 2*, 149–159.

Wekesa, V. W., Maniania, N. K., Knapp, M., & Boga, H. I. (2005). Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to the tobacco spider mite *Tetranychus evansi*. *Experimental & Applied Acarology, 36*, 41–50.

Chapter 23
Flight Behaviour and Activity Time of *Tuta absoluta* (Meyrick) Males Trapped on Tomato and Miscellaneous Crops, Gezira State, Sudan

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Abstract *Tuta absoluta* is an invasive and devastating pest of tomato, native to Latin America. It was reported in Spain in 2006 and spread to Euro-Afro-Asian countries. In Sudan, it was reported in 2010. We studied the flying behaviour of *T. absoluta* to understand the times of insect activity and the role of crops in insect attraction, using a pheromone (Tua optima 0.8 mg) and baited water traps. The timing of male activity was monitored every 3 h from 6:00 pm to 9:00 am the next day. The height of flying was studied by installing four traps (from 0 to 150 cm). All traps used in these studies were put upwind of tomato and miscellaneous crops. Temperatures and wind velocities were recorded during the study period. The males activity started at photo phase and continued for almost 1 h thereafter, ending at almost 30.0 min after sunrise. The height of flying for 90% of males was at ground level. Traps deployed upwind of tomato crop captured 70% of males (n = 1466). The findings confirmed that males of *T. absoluta* were attracted by pheromone and tomato, and traps should be placed at the ground level, before photophase.

23.1 Introduction

Tomato *Solanum lycopersicum* is an important vegetable crop for income, food and nutrition in Africa (Zekeya et al. 2017a, b). In Sudan, it is second to onion in terms of area cultivated. Tomato is grown in all states of the country, accounting for 18% of the total area used for cultivating vegetables (Mohamed 2008). Tomato-growing farmers in Gezira are currently stranded because of a lack of effective pest control options. They had almost abandoned tomato cultivation because of the high costs of inputs, such as seeds with tolerance to high temperatures and diseases, the costs attributable to employing labourers with good experience in tomato production and

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purchasing fertilisers, and also because of the risks of insect infestations and the lack of marketing security. Among the insect pests threatening tomato production is tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae), which is invasive and very devastating to plants. The pest is known under very many common names. Some of these are the South American tomato leaf miner (Biondi et al. 2013; Lobos et al. 2013; Jamoussi et al. 2013), leaf miner (Mouttet et al. 2013), tomato borer (Roditakis et al. 2013), tomato pinworm (Dias et al. 2013), and tomato moth (Savino et al. 2012). The insect originated in South America and was detected in Spain during 2006 (Arnó and Gabarra 2010). It was reported in France, Algeria and Morocco in 2008. During 2009, the pest was reported in Italy, Israel, Turkey, Tunisia, Malta and Libya. Furthermore, it was reported in Sudan during 2010 (Mohamed et al. 2015). Zekeya et al. (2017a) stated that there is a risk that most tomato-growing farmers would switch to other crops because of losses caused by the invasive tomato leaf miner. Moreover, invasive species represent a major threat to both natural and agronomic ecosystems. The damage caused by tomato leaf miner to tomato fields, in the open or closed farming systems, is devastating, because the plant is the most suitable host for the insect and can be infested at any developmental stage (Nicolas et al. 2010). Losses in production attributable to damage caused by tomato leaf miner could reach 80–100%, if left unchecked (Nicolas et al. 2010; Daniel and Bajarang 2017). Hence, yields and fruit quality will be reduced.

Many tools used for crop protection were tested to halt damage on tomato crops, in open and closed farming systems. Farmers resort to the use of insecticides because of they are easy to use, with reasonable costs, and cater for pest control in due course. However, the drawbacks of pesticides regarding pest resurgence, appearance of secondary pests, resistance and negative effects on the environment are of no concern for most farmers. The use of insecticides to control tomato leaf miner is practised in the area of its origin and in Mediterranean basin countries (Savino et al. 2012, Nicolas et al. 2010). Different groups of insecticides have been used to control tomato leaf miner, with insufficient results (Roditakis et al. 2013; Biondi et al. 2013; Consoli et al. 1998; Nicolas et al. 2010; Zekeya et al. 2017b; Arnó et al. 2009). The inefficacy of insecticides against tomato leaf miner arises because, at the damaging stage, its larva is protected within the plant tissue and no contact action will take place. Moreover, the insect has many alternative hosts among crops and wild plants. It has also the ability to develop resistance to many insecticide groups, which arises because the insect can attain 10–12 generations per year, depending on the availability of food, and hence is likely to undergo genetic changes (mutations), which in turn cause resistance to chemical insecticides (Daniel and Bajarang 2017; Arnó and Gabarra 2011). That is why the action of depending only on chemical insecticides (Lobos et al. 2013), as a tool to control tomato leaf miner, contributes a large portion of the costs in production (Dias et al. 2013).

However, the invasive and devastating characteristics of tomato leaf miner on tomato crops have resulted in calls for research into pest management in a more efficient and sustainable manner (Nicolas et al. 2010; Contreras et al. 2014).

This could be achieved through the understanding of the key elements (biotic and abiotic factors), active in a balanced natural environment. Then, the agronomic

ecosystem of tomato crops should achieve a presence of natural enemies under tolerant or resistant genotypes, together with encouraging cultural practices, for better tomato production and suppression of the tomato leaf miner. Many natural enemies have been tested to control the tomato leaf miner, including predators (Jaworski et al. 2013; Arnó et al. 2009; Urbaneja et al. 2009), parasitoids (Chailleux et al. 2013; Biondi et al. 2013) and insect pathogens (Contreras et al. 2014; Jamoussi et al. 2013). The most important tool for cultural practices appears the use of an insect pheromone. This is a technique that can be used for pest detection, population monitoring, mass trapping, and mating disruption (Daniel and Bajarang 2017).

However, the time of activity and height of flying of the pest, as well as the effects of various crops on *T. absoluta*, are not clear, and sometimes controversial. For deployment, Russell IPM indicates the height of a pheromone trap should be set at a level of 100 cm above ground level. However, other works proved that the pheromone trap is more effective in capturing males when deployed near ground level, at a height of 20–40 cm (Lobos et al. 2013).

Concerning the time of flying, studies showed that the adults of tomato leaf miner are active during the night and do not move during the day time (Abdul-Rassoul 2014; Daniel and Bajarang 2017). In some insect species, males recognise the females through the pheromones they emit (Chapman 1980). The tomato leaf miner is one of these insects, where mating and fertilisation take place when the males are attracted towards females through its pheromone production. However, the crop itself has a role in male attraction, because it has been found that the deployment of pheromone traps upwind of tomato fields captures more males of the insects (Lobos et al. 2013). Adults (males and females) are attracted towards tomato plants (Bawin et al. 2014) that emit solanaceous volatiles (Caparros Megido et al. 2014). No more data about other crops, whether attracting or repelling the tomato leaf miner, have been found. Information about the siting of pheromone trap deployment and the time of male activity, as well as the role of the crop, is needed to aid the formation and application of integrated control strategies against this pest (Cuthbertson et al. 2013).

The objective of this study is to evaluate the height that males fly at, the time of male activity, and the role of crops in attracting males.

23.2 Materials and Methods

23.2.1 Site of the Experiments

Experiments were conducted at the Experimental Farm, Faculty of Agricultural Sciences, University of Gezira, which located at Latitude 14.44°N, Longitude 33.39 °E and altitude 407 m above sea level.

23.2.2 Pheromone Baited Water Trap

Water bowl traps were used in these experiments. The trap is round, with a radius of 15 cm, and a volume of 3.5 litres. The bowls were made of red plastic material, baited with "Tua optima" sex pheromone of 0.8 mg, loaded onto a grey rubber septa produced by Russell IPM (UK), imported by Star Chemical Ltd. The pheromone was stored in a refrigerator until the time of use in the field. The pheromone rubber septa was renewed every 10 weeks. The grey rubber septa, impregnated with pheromone, was secured in a small porous, translucent, plastic vial (vol. 50 ml). The base of the vial and the screw cover were perforated for a tender wire to pass through longitudinally, and the vial was fixed in the centre of the round shape of the bowl by knotting the ends of the tender wire at the rim of the bowl.

The bowl was filled with water to a level just below the vial. The water surface tension was broken by drops of liquid soap. All bowl traps were deployed upwind of the crop concerned. The bowls, tender wire, liquid soap and others were purchased at local grocery shops.

23.3 Cultural Practices for Crops

23.3.1 Tomato

A disc plough was used for land preparation, followed by a disc harrow to break the soil clots, and the soil was then levelled before making plant beds at a width of 160 cm each. The area under the crop was pre-watered to allow for weeds to grow before using a grass killer (Glyphosate) at a rate of 1.5 litre per feddan, equivalent to 750 grams active ingredient (a.i).

Tomatoes were directly seeded at the line of water seepage on the top of the bed, at a distance of 30 cm between holes, and thinned to 2–3 plants at 3 weeks after sowing. Nitrogen in the form of urea was applied, at equal doses, 4 weeks after first irrigation and 1 month later to give a total dose of 2 N (80 kilograms urea, per feddan) (1 feddan = 0.42 hectare). Weeding of the plots was done when needed. Irrigation, depending on temperature, was practised every 7–10 days. No pesticides were used at the time of sowing, except for the seed dresser Gaucho 70 WP, which was applied at rate of 7 grams (4.9 grams a.i) per kilogram of seeds. The total area under the tomato crop was approximately one feddan. A similar area was put under miscellaneous crops.

23.3.2 Miscellaneous Crops

The miscellaneous crops were cotton, sorghum and cowpea. The crops were executed for other experimental purposes, without insecticidal spraying, and the traps were deployed in the upwind direction of these crops.

23.4 Activity Time for Males of Tomato Leaf Miner

Water bowl traps, baited with pheromone, were deployed weekly on tomato fields, at 6:00 pm, and the captured males were collected every 3 h until 9:00 am on the next day. At the peak of male capturing, the collection period was narrowed to every 15 min, and time of sunrise was considered. The traps were put at the ground level. The data of the captured males were recorded during the period from March 19, 2016 to May 27, 2016.

23.5 Height of Flying of Males of Tomato Leaf Miner

The insects height of flying was monitored by installing two vertical galvanised metal tubes (2.0 inches in diameter and 2.0 metres in length). Each metal tube supported 4 pheromone–baited water bowl traps at ground level, 50, 100 and 150 cm above ground level. Metal rings were welded on to the tubes to support the bowls. The traps were deployed weekly, before sunset, and the captured males were recorded at 9:00 am on the next day. The traps were deployed upwind, one for tomato and the other for the miscellaneous crops. Wind velocity was recorded at 8:00 am. The data of the captured males were recorded during the period from December 3, 2016 to May 28, 2017.

23.6 Results

23.6.1 Time of Male Activity

At the beginning of the research in March 2016, the number of tomato leaf miners was negligible, especially in the first 2 weeks, in all periods where traps were deployed. In the second week, pests began to appear and the trapped males numbered 8 per trap at 6:40 am to 7:40 am. Thereafter, the numbers increased in the third week, reaching 54 males per trap (17 from 6:40 am to 6:50 am, and 37 from 6:50 am to 9:00 am).

In April, the pests started to appear in a high numbers from the first week (n = 58) and reached the highest number in the fourth week (n = 80,). Thus, numbers reached a peak in April, with an average total number of 282 males per trap. The numbers of tomato leaf miner started to decline in May, reaching a total of 94. After that, the pests began to reduce in numbers in the last weeks of May, June and July. The numbers of captured males were summarised on a monthly basis for March, April and May, as seen in Figs. 23.1, 23.2 and 23.3 and in Table 23.1.

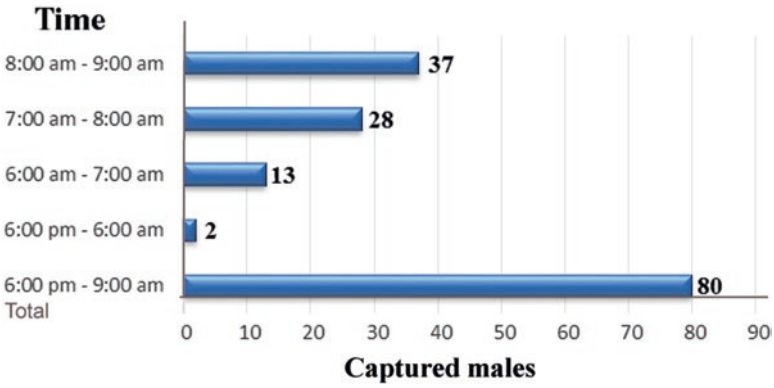

Fig. 23.1 Pheromone captured males of *Tuta absoluta* versus time (March 2016, sunrise: 6.46–6.51 am)

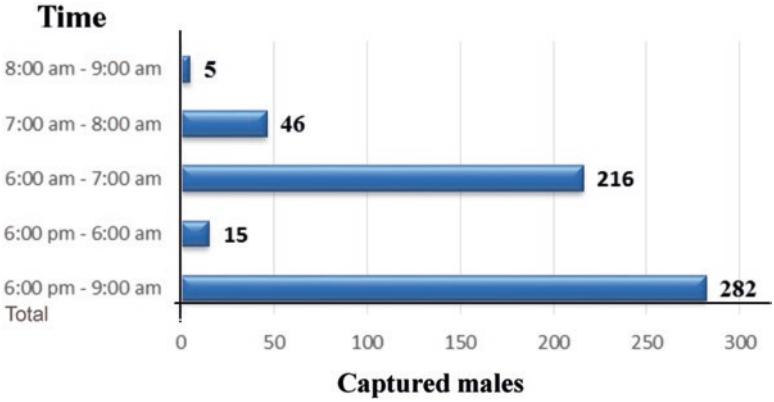

Fig. 23.2 Pheromone captured males of *T. absoluta* versus time (April 2016, sunrise: 6.24–6.32 am)

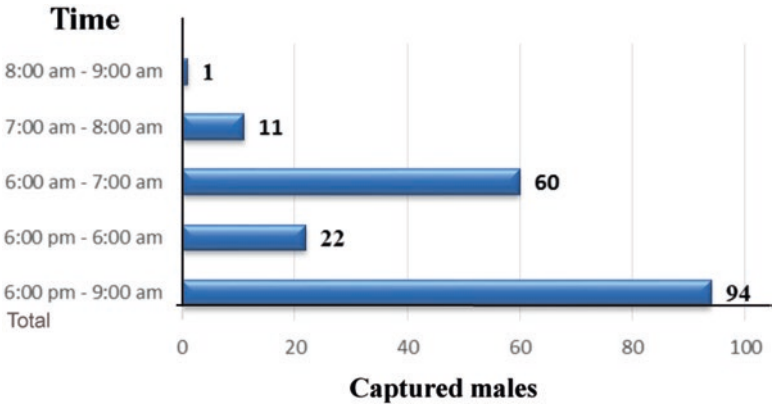

Fig. 23.3 Pheromone captured males of *T. absoluta* versus time (May 2016, sunrise: 6.16–6.21 am)

Table 23.1 Males of *Tuta absoluta* captured 30 min

Date (2016)		Sunrise	Total	Before	After		
				No.	%	No.	%
March	19	6:51	54	17	31	37	69
	26	6:46	26	13	54	11	46
April	16	6:32	60	39	70	17	30
	23	6:28	23	10	83	2	17
	30	6:24	80	39	84	7	16
May	7	6:21	26	9	41	13	59
	14	6:19	26	12	63	7	37
	21	6:17	21	14	82	3	18
	28	6:16	21	9	56	7	44
Mean			337	62	38		

Before and after sunrise (Gezira State, Sudan)

In March 2016, the total number of trapped males was 80 (Fig. 23.1). The lowest number obtained was 2 from 6:00 pm to 6:00 am, and the highest was 37 from 8:00 am to 9:00 am. Accordingly, the increasing numbers coincided with sunrise.

The numbers of Tomato leaf miner reached a peak in April (total number = 282), from 6:00 pm to 9:00 am. The insect appeared in small numbers (n = 5) from 6:00 pm to 6:00 am, with the largest numbers of males being trapped from 6:00 am to 7:00 am (n = 216). From 7:00 am to 8:00 am, the total started to decline in number (n = 46), until it reached 5 males per trap at 8:00 am to 9:00 am.

The total number of trapped males in May was 94. The lowest number obtained was 1 male per trap from 8:00 am to 9:00 am, and the highest was 60 males per trap from 6:00 am to 7:00 am.

Table 23.1 shows a clear variance in the times for sunrise during the period from March to May. when the sunrise occurred 35 min earlier than in March (6:16 vs. 6:51). Regardless the particular time of sunrise, 80% of the male activity took place within 1 h of sunrise. Furthermore, 60% of this 1-h activity (before and after sunrise) took place 30 min before sunrise, with 40% occurring within 30 min after sunrise.

23.6.2 Flight Behaviour

In December 2016 (n = 13) and January 2017 (n = 48), tomato leaf miner males appeared in very small numbers in the traps at the four levels (0, 50, 100and 150 cm), with the number of trapped males being concentrated at the ground level (0 cm). In February, the number began to increase (n = 294), and the largest number of males were captured at the ground level in the traps deployed on the tomato crop. The increase in number was very clear in March (n = 532) and April (n = 371), and then it started to decline in May (n = 196). It was observed that males have a tendency to

Table 23.2 Captured males of *T. absoluta* per month at different heights (year 2017)

	Miscellaneous crops						
Trap height	Jan.	Feb.	March	April	May	Total	%
0	5	126	102	89	83	405	88
50	2	1	14	5	6	53	12
100	1	2	5	3	2		
150	3	1	6	2	0		
Tomato							
0	29	155	365	248	96	893	91
50	3	4	24	19	6	90	9
100	3	4	11	3	2		
150	2	1	5	2	1		

Table 23.3 Response of *T. absoluta* captured males to wind velocity

	Wind velocity (m/sec.) vs. captured males			
Pheromone height (cm)	1–2	%	6–7	%
0	601	89	368	93
50	41	11	18	7
100	15		9	
150	15		2	
Total	672	100	397	100

fly at higher levels when the wind velocity was low (2 m/sec. or less) in the morning, while they were present in large numbers at ground level when the wind velocity was higher. Furthermore, the total numbers of males captured in the traps deployed in the tomato crop were by far greater than those deployed in the miscellaneous crops. The heights of flying for the captured males are summarised in Tables 23.2 and 23.3.

Table 23.2 shows that 90% of the male activity was experienced at ground level, whether the baited pheromones traps were deployed upwind of the tomato or the miscellaneous crops. However, the tomato crop attracted 68% of males, while the miscellaneous crops attracted 32% of the population.

It was noticed that the males had a tendency to fly at higher levels when the wind velocity was low (Table 23.3). Nevertheless, this tendency was limited and it was noted for around 10% of the population.

23.7 Discussion

Some insects may be controlled by a combination of practices that are not fully effective when used individually, as in the case of the tomato leaf miner which requires the use of more than one practice at a time, to achieve a better control (Daniel and Bajarang 2017). However, understanding the insect behaviour will aid

in the formation and application of integrated control strategies to apply (Cuthbertson et al. 2013). Pheromone-baited water traps provide an environment-friendly control measure. The mass trapping of tomato leaf miner proved to be economically viable (Lobos et al. 2013). The use of the pheromone somehow addresses the issue of the lack of natural enemies, which in this case are yet to be identified and introduced (Ferracini et al. 2012).

For this study, the pheromone-baited water trap was contained in shiny red-colour plastic bowls that proved to be more attractive to males of *T. absoluta* (Zekeya et al. 2017b). The traps were deployed upwind of the tomato and other miscellaneous crops, that presented an attractive site (Lobos et al. 2013). The results also revealed that the University Experimental Farm had been harbouring the tomato leaf miner, because the insect has no records of flying for long distances.

Most studies have described Tomato leaf miner as being nocturnal (Daniel and Bajarang 2017). However, some authors stated that the insect is active in the early morning and at sunset. This study revealed that 80% of the activity the tomato leaf miner is framed between 30 min before sunrise and 30 min after sunrise (Figs. 23.1, 23.2 and 23.3 and Table 23.1). No detection of activity was recorded at sunset. Moreover, 60% of the activity took place 30 min before sunrise (Table 23.1).

The question of the levels for the deployment of traps has been controversial, for instance for the fall armyworm and fruit flies the pheromone trap has to be hung at approximately 150 cm above the ground (Abdalla 2007; Day et al. 2017). However, for tomato leaf miner, height levels between 100 and 20 cm have been recommended by Russell IPM and other sources (Mohammed et al. 2015).

In this study, the results showed that 90% of the captured males were trapped at ground level in the tomato and other miscellaneous crops (Table 23.1). The main host plant for tomato leaf miner is the tomato plant, although the insect can be attracted by other solanaceous crops (Mohamed et al. 2015). However, solanaceous volatiles attracted both males and females of *T. absoluta* (Bawin et al. 2014; Caparros Megido et al. 2014). A similar result was achieved by this study, where it was found that 70% of males were attracted to traps deployed upwind of the tomato crop (Table 23.2), while 30% were attracted to traps deployed upwind of the miscellaneous crops.

In conclusion, the study showed that *T. absoluta* is not rigorously nocturnal, and it is rather active at photo phase. The height the males fly at was determined to be ground level, and wind velocity reflected weak effects upon the flying height of males. Some 60% of male activity was reported within the 30 min before sunrise. Pheromone-baited water traps should be deployed at ground level for monitoring, detection, mass trapping and mating disruption of *T. absoluta*. Since *T. absoluta* can infest tomato plants at growth and developmental stages, great attention should be given to tomato crops that coincide with the peak of infestation between March and April. The intercropping of a tomato crop with other crops might distract the attraction by the *T. absoluta*.

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References

Abdalla, A. M. (2007). *Monitoring, host range and economic impact of same tephritid fruit fly on mango in the river Nile state*. M.Sc. Thesis, Sudan Academy of Sciences, 64 pp.

Abdul-Rassoul, M. S. (2014). A new host record for Tomato Leaf Miner *Tuta absoluta* (Meyrick, 1917) in Baghdad Province, Iraq. *Bulletin of the Iraq Natural History Museum, 13*(1), 15–18.

Arnó J., & Gabarra, R. (2010). Controlling Tuta absoluta, a new invasive pest in Europe, Training in Integrated Pest Management – No.5 IRTA Cabrils, Spain.

Arnó, J., & Gabarra, R. (2011). Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). *Journal of Pest Science, 84*, 513–520.

Arnó, J., Sorribas, R., Prat, M., Matas, M., Pozo, C., Rodriguez, D., et al. (2009). *Tuta absoluta*, a new pest in IPM tomatoes in the northeast of Spain. *IOBC/WPRS Bullettin, 49*, 203–208.

Bawin, T., De Backer, L., Dujeu, D., Legrand, P., Caparro Megido, R., Francis, F., & Verheggen, F. J. (2014). Infestation level influences oviposition site selection in the tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae). *Insects, 5*, 877–884.

Biondi, A., Zappalà, L., Stark, J. D., & Desneux, N. (2013). Do biopesticides affect the demographic traits of a parasitoid wasp and its bio control services through sub lethal effects? *PLoS One, 8*, e76548.

Caparros Megido, R., Lara, D. B., Ettaib, R., Brostaux, Y., Fauconnier, M. L., Lognay, G., et al. (2014). Role of larval host plant experience and solanaceous plant volatile emission in *Tuta absoluta* (Lepidoptera: Gelechiidae) host finding behavior. *Arthropod Plant Interactions, 8*, 293–304.

Chailleux A., Biondi A., Han P., Tabone E., & Desneux N. (2013). Suitability of the Pest-Plant system *Tuta absoluta* (Lepidoptera: Gelechiidae) – tomato for Trichogramma (Hymenoptera: Trichogrammatidae) parasitoids and insights for biological control. *Journal of Economic Entomology, 106*(6), 2310–2321.

Chapman. (1980). *The insects – Structure and function*. Kent: Hodder and Stoughton Ltd..

Consoli, F. L., Parra, J. R. P., & Dr Hassan, S. A. (1998). Side-effects of insecticides used in tomato fields on the egg parasitoid Trichogramma pretiosum Riley (Hym. Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep. Gelechiidae). *Journal of Applied Entomology., 122*(1–5), 43–47.

Contreras, J., Mendoza, J. E., Martinez-Aguirre, M. R., García-Vidal, L., Izquierdo, J., & Bielza, P. (2014). Efficacy of enthomopathogenic fungus *Metarhizium anisopliae* against *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Economic Entomology, 107*, 121–124.

Cuthbertson, A. G. S., Mathers, J. J., Blackburn, L. F., Korycinska, A., Luo, W., Jacobson, R. J., & Northing, P. (2013). Population development of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under simulated UK glasshouse conditions. *Insects, 4*, 185–197.

Daniel, T. I., & Bajarang, B. S. (2017). Control and management of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). A review. *Journal of Applied Chemistry, 10*, 14–22.

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., et al. (2017). Fall armyworm: Impact and implication for Africa. *Outlooks Pest Management, 28*, 196–201.

Dias, D. M., Resende, J. T. V., Faria, M. V., Camargo, L. K. P., Chagas, R. R., & Lima, I. P. (2013). Selection of processing tomato genotypes with high acyl sugar content that are resistant to the tomato pinworm. *Genetics and Molecular Research, 12*, 381–389.

Ferracini, C., Ingegno, B. L., Navone, P., Ferrari, E., Mosti, M., Tavella, L., & Alma, A. (2012). Adaptation of indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. *Journal of Economic Entomology, 105*, 1311–1319.

Jamoussi, K., Sellami, S., Nasfi, Z., Krichen-Makni, S., & Tounsi, S. (2013). Efficiency and midgut histopathological effect of the newly isolated *Bacillus thuringiensis* KS δ-endotoxins on the emergent pest *Tuta absoluta*. *Journal of Microbiology and Biotechnology, 23*(8), 1099–1106.

Jaworski, C. C., Bompard, A., Genies, L., Amiens-Desneux, E., & Desneux, N. (2013). Preference and prey switching in a generalist predator attacking local and invasive alien pests. *PloS One, 8*, e82231.

Lobos, E., Occhionero, M., Werenitzky, D., Fernandez, J., Gonzalez, L. M., Rodrigues, C., et al. (2013). Optimization of a trap for *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) and trials to determine the effectiveness of mass trapping. *Journal of Neotropical Entomology, 42*, 448–457.

Mohamed, B. M. (2008). Guidance to agricultural engineers for vegetables production. In *Sudanese General Agricultural Engineers Union*. National Bookshop, Khartoum.

Mohamed, E. S. I., Mahmoud, M. E. E., Elhaj, M. A. M., Mohamed, S. A., & Ekesi, S. (2015). Host plant record for tomato leaf miner *Tuta absoluta* (Meyrick) in Sudan. *Bulletin OEPP/EPPO, 45*, 108–111.

Mouttet, R., Kaplan, I., Bearez, P., Amiens-Desneux, E., & Desneux, D. (2013). Spatiotemporal patterns of induced resistance and susceptibility linking diverse plant parasites. Plant-microbe-animal interactions. *Oecologia, 173*, 1379–1386.

Nicolas, D., Eric, W., Kris, A. G. W., Giovanni, B., Salvatore, A., Consuelo, A. N.-V., Joel, G.-C., Diana, C. R., Elisabeth, T., Jacques, F., Jeannine, P., Christine, P., Tomás, C., & Alberto, U. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. *Journal of Pest Science, 83*(3), 197–215.

Roditakis, E., Skarmoutsou, C., & Staurakaki, M. (2013). Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. *Pest Management Science, 69*, 834–840.

Savino, V., Coviella, C. E., & Luna, M. G. (2012). Reproductive biology and functional response of *Dineulophus phtorimaeae*, a natural enemy of the tomato moth, *Tuta absoluta*. *Journal of Insect Science, 12*, 1–14. https://doi.org/10.1673/031.012.15301.

Urbaneja, A., Montón, H., & Mollá, O. (2009). Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus caliginosus* and *Nesidiocoris tenuis*. *Journal of Applied Entomology, 133*, 292–296.

Zekeya, N., Chacha, M., Ndakidemi, P. A., Materu, C., Chidege, M., & Mbega, E. R. (2017a). Tomato leaf miner (*Tuta absoluta* Meyrick 1917) threat to tomato production in Africa. *Journal of Agriculture and Ecology Research International*, 101–110.

Zekeya, N., Ndakidemi, P. A., Chacha, M., & Mbega, E. R. (2017b). Tomato leaf miner, *Tuta absoluta* (Meyrick 1917), an emerging agricultural pest in sub-Saharan Africa: Current and prospective management strategies. *African Journal of Agricultural Research, 12*, 389–396.