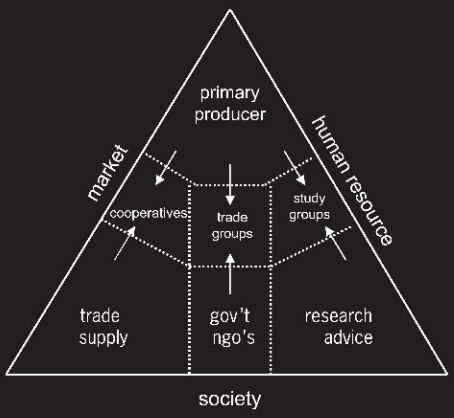
Progress in Biological Control

An Ecological and Societal Approach to Biological Control



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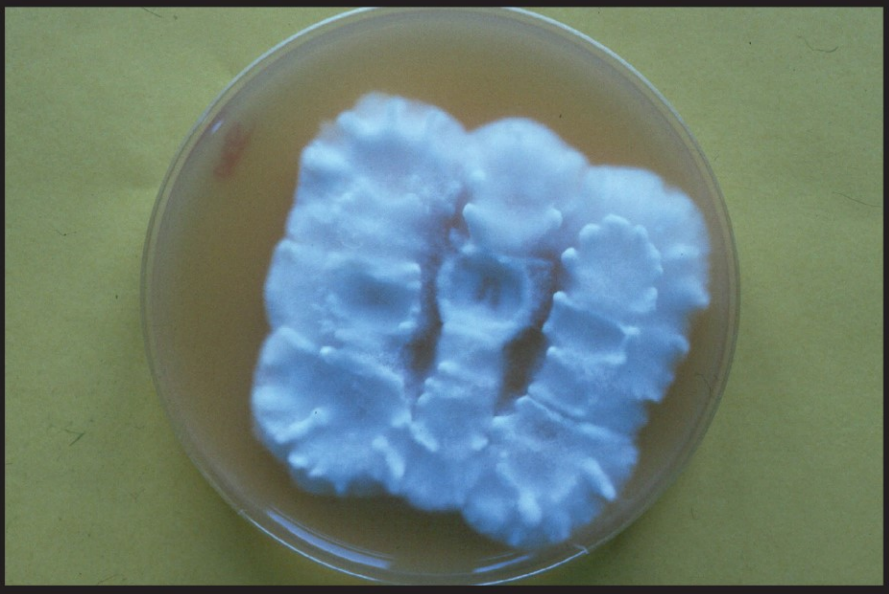

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AN ECOLOGICAL AND SOCIETAL APPROACH
TO BIOLOGICAL CONTROL

An Ecological and Societal Approach to Biological Control

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Insect Pathogenic fungi are among the organisms, which are used for biological control. An example is the fungus *Beauveria bassiana* and the photo shows an isolate of this fungus on artificial growth medium.
Photo Credit: Department of Ecology, The Royal Veterinary and Agricultural University, Denmark

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PREFACE

Biological control is among the most promising methods for control of pests, diseases and weeds. It has shown its potential in many agricultural, horticultural and forestry systems and also in situations where the targets are vectors of human diseases or nuisance pests. Yet biological control has not reached its full potential. Several recent textbooks have addressed issues of relevance for the success of biological control: selection of candidate organisms, application methods, formulation of products, and non-target effects. Our approach in this book is to evaluate biological control from an ecological and societal perspective. In an ecological approach the aim is to evaluate the significance of certain biological properties like biodiversity and also to look on habitats as natural reservoirs. Further, it is important to see biological control from an organic (or ecological) farming point of view. The reason for the societal approach is also obvious: terms like 'consumer's attitude', 'risk perception', 'learning and education' and 'value triangle' are recognised as very significant for biological production and human welfare and biological control should be subjected to studies from these perspectives.

We have carefully selected authors to cover the above mentioned themes. We chose to focus on European conditions, so the specific cases as well as the author's affiliations particularly reflect aspects of biological control in this region. This is not to ignore the interesting cases and experiences from other parts of the world. We feel, however, that there are so many valid stories of global significance from Europe that they deserve to be highlighted.

Chapter 1 outlines the general concepts for biological control. The four complementary strategies are described and further, this chapter was used by all authors as a reference to ensure a uniform use of terms throughout the book. Chapter 2 reviews the socioeconomic benefits of biological control and examples of societal benefits are given.

In modern agriculture, organic farming is a very successful environmentally friendly production method. Is biological control always an integral element in organic farming, or is it only recommended in certain cases? This very interesting question is discussed in chapter 3. The consumer is regarded as a driving force in technological development and chapter 4 will, for the first time, provide an insight into how consumers perceive biological control. Then, chapter 5 analyses educational aspects at a university level and experiences from Denmark are presented. The competences of students who have participated in biological control courses are described in a broad context.

Turning to the agricultural production and the farmer's attitude, chapter 6 outlines the experience from the Netherlands, where there has been a long history of implementing biological control within integrated pest management in glasshouses. Chapter 7 keeps the focus on glasshouses, although the ecological potential and limitations are reviewed here. The next two chapters, 8 and 9, evaluate the soil as a reservoir for naturally occurring beneficial

organisms. The soil is a fantastic reservoir for both antagonists to plant diseases and for natural enemies of insects. Despite the natural potential of, for example, entomogenous fungi, there are certain biological limitations, for example attenuation, which is illustrated in chapter 10.

Three chapters are novel case studies, illustrating rather different challenges and approaches. Mosquitoes, which are nuisance pests in the Rhine Valley, are successfully controlled using *Bacillus thuringiensis.* The case, which is reviewed in chapter 11, obviously included many societal questions to solve: how to organise the application at the regional level and how to get the control financed. The theme of chapter 12 is some high value crops, Christmas trees and greenery plantations, which have recently been subjected to biological control. The high product price for producers and the high public attention to these crops support a future biological control. The aims of a recently initiated EU co-operation project on biological control of plant diseases and weeds are described in chapter 13. Such co-operative projects are complex and the partner's need, besides addressing the biological challenges, to consider carefully the management and dissemination of results.

Finally, we included two chapters paying attention to problems of increasing significance for the public acceptance of biological control. Chapter 14 explores whether handling and using biological control agents pose a risk because of the exposure of humans. Further the chapter reviews certain aspects of the EU registration procedure for biological control agents. A totally new challenge is presented in chapter 15. Since spring 2005, a national research programme in England, including scientists and the public, aims to obtain data to document whether a ladybird beetle used for biological control in continental Europe has become an invasive species in England.

Our hope is that the book will stimulate people from many branches to develop biological control further. Beyond that, we hope that our book will contribute to an understanding that future biological control is heavily dependent on both ecological and societal elements. It is our hope that the thoughts and theories presented in this book will stimulate further multidisciplinary work addressing the concepts in greater detail than is provided here.

Per Jørgensen, secretary at KVL, is warmly thanked for extensive, skilled technical assistance.

Jørgen Eilenberg and Heikki Hokkanen, Copenhagen and Helsinki, September 2005

CHAPTER 1

CONCEPTS AND VISIONS OF BIOLOGICAL CONTROL

Jørgen Eilenberg

1. The vision

Biological control is one of several strategies used to control pests to avoid economic damage on crop plants, in husbandry, or on recreation areas. It is also used against nuisance pests. In this chapter, I use the terms 'pest' and 'pests' for insect, mites and vertebrate pests, plant diseases, and weeds.

Biological control (or biocontrol, which is synonymous) has been defined a number of times. A recent definition by Eilenberg *et al.* (2001) is:

> '*The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be*'

It should be stressed that the definition clearly states that 'living organisms' are used. This definition includes predators, parasitoids, nematodes, fungi, bacteria, protozoa, and viruses, while genes or gene fragments without a living organism are excluded. Metabolites from various organisms used for pest control, but applied without the organisms producing them, are not included in biological control, but should merely be grouped as 'biorational control'.

It should also be stressed that in the definition above, biological control is not strictly linked to the term 'natural enemy', which was the case in many earlier definitions of biological control (DeBach 1964). Irrespective of this, biological control is normally understood as a natural way to achieve control and people will reflect positive to the word (Jetter and Payne, 2004).

Much research towards biological control has never led to practical usage due to obstacles which have not yet been overcome, for example mass production of a potential biocontrol organism. It should, however, also be mentioned that much natural regulation of pests is working in each crop everywhere in the world at all times. The natural regulation of pests is namely one main reason why, for example, most insect species feeding on crop plants are not pests; their populations are kept from increasing by predators, parasitoids, and insect pathogens.

Biological control can be seen as a vision of an almost perfect ecological balance, based on observations, which lead to a management of the interactions between the pest and its natural enemies. DeBach (1964) gave this introduction to the vision: '*we would point out that people fortunate enough to have witnessed a striking example of biological control taking place usually become* '*true believers*'*, but some of those, who happen later to see only the final result can be unimpressed if not downright sceptical*'. This vision can be seen as positive in the sense that most scientists and extension officers dealing with biocontrol are enthusiastic because they

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really believe that biocontrol is powerful. The vision can, however, also be viewed in a more sceptical light; Is it really necessary to observe the striking action of natural enemies personally? Isn't it sufficient just to evaluate the final result? Is there a risk that biocontrol will stay forever as a branch of 'true believers'?

Biological control is in any case defined and understood from a utilitarian perspective; the end goal is to use biology to serve man. The vision is therefore to use biology in an environmentally friendly way to ensure healthy crops or other products in agriculture, horticulture, forestry or husbandry or to minimize nuisance pests.

2. Classical biological control

Classical biological control is defined by Eilenberg *et al*. (2001) as:

> '*The intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control*'

The main principle of classical biological is shown in figure 1. When an organism is introduced either intentionally or accidentally into an area in which it did not occur previously, it can sometimes increase to a high population density and become a serious pest. This population increase is mostly due to the fact that the pest was introduced without its natural enemies (predators, parasitoids or microorganisms). In classical biological control, one or more of these enemies are collected in the area of origin of the pest species and released as biocontrol agents in the pests non-native habitat (time T on figure 1). The goal is for the natural enemy to establish and spread with the result that the pest population decreases in population density, hopefully below the economic injury level of the pest. The time scale on figure 1 can be years.

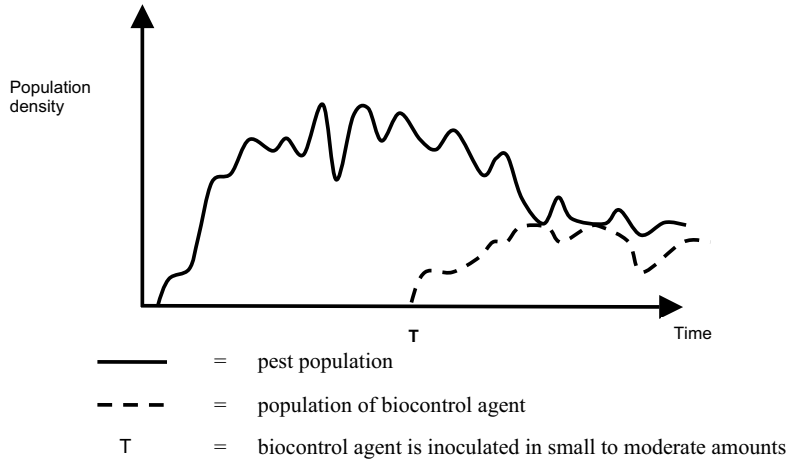

Figure 1: Classical biological control

Classical biological control has been a very significant strategy within biological control since the striking success with the introduction of the 'Vedalia beetle' to control scale insects in California in the late 1880'ies (see van Driesche and Bellows, 1996). The early successes were the reason for the term 'classical', which cannot be understood without this historical dimension. For most people the word will immediately be associated with positive attributes like 'naturalness' 'ecological balance' etc. It was (and still is) among the most successful methods to manage introduced pest species in North America and other parts of the world, while it has never been a significant element in biological control in Europe. This is due to two reasons: first, the major bulk of European pests are native and their natural enemies are already present, and secondly, classical biological control needs a strong, regional co-ordination of the efforts, which has normally not been the case in Europe.

Classical biological control is often, seen as an ideal ecological (re)establishment of a balance, which man temporarily had disturbed. In a table about disadvantages of classical biological control and chemical control, DeBach (1974) stated that there were no disadvantages of classical biological control related to environmental effects, for example danger to non-target organisms. The vision of classical biological control especially as an ideal ecological tool was highlighted recently by Waage (2001), who wrote '*the capacity of introduced natural enemies to persist in the environment, to reproduce there and to spread gives biological control its unique advantage as a pest control method'.*

We should, however, be aware of linking any method (biological or non-biological) to a suggested human perception of 'naturalness'. In principle (and also seen in practice), classical biological control may also have drawbacks. Nowadays, the authorities in EU and elsewhere evaluate classical biological control as a possibility among other methods of pest management, and finally approving or rejecting the suggested introduction of exotic agents.

3. Inoculation biological control

Inoculation biological control is defined by Eilenberg *et al*. (2001) as:

> '*The intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently*'

The main principle of inoculation biological control is shown on figure 2. A pest population increases in size but in due course, before this population density has reached the potential maximum, a biocontrol agent is inoculated in small to moderate amounts (Time T on figure 2). The goal is for the natural enemy to increase in population size and control the pest over a period of time. The inoculated biocontrol organisms will, however, not establish permanently at a sufficient high population density. The pest will therefore increase in population size after a period of time and a new inoculation would then be needed. The events in inoculation biological control are often limited to one cropping season, so the time scale on figure 2 is weeks or months.

Inoculation biological control has much in common with other inoculation practices, as seen from figure 2. The major factor is that the biocontrol organism is expected to proliferate, at least temporarily. Conceptually it is therefore comparable to classical biological control but with the main differences that 1) inoculation biological control uses mostly organisms which

already occur in the area of application and 2) only temporary establishment is achieved. Typical examples are the releases of *Encarsia formosa* and other parasitoids in glasshouses (van Lenteren, 2000) and the inoculation of soil with the insect pathogenic fungus *Beauveria brongniartii* (Enkerli *et al*., 2004).

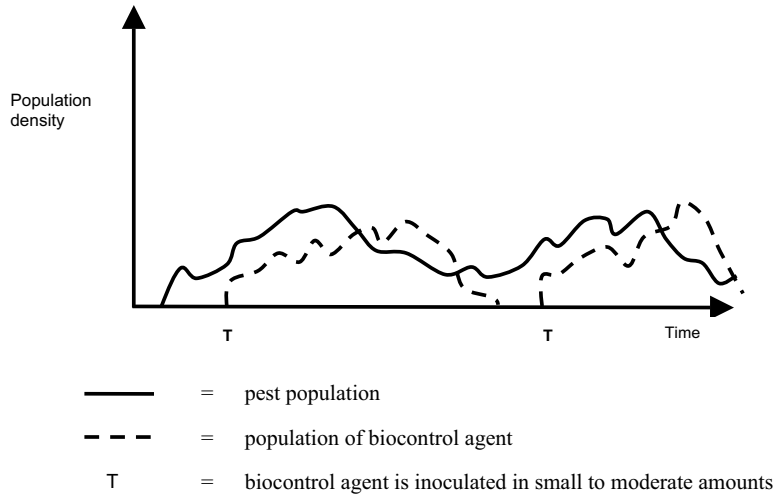

Figure 2: Inoculation biological control

It can also be postulated that inoculation biological control represents a reestablishment of a natural balance, temporarily distorted by man. Soil for cropping is for example inoculated with other additives to enhance growth (mycorrhiza for example) and inoculation with a biocontrol agent can be seen as a moderate help to speed up a natural process. We should of course not take for granted that inoculation *per se* always mimics a natural process. The level of naturalness must be proven in each case. Inoculation biocontrol has always, however, the advantage of being closely linked to monitoring pest populations and thus understanding population interactions. In glasshouses, a successful inoculation of biocontrol agents requires proper diagnosis of the the pests present and in due course, release of the correct agents at the optimum density and time. Recent books to educate glasshouse growers in Europe to diagnose pests and biocontrol agents can be of high quality with condensed biological information (Malais and Ravensberg, 2003). The education aspect is an integral element in inoculation biocontrol: without sufficient education of end users, there will be no success.

4. Inundation biological control

Inundation biological control is defined by Eilenberg *et al*. (2001) as:

'*The use of living organisms to control the pests when control is achieved exclusively by the released organisms themselves*'

The main principle of inundation biological control is shown on figure 3. A pest population increases in size, but at a certain time (Time T on figure 3, for example when the economic injury level has been passed) a biocontrol organism is applied in large amounts ('inundated'). The pest is quickly controlled and the population density of both the pest and the biocontrol agent decrease over time. The pest population will, after a period of time, increase again and a new application of the biocontrol agent is needed. The events in inundation biological control are often limited to one cropping season, so the time scale on figure 3 is weeks or months. A typical example of inundation biological control is the widespread use of *Bacillus thuringiensis* to control lepidopteran and dipteran insects.

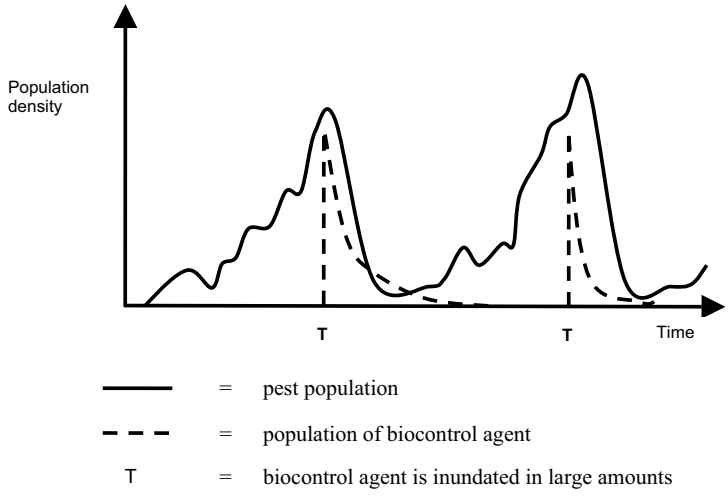

Figure 3: Inundation biological control

The term 'biopesticide' is often associated with inundation biological control, linking the concept rather closely to the use of chemical pesticides. The association is in parts correct; for both inundation biological control and application of pesticides, the two main points are 1) application is done when the pest population is, or is expected soon to be, above economic injury level, 2) a high dosage of the biocontrol agent or the chemical compound is used, and 3) the biocontrol agent or the chemical are expected to disappear over time. Still, however, inundation biological control is based on a living entity and not a chemical compound and further, the term 'biopesticide' is not limited to biocontrol agents, but also refers to the use of natural chemical compounds (Copping, 1998). In general, I suggest avoiding the use of the term 'biopesticides' for biological control agents.

Inundation and inoculation biological control are often termed together as 'augmentation' (Hajek, 2004), and there are several good reasons for this. First, in both cases, biocontrol organisms (often commercially available) are released at more or less regular intervals, with the aim to augment the population of the biocontrol agent. Secondly, it can be difficult to know exactly whether the effect on the target was due to the released organisms themselves or their progeny.

We should, however, as much as possible, distinguish 'augmentation' biological control as either inundation or inoculation due to the obvious differences in expectations and thus medium to long-term effects on targets and non-targets.

Inundation biological control with its strong resemblance to chemical control can be perceived as 'less natural' than the other biological control strategies, especially when using a microorganism for biocontrol. The amount of the control agent to be applied is often several magnitudes higher than would ever occur under so-called natural conditions. The presentation of the inundation biological control agent gives association to chemical pesticides; for microorganisms to be used in biocontrol, the product label has the appearance of a chemical product with information about the concentration and application rate expressed per square unit.

Nevertheless, we should look upon inundation as one strategy, which may provide excellent results in many cases, in full accordance with ecological acceptability. Further, the concept is very easy for everyone to understand as biocontrol. Finally, the evaluation of non-target effects by the authorities can be simplified by the fact that the biocontrol agent is expected to return to background levels over time.

5. Conservation biological control

Conservation biological control is defined by Eilenberg *et al*. (2001) as:

> '*Modification of the environment or existing practices*
> *to protect and enhance specific enemies or other organisms*
> *to reduce the effect of pests*'

The main principle of conservation biological control is shown on figure 4. A pest occurs at high population levels due to insufficient effects of the natural enemies. Natural enemies include all kinds of biological regulation: macro- and microorganisms controlling invertebrates, weeds and plant diseases, including the antagonistic microorganisms responsible for 'suppressive soils'. At time T on figure 4, the environment is modified or the practice is changed in order to enhance the natural enemies, which are already present. They increase in population size and their effect results in a lower pest population. The time scale on figure 4 can be years.

Conservation biological control is thus completely different from the three other biological control strategies, since no organisms are released. Only organisms, which are already present are enhanced in order to avoid damage. It is important to keep in mind that the definition allows both passive and active conservation. An example of passive conservation is the avoidance of actions which disfavour the natural enemies, for example spraying with certain chemical pesticides. An active conservation could be the initiation of actions to support the natural enemies actively by establishing for example 'beetle banks' (Landis *et al*., 2000). In

Barbosa (1998) and Pickett and Bugg (1998), many other examples of habitat manipulation at different levels are found, from landscape to crop plants. Among the four biological control strategies, conservation biological control can be seen as the most tightly connected to the main principles of organic farming, which have the protection of the existing natural enemies as one of the main principles (Anonymous, 2002).

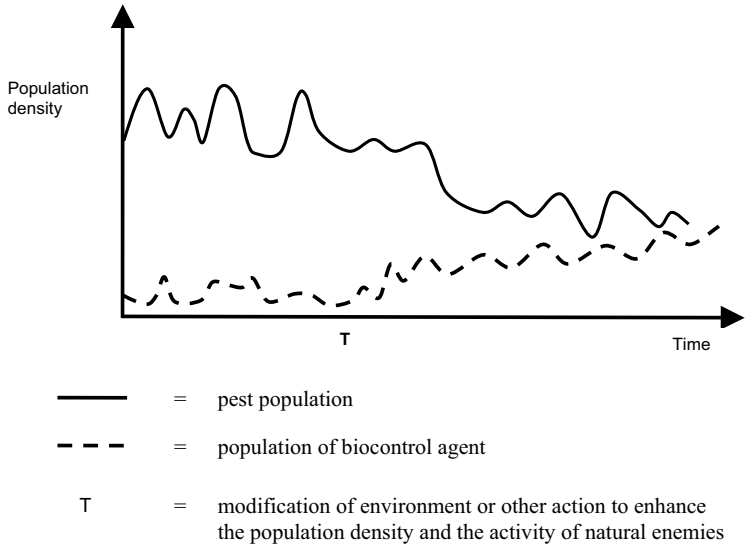

Figure 4: Conservation biological control

There is a tight connection also to 'conservation biology' (Letourneau, 1998), since conservation biological control to large extent builds on ecological theory about metapopulations, spatial fragmentation, and fate of species in a habitat. Conservation biological control can thus be seen as an example of habitat restoration with the specific purpose of supporting natural enemies to control pests.

6. The interface between biological control and society

Much work to initiate biocontrol in the target – biocontrol agent system under consideration starts with autecological studies of the biocontrol organism and with studies of the interaction between target and biocontrol agent. Thus, the initial studies are limited to a two-organism system. After successful experimental work at the laboratory scale, semi-field and finally field scale experiments are added. If successful, development towards a commercial product may be

initiated. More studies are added, including studies on the formulation of product, non-target effects and finally, economic feasibility.

The above listed progression from the initial discovery of a potential agent to the final, successful biocontrol agent, almost never takes place in its 'pure' form. Normally, some economic considerations are included from the very beginning, to evaluate if the idea of biocontrol is at all realistic. Biocontrol products for a small niche market are difficult to develop, because such biocontrol agents will not be attractive to a producer due to the limited economic potential. Nowadays, studies to determine potential effects of a biocontrol agent on non-target organisms in the environment as well as on human health are often initiated at an early stage in the process towards practical use. Overall, the ecological and societal components must be strong from the beginning and never be forgotten throughout the process towards implementation of the biological control strategy.

Perkins and Garcia (1999) addressed the interface between biological control and society. They stated, correctly, that most scientific work and products are subjected to political and economic considerations, which have little to do with the scientific subject matter. The political/societal involvements are increasingly present for all kinds of biocontrol. Obviously, inundation and inoculation need the involvement of national of regional authorities to evaluate health and environmental effects. A release of any exotic organism, in principle regarded as an alien, can be regarded as controversial.

Figure 5 illustrates the different components. Central are studies of the organisms (targets and agents), but as part of integrated plant protection, interactions between other pests, crop and control methods must also be included. The eco-system for application must be considered and encompass the crop, the pests and the agents. Encompassing everything is, however, the society, since the final decisions about the usefulness (or lack thereof) and thus the success of a biocontrol agent will depend on societal factors.

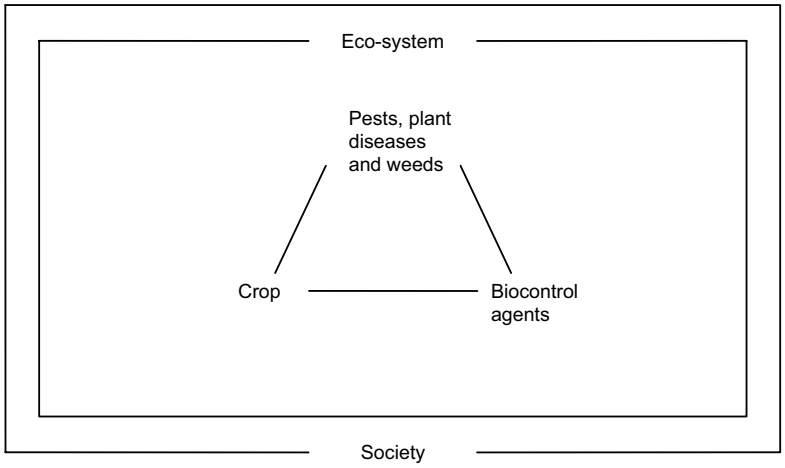

Figure 5: The relationship between biological control and targets, crop, eco-system, and society

The approaches to biological control have been divided into four eras (Gurr *et al*., 2000): 1) Pre-scientific era (pre-1880), 2) Classical era (1880-DDT (1939)), 3) Chemical era (DDT – 'Silent Spring' by Carson (1962), 4) Integrated era ('Silent Spring'– present). The last era, the integrated era was defined by the evolution of a much wider use of biocontrol (targets and crops) and strategies other than classical biocontrol. In their description of the integrated era, the authors did, however, pay most attention to the biological/ecological elements. Several societal (but not necessarily governmental) matters need to be incorporated in the future concept for biological control at the European level, for example company structure, market structure, consumer's attitude and political movements. Organic farming can be seen as a political movement, which will strongly influence the future of biological control in Europe.

The company structure most successful for biocontrol in Europe and elsewhere seems to be small to medium sized enterprises (SME), often operating at the national or regional level. Throughout Europe (and elsewhere), a number of such companies form the backbone of biocontrol producers and distributors. We should take into account how the development of new biocontrol agents can become attractive to such SME's.

The attitude of consumers to biocontrol has only recently been subjected to detailed scientific studies (Jetter and Payne, 2004). Consumers in the focus area in California were asked about their ratings of control of a snout beetle damaging Eucalyptus trees in the urban landscape. The focus group was generally positive to biocontrol, but people rated different biocontrol agents differently. Most consumers in the study preferred parasitoids to *Bacillus thuringiensis* (in the study this option was termed 'biorational' control) for insect control, while both types of organisms were rated over chemical control. The bulk of the interviewed consumers would accept to pay more (in taxes) to support biological control in their environment.

We should take into account how consumer's attitude will influence the willingness to choose vegetables produced using biocontrol in supermarkets. In Denmark, for example, tomatoes produced using biocontrol, are often labelled 'produced by biological control', with the expectation that the consumers have a positive attitude to this label.

Organic production has increased over the last years in the EU. The guidelines for organic farming are compiled in IFOAM principles (Anonymous, 2002). Here, it is explicitly stated that biocontrol agents like predators, parasitoids and microorganisms are allowed. Above all, however, is the vision that organic producers avoid as much as possible the use of additives, biocontrol included. Thus, organic production seems potentially to favour much conservation biological control and inoculation biological control, with growers relying less on inundation biological control, using it sparingly and as a 'last option'. We should increase the dialogue with the organic farming system to ensure that elements of biological control will still be seen as integral elements of organic production.

In conclusion, the future of biological control will still be based on further expansion of the biological knowledge. We need to study the basic interactions between target and biocontrol agent. We need, however, also to ascertain that the ecological and societal components are strongly represented in the approach. Concerning the ecological components, these include both elements from the scientific discipline ecology and also elements from the currently organic or ecological cropping systems. Concerning the societal components, initiatives from governments and bodies like the EU as well as non-governmental societal components like the consumer's attitude, will play an increasingly important role.

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CHAPTER 2

SOCIOECONOMIC SIGNIFICANCE OF BIOLOGICAL CONTROL

Ingeborg Menzler-Hokkanen

1. Introduction

The different approaches to biological control (see Eilenberg *et al*., 2001) and their applications for widely varying target situations provide a wealth of opportunities for economic and societal analysis. While some applications are attractive even to big business (e.g., biopesticides based on *Bacillus thuringiensis*), and can be considered from a strictly economic point of view as any other saleable product, many others have no commercial value at all, but can provide huge public benefits (e.g., classical biological control preventing national parks from being overrun by exotic weeds). Sometimes biological control can save an industry after chemical pesticides have failed, and often biological control can be integrated into a farming system to complement the actions of other control measures.

2. Economics of pest management

2.1. Farm-level considerations

A farmer's choice of the pest management method is influenced by many factors. Sometimes there is not even a choice: if a crop is grown on a contract, the contractor often determines how the crop is to be treated. In Europe this is an increasing trend, with large wholesale chains specifying more and more precisely the quality standards for the products which they agree to buy. If the farmer has a choice, at least the following factors will affect how pests ultimately will be managed:

- pest pressure at the time when crop is susceptible, and damage potential
- direct expense of control (e.g., price of pesticide treatment/ha)
- indirect expenses (e.g., equipment, fuel)
- time constraints (e.g., is there time to carry out treatments at the right time)
- compatibility of pest control method with other farm operations (e.g., weed and disease control)
- knowledge of factors affecting efficacy of treatment
- expected efficacy of control treatments
- expected change in crop value as a result of pest management
- expected development of market value of the commodity (including price elasticity)
- overall economics of pest management

J. Eilenberg and H.M.T. Hokkanen (eds.), An Ecological and Societal Approach to Biological Control, 13–25.

Several computer and internet-based decision support systems have emerged to assist farmers in making choices particularly regarding the timing and need of pesticide treatments; these seldom, however, take into consideration alternative pest management options. At the farm level, the over-riding factor in deciding which pest management method to use, is the net economic benefit from the pest management operation (Mumford & Norton, 1984), combined with perceived reliability of the method (avoidance of crop failure, sometimes leading to 'insurance' treatments). Although in theory numerous control alternatives exist (such as host plant resistance, cultural control methods, etc.), the considerations as listed above currently usually lead to straightforward applications of chemical pesticides, where the fine-tuning comes from choosing the active ingredient, when and how to apply it, and how many treatments are necessary. Overall, it has been estimated that using pesticides results in improved crop revenues in the USA at the rate of about four dollars for each dollar invested (Pimentel *et al*., 1997); similar data have been presented for German agriculture (Waibel *et al*., 1998). For the UK, benefits at the farm level from pesticide use vary greatly, being in commercial apple production about ten times greater than the cost (Webster & Bowles, 1996), but in wheat production hardly matching them (Webster *et al*., 1999). Similarly in Finnish cereal production the private costs of pesticide treatments are barely recovered by the increase in crop value; indeed, in many cases negative balance is obtained (Kurppa, 1990).

At the farm level, short-term private benefits dictate which method of pest control will be used. Biological control cannot seriously as yet compete with chemical control in most crops, either because suitable methods have not been worked out, control agents are not available, or because farmers do not consider that they provide reliable enough control at an acceptable level. A notable exception is the greenhouse industry, where on vegetables in particular biological control is the rule rather than exception. Under the relatively simple, controlled conditions existing in a greenhouse, biological control has proven to be also economically superior to other forms of pest management, and therefore has gained overwhelming farmer acceptance and level of adoption in particular in Western Europe.

2.2. Societal considerations

Pest management decisions do not only provide private benefits and costs to the farmer, but also affect the society at large. Benefits arise from improved farm economies and increased output of agricultural products, affecting welfare of the farming sector. Negative impacts on the society are mainly related to changes in pesticide usage, which involves at least two major categories of externalities. Firstly, human health can be affected by pesticide use. Particular groups at risk include those who apply pesticides, bystanders, and the consumers of food containing pesticide residues (Bowles & Webster, 1995). Secondly, natural ecosystems may also be at risk, through effects on non-target organisms, and subsequently on other members of the ecosystem via the food chain. Indirect effects of pesticides may reduce the biodiversity and resilience of the ecosystem. Valuing these externalities is a difficult and complicated task. Webster and his co-workers have considered these in a series of papers analysing the economic benefits of alternative pesticide usage scenarios in the UK for wheat and apple production (e.g., Bowles & Webster, 1995; Webster & Bowles, 1996; Webster *et al.*, 1999). The ratio between private and society benefits in their example on UK wheat production is illustrative: for every £1 gained by farmers in private benefits in a move from conventional to integrated farming (with reduction in pesticide usage), there would be £6 worth of benefits to society. The authors

conclude from this that the government may have a role in the promotion of reduced pesticide strategies.

Another series of papers by Pimentel and co-workers analyse the environmental and socio-economic costs of pesticide use in the USA (summarised by Pimentel & Greiner, 1997). They calculate that these costs amount in the US to about $8.3 billion every year (roughly $30 per person per year). This clearly exceeds the purchase value of all pesticides, which is about $6.5 billion per year. Thus the real costs of applying pesticides is more than double of that what is paid by farmers, and could be viewed as society subsidies to support this form of pest management. In the estimates by Pimentel & Greiner (1997) the highest cost from pesticide usage was calculated to arise from bird losses ($2.1 bn/a), followed by costs of groundwater contamination ($1.8 bn/a), costs of pesticide resistance ($1.4 bn/a), and public health impacts ($0.93 billion/a). These authors conclude that if it would be possible to measure the full environmental and social costs of pesticide usage, the total cost would still be significantly greater than their estimate of $8.3 billion/year in the USA.

Replacement of chemical pesticide treatments by biological controls would therefore bring immense socio-economic benefits to the society: the benefits from controlling the pests would still accrue, but the negative externalities would disappear. Biological control methods are not known to pose any health hazards to the application personnel, nor to the consumers because there are no toxic residues on the products. Negative impacts on the environment from biological control treatments usually do not exist (van Lenteren *et al.,* 2003; 2006; Hokkanen & Hajek, 2003), nor any other of the socio-economic costs similar to those associated with the use of chemical pesticides (see Pimentel & Greiner, 1997).

3. Promoting biological control

The benefits that could be accrued by the society from a higher degree of adoption of biological control methods should be incorporated into the decision making and support structures, which determine the farmer's choice of pest management methods. The development of new biological control methods for situations where satisfactory solutions do not currently exist should be strongly supported by governments, as well as the market entry of biological plant protection products. Because of the benefits to the society associated with the replacement of chemical pesticides by biological controls, there should be mechanisms of price support in favour of the biologicals; currently this price support is in favour of the chemical pesticides at least via their indirect costs to the society. To balance this out, these external costs should be incorporated directly into the price of chemical pesticides, which would more than double in price. Because farmers primarily make pest management decisions based on expected private benefits from the treatments (cost vs. revenue), this distorts the choice between chemical pesticides and biological controls, and results in the current overuse of chemicals. Under the current competitive situation, biological control methods have successfully been able to replace chemical pesticides only in very few cases: of the global sales of pesticides, only about 1-2% accounts for biological products.

A major obstacle in the development of economically competitive biological control methods has been the requirement in the major markets to register microbial control agents following the rules originally intended for chemical pesticides. Many efficient microbial control agents have been developed, but they are not commercially available. Markets are usually too small to justify the registration, which is not only costly but also time-consuming. For example,

the bacterium *Pseudomonas chlororaphis* for treatment against seed-borne diseases of barley and wheat, was developed by a Swedish company and submitted for registration following the EU directive 91/414 in January 1996. It was finally approved in April 2004 after more than 8 years (Ehlers, 2005). These conditions cannot attract venture capital to be invested into small or medium-sized companies developing biological control products. Therefore, only large companies with interest in biological control products are in the position to register microbial products which have been developed in Europe. If the access to the market will continue to be difficult, even large companies may loose their interest in the development of biological control.

There is a strong public interest in finding alternatives to the use of chemical pesticides. The EU has supported research and development work by providing funds to networks such as COST Actions on biological control (e.g., 830, 842, 850 or 862) and many RTD projects. An increased substitution of chemical control by biological control would significantly reduce the problems of current pest management. Progress in this area, however, is hampered because of restrictions implemented by regulation requirements. Less costly regulation procedures would enhance commercialization of biological control agents, as can be exemplified by the commercial success invertebrate agents. Unlike microbials, these have been exempted from registration in most EU member states. Within the past two decades the market for macrobials has increased from almost zero to a volume >100 million € turnover per year, with the EU being a global leader in this area (Ehlers, 2005). Complete biological control systems are available to control all major pest problems in vegetable and ornamental production in greenhouses, facilitating replacement of broad-spectrum chemical insecticides. Conditions of low regulation have produced a healthy working environment particularly for those working in protected crops, and have provided sustainable control measures because resistance to parasites and predators has never been observed to develop. These benefits from the use of microbial control agents have not caused any measurable damage to the environment so far, and hazards related with the production of insects or mites (allergies) can be managed and avoided without major costs (Ehlers, 2005). Existing and threatening over-regulation of the biological control market in the EU also contradicts the objectives of developing sustainable, ecologically and economically sound agriculture and forestry management systems.

4. Biological control as an economic activity

Different types of biological control are from the economic point of view completely different. Classical biological control is an activity typically carried out by, or on behalf of, national or regional governments and public research organizations. In some cases international aid agencies provide significant funding for such work. Beneficiaries from the R&D activity involving classical introductions are to a large extent the researchers employed by the governmental or international agencies. Several thorough economic assessments of classical biological programs have been carried out, indicating spectacular efficiency with a benefit to cost ratio, overall, in the range of 30-40 to 1 (e.g., Cullen & Whitten, 1995; Greathead, 1995; Lubulwa & McMeniman, 1998).

Conservation biological control usually requires public support for research and farmer education, but at the implementation level no further government involvement is necessary (Perkins & Garcia, 1999). Often, measures that could contribute to conservation biological

control, are eligible for specific subsidies in the EU. Economic analyses concerning the benefits and costs of establishing and operating for example beetle banks, are currently not available.

Inundative biological control involves usually purchased inputs by the farmer, leading to an expected increase in crop productivity. The inputs – biological control agents – are produced and marketed by commercial companies, although often the basic research stems from work carried out at universities and research institutes (Törmälä, 1995). This form of biological control thus also supports private enterprises and the associated economic activities. Markets for inundative biocontrol have changed significantly during the last decade. Their overall share of the total plant protection market has increased from 1% to current 2%, with an annual turnover of approximately 150 million € in 2004, and annual increase between 9 and 13% (Frost and Sullivan, 2001). In the past, *Bacillus thuringiensis* had an 80% market share of all biopesticides (Lisansky and Coombs, 1994), but in 2000, 55% were products based on macrobial agents (insects, mites and nematodes). The sales of Bt have not decreased, but the developments with the macrobial agents have been dramatic without the regulatory hurdles. This trend therefore is a likely result from the difficult registration situation with microbial agents (Ehlers, 2005). The revenues in the microbials market are severely restricted by the requirements to register new products (Frost and Sullivan, 2001).

Many biocontrol companies in the USA have economically failed because of the expectations on quick returns on the investments by share holders (Ehlers, 2005). In Europe 20% of the inundative biocontrol market belongs to small companies, which are often family-owned, and not only fixed on shareholder value. Commercial biocontrol started in Europe in 1968 with two companies, and at least 26 producers in Europe, and 64 worldwide were recognized in 1997 (van Lenteren *et al.*, 1997). The actual numbers of companies involved in commercial biocontrol is much higher, if all companies selling the agents are counted: there are some 600 suppliers in the USA (calculated from The IPM Practitioner), and over 200 in Europe (calculated from the Biopesticide Manual). The total employment in Europe is about 750 persons, but only three companies employ more than 50 persons (Ravensberg, personal communication, 2004).

5. When does biological control make a difference? Illustrative case studies of problem situations

5.1. California citrus industry

Citrus has had a profound impact on the history and development of Southern California (Anon., 2005). California currently is by far the biggest producer of fresh market citrus fruits in the USA with a crop value close to 1000 million USD per year, while in Florida the total citrus production is much bigger but mainly for the processing industry (USDA 1991, 2002). Overall, US is the second largest orange producer in the world (after Brazil), and the industry employs about 90,000 persons in Florida alone (Burden, 2003).

Two hundred years ago, there was no Orange County in California. The first groves were planted in 1804, and the first commercial citrus in 1841 in what now is downtown Los Angeles (Webber, 1967, Anon., 2005). The California citrus industry did not get started until a new orange variety, the ”navel” orange appeared in 1870s. At the same time, the completion of the three transcontinental railways between 1876 and 1885 allowed an efficient and economical

shipping of the fruits, enabling the commercial citrus production to develop (Webber, 1967, Anon., 2005).

The fledgling industry was almost destroyed by an exotic pest, the cottony cushion scale *Icerya purchasi*, which invaded California around 1869 on Australian acacia trees. It spread within a decade through the orchards, and by 1886 was devastating the young citrus industry in Southern California. The pest has a wide host range and is notorious for its ability to severely debilitate and even kill mature trees (Kennet *et al*., 1999). Fumigation with hydrocyanic gas was first attempted, with obvious hazards and little effect on the pest, forcing many orchardists to destroy their trees (Webber, 1967; Kennett et al., 1999). In desperation, biological control was attempted and the ladybird beetle *Rodolia cardinalis* was introduced from Australia and New Zealand in 1888. The rest can be read from any textbook on biological control: by late 1889, within only a few months, the predator virtually cleared all trees from the cottony cushion scale, and provided thus the most spectacular case in classical biological control to date. The pest has been kept under complete biological control ever since, allowing citrus production to continue not only in California, but in some 55 other countries and regions around the world, where the same dramatic success has been repeated subsequently (Kennett et al., 1999).

This success also has been hailed as the start of the science of biological pest control, as it led to the establishment of permanent research programs by governmental agencies in the USA and other countries (Federici, 1999). I am not aware of specific socio-economic analyses of this biocontrol success; already the few data that are available pose difficult analytical problems. The biological control agents were imported to California at a cost of a few hundred dollars when the industry was at the verge of collapse. One year later, citrus fruit shipments from Los Angeles County had tripled (Gutierrez *et al*., 1999), and now the crop in California alone is worth around one billion dollars annually. How could one estimate the economic value of such a program – the benefits of which continue to accrue still today? And what about the social impact of this single successful case: how would California have developed if it would have had to cope with the cottony cushion scale in some other way? Or other citrus growing areas of the world? Without biological control, maybe citrus only could be grown successfully in Australia, where the pest would be under perfect natural control by *R. cardinalis* – almost everywhere else the pest would prevent the growing! Without classical biological control, the global division in agricultural production might look quite different than what it does now, and certainly, the history of California would have to be rewritten.

This case illustrates how classical biological control has the potential to benefit societies in a sustainable way over decades, even centuries, and how it can affect economic decisions and social welfare by allowing other production factors than pest management to decide where to locate economic activities. Australia alone hardly could produce enough citrus for the whole world; thus the durable and efficient control of the cottony cushion scale by biological means also has led to profitable production of citrus around the world, and allowed access to vitamin-rich, delicious citrus fruits for a much higher proportion of mankind than would otherwise have been possible.

5.2. Cassava pests in Africa

Introduced from Asia, and originating from Latin America, cassava (*Manihot* spp.) is grown over an area of 9 million ha in Africa (Zeddies *et al*., 2001). More than two-thirds of the total

cassava production is used as food for humans - it is the staple food of more than 200 million Africans. Smaller amounts are used for animal feed, and increasingly, for industrial purposes. Most cassava in Africa is grown by small-scale, semi-subsistence farmers, who have little access to external inputs either because they cannot afford them, or because they live in remote areas – or, usually, both. Under these conditions, biological control is not an alternative to synthetic pesticides, but apart from plant resistance the only available option in plant protection (Langewald and Neuenschwander, 2002).

Two major pests, the cassava mealybug, *Phenacoccus manihoti*, and the cassava green mite, *Mononychellus tanajoa*, spread to Africa in the early 1970s and, by 1987, had invaded 31 countries across the continent. The mealybug was first discovered in Zaire in 1973, but it rapidly spread through the cassava belt. It can alone cause yield losses of up to 80 percent, and thus posed a severe threat to African food security. A multinational collaborative research project was established in 1981 to combat the pest through classical biological control. The parasitoid *Apoanagyrus lopezi* was found on a mealybug from Argentina, and was introduced into Africa. It dramatically reduced the mealybug threat, maintaining the pest numbers below levels that cause economic damage. From 1981 on, *A. lopezi* was released in about 150 sites in 20 countries (Neuenschwander and Markham, 2001). The impact on cassava was slow and stable biological control was achieved only after several years. But by the end of the decade, the agent had spread to all major mealybug infestations in 27 countries, and had brought the pest under control in 95 percent of all fields—at a relatively low cost to the public sector, no cost to farmers, and without any use of chemical pesticides (Langewald and Neuenschwander, 2002). A study of the economic benefits of cassava mealybug biocontrol over a 40-year-period (1974-2013) estimated a benefit-cost ratio of about 200 at world market prices, and 370-740 when inter-African prices were considered (Zeddies *et al*., 2001).

The cassava green mite appeared first in Uganda in the early 1970s, and spread over the cassava growing areas rapidly, infesting 27 countries and causing 30-50% reductions in yield (Yaninek, 1997). It threatened production in many marginal areas where cassava often is the only crop available, after all other crops have failed. It became the most serious arthropod pest of cassava after the successful biological control of the mealybug *P. manihoti*. Biological control of the green mite was attempted without success already in 1970s. The efforts were continued and at least 7 species of predatory mites were released at 341 sites in 10 countries – none of them ever became established (Yaninek, 1997). Further 5 species were released between 1989 and 1995; three of them have now established. In 1993 a Brazilian predatory mite species, *Typhlodromalus aripo*, was first released, and by 1997 it was established in more than 1000 locations in 11 countries. This predator spreads at a rate of about 12 km in the first, and up to 200 km in the second season, and covers now an estimated 500,000 km^2 mainly in West Africa. *T. aripo* reduces the green mite population by >50%, once established, and increased crop yields by 32% in an impact trial (Yaninek, 1997). One major advantage of this predatory mite is that it does not require a mass breeding programme. It can be transferred to new locations on the cassava shoot tips, established in the field for multiplication, and later transferred to the release sites. This makes it very easy for national programmes to organise and implement a classical biological control campaign.

The Africa-Wide Biological Control Programme for the control of cassava pests has been the largest biological control campaign ever, and it has been subjected also to thorough economic analysis (e.g., Norgaard, 1988; Zeddies *et al*., 2001) as well as environmental impact analysis (e.g., Neuenschwander and Markham, 2001). The economic analyses have ignored

environmental and social benefits from the successful biological control of the major pests, but nevertheless yield handsome benefit to cost ratios: 200-740 –fold, depending on the points of reference (Zeddies *et al*., 2001). This study also considered alternative scenarios to the successful biological control. One scenario would be partial cassava crop failure (in the absence of effective control of the pests), leading to cassava or maize imports (food aid). Alternatively, another crop (maize) might be planted (which is not always possible). In all scenarios, the benefits of the successful biological control accumulated over 40 years reached from 8 billion USD (when maize would be grown as alternative) to over 20 billion USD (crop failure leading to food aid program), for the 27 countries analysed (Zeddies *et al.*, 2001).

Recent developments highlight the critical economic and social importance of the successful biological control of the cassava pests in Africa. A report by FAO (2000) points out that in contrast with the general trends, several countries in Africa were recently able to reduce the prevalence of undernourishment significantly. Both Ghana and Nigeria reduced it by over 30 percentage points between 1980 and 1997 (Ghana from 62% to 10%; Nigeria from 44% to 8%). The report points out that an important underlying factor was the rapid increase in the supply of cassava products during that period, which especially benefited the poor and undernourished. Cassava's importance rose also after the widespread drought over much of Africa in 1982-83, which forced many farmers to turn to cassava from other crops (FAO, 2000). Cassava production and consumption both in Nigeria and in Ghana doubled in a short time, and now cassava is the largest agricultural commodity produced in Ghana, representing 22% of agricultural GDP (1998).

Furthermore, cassava is rapidly becoming an important cash crop and a major raw material for many industrial products such as starch and its derivatives (glue, adhesives, modified sugars, organic acids, ethanol, etc) (Nyerhovwo, 2004). Cassava demand is projected to grow, worldwide, from 173 million tons in 1993 to 275 million tons in 2020, and the major beneficiaries from this expansion are expected to be countries in Sub-Saharan Africa (Nyerhovwo, 2004). Without the permanent and inexpensive biological control solution to the cassava pest problems, these positive developments could not have taken place, but rather, would have left some 200 million people in Africa struggling for their subsistence, and without a hope for a better future.

It also should be remembered that worldwide, cassava feeds 600 million people (FAO, 2000), and that if the cassava mealybug and the green mite ever should invade the cassava growing areas in Asia, the biocontrol solution, which already has proven its value in Africa, can be expected to be relatively easily transferable to the conditions in Asia.

Beyond the socioeconomic considerations discussed above, in Africa some ecological studies have been carried out to assess the impact of biological control on the environment. Positive overall effects on biodiversity could be demonstrated. Ecological studies after the introduction of *A. lopezi* indicate only transient effects on indigenous competing predators and parasitoids. A food web study of 135 species found that *A. lopezi* was specific to cassava mealybug, and did not affect other species. The biocontrol measures are considered to have had a large, though as yet unmeasured, impact on habitat protection, by precluding the need for farmers to clear large areas of additional land to compensate for mealybug destruction of cassava fields (Neuenschwander and Markham 2001).

This case study illustrates how biological control can cover unbelievably vast areas, reach also the most remote locations, and can provide efficient, sustainable pest control at no cost to the farmers (who seldom may even be aware of the control taking place). It also stresses the

role of international cooperation in tackling problems of this magnitude, and the long-term commitment which sometimes is needed to obtain the success. This case demonstrates that biological control is able to solve some of the most pressing problems facing humanity: that of giving a possibility even to the poorest people to grow successfully their own food.

5.3. Water hyacinth in Lake Victoria

The water hyacinth, *Eichhornia crassipes*, is a floating plant native to the Amazon areas of South America. Considered to be the world's worst aquatic weed, it apparently was introduced into Egypt as an ornamental plant already between 1879 and 1892. The presence of water hyacinth in Lake Victoria was first reported in Ugandan waters in 1988, but it may have been there as early as 1981, with infestations probably originating from several sources (Bugenyi and Balirwa, 1998). In Lake Victoria water hyacinth is particularly concentrated in the Ugandan side of the lake, mainly because the prevailing southerly winds blow mats from the mouth of the Kagera River northwards to Uganda. The location, size, and form of water hyacinth mats in Lake Victoria are highly variable, with some mats reaching 300 hectares in cover, others infesting entire bays. Bugenyi and Balirwa (1998) list the main negative effects of the increasing infestations of water hyacinth as:

- A reduction in fish populations caused by smothering of breeding grounds, extensive de-oxygenation in some areas, and increased debris loads over feeding grounds;
- An increased habitat for disease vectors (*Biomphalaria* bilharzia snails), mosquitoes, snakes, etc; and
- An alteration of the natural wetland fringe through successional patterns, and elimination of underwater plants and enhydrophytes in general.

They also list a variety of socioeconomically detrimental effects of water hyacinth, which include:

- Physical threats to water-based utilities, especially the national hydroelectric power station in Uganda, and to water intakes, in addition to increased operational costs for purifying and pumping the water;
- Physical interference to water supply for rural communities;
- Physical interference with fishing operations (entanglements or loss of nets), especially in fishing grounds, at fish landings, and around piers;
- Blockage of commercial transport routes and communications between islands; and
- Increased operational costs for commercial vessels.

In 1999 the weed covered already over 12,000 hectares along the shores of Kenya and Uganda. Fishing villages were being abandoned and millions of people faced dislocation and hunger, because fishing vessels could not any more reach open waters. Similarly, rail-ferry links were often broken for weeks, because the ships could not dock at their wharves (Collis, 2000). Fishing industry, which still in 1989 caught about 500,000 tons of fish from the lake, was declining (Bugenyi and Balirwa, 1998). Fisheries sector employed directly 300,000 people, and in each riparian country at least 10 fish processing plants had been established, targeting for exports to other countries, mainly Europe. These investments provided the much-needed foreign exchange earnings at the level of 100 million USD for these countries annually

(Bugenyi and Balirwa, 1998). The prospect of losing this economic activity and the source of food, employment, and income, because of the invasive water hyacinth, was alarming.

In addition, Lake Victoria was also experiencing what is called the greatest extinction of vertebrates in modern times: 30 years earlier there were about 500 fish species in the lake, and more than half of them went extinct, including *Oreochromis esculentus*, which used to be the main species caught for food. In total, the existence of some 30 million people along the shores of the lake was endangered (Collis, 2000).

Water hyacinth control options were debated, and first mechanical and chemical strategies were employed. Several multi-million dollar harvesting machines were sent to the lake from Europe (and later from the USA), with hardly any effect on the weed: working with maximum efficiency, they could clear about 300 hectares (Collis, 2000). The World Bank allocated 9.3 million USD for solving the problem, and various chemical companies set up offices in the capitol of Uganda in the hope of attacking the weed with herbicides. Biological control was considered, but political opposition to it was strong, and the option was ridiculed by the (then) Uganda Minister for Agriculture. In 1998 also Bugenyi and Balirwa (1998, p. 18) still believed that

> "*Biological control may be regarded as a viable option, especially if systematically introduced in the entire great lakes region and upper Nile basin. However, this option is expensive and takes many years to show impact.*" They also discuss the other options, but conclude that "*identifying the most efficient, viable and environmentally friendly option combination for Lake Victoria remains elusive*"

The use of chemicals to control the weed was tried in some countries, but there were concerns about its environmental, socioeconomic, and political implications. These include contamination of water for domestic and livestock use, as well as food chain effects on fishing. A major worry was uncertainty relating to fish export markets, e.g., whether the Europeans would reject the products because of chemical use in the lakes (Collis, 2000).

Nevertheless, while the official attention was fixed on the debate over herbicides and mechanical harvesters, a number of Ugandan and Kenyan scientists were trained in Australia in the techniques of biological weed control, and local communities were given courses on how to raise the small weevils in drums and tanks (Collis, 2000). In 1997 the first water hyacinth weevils *Neochetina eichhorniae* and *N. bruchi* were released onto Lake Victoria off the coasts of Uganda and Kenya. By the end of 1999 the weevils had not only firmly established viable populations in the release areas, but had practically wiped out the plant, cleared the waterways, and dramatically changed the view on Lake Victoria within two years. This successful control of water hyacinth is now emerging as one of the world's great biological control success stories, and as a rare humanitarian triumph (Collis, 2000).

This case shows how classical biological control is of utmost importance in present times, when ever increasing numbers of exotic organisms invade at accelerating rate new regions of the world, often causing vast economic and social problems and threatening the livelihood and subsistence of millions of people. It also shows how – sadly – various quick-fix, short term solutions to ecological and environmental problems are constantly preferred by politicians and other decision-makers, over the more subtle, long term or even permanent solutions such as biological control.

5.4. Fruit production in South Asia

An interesting set of case studies was published by Lubulwa and McMeniman (1998) on the classical biological control projects carried out in South Asia to combat pests affecting fruit production in the area. In total, ten projects were evaluated, and of them, only three failed to generate significant economic impacts. Even more interestingly, two of the 'failed' projects did not fail because the biological control would not have worked, but because the industry, which they were helping, was not economically viable and disappeared for other reasons. These analyses clearly showed that the impact of biological control on an industry can vary depending on its overall economy. If the business fails due to other reasons, even a highly (ecologically) successful biological control will not provide much obvious benefits (e.g., the passion fruit white scale in Samoa, Lubulwa & McMeniman, 1998). However, if the industry is stagnant and barely surviving, then a successful biological control project can make a big difference (e.g., banana skipper in Papua New Guinea, Lubulwa & McMeniman, 1998). If the industry is healthy and growing, then biological control can easily provide great economic benefits (e.g., control of the fruit-piercing moth in Fiji, Western Samoa, and Tonga; Lubulwa & McMeniman, 1998).

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CHAPTER 3

BIOLOGICAL CONTROL IN ORGANIC PRODUCTION: FIRST CHOICE OR LAST OPTION ?

Bernhard Speiser, Eric Wyss and Veronika Maurer

1. Introduction

'Biological agriculture' is a synonym for organic farming, but the term was developed independently from 'biological control'. Therefore, it cannot be taken for granted that all methods of biological control are acceptable or even a first choice in organic farming. In this chapter, we explore the attitude of organic farming towards methods of biological control. Although organic farming has become popular during the last decade, organic farms are still a minority in all countries (Willer & Yussefi, 2004). Furthermore there is a lack of profound knowledge about the regulatory framework for organic farming (i.e. public regulations and private standards). Therefore, we will firstly give a brief introduction to organic farming.

1.1. What is organic farming?

Many people primarily think of organic farming as 'farming without chemicals' (Lampkin, 1990). This oversimplified view suggests that organic farming substitutes 'agro-chemicals' with 'organic inputs'. In the present context, this would mean that pesticides or veterinary drugs are substituted with biocontrol agents.

Organic farming defines itself primarily by what it is doing, and not by what it is avoiding. The IFOAM Basic Standards (see below) define organic farming as a *system approach* resulting in 'a sustainable ecosystem, safe food, good nutrition, animal welfare and social justice', which is 'more than a system of production that includes or excludes certain inputs'. This will become evident in the following discussion. For a thorough introduction to organic farming see Lampkin (1990).

Organic farming is characterized by a number of *general principles*, which are 'intended goals of organic production and processing' (IFOAM, 2002). These principles indicate how production methods should be designed and evaluated, and whether they are the first choice or a last option. By contrast, *standards and regulations* are minimum requirements that a farm must meet to be certified organic. Regulations are precise instructions, for example whether a certain input is allowed or prohibited.

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1.2. Development of organic farming in a socio-economic context

Organic farming principles and standards/regulations reflect the current state of agriculture and society and should not be seen as a final statement, but rather as a work in progress (IFOAM, 2002). This is illustrated by the following, brief history of organic farming.

The roots of organic farming can be traced back to the 1920s, when a few pioneers searched for alternative methods of agricultural production. Their goal was to develop a production method which was appropriate for living systems and which could promote human well-being and harmony between humans and the cosmos. They objected to 'industrialized' agricultural production, and as a practical consequence rejected the use of mineral fertilizers. In the following decades, these ideas were further developed in practice (Vogt, 2000). At that time, the guidelines were laid down in the form of general principles, which left some freedom to the farmer how to fulfil the principles.

For the control of pests and diseases, preventive measures were considered as the most appropriate tools, but the use of very few pesticides available at that time (mainly copper and sulphur) was tolerated when needed. However, when synthetic pesticides became popular in the 1950s and 60s, their use was explicitly banned from organic farming.

In 1976, the International Federation of Organic Agriculture Movements (IFOAM) decided to work on common, international standards. In 1980, the first IFOAM Basic Standards were published (still more in the form of general guidelines). Also in 1980, the first Swiss standards for organic production were published, based on an agreement between five producers' organisations and the Research Institute of Organic Agriculture (FiBL). Originally, these standards all contained mainly guidelines for crop production, with only a small section about animal husbandry and animal feeding. Later, during the 1980s and 1990s, animal husbandry became more important. The standards clearly stated, with a positive list, whether a given agricultural practice, for example the use of a pesticide, was allowed or prohibited. Once such standards were in force, organic farms could be inspected and certified. Growing public awareness about environmental pollution, animal welfare and food scandals contributed to an increasing demand for certified organic produce by consumers.

Genetic engineering of crops and livestock (GMOs) progressed in the 1990s, causing great public concern, especially in Europe. GMOs and all derivatives of GMOs were considered 'unnatural' and therefore banned from organic agriculture (Schmitt and Haccius, 1992).

Towards the end of the 1980s, some governments discovered that promotion of organic farming combined the efforts for reducing overproduction and conservation of the environment. Already in 1987, Denmark and the German federal state Saarland had started to pay subsidies for conversion to organic farming. Later, various countries started 'organic programmes' with financial, educational and legislative incentives for organic production and marketing. As a consequence, a broad range of organic products became available in larger quantities and with better quality. Inspection and certification systems were further developed to give consumers a guarantee that the production method is followed. Large retailers began to sell organic products, but also prices began to sink. Today, retailers have become important key players, who influence the development of the organic food sector. Organic products are now marketed as premium products with an 'added value' of environmental friendliness, animal welfare and high product quality and safety. At the marketing level, there is a trend to combine these attributes with other 'added values' such as fair trade, convenience, and fully transparent product declaration to the consumer, all of which are characteristic of 'premium products'.

2 The major organic farming standards

2.1. IFOAM Basic Standards

The International Federation of Organic Agriculture Movements (IFOAM) is a worldwide umbrella bringing together organizations of organic farmers and growers, traders and consumers. It represents some 700 member organizations in over 100 countries. The 'IFOAM Basic Standards for Production' (hereafter called 'IBS') were first published in 1980 and were updated until now biannually, and in the future every three years (Blake, 2004; O. Schmid, FiBL, pers. comm.); our discussion is based on the 2002 edition (IFOAM, 2002). The IBS are 'standards for standards', which means that they can only serve as a basis for developing regional standards which can then be used for certification of organic farms (Blake, 2004). As a private initiative, the IBS have no legal standing but their political and practical impact has been huge (Blake, 2004). For example, they are the basis of a private accreditation programme with more than 20 member organizations. Although the IBS include lists of allowed inputs, their focus is on general principles and on criteria for evaluation of novel inputs.

2.2. Codex Alimentarius guidelines

The Codex Alimentarius is a joint food standards programme of FAO/WHO (United Nations' Food and Agriculture Organization and World Health Organization). The Codex Alimentarius is a collection of internationally adopted food standards. Their purpose is to protect the health of consumers and to ensure fair practices in food trade (Codex Alimentarius Commission, 1999/2001). The 'guidelines for the production, processing, marketing and labelling of organically produced foods' (hereafter called 'Codex guidelines') were published in 1999 and revised in 2001. These guidelines were the result of extensive consultations of the delegates, which were mainly representatives of national governments and IFOAM as a private organization. The Codex guidelines for organically produced food therefore represent a broad international consensus about the nature of organic production. The requirements are comparable with the EU Regulation 2092/91 (see below) and the IBS (Schmid, 2002). Codex Alimentarius guidelines are themselves not legally binding, but they have a strong influence on national and international regulations. In the last years, a major activity was the revision of the criteria for admission of new inputs and of the list of allowed inputs.

2.3. European Council regulation EEC 2092/91

The European Council regulation on organic farming EEC 2092/91 (hereafter called 'Reg 2092/91'), was issued in 1991, and has been amended several times. In particular, it was supplemented by the European Council regulation (EC) Nr 1804/1999 to include livestock production in 1999. It provides legally binding standards for organic production, processing and marketing of organic products. The regulation reflects the political consensus between the EU member states, and not so much the principles of organic farming. It contains general principles of production and detailed lists of allowed inputs, additives and processing aids for organic food processing. Reg 2092/91 offers little flexibility with respect to including new inputs. For a

comprehensive overview of this regulation, see Schmidt & Haccius (1992) and Graf *et al.* (1999).

2.4. United States' National Organic Program

The United States' National Organic Program (hereafter called 'NOP') was first proposed in 1997, and has been amended in 2000. For a brief history, see Baker (2004). It provides legally binding standards for organic production, processing and marketing of organic products. NOP takes a different approach to inputs than the other regulations: All natural ('nonsynthetic') inputs are allowed, unless they are explicitly prohibited, and all synthetic inputs are prohibited, unless they are explicitly allowed.

Table 1: Terminology of biological control methods (adapted from Eilenberg et al., 2001). BC = biological control

Term	*Description*
Conservation BC	Deliberate modification of the environment or management practices to enhance specific, natural enemies.
Classical BC	Introduction of an exotic BC agent for permanent establishment.
Inoculation BC	Release of a BC agent for extended, but not permenent control.
Inundation BC	Use of a BC agent, where only the released organism provides pest control.

3 Evaluation of biological control methods

The evaluation of whether a given input (e.g. a biological control agent) can be used in organic agriculture involves weighing its advantages against its disadvantages (for an overview of the procedures involved, see Speiser & Schmid (2004)). Because these aspects may have different priorities for different stakeholders, input evaluation sometimes provoques long-standing discussions and controversies. The evaluation procedure is as such for different types of pest and diesease control products. However the outcome of the assessments can be quite different. This applies also to various biological control methods, which are described below in Table 1. To remind the reader: such discussions take place when standard-setting organizations evaluate inputs. However, there is no scope for such discussions at the farmer's level; the standards currently in force have to be followed strictly.

3.1. Allowed or not? – Check against the evaluation criteria

Some standards for organic farming provide criteria for the evaluation of inputs. In the following, it is described how biological control methods are evaluated against the criteria given in Appendix 3 of the IBS (necessity; nature and mode of production; environment; human health and quality; ethical aspects; socio-economic aspects) plus one additional criterion given in the Codex guidelines for organically produced food ("consistency with priniciples of organic production").

3.1.1. Necessity for intended use

Inputs must be necessary. Necessity has two components: (i) the input must have a positive effect on yield, product quality, environmental safety, ecological protection, landscape or human and animal welfare; (ii) the same effect cannot be achieved with other, more acceptable methods (including cultural practices, varietal choice etc., as well as other inputs). Whether a biological control agent is necessary must be determined on a case-by-case basis and may vary regionally and from crop to crop, depending on the availability and practicability of alternative methods.

3.1.2. Nature and mode of production

Inputs should be organic (vegetative, animal, microbial) or mineral. This is clearly the case for all biological control agents. However, genetically modified organisms (GMOs) are considered unnatural, and thus incompatible with organic farming, irrespective if the organisms are used for biological control or for another purpose (Schmidt & Haccius, 1992); see also 'socio-economic aspects' below.

3.1.3. Environment

Inputs should not be harmful to the environment. In many cases, biological control is the most environmentally friendly solution in crop protection and animal husbandry. Nevertheless, a few species with a broad host range and non-native predators or parasites may raise concerns over side effects on non-target species. Thus, environmental impact should be assessed on a case-by-case basis. Where biological control agents have to be registered, environmental impact is assessed during registration.

3.1.4. Human health and quality

Inputs should not be harmful to human health and they should have no negative effects on the quality of the products. With respect to residues on the harvested products, biological control methods usually perform much better than their alternatives. In the case of microbial control agents, concerns over food safety have to be considered on a case-by-case basis.

3.1.5. Ethical aspects – animal welfare

Inputs shall not have a negative influence on animals kept at the farm. This requirement is fulfilled by all biocontrol agents; indeed, some serve to improve animal health and/or welfare.

3.1.6. Socio-economic aspects

Inputs should not meet resistance or opposition from consumers, and they '… should not interfere with a general feeling or opinion about what is natural or organic – e.g. genetic engineering'. This criterion includes perception by producers, consumers and the media, and depends on the social context. It may thus vary from one region to another, and also over time. For example, granulosis viruses have been used in Switzerland for many years (see below) without raising any public concern, while the spraying of any viruses whatsoever would meet presently great opposition in the UK (and is therefore not practised). On the other hand, the release of the Japanese ladybird beetle *Harmonia axyridis* (Pallas) has met opposition in Switzerland, because it was suspected to attack the native fauna (see below). As a broad consensus, the use of genetically modified organisms (GMOs) is prohibited by all standards. In the context of biological control, this concerns at present genetically modified strains of *Bacillus thuringiensis*, which are now used in the USA. All standards allow only the use of the naturally occurring strains of *B. thuringiensis.*

3.1.7. Organic farming principles

The Codex guidelines contain similar criteria as the IBS (see above), but in addition they require that inputs must be consistent with principles of organic production (Codex guidelines, Section 5). As 'biological methods' are part of these principles according to the Codex definition, biological control methods are allowed.

3.2. First choice or last option? – Check against general principles

The evaluation criteria described above serve to determine whether or not an input is *allowed* in organic agriculture. General principles are 'intended goals of organic production' (IBS), and help to create an understanding of organic farming practices. They indicate the desirability of methods, i.e. whether an input is a *first choice or last option*. Based on these principles, individual certification bodies may decide to prohibit the use of an input by their producers, or restrict it to certain conditions. For example, Reg 2092/91 allows many inputs only under the condition 'need recognized by the inspection body or inspection authority'. In the context of biological control, the following principles are relevant:

3.2.1. Sustainable production of sufficient quantities of good quality food

The overall aim of organic farming is to produce sufficient quantities of high quality food, fiber and other products (IBS, section B 1). According to the Codex guidelines (section 2.1), organic farming systems should seek to achieve sustainable productivity. In other words, organic farming seeks the best trade-off between the farmers' need for economic production, the consumers' demand for high quality products, the markets' need for sufficient supply at the right time, environmental protection and ethical issues such as animal welfare. In the evaluation process, these aspects have to be weighed against each other.

The use of biocontrol agents is thus desirable, if they contribute to yield increase, yield stability, product quality or animal health and/or welfare, or if they substitute other, less favourable compounds.

3.2.2. Principle of prevention

Organic farming considers problems with pests, diseases or parasites as indicators of an inadequate management system. Therefore, the main challenge in organic production is to optimize the management system, including (but not restricted to) all measures by which the crops or animals become more healthy, or the living conditions for pests and diseases become less favourable. This approach called 'indirect crop protection' or 'preventive animal-health management' is typical for organic farming. Only when this approach is insufficient, may the organic farmer resort to 'direct crop protection' such as spraying of allowed insecticides or fungicides (including biocontrol agents) or to veterinary treatments such as the use of anthelmintics.

3.2.3. Working with natural cycles

In organic farming, the agroecosystem is considered as a whole. All living organisms within the 'farm ecosystem' are considered to be in a dynamic equilibrium with each other. This concept applies to crops, pests and their natural enemies, as well as to farm animals, wildlife or microorganisms in compost and soil. It is applied regardless of the underlying mechanisms (predator-prey relationship; parasite-host relationship; competition for substrate, light, space etc.). The equilibrium can be influenced by appropriate management practices, which are themselves part of the natural cycles (indirect control). This is preferable to direct control of pests or diseases, which represents an intervention from outside the agroecosystem. In other words, the difference is whether the farmer *lets and helps nature* find a new equilibrium between pests and beneficials, or whether he *himself attempts* to control the pest (by 'spraying'). Farm animals and their health are also considered in the context of the entire farm ecosystem and the same conclusions apply.

Another implication of the principle is that all measures taken should have as little impact on natural cycles as possible. This applies particularly to effects of crop protection measures on non-target organisms, and to the side-effects of veterinary drugs on animals, and on the environment after excretion.

This principle also emphasizes the importance of the flow of materials within the 'farm ecosystem', which is also the unit that is subject to inspection and certification. Materials originating from outside the farm are called 'off-farm inputs' or simply 'inputs'. The use of inputs always means an open cycle on the farm and should be minimized (although it can never be zero). If inputs have to be used, they should preferably come from other organic farms, thus closing the cycle on a wider scale.

3.2.4. Precautionary principle

Organic farming avoids methods or products for which there is a doubt about negative effects, until they are proven to be harmless. The burden of proof therefore lies with the proponent of an activity, and not with the public or the organic farming community. This principle has always been the standing practice; currently IFOAM are in discussions to mention it explicitly in the next issue of the IBS under the name 'precautionary principle' (O. Schmid, FiBL, pers. comm.).

3.3. Implications for various biological control agents

From the point of view of organic farming, three categories of biological control agents must be considered separately: predators and parasites, natural microorganisms and genetically modified microorganisms.

3.3.1. Predators and parasites

This category includes mainly predatory and parasitic insects which attack crop pests, but also predatory mites, other arthropods and nematodes for the control of insects or molluscs. All standards mention the use of predators and parasites in crop production. Because predators and parasites are not considered as pesticides, they are not listed individually in the annexes.

The IBS list in appendix 2 '…release of parasites, predators …'. The Codex guidelines mention '…release of predators and parasites…' in Annex 1, section A 6. Reg 2092/91 lists in Annex I, section A 3 'protection of natural enemies of pests through provisions favourable to them (e.g. hedges, nesting sites, release of predators)'. The NOP mentions in § 205.206 (a) 'augmentation or introduction of predators or parasites of the pest species'. In conclusion, the use of predators and parasites is not only allowed, but even *recommended* by all these standards.

In the context of animal husbandry, the use of biocontrol agents is not specifically mentioned in any of the standards, but the general principles concerning animal management and health does make it clear that their use is also desirable in this context. For example, in section 5.1 the IBS emphasize the use of various indirect control methods to 'prevent disease and parasitism, and avoid the use of chemical allopathic veterinary drugs'.

3.3.2. Natural microorganisms

This category includes mainly bacteria, the most widely used being *Bacillus thuringiensis*, but also certain fungi and viruses. All standards list these biocontrol agents in an annex, together with plant and mineral-based crop protection products. The listed products are allowed, but not recommended.

The IBS list in appendix 2 as allowed '…fungal preparations; bacterial preparations (e.g. *Bacillus thuringiensis*); […] viral preparations (e.g. *granulosis virus*)…'. The Codex guidelines (Annex 2, table 2 III) list 'Micro-organisms (bacteria, viruses, fungi) e.g. *Bacillus thuringiensis*, Granulosis virus, etc' as allowed. Reg 2092/91 lists as allowed in Annex II, section B II 'Microorganisms (bacteria, viruses and fungi) e.g. *Bacillus thuringensis*, *Granulosis virus*, etc'. The NOP considers natural microorganisms as 'nonsynthetic'. Their use is therefore allowed without explicit mentioning.

In the context of animal husbandry, the use of these biocontrol agents is again not specifically mentioned in any of the standards, but as for predators and parasites, their use is obviously desirable (see above).

3.3.3. Genetically modified microorganisms

The use of genetically modified microorganisms as biocontrol agents is prohibited by all standards, as detailed above.

3.3.4. Discussion points

As described above, the major standards for organic farming *recommend* the use of predators and parasites, while the use of microbial control agents is only *allowed*. The authors have some reservations against this hierarchy: (i) many of the predators and parasites which are commercially available originate from industrial production almost as much as microbial control agents; (ii) some of the commercially available predators and parasites are not native species; (iii) some predators will attack a large number of non-target species, while many microbial control agents have a narrow host range and thus less impact on non-target species.

From the authors' point of view, biological control with macro- and microorganisms is in principle equally compatible with organic farming, but case-by-case evaluations are necessary to eliminate unwanted cases. Whenever biological control (with macro- or microorganisms) has less side-effects on the environment than plant- or mineral based crop protection products, their use should be favoured. The strategies for crop protection and animal husbandry outlined below are based on these considerations.

4. Integration of biological control into organic crop protection strategies

4.1. Outline of organic crop protection strategy

It was not until the 1980s that crop protection researchers developed specific crop protection strategies for certain crops. Today, the most advanced strategies involve science-based and ecologically sound measures which are compatible with organic farming standards. Nevertheless, there is still much scope for improvements in this complex field. These strategies include several measures which should be used with decreasing preference; thus, the least preferred measure should only be used if all measures of higher preference are not successful (see figure 1 A).

As a first step, preventive measures should be taken, such as optimizing crop rotation, using cover crops, planting of hedgerows and wildflower strips, avoidance of host plants of pests and diseases, thinning the canopy to allow quicker leaf drying, soil amendments (manure, slurry, composts, green manure), soil cultivation and choice of adapted species and varieties. This includes also conservation biological control measures.

In second priority, biological control should be used. In Switzerland, 30 species of predators and parasites and 10 species or strains of microorganisms are currently allowed for use (Table 2 A).

In third priority, plant- or mineral based products can be used for the control of crop pests or diseases. Products with the lowest possible impact on the environment and non-target organisms should be selected, and the choice of products is always restricted to the listed compounds. Synthetic pesticides are generally prohibited. For crops, this approach has been described in more detail by Tamm (2000).

Finally, in exceptional cases the EU regulation 2092/91 allows a few chemically synthesized substances, but only for use in traps or dispensers, so that they do not come into contact with the crops or the soil.

4.2. Case study: importance of biological control in organic apple orchards

Organic apple growers face the same severe plant protection problems as their colleagues in conventional or integrated pest management systems: apple scab, powdery mildew, fire blight, codling moth and rosy apple aphid. But, in contrary to them, the organic farmers have a very limited range of approved products to control these problems. Thus, the approaches to pest management in organic apple orchards rely largely on preventative measures as direct, or re-active control methods are rare. Below, the concept of organic pest management in apple orchards is explained for some important pests and diseases, and the role of biocontrol is highlighted.

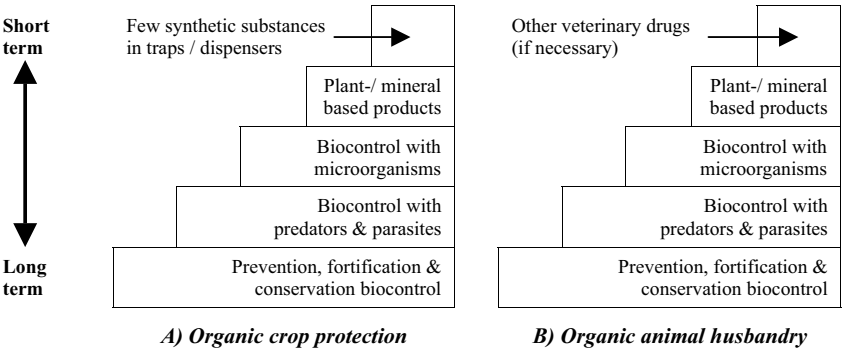

Figure 1: Integration of biological control into organic management strategies for crops (A) and animals (B). Methods shown at the bottom have a long-term effect, while methods shown at the top have a short-term effect. In organic farming systems, methods with a long-term effect are the basis of crop production and animal husbandry, and should be used with preference, while methods with a short-term effect should be used in emergencies only. For discussion see text

4.2.1. The rosy apple aphid

The rosy apple aphid, *Dysaphis plantaginea* Pass., is one of the most severe insect pests in apple production. This pest is also a good example to show the range of solutions within an organic plant protection strategy.

In a first phase the grower has the possibility to choose apple varieties tolerant or resistant to aphids. Some varieties are known to be more resistant to the rosy apple aphid than others, for example: Ariwa, Delorina, Florina, FloRub, Goldrush, Reanda, Red Devil, Renora, Rewena, Rubinola, Saturn (Habekuss *et al.*, 2000; Würth *et al.*, 1999; Würth *et al.*, 2002). However, this range of varieties is too small to fulfil all agronomic and quality demands and therefore, susceptible varieties are still grown. Cultural practices including selective pruning, soil management and adapted organic fertilization are important tools to lower the risk of aphid calamities.

In a second phase, habitat management in the sense of conservation biocontrol (Eilenberg & *al.*, 2001) is implemented by sowing flowering weed strips to enhance populations of aphid predators. These strips consist of a mixture of indigenous annual and perennial plants adapted to the needs of the beneficial insects (Wyss, 1995). At least three meter wide strips are installed in the alleyways or at the border of the orchards. They provide pollen and nectar during flowering and serve as important over-wintering sites. Some of the sown plant species also host aphids when they are rare on apple trees. Therefore, pollen, nectar and aphids are available to a great number of aphidophagous species throughout the year. These weed strips attract and give shelter to a significantly higher number of aphid predators than in orchards without weed strips (Wyss, 1995). During spring and summer, tree inhabiting spiders benefit from the high number of non-pathogenic insects attracted by the weed strips to build up their populations. In autumn, they are the dominant predators and significantly reduce the number of rosy apple aphids returning from their summer host plants (Wyss *et al.*, 1995). Similarly to weed strips, hedgerows are planted at the borders of orchards to encourage natural enemies of aphids. However, habitat management does not always provide sufficient control, particularly in years with high aphid populations (Wyss, 1997).

If indirect measures are insufficient, biocontrol agents could be used in a third phase, but these methods are still under development. A few years ago, the use of the Japanese ladybird beetle *Harmonia axyridis* (Pallas) against different aphid species on fruit trees was considered. However, this was rejected because there were concerns that this species would outcompete native ladybeetle species. (Wyss, Villiger, & Müller-Schärer, 1999) showed the potential of three native predators to control the rosy apple aphid and continued working on mass releases of the most promising predator, the ladybird beetle *Adalia bipunctata* L. Releases of larvae either in spring or in autumn significantly reduced the rosy apple aphid (Kehrli & Wyss, 2001; Wyss *et al.,* 1999). Autumn applications against the gynoparae, females and males seem to be promising, but more research is needed to establish a valuable and practical biocontrol of the rosy apple aphid.

The next preferred solution would be repellent agents against aphids. For example, autumn treatments with a processed kaolin product hindered the gynoparae and sexuales from landing on apple trees (Wyss & Daniel, 2004).

If in spring there is still an acute risk of an aphid calamity in apple orchards, organic fruit growers may use insecticides of natural origin as a last option. In some European countries rotenone, pyrethrin and the more selective azadirachtin (extract of neem tree kernels) are allowed to be used against spring populations of the rosy apple aphid (Wyss, 1997).

4.2.2. Lepidopteran pests

Until now, little work has been done to evaluate conservation biocontrol strategies to enhance the rate of parasitism of lepidopteran pests. (Pfannenstiel & Unruh, 2003) report that wild roses planted nearby apple orchards enhanced a specific leafroller parasite by providing an overwintering host. As a consequence, parasitization of leafrollers was significantly increased in the neighbouring orchards.

The codling moth, *Cydia pomonella* L., is mostly controlled by mating disruption with the specific pheromone (Brunner *et al.*, 2002; Zuber, 1999). However, this technique is only efficient enough on large surfaces and when less than 2 % of the apples were attacked in the previous year. In orchards with higher populations, a very efficient inundation biocontrol agent may be used: the codling moth specific granulosis virus CpGV. It is most efficient against

young larval stages and must be repeatedly applied during the entire flight period of the codling moth. Mating disruption and granulosis virus are often combined for better efficacy.

In organic apple production, two other biocontrol agents are often used: the granulose virus AoGV against the summer fruit tortrix moth *Adoxophyes orana* (Fischer von Rösslerstamm) and *Bacillus thuringiensis* var. *kurstaki* against the winter moth *Operophtera brumata* (L).

4.2.3 Woolly apple aphid and San José scale

Both the woolly apple aphid (*Eriosoma lanigerum* Hausmann) and the San José scale (*Quadraspidiotus perniciosus* Comstock), were brought to Europe in the last century. Some work has been done on breeding resistant rootstocks (against *E. lanigerum*) and many insecticides were tested with varying degrees of success (Häseli *et al.*, 2000). Some interesting results were achieved by classical biocontrol. In the 1930s, the parasitic wasp *Aphelinus mali* (Haldeman) was introduced to control the woolly apple aphid on the aerial part of the tree. Due to climatic factors, *A. mali* has never continuously controlled the aphid. However, new studies indicate that Canadian strains might be more efficient under European weather conditions (Mols & Boers, 2001).

Similarly, the antagonist of the San José scale, *Prospaltella perniciousi* (Tower), was introduced to Europe. In most releases, the efficacy ranged between 20 and 80 % and is limited due to climatic factors (Benassy *et al.*, 1968; Mathys & Guignard, 1965; Neuffer, 1990).

Therefore, both pests periodically still cause some problems in organic apple orchards. In this case, new releases or releases of better adapted strains would be the preferred solutions in organic agriculture. At the moment, this cannot be practised due to a lack of companies providing these species.

4.2.4. Fire blight

Fire blight is a recent problem in Europe. Since the 1960s this detrimental disease caused by the bacterium *Erwinia amylovora* (Burr.) spread through most European countries (van der Zwet, 2002). Most countries took measures like uprooting diseased plants and prohibition of planting susceptible host plants (e.g. *Cotoneaster* ssp., *Crataegus* ssp.). Due to these efforts, the incidence of fire blight infections has decreased by more than 90 % (van Teylingen, 2002).

During the invasive phase of fire blight, some countries allowed the use the antibiotic streptomycine, while organic farmers avoided this synthetic and problematic substance. Simultaneously, researchers worked hard to find alternatives to streptomycine. Some apple varieties and certain rootstocks were detected to be less susceptible or completely resistant to this bacterial disease (Mohan *et al.*, 2002; Norelli *et al.*, 2002; Richter & Fischer, 2002). In addition, essential oils, plant extracts, and clay minerals were found to have inhibitory effects against fire blight (Römmelt *et al.*, 1999). Furthermore, the inhibitory effect of some bacteria was tested for a possible use as biocontrol agents against fire blight: *Pseudomonas* ssp., *Rahnella aquatilis*, *Pantoea agglomerans*, and *Bacillus subtilis* (Alexandrova *et al.*, 2002; Holtz *et al.*, 2002; Laux *et al.*, 2002; Vanneste *et al.*, 2002). Today, products like Serenade® or Biopro® with *B. subtilis* as the active ingredient are registered and used by fruit growers to prevent fire blight.

4.2.5. Limits of biocontrol in organic apple production

Eilenberg *et al.* (2001) suggest a stringent terminology of classical, inoculation, inundation and conservation biocontrol (see table 1). Organic fruit growers try to do best with cultural practices and conservation biocontrol measures (which they collectively call "indirect measures") to protect and enhance pest antagonists.

If indirect measures are not sufficient, fruit growers apply indigenous, species specific organisms for inundative or inoculative biocontrol whenever there are commercial products available. Classical biocontrol measures are not specifically linked to organic agriculture but are also part of an organic plant protection strategy. However, knowledge on biocontrol strategies in apple production is still limited and for the key diseases, such as apple scab and mildew, no biocontrol solutions are on the market yet.

5. Integration of biological control into organic animal husbandry

5.1. Outline of strategies for disease and parasite control in organic animal husbandry

Until recently, organic animal husbandry has relied largely on conventional veterinary approaches for the control of diseases and parasites. Today, the outlines of organic approaches to these problems are emerging, but much more work needs to be done in this area. The basic approach is very similar to the approach to crop protection, as illustrated in figure 1 B.

As a first step, preventive measures should be taken. This includes selection of animals adopted to the farm conditions, appropriate herd size, holding system, feed and proper use of technical installations. Also, hygiene, milking technique and grazing management should be adapted specifically to reduce diseases and parasites.

In second priority, biological control can be used. At the moment, one microbial biocontrol agent and three predators/parasites against house and stable flies are commercialized. Biocontrol agents against other pests of cattle are under development. In addition, *B. thuringiensis* var. *kurstaki* is allowed for use against the wax moth, *Galleria mellonella*, in apiculture (Table 2 B).

As a next step, complementary medicine and direct control measures by means of natural compounds should be applied. Complementary medicine (mainly homeopathy and phytotherapy) can be applied in case of infectious or metabolic diseases and accidents. A number of natural compounds are available for the control of parasites: pyrethrins, plant extracts, silica, and several acids against varroa mites.

As the last step only, recurse may be taken to chemically-synthesised veterinary products or antibiotics, if other methods are not successful. Although these products are not of natural origin, their use as a last option is sometimes necessary for the sake of animal welfare.

Table 2: Examples of biocontrol agents allowed in organic farming (see Speiser et al., 2005)

A) Crop protection

Insects

Adalia bipunctata
Anthocoris nemoralis
Aphelinus abdominalis
A. colemanii
A. ervi
Aphidoletes aphidimyza
Cryptolaemus montrouzieri
Dacnusa sibirica
Diglyphus isaea
Encarsia formosa
Feltiella acarisuga
Leptomastidea abnormis
Leptomastix dactylopii
Macrolophus caliginosus
Metaphycus helvolus
Microterys flavus
Orius insidiosus
O. laevigatus
O. majusculus
Pseudaphycus maculipennis
Trichogramma brassicae

Mites

Amblyseius cucumeris
A. barkeri
Hypoaspis aculeifer
H. miles
Phytoseiulus persimilis

Nematodes

Heterorhabditis bacteriophora
Phasmarhabditis hermaprodita
Steinernema carpocapsae
S. feltiae

Microorganisms

Ampelomyces quisqualis
Bacillus subtilis
B. thuringiensis var. *israeliensis*
B. thuringiensis var. *kurstaki*
B. thuringiensis var. *tenebrionis*
Beauveria bassiana
B. brognartii
Coniothyrium minitans
Granulosis virus AoGV
Granulosis virus CpGV

B) Animal husbandry

Insects

Muscidifurax zaraptor
Nasonia vitripennis
Ophyra aenescens

Microorganisms

Bacillus thuringiensis var. israeliensis
B. thuringiensis var. *kurstaki* (against wax moth)

5.2. Case study: importance of biological control in organic husbandry of cattle

Organic cattle production is concerned by two main health problems: mastitis in dairy production and internal and external parasites in beef and dairy cattle. Prevention is the key to the control of both disease complexes. In addition to preventive measures, the free living parasite stages are accessible to biocontrol agents. Below, this approach is explained for external and internal parasites (flies and gastro-intestinal parasites, respectively).

5.2.1. Mastitis

In mastitis control, preventive measures such as selection of appropriate breeds, hygiene in general, selection of bedding material, quality of feeding and proper milking technique, will lead to a substantial improvement of the situation (Walkenhorst *et al.*, 2004). In addition, it might be possible to create an unfavourable environment for pathogens e.g. by dipping teats with lactic acid bacteria preparations. Besides the development of prophylactic measures, considerable efforts are undertaken to replace allopathic treatments with 'conventional' medicines by homeopathic treatments or by treatments with natural substances. However, intramammary applications of living organisms are not possible, which excludes biocontrol in the udder.

5.2.2. House and stable flies

House flies (*Musca domestica*) and stable flies (*Stomoxys calcitrans*) present an important hygiene and animal welfare problem associated with animal holdings. The immature stages of both species develop in organic material such as humid feed, deep litter or the solid layer on top of slurry. Adult house flies also feed on those materials, whereas adult stable flies are blood-sucking. Both species transmit pathogens of humans and animals and can act as vectors or intermediate hosts for parasites of farm animals (Kettle, 1995). The economic importance of house and stable flies is mainly due to irritation of the animals, resulting in reduced weight gain (Catangui *et al.*, 1993) or milk production (Marchand, 1984).

The first measure to be taken is strict hygiene management. Cleaning the stables thoroughly in spring reduces the over wintering fly population. Farmyard manure and other organic waste should be removed to eliminate feeding and breeding areas. A dry and well compacted deep litter area presents an unfavourable breeding place for the flies. The solid layer on liquid manure is an important breeding place for the flies, and should be destroyed regularly by pumping or stirring. Several kinds of baited or sticky traps are available for the prevention of massive development of fly populations, but they have the disadvantage of catching only adult flies.

In the sense of conservation biological control (Eilenberg *et al.*, 2001), measures should be taken to enhance and to protect insect-feeding swallows in stables. The barn swallow (*Hirundo rustica*) occupies nesting places in the buildings, whereas the house martin (*Delichon urbica*) breeds on the external facade of buildings. Both species readily accept artificial nests. Four nestlings of *D. urbica* eat about 150'000 insects during their rearing time (Schweizerische Vogelwarte, 2004). A number of predatory or parasitic arthropods are usually associated with the breeding places of house and stable flies. These natural enemies are at risk by other fly control measures: sticky traps present a severe danger for swallows, if they are not protected; swallows as well as litter inhabiting mites and insects may also be poisoned by insecticides applied against adult flies.

Biocontrol agents can be used as a next step. Many species of natural enemies have been released to control *M. domestica* worldwide before the use of synthetic insecticides started in the 1940s, and when the development of resistance to chemical insecticides had become an important problem in the early 1960s (Legner *et al.*, 1974). Today, three biocontrol agents are commercially available in Europe: (i) in deep-litter systems, *Bacillus thuringiensis* can be applied to the breeding regions of the flies; (ii) the pteromalid pupal parasites *Muscidifurax zaraptor* and *Nasonia vitripennis* are commercially available for release in deep litter systems; (iii) in systems with slatted floors, larvae of *Ophyra aenescens* live predaceously on house fly larvae in the solid top layer of liquid manure.

As a last step, the use of insecticides of natural origin against adult flies may be considered. However, this should be the last option since natural enemies and insects released as biocontrol agents are at risk by these products, mainly pyrethrine (see above).

5.2.3. Gastro-intestinal nematodes

Gastro-intestinal nematodes are a major health problem in grazing cattle, particularly in first grazing-season animals. In the past decades, control of gastro-intestinal nematodes has almost entirely relied on the use of anthelmintics. On organic farms, the preventive use of these substances is not permitted, and other control strategies have to be developed.

Grazing management is the base of such a strategy; it makes use of the facts that older cattle acquire resistance to gastrointestinal nematodes and that many helminth species are host-specific. Thus, not only evasive grazing (turning out highly susceptible animals on parasite-free pastures), but also mixed grazing of first grazing-season cattle with older cattle and mixed grazing of cattle with other species are effective preventive strategies applied readily on organic farms (Thamsborg *et al.*, 1999, Hördegen *et al.*, 2005).

Additional non-chemotherapeutic strategies are currently under development. Biological control by means of nematophagous fungi is a promising element to be incorporated as a first step into a future control strategy against gastro-intestinal nematodes. Attempts to control parasitic nematodes of livestock by nematode destroying fungi have been made since the 1930s. At present, the nematophagous fungus *Duddingtonia flagrans* is the most promising biocontrol agent (Larsen, 1999). The thick-walled chlamydospores of this fungus survive passage through the gastro-intestinal tract of livestock and are capable of germinating in the faeces. There, the fungus traps larvae of parasitic nematodes, thus reducing pasture infectivity. Side-effects of *D. flagrans* on free-living nematode populations in and around treated dung pats have not been observed (Yeates *et al.*, 1997); various environmental impact studies are ongoing (Yeates *et al.*, 2003). The lack of simple and reliable application systems is a major problem to be solved before the introduction of this biocontrol agent into practical control strategies.

A second component of a non-chemical control strategy is the use of plants with anthelmintic properties. These can either be fodder plants with high contents of condensed tannins (Niezen *et al.*, 1996) or medicinal plants which are applied in schemes similar to conventional anthelmintics (Danø & Bøgh, 1999; Hördegen *et al.*, 2003).

As a last step, the therapeutic (but not the preventive) use of conventional anthelmintics is permitted with a number of restrictions (IBS and EU Reg 2092/91 at least double withholding period than required by law).

6. Concluding remarks

Organic farming emphasizes integrated strategies, rather than individual control methods, both in crop protection and animal husbandry. Biological control methods may be components of such strategies. Conservation biological control and the use of predators and parasites are favoured methods. However, non-native predators and parasites should only be used if this causes no threat to the native fauna. The use of microbial control agents is also possible, but is not favoured by the major regulations and standards. In the authors' personal view, the use of microbial control agents can be preferable to the use of plant or mineral derived pesticides, in cases in which this causes less side-effects on the environment. In contrast, the use of genetically modified biological control agents is not allowed.

Strategies for organic crop protection are available for a few crops, but are still lacking for many others. Strategies for control of diseases and parasites in organic animal husbandry are even more scarce. In conclusion, there is a need for research in organic crop protection and animal husbandry practices – including, but not limited to, biological control methods.

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CHAPTER 4

FOOD CONSUMPTION, RISK PERCEPTION AND ALTERNATIVE PRODUCTION TECHNOLOGIES

Christopher Ritson and Sharron Kuznesof

1. Introduction

Biological control is one of a number of strategies, (including mechanical control, the use of conventional pesticides, and transgenic plants genetically modified to be resistant to specific predators) available for the management of pests. According to Kogan (1998), within the integrated pest management framework, the selection and use of pest control tactics should take into account the interest of and impact on producers, society and the environment. From a societal or consumer perspective, there is significant empirical research on consumer attitudes to the use of pesticides and genetic modification in food production, but very little in relation to biological control. This chapter is concerned with the potential impact of the behaviour of food consumers on the use of biological control technology in agricultural production.

We begin by making three important distinctions. First, the word 'consumers' is sometimes rather loosely used to mean something like 'society' or 'the public'. Public opinion can impact upon production activity in a variety of ways. Concern, for example, over the environmental impact of a particular production technology, the working conditions for employees, or the quality of what is produced, can lead to pressure for change, via say a media campaign, direct protest action, or more generally via the political process and legislative control; or, the concern might influence the willingness of consumers to purchase the product of that production technology. In this chapter we consider only the latter- that is, the relationship between biological control and consumers, *as consumers of food.*

Second, in the case of food consumption, a useful distinction to make is between aspects of the production method which influences consumption because the technology is perceived to affect some feature of the quality of what is consumed; and aspects of production which lead people explicitly to purchase (or not purchase) because of a view relating to some other kind of benefit (or disbenefit) associated with the production (Wier *et al*., 2004); for example, a belief that a purchase will benefit small local producers, rather than 'big business', or that eggs have come from hens kept in cages.

Third, food consumption is about choice (Ritson and Hutchins, 1995). Thus the decision to purchase a food produced using a particular technology may be influenced by a positive attitude to that technology; or, a negative attitude to another technology associated with an alternative food product. Thus a consideration of the food consumer and biological control must be placed in the context of a broad view of the factors influencing food choice.

The chapter therefore begins with a brief overview of the factors known to influence food selection. This is followed by a more detailed consideration of the issue likely to be of most

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direct relevance to the link between new production technology and food consumption, public perception of food safety. The theory of perceived risk helps to provide a systematic explanation of those aspects of production technology likely to influence the perceived safety of the resulting food product. Although there appears to be no research specifically directed towards consumer attitudes to biological control technology, a substantial body of work has now accumulated on consumer attitudes and behaviour in the context of, respectively, genetic modification, and organic foods. An overview of this work is therefore presented, and inferences drawn on food consumption and biological control.

2. Factors affecting food choice

One way of characterising the subject matter of this chapter would be to say it is attempting to address the question of whether the use of biological control technology in agricultural production is sufficiently important to consumers to have a significant affect on food choice. Assessing the relative importance of the factors influencing food selection is, though, a complex issue. If a single food purchase decision is viewed in isolation, then no sensible meaning can be attached to the question of what factors influenced that decision, or their relative importance. To do that, some kind of comparison has to be made.

First, the decision to purchase a food product will typically involve the choice of one product rather than another. Take a very simple example, in which two food products are identical in every respect- price, location, taste, and so on, except that they are produced using different technologies. Then a conscious decision to purchase one rather than the other implies that the production technology used *is* a factor which influences food choice- though not how important a factor it is. Importance begins to have meaning when the products differ also in some other respect. For example, the preferred technology may be associated with a higher price or inferior taste. Then, the extent to which the chosen product is inferior with respect to other factors gives some indication of the importance of the production technology.

Second, food preference patterns of course differ between individuals, so another way of considering 'importance' would be if one individual chose the cheaper, but less preferred technology, and the other did not. Then, the production technology was more important as a factor influencing food choice for one consumer compared to the other.

Preference patterns can also differ over time for a particular individual, so than one product might be chosen one week, the other the next; the factors influencing food choice have changed in importance over time. Sometimes the relative importance of factors can change in a systematic and sustained manner, and involve large sections of the population. For example, Ritson and Hutchins (1991) argue that statistical analysis of National Food Survey data in the UK implies that changes in patterns of food consumption in the UK over the previous 40 or so years were first dominated by growth in incomes, subsequently by price changes, and more recently by consumer preferences for product characteristics, such as convenience and health perception; in other words the relative importance of the factors influencing food choice had changed substantially over time. At other times, the relative importance of the factors affecting food choice can change in an unpredictable and short term manner, such as change in purchasing patterns in response to food scares (Frewer & Miles, 2001).

The above discussion underlines the fact that assessing the importance of a particular factor influencing food choice must be seen in the context of other factors, and that it would be helpful to have for a context for the consideration of biological control, some broad

classification of these factors. One distinction that is sometime made is a crude division between economic factors and non-economic factors (Ritson & Petrovici, 2002). This follows the economics model of consumer behaviour in which patterns of consumption can be explained by economic factors- broadly prices and incomes, which can be measured, and consumer tastes and preferences, (which are much less easy to measure independently). 'Tastes and preferences', however is a catch-all for a very broad range of factors, and in order to place biological control in context, a sub-classification is necessary.

In addition to economics, other social science disciplines, in particular psychology, sociology and anthropology, contribute explanations of food choice. Various models of food choice emerge which, like the economics model, concentrate on food selection factors which relate to the discipline. Thus, as with the economics model, none provide a comprehensive account. To do that requires a synthesis of perspectives, such as that provided by Ritson *et al*., (1986) or Frewer *et al*., (2001). The latter attempts a comprehensive interdisciplinary account of 'the exact determinants of food perception, liking and food choice'. Drawing on specific chapters in this book, we suggest that the following capture the contribution of the various disciplines, as a classification of factors affecting food choice.

1. Economic factor, such as prices and incomes.
2. Sensory aspects of eating quality.
3. Perceptions relating to health, nutrition, and food safety.
4. Lifestyle factors, such as convenience and shelf life.
5. Perceptions relating to geographic origin of produce.
6. Beliefs associated with agricultural production methods.

Biological control clearly forms part of group six. Production technology is also likely to be a major factor in perceptions of food safety, and it is this to which we now turn.

3. Public perceptions of food safety

Over the past two decades, the perceived safety of food has had a significant impact on food purchasing decisions of consumers. Food scares have heightened the public's awareness of the safety of particular foods and many individuals change their food purchasing patterns in response to these scares (see for example Kuznesof and Brennan, 2004). At an aggregate level, these changes in behaviour are short term, usually lasting the duration of media attention devoted to the scare (Reilly and Miller, 1997). However, food scares, most notably bovine spongiform encephalopathy (BSE) and its causal link to variant Cruetsfeld Jacobs disease (vCJD) (the human form of the disease) have longer term impacts such as reduced public confidence and trust in agricultural production and food processing industries, which are the sources of the scares, and also in the regulatory authorities for their perceived inability to regulate the food supply industries and protect public health. Although many consumers are dislocated from food production processes, there is nevertheless a widespread interest in how food is produced and processed. This public interest is expressed through consumers choosing to purchase products with particular production attributes such as 'organic' or alternatively negative attributes where labelling may describe the omitted property as 'free', for example 'GM free' or 'pesticide free'. It is therefore important to examine the food safety issues that may be inherent in novel foods and food production methods to determine public acceptance.

This section will therefore examine food safety concepts and consider their application to biological control measures.

Food safety is often defined as the inverse of food risk, where food risk is defined as the probability of an adverse effect and the severity of that effect, as a consequence of a hazard in food (FAO, 1995). Hazards can be categorised as biological, chemical or physical agents 'in' or 'as a condition of' food with the potential to cause an adverse health effect (FAO, 1995). These scientifically accepted definitions, however, need to be understood from a consumer or 'lay' perspective. Public concerns about the safety of food covers a range of factors relating to the each stage of the food supply chain such as the inclusion of animal products in animal feeds, the use of hormones and chemical pesticides in animal husbandry and agronomic practices respectively, and specific issues such as genetic modification, food irradiation and providing children with a nutritionally adequate diet (Miles *et al*., 2003). Studies comparing 'expert' and 'lay' perspectives of food risks indicate an inverse relationship between expert and scientific perceptions of food risks, as shown in Table 1.

Table 1: Expert and public risks associated with food (ranked in order of importance)

Expert/Scientific	*Public*
1. Microbial contamination	1. Food additives
2. Nutritional imbalance	2. Pesticide residues
3. Environmental concerns	3. Environmental concerns
4. Natural toxicants	4. Nutritional imbalance
5. Pesticide residues	5. Microbial contamination
6. Food additives	6. Natural toxicants

Source: Smith, 1997.

Comparing the number of deaths in the UK attributable to food consumption as shown in table 2, indicates that expert perceptions of risk more accurately reflect 'real' food risks than public perceptions of food risks. Insights into public perceptions of food risks can be derived from psychological and behavioural theories of risk, and these are discussed in the following section.

Table 2: UK deaths per year related to diet and food

Risk	*Number of Deaths*
Cardiovascular disease*	73,000
Cancer*	34,000
Food borne illnesses (estimated)	50
Food allergy	20
vCJD**	15
Genetically modified organisms, pesticides, growth hormones	0

Source: Krebs, 2000

* assumes one third and one quarter of total CHD and cancers respectively, are diet related.

** vCJD, or variant Cruetsfeld Jacobs disease is commonly known as the human form of BSE (bovine spongiform encephalopathy or 'mad cow disease'

4. Theories of perceived risk

Psychological and behavioural theories of risk provide a framework to enable informed hypotheses to be made about how consumers may characterise and perceive the risks in biological control measures. These theories will be discussed and then applied to two contrasting case studies, namely GM foods and organic foods.

4.1. Psychological theories of perceived risk

Risk perceptions are 'socially constructed' or shaped by the attitudes and behaviours of individuals within a particular social and cultural environment. The way individuals respond to risk is driven by their beliefs and perceptions and not by scientifically-based technical risk estimated of experts (Frewer, 1999). Within social psychology, substantial research has been undertaken to help understand the factors affecting the public's perceptions of risk and the context in which they are created (see for example Starr, 1969; Slovic, 1987, 1992; Fischoff, 1995). Research that seeks to explain why some risks invoke more alarm, outrage, anxiety or dread than others regardless of scientific estimates of their seriousness are referred to as the 'psychometric paradigm'. Table 4, provides a summary of these key 'risk amplification' factors, many of which are often referred to as 'dread' or 'fright' factors. These are factors that are believed to pose a greater threat than technical risk estimates would suggest. The table also lists 'risk attenuation' or 'comfort' factors that reduce perceptions of risk. Although these factors are not predictive, they can give an indication of how individuals may react to a particular perceived hazard.

Table 3: Risk amplification and risk attenuation factors

Risk Amplification Factors	*Risk Attenuation Factors*
• Risk is involuntary	• Risk is voluntary
• Third party control	• Individual control
• Inequitable	• Equitable
• Inescapable	• Avoidable
• Unfamiliar or novel	• Familiar
• Man-made	• Natural
• Effects unknown	• Effects known
• Long term effects	• Short term effects
• Irreversible damage	• Damage is reversible
• Danger to vulnerable groups or future generations	• Population equally affected
• Risk poorly understood by scientists	• Well understood by scientists
• Contradictory statements from responsible sources	• Consistent statements from responsible sources

Source: Bennett (1999)

Table 4 provides some explanation for seemingly irrational and contradictory attitudes individuals may be believed to hold with respect to food risks. For example, despite there being no deaths attributed to the consumption of GM foods, some consumers are alarmed by the foods because they believe the production process is unnatural, they are involuntarily exposed to the (perceived) risks, they can not control their consumption of GM foods (particularly in the absence of labelling to enable consumers to make choices in their food purchases), and they believe it may cause irreversible environmental damage over a sustained period of time. As a basis of comparison, the often quoted corollary to this is cigarette smoking. An estimated 114,000 deaths are attributed to cigarette smoking in the UK per annum (Peto *et al*., 1994). However, many people accept the risks of cigarette smoking because (addiction aside), the risk is voluntary, people can choose to smoke, smoking is a familiar passtime and the (negative) effects are known, i.e., people consider they have a degree of 'control' in their smoking habit. If the above risk characteristics are applied to biological control methods as shown in Table 5, it may be hypothesised that techniques will be seen as largely natural and with short term effects. Both these characteristics are 'comfort' factors and biological control methods may be perceived as less risky than conventional chemical pest control counterparts or novel technologies such as genetic engineering.

Table 4: Risk characteristics of different types of biological control

Biological Control Mechanism as defined by Eilenberg et al., 2001	*Risk Characteristics*
Classical Biological Control	
The intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control	familiar natural
Inoculation Biological Control	
The intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently.	familiar natural effects known short to medium term
Inundation Biological Control	
The use of living organisms to control pests when control is achieved exclusively by the released organisms themselves.	natural effects known short term
Conservation Biological Control	
Modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests.	natural effects known

4.2. Behavioural Theory of Perceived Risk

Behavioural theories of perceived risk complement psychological theories by examining risk from the perspective of individual purchasing decisions. All forms of consumer behaviour have been described as 'risk-taking' behaviour to the extent that the consequences of any purchasing or consumption action can not be foreseen with complete certainty (Bauer, 1967). Seven types of 'risks' or 'losses' have been associated with the processes of purchasing, consuming and disposing of products. These 'risks' or losses are i) physical or safety risk such that the product may cause potential harm, ii) performance risk such that the product does not live up to prior expectations, iii) social risk such that the product may potentially harm social standing, iv) psychological risk such that the product reflects negative self-concept, v) financial risk such that money spent on the product is wasted, vi) time risk such that time spent considering, purchasing and consuming the product may have been wasted and vii) opportunity loss, in terms of the opportunities missed in the situation of a product failing to meet expectations.

Research indicates that individuals' perceptions of risk and their subsequent purchasing behaviour are causally linked, with risk perceptions an important explanatory variable of

purchasing behaviour (Eom, 1994; Huang, 1993; Mitchell & Greatortex, 1990). However, as with all forms of consumer behaviour, risk is temporal, being associated with a particular product, unique to a particular person at a particular point in time. As demonstrated earlier, many routinised food purchases have been disrupted following a food scare.

There are many individual differences in approaches to food safety. Some individuals exhibit both risk-averse and risk-seeking behaviour across a wide variety of situations. Consumers have inherent predispositions to seek or avoid risk in purchase situations (Dowling, 1986). Depending upon an individual's tolerance to risk, there may be situations under conditions of boredom, curiosity or variety seeking, where a less 'risky' product may be rejected in favour of a more 'risky' product. To the extent that consumers may meaningfully purchase products with respect to perceived riskiness, trade-offs between product purchases can be made according to the benefits sought.

Consumers perceptions of risk therefore stimulate information search and risk handling. When faced with a potentially risky purchasing decision, consumers may attempt to reduce the risk involved by developing strategies to reduce perceptions of risk and enable them to act with relative confidence in uncertain situations. Four generic strategies to resolve or reduce perceived risk include (Roselius, 1971):

1. Reduce the perceived uncertainty about the product, or reduce the severity of real or imagined loss suffered if the product does fail;
2. shift from one type of perceived loss towards one for which there is more tolerance;
3. postpone the purchase;
4. make the purchase and absorb the unresolved risk.

Risk relieving strategies can also be initiated by sellers, and these include adopting quality assurance schemes, labelling and providing product information (for further examples, see Yeung & Morris, 2001). From the perspective of consumers selecting foods produced using biological control measures, final product choice will depend upon a number of factors and the degree to which these factors match consumer expectations.

5. Case study 1: Public perceptions of GM foods

From a strategic perspective, biotechnology is regarded as 'one of the most promising frontier technologies for the coming decades' (CEC, 2002). Defined as 'the application of biological organisms, systems and processes based on scientific and engineering principles, to the production of goods and services for the benefit of man' (Bull *et al*., 1982), biotechnology incorporates within its definition the socially sensitive 'gene technology'. Gene technology has been described as 'the manipulation of an organism's hereditary material using artificial techniques with the aim of incorporating or deleting specific characteristics into or from the organism' (CEGMFU, 1993). The science is potentially pervasive in that it has applications in agricultural and food production, medical and environmental spheres. Gene technology is also a science-driven (rather than market-let) technology, a 'man-made' means of production enabling the transfer of genetic material across species boundaries, a phenomenon that would not occur in nature. In addition to being novel, the technology is also complex. As an enabling technology, genetically modified (GM) agricultural and food products can be classified as a

GM food, GM ingredient, GM-derived ingredient, GM processing aid or GM ingredient in animal feed. Understanding the technology, its applications and output can be difficult to comprehend. However, to fulfill its strategic potential, broad public support of gene technology is regarded as essential (CEC, 2002; AEBC, 2003).

There is a large and growing body of research on public attitudes towards gene technology and genetically modified foods. When the technology was in its commercial infancy in the early 1990's, it was estimated that nearly 70,000 people worldwide had been asked their opinion about biotechnology and gene technology (Zechendorf, 1994) and in an environment of little or no product knowledge (Tait, 1994). Although awareness of gene technology was low in the early 1990's, it has increased in the intervening decade. This increase has however, not improved perceptions of the technology and European consumers remained negatively predisposed towards the gene technology and GM foods (Bredahl, 1999; Gaskell, 2003; INRA, 2000).

Qualitative focus group research undertaken by Kuznesof and Ritson (1996) suggests that there are three types of potential consumers of GM foods. First, are the 'refusers', who reject the technology on moral and animal welfare grounds and indicate they would not purchase products of the technology. This category although in the minority represents individuals whose purchasing decisions are influenced by production method. Their attitudes are closely related to personal value systems and are firmly held. This finding can be further explained by the 'top down' and 'bottom-up' processes of attitude formation which have been explored by Scholderer & Frewer (2003) in relation to GM foods. The top-down process of attitude formation suggests that attitudes are formed based upon 'a system of general attitudes and values' (Scholderer & Frewer, 2003). This 'general attitudes' function guides the way in which individuals develop attitudes to novel objects. One implication of this is that attempts to change attitudes through the provision of information are likely to fail. In fact for this group of people, the provision of information is likely to strengthen negative attitudes (Scholderer & Frewer, 2003).

The second category of consumer, of equal size to the 'refusers', was labelled 'triers'. Two groups of consumer within this category were identified. 'Enthusiastic triers' were positively predisposed towards the technology and were interested in sampling genetically modified foods and judging its merits based upon product trial. The second group, the 'traditional triers' were typified by consumers with low disposable incomes and for whom price was a major factor in food purchasing decision-making process. Thus in a situation where GM foods were cheaper than conventionally produced counterparts, the GM food offering would most probably be purchased.

The third category of 'undecided' consumers represented the majority view. For the members of this group the decision to accept or reject GM foods was dependent upon a variety of factors. For example, the perceived beneficiaries of the technology were important. Benefits to the consumer were viewed as more acceptable than producer benefits (Kuznesof & Ritson, 1996; Frewer *et al*., 1996), remote societal benefits are not found to be important promoters of acceptance (Grunert *et al*., 2001). There is also a 'scale of acceptance' related to the product being modified (Hamstra, 1993). In descending order of acceptability, the modification of fruits and vegetables is more acceptable than fish, poultry and red meat (Sparks & Shepherd, 1994; Kuznesof & Ritson, 1996; Frewer *et al*., 1997; Saba *et al*., 1998). The nature of the gene transfer was also found to be important with interspecies transfer of genes more acceptable than intraspecies transfer (Kuznesof & Ritson, 1996). Although gene

technology was viewed as the antithesis of 'organic foods', organic foods being perceived as 'natural' and 'wholesome' and GM foods as 'unnatural' and 'unethical', GM foods were perceived as more acceptable than foods produced using 'chemicals'. Thus many factors are likely to influence the decision to purchase or not purchase GM foods.

For many 'undecided' consumers, attitudes to GM foods can be assumed to be based upon a 'bottom-up' process of attitude formation. In this situation, attitudes towards an object are based upon knowledge about the object and its perceived characteristics. Knowledge is based upon 'information' and 'experience', where 'own-experience' is believed to have a stronger impact on attitude than information. Although few European consumers have had direct experience of GM food, research by Grove-White et al, (1997) identified that in the absence of knowledge and information about gene technology and GM foods, the public turn to related frames of reference or 'conceptual templates' in forming attitudes. In the UK, the commercialisation of GM foods coincided with the public inquiry into the BSE food scare. Undermined public confidence and trust in the Government at that time were well-documented (see for example Frewer & Shepherd, 1994, Frewer *et al*., 1996, Marlier 1992, INRA, 1993, 1998) and still exist (INRA, 2000; Gaskell *et al*., 2003). BSE is still used as a 'conceptual template' during discussions about GM foods and of regulatory capabilities in the face of uncertainty and incomplete information (see for example Frewer *et al*., 2001, AEBC, 2003).

Issues of the perceived risk characteristic 'control' of GM foods are intertwined with governmental and regulatory trust. Trust in government and industry is an important determinant of attitudes towards gene technology (Frewer & Shepherd, 1994). One problem arising from lack of trust is that control of the technology is seen at the level of society (rather than within the scope of the individual to control) and therefore, determined primarily by science and government. With control at this 'third party' societal level, it is viewed as important that a regulatory framework for GM that can inspire confidence is in place to protect consumers and the environment (AEBC, 2003).

A number of issues arise out of this case study, which have relevance to consumer perceptions of biological control. Consumers are a heterogeneous collection of individuals with different values, experiences, attitudes and perceptions and these differences will be reflected in their food purchasing selection decisions. Food production method can be influential in food purchasing decisions. Where 'control' of the technology is viewed as outwith the level of the individual, instead at the level of society, trust and confidence in the regulatory processes governing the technology will be important in determining broader public acceptance.

6. Case study 2: Public perceptions of organic foods

The reason for considering consumer attitudes and behaviour with respect to organic products (sometimes known as Ecological or Bio Products) is similar to that for GM products- that certain aspects of biological control technology may trigger consumer responses consistent with those known to be associated with organic agriculture. There are, though, important differences which allow the messages that may be derived from the second case study to complement those from the first.

Typically, the GM food product differs from the non-GM product only in whether GM technology has been involved in its production. The same applies when considering the implications of the use of a biological control technique. Of course the technique may have

some impact on – say- product quality or appearance, which may influence consumers; but the distinction is clear cut. Will the use of the technology have any impact on a consumer choosing to buy a product which uses the technology compared to one which does not?

In contrast, an organic product is a bundle of attributes. Lampkin and Measures (2001) describe organic farming as:

> 'an approach to agriculture where the aim is to create integrated, humane, environmentally and economically sustainable agricultural production systems. Maximum reliance is placed on locally or farm-derived, renewable resources and the management of self-regulating ecological and biological processes and interactions in order to provide acceptable levels of crop, livestock and human nutrition, protection from pests and diseases, and an appropriate return to the human and other resources employed. Reliance on external inputs, whether chemical or organic, is reduced as far as possible.'

All of this is backed up by a complex, and certified, set of rules relating to farm production, and to some extent food processing. Thus the consumer of an organic product buys a package and is not in a position to choose a variety of different 'quantities' of organic product attributes, which might indicate the most important features of the package. But if we combine two pieces of evidence we can infer important messages for the use of biological control technology.

First, 2000 consumers in Germany were asked what they most associated with Bio (organic) products. The various responses are shown in Table 6, the responses ranked from most frequently mentioned association.

Table 5: Association with the stimulus 'bio-products'

Association
1. Without chemicals
2. Natural products
3. Without artificial fertiliser
4. 'Biological' farming
5. Healthy
6. 'Ecological' farming
7. Caring animal husbandry
8. Not sprayed
9. Environmentally friendly
10. Expensive
11. No pesticides
12. Controlled farming
13. Not containing noxious agents
14. Not genetically modified
15. Natural manure
16. Free range animals
17. Negative associations

Source: Alvenslaben, 2000

Second, in a survey of 1000 British consumers, respondents were asked 'how worried' they were about a series of potential food safety issues previously identified from focus groups as things which concerned consumers about food consumption. In Table 7 the 'worries' are now ranked from most to least worried (percentage of the sample which said they were either highly or extremely worried).

Table 6: UK public concerns about food

Concern
1. The use of hormones in animal production
2. The use of antibiotics in food production
3. The use of pesticides in food production
4. Animal welfare standards in food production
5. Eating genetically modified food
6. Safety of meat products produced by intensive farming methods
7. The use of additives in food
8. Quality of food using intensive farming methods
9. Conflicting information on food safety
10. Lack of information about food from Government
11. Hygiene standards in the food industry
12. Hygiene standards in restaurants and take-aways
13. Being able to afford good quality food
14. Amount of fat in your diet
15. Information about what foods are good for you keeps changing
16. Knowing what to do when there is a food scare
17. Getting food poisoning
18. Hygiene standards in your home

Source: Miles et al., 2003.

The striking observation is that many of features of food consumption which seem to cause most concern to consumers – pesticides, hormones, antibiotics, additives, intensive farming and poor animal welfare- represent negative characteristics thought to be absent from organic products (without chemicals, without artificial fertilisers, no pesticides, not sprayed, caring animal husbandry.) Thus, without doubt, the major positive feature of a food product which has been produced using biological control technology is that it may allow the product to share an element of the organic 'without' package.

These valued 'without' characteristics of organic products have two dimensions. First they are associated with a better quality and safer product; second a more 'environmentally friendly' production system. Wier *et al.* (2004) describe these as 'use' and 'non-use' values. From analysis of Danish consumer panel data, they conclude that although consumers recognise the merits of the non-use values of organic products, belief in the value of these attributes does not appear to explain a greater tenancy to purchase organic products. In contrast 'We find that household propensity to purchase organic foods increases significantly with the household's stated importance of private good attributes' (use values).

This confirms a number of studies which indicate that consumers say they primarily buy organic foods because of health considerations. A second conclusion follows- that it is the capacity of biological control to allow a food product to be perceived by consumers as 'more healthy' than products which have used chemical control that is likely to be the most significant positive consumer attribute.

What might be described as the 'positive side' of the 'without' attributes is the common association of organic with 'natural'. Interestingly, there do not appear to be any strong associations with specific 'organic approved' production methods- it is just a general view- biological/ecological/controlled/animal friendly farming.

This raises the issue as to whether being explicit about – say – that parasitoids and predators had been deliberately released into glasshouses, would be regarded as 'natural production', and we know of know research which has explored this issue.

We have described organic products as a package. Sometimes consumers perceive the package to contain attributes which lie outside organic rules. In particular, values such as locally produced and small scale production are associated with organic; that is, even if consumers do not necessarily believe that all organic produce possesses these attributes, we find that most organic consumers are also the people who value these attributes in their patterns of food purchase. (Wetherell *et al*., 2003).

'Organic' has been described as a very successful food 'brand'. Consumers recognise it, know (or think they know) what they are buying, and the purchase of an organic product will almost always be a deliberate, positive, choice. This leads on to the issue of communication in the case of a product produced using biological control technology, but not marketed as organic. Three cases can be distinguished.

a) If on balance the production technology has potential negative associations for consumers, but can supply produce identical (in consumer terms) to that produced by modern conventional technology, then communication will be avoided; the issue of consumer acceptability only emerges if (like GM foods) media attention forces the issue into the open.

b) If however, the technology has positive associations, this has to be communicated ('produced with minimum use of chemicals') if consumers are to be influenced into buying in preference to conventionally produced produce.

c) The technology may, though, be associated (as with organic) with higher cost production, and this leads to the fundamental question of to what extent consumers will be willing to trade-off the positive association with the negative one of higher cost.

Table 8 shows the price premiums for organic produce averaged across EU member states. These premiums are though very sensitive to quantity supplied and there are recent examples of severe 'erosion' of price premiums and organic produce being diverted into conventional marketing channels.. Thus consumers vary greatly in the extent to which they are willing to pay a premium. Research in Spain indicated that consumers were willing to pay a premium which varied from 5-10% to 50-60%; and that the proportion of consumers who would buy organic varied as a consequence from 5% to 90% (Soler *et al*., 2002). Similarly, in Denmark, researchers found that a change in the price premium of 10 percentage points might influence the organic market share by between 2 and 5 percentage points.

Table 7: Farmer and consumer price premiums for organic products, 2000

Farmer Price Premiums (%)		*Consumer Price Premiums (%)*	
Cereals	102	Bread	61
Potatoes	257	Potatoes	91
Milk	22	Milk	39
Beef	34	Steak	40
Sheep	43	Apples	45
Pork	69	Carrots	51
Poultry	182	Chicken	113
Eggs	167	Yoghurt	69

Source: Hamm et al,2003

There are two messages for biological control technology. First, positive consumer associations must be communicated if production costs imply price premiums over conventional technology. Second, it is not possible to be specific concerning the extent to which higher production costs could be recouped from the market, because of the sensitivity of price premiums to supply balances.

7. Inferences from public food risk perception for biological control

The major attribute of biological control technology from a consumer perspective is the capacity it provides for food products to be supplied without the negative perceived attributes of production technology involving the use of chemical control of pests and diseases. As a production technology that may be perceived as 'natural' with 'short-term' consequences, biological control may be hypothesised to have low perceived risk characteristics. If the technology is, however, associated with other negative consumer attributes, such as higher price, or inferior appearance, the fact that the product has been produced using biological control technology must be communicated to consumers.

This raises the problem of trust - can the technology be incorporated into farm assurance schemes or retailers provide their own assurance? There is evidence that, even with organic, consumers may doubt the authenticity of labelled produce.

In the context of the theory of perceived risk, biological control, in contrast to chemical control or genetic modification, appears to map quite well on the food product characteristics associated with risk attenuation, rather than risk amplification. However, the main threat to sustained adoption of a particular technique is if it should acquire an aura of being 'unnatural'- the negative association of 'man playing with nature'. The other potential consumer related impediment to sustained application and development of biological control is if a particular technique should be linked to an outbreak of food borne disease leading to a 'food scare'.

Clearly, consumers can determine the success, failure or impede the diffusion of novel technologies, as in the case of GM foods. However, they will also make food purchasing decisions according to a number of often competing criteria. Thus, although a novel technology is 'acceptable' to consumers, acceptance does not automatically equate to purchase. This chapter therefore, implicitly raises the need to research consumer perceptions of the use of biological control in food production.

8. Notes

1 In a study of Californian residents' preferences for pest control, of three pest management options presented, namely i)chemical pesticide, ii) biorational insecticide and iii) the introduction of a natural enemy, the latter was the preferred choice (Jetter and Paine, 2004). Although the authors did not speculate as to the reason for their respondents stated preferences, the degree of 'naturalness' of the 'natural enemy' option implicit in the research design may provide some explanation.

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CHAPTER 5

EDUCATION IN BIOLOGICAL CONTROL AT THE UNIVERSITY LEVEL AT KVL

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1. Competence

Why should we be concerned about education in biological control? It can be argued that most people working with this subject (scientists, extension officers etc.) do not need a particular education, but need solely a strong background in one discipline relevant for their particular approach. For example, scientists can have a background in applied entomology, plant pathology, microbial fermentation or legislation.

At many universities worldwide biological control is one among other elements to be taught at courses in applied entomology, plant pathology or weed control. Students are provided with an overview, for example by having a lecture or two on the subject. Such overview lectures are mostly closely related to the application of biological control and can be excellent introductions to the subject. Such introductory lectures will potentially stimulate students to learn much more in depth and thus to obtain real qualifications in biological control.

We believe that education at the university level in biological control has not yet reached its potential, but should be devoted much more attention as a subject in its own right. Students should get a chance not only to get a brief overview, but they should be able to understand fully the concept and practical possibilities. Also, we believe that the strict separation between biological control of pest insects, plant diseases and weeds is a hindrance for future scientists and other people involved in the protection of plants and husbandry, to develop a broad perspective on biological control. Therefore, we suggest that education in biological control should be based on a strong, broad view, and that this education should include as much as possible biological control of both pest insects (and other invertebrates), plant diseases and weeds. Education in biological control must be closely linked to the needs of the end-users, but should also include fundamental aspects.

At the Royal Veterinary and Agricultural University (KVL) in Denmark, overview lectures on biological control have been given for many years. Since 1988 our student have had the opportunity to choose courses devoted solely to biological control and thus to obtain defined competences in biological control. The first course was a laboratory course in biological control of insects, later a laboratory course in biological control of plant diseases and a theoretical lecture course in biological control of insect pests, plant diseases and weeds were added. The following describes the most important experience we have obtained over these years by having laboratory and lecture courses.

J. Eilenberg and H.M.T. Hokkanen (eds.),An Ecological and Societal Approach to Biological Control, 65–71.

Our aim is to develop an education scenario based on an analysis and description of the competences to be obtained by the participants. In other words: which kind of problems should the students be able to solve after participating in a KVL course in biological control?

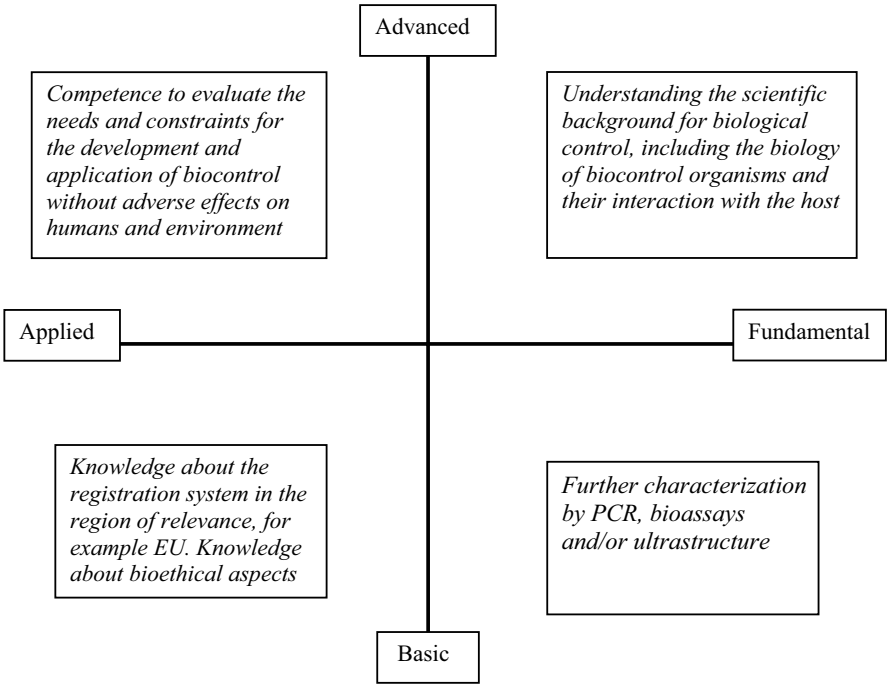

Figure 1: Competences obtained by participants in courses at KVL in biological control. See text for further explanation

On figure 1 is shown the main competences to be expected from a student who has passed our biocontrol courses. The figure is an example on the KVL implementation of the ideas of the sociologists Pierre Bourdieu and Emile Durkheim (Høyen, 2003). All course responsibles at KVL must nowadays describe the competences obtained by participants. Based on the diagram teachers are able to implement lectures, exercises, discussion and other activities, which together ensure that the competences are actually obtained.

The upper left corner on fig. 1 gives information about the most significant competences obtained in our courses in biological control. The competences are acquired at an advanced level and with the focus on applied aspects, since biological control should relate to applied problems of real significance to man. Our aim is thus to ensure that students can analyse the needs and constraints for the development of biological control. Further, students should be able to do this with sufficient ecological care and without adverse effects on man (producers, end users, and consumers). These qualifications must be obtained by advanced lectures detailing scientific problems, student analysis of primary scientific literature, in both cases with

discussions among the participants and teachers. Also, students should on their own seek information about practical experiences with selected biocontrol agents.

The competences should also be based on experimental work in the laboratory. Such laboratory work should at the best be a progression of a project rather than a series of prefabricated exercises. Students should develop their own experimental approach using their supervisor as consultant.

The upper right corner gives information about competences at a high level, although more fundamental. Students must understand the basic biological interactions between for example target insect and predator or plant disease and antagonist. They must acquire the elementary glossary on population ecology, infection processes and other subjects of particular relevance for biological control. In our courses in biological control, these elements are integral parts of lectures and student group discussions.

In the lower left corner are shown elements, which are parts of the education in biological control, but at lower levels. Students are expected to have some level of knowledge about these elements but not sufficient to analyse complex situations. For example, a lecture will provide students with information about the registration system for microbial control agents. Students will learn about the status in EU (or elsewhere), but are not expected to have a competence in EU legislation.

Finally the lower right shows some additional benefits for students attending our courses in biological control. They learn at a fundamental level about biological characters of some major taxonomic groups of biocontrol agents, and they get experience about the correct behaviour in a laboratory when performing scientific studies. The latter element can be regarded as general and can of course be obtained in other courses not related to biological control. Yet, experimental work in biological control will add to the total student competence in laboratory work and how to progress.

2. The student's background

Our courses are held in English and are attended by students from all parts of the world. This gives some additional challenges. First, it can be hard, if not impossible, to check the level of each applicant student. We normally recommend that a student should have passed courses in applied entomology, plant pathology and/or microbiology. In reality, however, students from foreign universities have various backgrounds with more or less emphasis on elements we regard as important. This is not necessarily a problem and we have experienced that it can sometimes be regarded as an advantage that students have complementary skills when starting.

Depending on the region of origin for a student, they know specific insect pests and plant diseases. Since our courses aim to cover general aspects and not region-specific problems, we advise often the students to pay attention to the general aspects and not species-specific aspects. Biological control of aphids, for example, has something in common worldwide, irrespective of the specific aphid species in focus.

Cultural differences among students do also exist. Some students are already familiar with group discussions and mutual analysis of problems (in general or for example through specific methods like '*problem based learning'*) while others have no such experience. To challenge the cultural differences the teachers are active and often decisive in the formation of student teams to ensure a sufficient mixture in each case.

3. The conceptual framework

Many textbooks on biological control do not provide the reader with a conceptual framework. This is urgently needed before starting any course in biological control. We need to define, how we understand biological control and which elements are parts of or are not parts of biological control. We need as much as possible to homogenize terms and to understand discipline specific terms, for example terms used in plant pathology while not in entomology.

For this purpose we use the first session in our theoretical course to discuss the conceptual framework with the students. They need all to understand exactly what is biological control and what is not. Table 1 is a list of terms we give the students. In groups of four to six the students are asked to organize these terms by cutting and pasting (by paper and tape or by computer). First, they should define what *biological control* is part of, namely *integrated control*. They should learn that biological control and *biocontrol* are synonymous. Then, they should find the core elements included in biological control, for example organisms like *parasitoids* (used in entomology) or terms like *suppressive soils* (used in plant pathology). Then, they should discuss and define how biological control is related to terms like *organic farming* and *risk assessment*. Last, but not least, they should learn which terms are not at all defined in relation to biological control but merely reflects a vision, for example the term *environmentally friendly control*. It is on purpose that one or more squares are left blank. Some students may find that some terms are missing and can suggest these to be added. Student put up their solutions on cardboard posters or they upload files on the Internet. Each team presents their solutions to the other teams and to the teachers, the suggestions are discussed, and a consensus is decided.

Table 1:

Students exercise to learn about terms of relevance for biological control. The students get an unorganised list of the terms. Groups of students must then organise the terms in order to clarify the definitions and their relationships

Biological control	Biocontrol	Environmentally friendly control	Genetically modified organisms
Induced resistance	Microbial inoculants	Antagonists	Non chemical control
Bacteria	Virus	*Bacillus thuringiensis*	Predators
Fusarium	Protozoa	*Pseudomonas*	Integrated control
Parasitoids	Antibiosis		*Trichoderma*
Sterile males	Crop rotation	Suppressive soils	Fungi
Natural control	Organic farming	Nematodes	Risk assessment

We find this exercise extremely useful. Each year, lively discussions take place. For example, we spend time to discuss, why biological control is not always environmentally friendly. We also spend time to clarify, that biological control is *per se* not a subset of organic farming but can be used in all types of farming systems. Finally it is challenging to discuss with the student that biological control is not at all excluding the use of GMOs. Based on these

discussions all students understand the necessary conceptual framework, they use the terms the same way, and they can analyse primary literature much better.

4. Student progression in experimental work

As mentioned our aim is to allow each team of students to obtain qualifications in the progression of experimental work. The process is illustrated on fig 2, using insect pathogenic fungi (the genera *Beauveria*, *Metarhizium* or *Paecilomyces* as an example. The principle is that a group of students starts with field sampling in order to obtain some novel isolates of these fungi. The students thus learn about sampling and diagnostics of insect pathogens. Selected fungi are isolates *in vitro* and used for experimental work. The students characterize the fungi by classical morphological methods, using microscopes. The group then decides with their supervisor how to progress. Should they go for PCR characterization? Should they perform infection experiments like dose response relationships? Should they study the behaviour of the target host in relation to the fungus? Should they study autodissemination? Should they perform one replicate of several types of experiments, or should they focus on very few types of experiments, but with more replicates?

The students must throughout the course perform experiments, evaluate, analyse and take decisions about the next experiments to be done. This is often not easy, and students need guidance and support, yet still allowing the progress to be decided by the students, the supervisor rather being a consultant. The final report should include an analysis of own work and the perspective of the tested fungal isolates. The fact that the fungi used by the team are 'their own' isolates never studied before is of major benefit. The students learn really how to work with biological control from nature (or cropping system) to laboratory and back to nature (or cropping system) again.

The example on fig 2 is related to insect pathogenic fungi and the ecological cycle of such organisms. The subjects of student teams have, however, covered a very broad range of organisms: Bacteria (*Bacillus thuringiensis*), predators (*Orius*, *Anthochoris* etc), parasitoids, and nematodes (*Steinernema*). The approaches have also different: student teams have focussed on behavioural aspects, morphology, bio-assays or genetically characterization. Some student teams have been involved in quality control experiments in co-operation with biocontrol companies. Concerning student groups involved with experimental work in biological control of plant diseases they will focus on selected problems, for example the efficacy of *Clonostachys* to control leaf spot.

The balance between elements planned by the teachers beforehand and decisions taken by the students as part of their progression is crucial. Obviously, some elements must be ready before the course starts: some insects, some plants, some biocontrol agents, some petri dishes, and some description of methods. The students should, however, be encouraged to be innovative and develop their own ideas and ask for additional support by the teachers. For example, we can add electron microscopy if wished by a group, but the students must define first why they want this element added. For example SEM can be a nice tool to study the mandibles of predators and by this students obtain a deeper understanding about attack and handling rates of the organism studied.

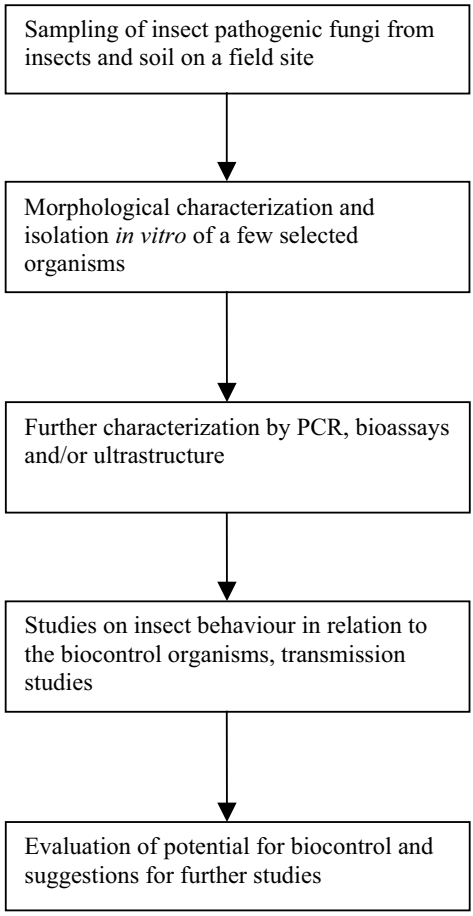

Figure 2:

An example of progression of a group of students performing experimental work in biological control

All in all, the student work tends to be as scientific as possible under the circumstances given. The examination reflects this. Students present their findings in a short and concise report (Student reports 1988-2004), a proceeding manus similar to the style used in IOBC, or as a poster. In all cases they present and discuss their results with the other teams and the supervisors.

Obviously this sort of student work in the laboratory has some drawbacks. Each team of students will only get the chance to work with a very limited number of organisms and with a

limited number of methods. We feel, however, that student can easily extrapolate and learn species-specific methods afterwards, when needed. It is more important that they have obtained qualification in biological experimental work related to biological control and have a realistic idea how such progression takes place. Based on this they have competence to analyze realistically the potential of new biocontrol agents.

5. The future: internet based teaching or 'hands on'?

In 2004, we tried for the first time to incorporate elements in our lecture course as e-learning. The Internet gives new challenges to education in biological control. We see e-learning as particularly useful for education in biological control.

First, biological control is not solely a biological discipline but includes political and ethical aspects. Such aspects can be presented and discussed on Internet conferences among student from different parts of the world, since such principles are universal. An example from autumn 2004 was an exercise devoted to *visions and limitations* of biological control. The activity was set up as a web-conference for students and teachers. The students were located home or at computers at KVL and were asked to suggest visions and limitations on a special set up designed for this purpose. The suggestions were grouped and discussed, by use of the web.

These novel possibilities will be incorporated in our courses in the future. These new aspects are obviously needed in the future world of seeking information on the web and communicating by use of the web as well. We feel although that 'hands on' in the laboratory, will still be essential for obtaining competences of high value. Also, we feel that face-to-face discussions are important and will stay important.

Acknowledgements

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CHAPTER 6

IMPLEMENTATION OF BIOCONTROL AND IPM IN DUTCH HORTICULTURE

Abco J. De Buck and Ellen A.M. Beerling

A socio-economic and technical innovation process

1. Introduction

The application of biocontrol in Dutch glasshouses has increased tremendously from its rediscovery in the 1960's up to now. In the last decade, the number of different natural enemies sold to Dutch growers increased from 7 in 1992 to 26 in 2001 (LTO Nederland, vakgroep Glastuinbouw, 2003). Integrated pest management (IPM) is practised on a large scale in all main vegetable crops. At the end of the millennium more than 90% of all tomatoes, cucumbers and sweet peppers were produced under IPM in The Netherlands (Van Lenteren, 2000). Also the area of glasshouse ornamentals grown under IPM increased. In 1998 biocontrol was applied in more than 10% of the Dutch ornamental crops (Van Lenteren, 2000). This increase is mainly accounted for by gerberas, roses, orchids and potted plants (LTO Nederland, vakgroep Glastuinbouw, 2003). According to Van Lenteren (2000), natural enemies were released on 78% of the area down to gerberas.

The expansion of glasshouse area on which biocontrol is applied has, however, now come to a halt. In some crops, like gerbera, the number of biocontrol species released has even declined seriously. In general growers mention the following reasons for discontinuing biocontrol: disappointing results with natural enemies, new pesticides which made biocontrol 'unnecessary', the lack of selective pesticides against new pests and the restriction of other selective pesticides.

The Dutch government aims to make crop protection more sustainable: by 2010 the environmental 'burden' should be reduced by 95% when compared to 1998 (Dutch Ministry of Agriculture, Nature and Food Quality, 2004). The government regards IPM and the application of biocontrol as the approach to achieve this reduction and has taken on the responsibility to ascertain knowledge on IPM and how it is developed and implemented (Dutch Ministry of Agriculture, Nature and Food Quality, 2004).

The flow of (new) knowledge from research to grower is one of the main concerns of the Ministry of Agriculture. Traditionally, co-operation between Research, Extension and Education took care of the development and implementation of knowledge. This so-called triptych (Figure 1) had been very successful in improving productivity of plant production in the periods of re-construction, mechanisation and computerisation (Table 1; Van Doesburg *et al.*, 1999; Buurma, 2001). Trading via co-operative auctions encouraged collaboration, which manifested itself in the formation of horticultural study groups. These study groups played an

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important role in spreading horticultural knowledge and were an invaluable link between the triptych and the individual grower. The knowledge exchange between growers is regarded as one of the main reasons for the leading position of Dutch Horticulture in the past and present (van Doesburg *et al.,* 1999).

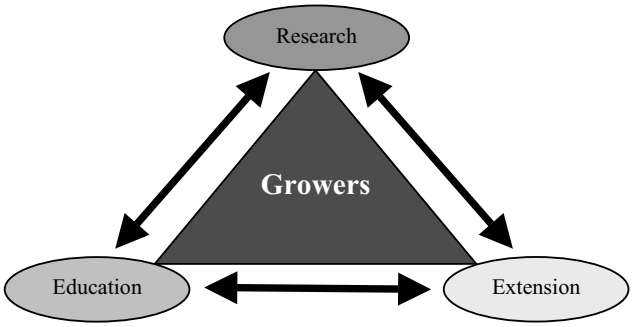

Figure 1: The triptych of research, extension and education

Table 1: Major developments after World War II in glasshouse horticulture in The Netherlands; their characteristics and corresponding knowledge system (adapted from: Van Doesburg et al., 1999)

Period	**Revolution**	**Characteristics**	**Knowledge system**
1946 - 1965	reconstruction	horticultural study groups, chemical control of pests and diseases, cultivars, growth control	'triptych'
1965 - 1980	mechanisation	glasshouse constructions, labour efficiency, heating, concept of IPM	
1980 - 1993	computerisation	application climate control, artificial substrate, CO_2	
1993 - 2000	chain reversion	product quality, quality assurance, new producer organisations	'mobilisation of stakeholders'
2000 - present	sustainability	emission, recycling, CO_2 balance, regional functions	

However, the situation changed in the nineties - the era of chain reversion - when the organisation of transactions involving agricultural products underwent a shift from production orientation to market orientation (Table 1). Trading via co-operative auctions became less important in favour of quality assurance programmes, set up by supermarket chains and apparent in contractual arrangements between buyers and producers organisations. The triptych of knowledge transfer did not change its' agenda accordingly, but was held together by government finance. A few years after the start of chain reversion, the Dutch government withdrew itself from the triptych. This triggered the fragmentation of research, extension and education. The new setting urges for a new knowledge system as a successor of the triptych, which has now been discarded after a long period of success.

The current knowledge system in Dutch agriculture is not sufficiently able to bridge the developments in the primary sector and the demands of society. As these demands are not addressed, each stakeholder follows its own strategy and there is a lot of disagreement between for instance growers, environmental organisations and supply chains. This hampers the transition to a sustainable production system (Table 1). The knowledge organisations have to search for a new knowledge system that meets the interests, visions and strategies of a group of stakeholders. Knowledge and its applications have to suit its stakeholders and are no longer straightforward. Hence, the development that should be predominant for the current decade can be denominated as mobilisation of stakeholders' interests, visions and strategies.

The setting that is outlined above is the background for the implementation and adoption in horticulture of sustainable production practices in general and biocontrol in particular. In the following paragraphs we first discuss the key stakeholder in the process, the grower, and his motives whether or not to apply biocontrol and changeover to IPM. Next, the stepwise implementation and improvement of IPM itself is described, and finally we explain how to speed up the innovation process by using networks as a modern follow-up of the traditional triptych.

2. The horticultural entrepreneur as the key-stakeholder

2.1. Motives for growers to (not) changeover to sustainable production systems

In the current structure of horticultural firms, the entrepreneur himself mostly takes decisions related to crop growth. Hence, the grower is the key-actor in crop protection. De Lauwere *et al.* (2003; English summary: De Lauwere *et al.* (2004)) conducted an interview-based study on the motives of agricultural entrepreneurs to changeover to Integrated Farming Systems (which comprise IPM) or to Organic Farming (SKAL guidelines).

Three different kinds of external motives were found to be important for changeover, or not, to sustainable production systems in general (Table 2). Firstly, technical factors were predominantly mentioned as a motive to not changeover; such as problems with certain diseases or pests, the complexity of biocontrol, and incompatibility with labour supply. Technical factors may also be the very reason to favour the changeover. (Impending) pesticide resistance to spider mites or leaf miners is for many chrysanthemum growers the main reason to start using natural enemies against these pests and inevitably against other pests as well. Plant-growth inhibition caused by chemicals, *e.g.* in roses, may be another reason growers are more inclined to apply biocontrol.

Table 2: External and internal motives important to (not) changeover to sustainable production (De Lauwere et al., 2003)

External motives	Internal motives
• Technical factors • Institutional factors • Economic factors	• Firm characteristics • Personal characteristics • Idealistic factors

Secondly, institutional factors were mentioned, such as the government, the professional network of advisers, traders and knowledge workers, and societal organisations. The national government plays a double role in this respect. On one hand it encourages knowledge development, it subsidizes a changeover to Organic Farming and it favours the changeover to sustainable production as elaborated in the Agreement on Crop Protection (in Dutch: Convenant Gewasbescherming). On the other hand, the severe legislation of the Dutch government was found discouraging since it is too far ahead of EU policy. Moreover, the government was found to not operate clearly and reliably. Another institutional factor is the professional network of advisers, traders and knowledge workers around the grower. The grower may have to change professional contacts when he changes the production system. Other factors are: the pressure, enforced by consumers or society towards sustainable production, the attitude of the social network of the grower and the image of the agricultural sector.

Thirdly, economic factors are important in the decision to changeover. Stability of income, now and in the future, was a decisive factor in the growers' choice. A major hamper is that the entrepreneur does hardly receive any reward on the market for his efforts on sustainable production. In fact, in some cases it may even lower his income and/or damage his image at the auction, for instance when flowers hold parasitized aphids (mummies). On the cost-side, the extra labour requirement was mentioned as a hampering factor. A specific problem of changing to organic farming is the transition period, in which the grower has to meet all requirements, while the produce cannot be traded as organic.

A fourth group mentioned in De Lauwere *et al.* (2003) was defined as person- and company-specific factors, sub-divided into the set-up of the company, personal characteristics and idealistic factors (Internal motives, Table 2). They all appeared to play a major role as well. The set-up of the company refers to the financial situation, the acreage, the developmental stage of the company and the crop that is grown. The personal characteristics of the entrepreneur refer to the type of entrepreneur, entrepreneurial capacities, risk attitude, risk perception and the willingness to experiment. Idealistic factors are related to the intrinsic drive of the grower; examples are: philosophy of life, health, contentment and contact with customers. Sometimes, a certain event (a diagnosis of cancer or an accident with a pesticide) triggers the changeover. In the past, idealistic motives were predominant in the decision to change to organic farming. Nowadays, idealistic motives were found to be less manifest in the decision to the change to organic farming or IPM. Idealistic factors were also used to motivate the decision not to changeover; *e.g.*, the conviction that the current way of growing is the right way.

2.2. Types of entrepreneurs

Each grower makes his own assessment of the previously mentioned groups of factors and partially uses them in a motivation to changeover – or not. Sometimes, motives to changeover are even the same to motives to not changeover. Where one grower feels a threat another sees an opportunity. Where one grower is convinced that, on the long term, only sustainable production will provide him with an income, the other does not believe that the market will ever pay for sustainability. Regularly, growers mentioned technical problems of organic farming and the fact that they had lost their professional network as an argument not to change. These aspects were not mentioned by their organic colleagues because they found a solution for it. Each entrepreneur responds differently to an innovation like IPM.

In the old triptych of education, extension and research, the diffusion of innovations was assumed to take place according to the 'trendsetter model' (Rogers, 1995; Van Broekhuizen & Renting, 1994). In this model, a small number of 'first innovators' implement the latest knowledge from research, which is adopted by followers after the innovation has proven its value and has been facilitated by extension officers. Innovations with respect to sustainability are complex and have no clear value to the entrepreneur. Biocontrol and IPM are clear examples of such innovations. The innovation of IPM does not act in accordance with the traditional 'trendsetter model': first innovators are hard to find and adoption by followers might even be more difficult.

In order to understand the adoption process of such complex innovations that do not come with clear financial returns, another model is required. In several studies on the Dutch agricultural sector, entrepreneurs were divided into different categories (Table 3). Three studies imply that there is no homogenous group of first innovators. The entrepreneur that invests in the latest robotisation technology is another type than the entrepreneur that adopts the newest biocontrol strategies. According to Van der Ploeg (1999) in his survey on dairy farmers in the region of Friesland in the Netherlands, for instance, the first can be denominated as an 'intensive farmer' and the second one as a 'fine-tuner'.

Table 3: Classification of agricultural entrepreneurs according to three different sources

Source	**Classification of agricultural entrepreneurs**
Van der Ploeg (1999)	large farmers intensive farmers cow farmers (*cf.* plant growers in plant production) fine tuners
De Lauwere *et al* . (2003)	societal entrepreneurs (focus on society; activities with a high added value) traditional growers (expansion and intensification of existing production) new growers (expansion and intensification with focus on society) low-cost entrepreneurs
Theuws *et al.* (2002)	daring entrepreneurs calm entrepreneurs threatened entrepreneurs

Each type of entrepreneurs exhibits a specific interaction with society and has a specific relation with the knowledge network. Entrepreneurs that are open for biocontrol measures and IPM strategies might be found in the groups of for instance: fine tuners (Van der Ploeg, 1999), daring entrepreneurs (Theuws *et al.*, 2002) or societal entrepreneurs or new growers (De Lauwere *et al.*, 2003).

3. Stepwise implementation and improvement of IPM

3.1. Relationship between crop species and biocontrol or IPM

The Dutch government proposes that all growers have switched to IPM by 2010 (Dutch Ministry of Agriculture, Nature and Food Quality, 2004). This started a discussion on IPM and its relation to biocontrol. Successful implementation of biocontrol is highly dependable on crop-specific features. Van Driesche & Heinz (2004) predict that 'biological control is likely to be easier: 1) in long-term rather than short-term crops, 2) in vegetables rather than ornamentals, 3) in crops having few pests other than the one targeted for biological control, 4) in a crop in which the target pest does not attack the part of the plant that is sold, and 5) in a crop in which the targeted pest does not transmit diseases in the crop'. The difference between ornamentals and vegetables is especially noticeable. Several publications discuss the reasons why biocontrol in ornamentals in general is more difficult (e.g., Fransen, 1992; Van Lenteren, 2000; Lindquist & Short, 2004). The most mentioned causes are: 1) a zero tolerance for pests (and beneficials) on export products, 2) low damage thresholds due to cosmetic demands and because often the whole crop is harvested, 3) crop production systems, e.g., no crop-free period, 4) the large number of different plant species and cultivars, and 5) more registered pesticides.

Aforementioned factors determine the kind and number of biocontrol measures that may be part of an IPM strategy for a specific crop. In fact, even when the same crop species is grown there may be significant differences between locations, due to choice of cultivar or growing medium, but also neighbours, pest- and disease history, climate, etc. Hence, custom-made IPM strategies are required. Detailed information on biocontrol and IPM in different types of glasshouse crops is beyond the scope of this chapter. Interested readers should refer to for instance, Heinz, Van Driesche & Parella (2004).

The minimum requirements for IPM are established in a Royal Ordinance about good crop-protection practice (Besluit beginselen geïntegreerde gewasbescherming, 2004). The aim is to work towards the so-called 'best practices' of crop-protection. Both 'good practices' and 'best practices' will change over time due to advancing possibilities and understanding, thus accomplishing a stepwise improvement of IPM.

3.2. Good crop-protection practice

The Ordinance of the Dutch government about the principles of good crop-protection practice and IPM, determines that the use of pesticides is reduced to the very minimum necessary to control pest populations below the economic-damage threshold (Besluit beginselen geïntegreerde gewasbescherming, 2004). The definition of good crop-protection practice depends on the feasibility of crop-protection measures for 80-90% of the growers of a particular crop, and may change in time.

Insight into measures of good crop-protection practice must be given in a crop-protection plan and a log. The crop-protection plan should address measures with respect to prevention, to establishment the necessity of control, to non-chemical control measures, and to chemical control measures (details in Table 4). Aberrations to the plan should be written down in a crop-protection log. The plan and log are mandatory from 2005 onwards, but at present growers are not yet forced to implement the measures as summarised in Table 4. The aim of a crop-protection plan is to raise consciousness and induce a behavioural change in growers.

Table 4: The crop-protection plan should at least give information about the following crop-protection measures (Besluit beginselen geïntegreerde gewasbescherming, 2004)

Class of measures	**Indicated in crop-protection plan**
1. Prevention	a) list of soil-born diseases, pests and weeds b) use of disease- and pest-free seeds/cuttings c) use of resistant cultivars d) hygienic measures e) nematode control measures
2. Establishment control necessity	a) scouting measures
3. Non-chemical control	a) use of natural enemies (of diseases and pests), and measures for their conservation and promotion b) mechanical and other weed-control measures
4. Chemical control	a) pesticide use for seed coating, or treatment of cuttings and young plants b) choice of pesticides based on environmental effect and selectivity, and protection of applicant c) local use of pesticides on local pests or diseases d) use of low-dosage systems for herbicides

3.3 Best practices

On request of the government, the research institution Applied Plant Research has described ‘best crop protection practices’ (for glasshouse crops: Dik & De Haan, 2004). ‘Best practices’ are the most important crop protection measures that will potentially contribute to a reduction in the environmental burden. ‘Best practices’ are not yet generally implemented and practical experience is lacking. Almost all ‘best practices’ identify obstacles that need to be removed before implementation is possible, or those needing further study. Therefore, ‘best practices’ are not mandatory to the growers, but this set of potential measures is a guide for research funding organisations (like the government) and growers' organisations.

For each crop ca. 7-11 ‘best practices’ are described. Each measure is classified according to degree of adoption (a), the obstacles (b), and their contribution to reduce the environmental impact (c) (Table 5). The list of ‘best practices’ is dynamic due to advancing possibilities and understanding. Ideally ‘best practices’ become ‘good practices’ and are thus implemented by all growers. The list of measures should be revised regularly and new ‘best practices’ should be

added, in order to continuously improve IPM. The present list of ‘best practices’ for each crop is discussed with groups of growers for feedback.

Table 5: Classification of ‘best practices’ (Dik & De Haan, 2004)

a) Degree of adoption	b) Obstacles	c) Contribution to reduce environmental impact
1. generally implemented (> 20% of growers) 2. only by trendsetters (< 20% of growers) 3. only in experimental situations 4. strategy in the making	1. costs 2. labour 3. risk 4. risk perception and unfamiliarity 5. no registration	1. no use of pesticides 2. large 3. moderate 4. small 5. unknown

4. Mobilisation of stakeholders in knowledge networks as an alternative to the former knowledge triptych

4.1. Speeding up the innovation process of biocontrol and IPM by network formation

The innovation process of biocontrol and IPM is complex, not only in technical but also in socio-economic sense. As explained in the introduction, the present environment for such innovations requires ‘mobilisation of stakeholders’. The stakeholders of a specific innovation, including growers themselves, are responsible for knowledge, engineering, motivation and support. These parties include suppliers and buyers, knowledge workers and advisers, sector organisations, producers', organisations and government. Recently in The Netherlands two types of networks have been developed based on this principle of collaboration of all parties: ‘growers' networks’ and ‘socio-technical networks’. Both types of networks aim to generate interactive knowledge and are formed in order to speed up the innovation process. Growers' networks have a practical approach and are focussed on the changeover to IPM and the awareness of the necessity to implement the latest feasible ‘best practices’. The socio-technical networks have a theoretical background and aim at a practical implementation of an innovation agenda for sustainable development. This agenda is fully decided on by growers and stakeholders, without a specific focus beforehand.

4.2. Growers' networks

4.2.1. The start

In 1999 a project funded by the Dutch Ministry of Agriculture, Nature and Food Quality, known as Farming with Future (in Dutch: Telen met toekomst) started. The aim of this project is the large-scale promotion of the application of sustainable crop protection and fertilisation. For this purpose growers' networks were formed, starting in 1999 with the ‘unprotected crops’: arable crops, field vegetables, flower bulbs, nursery stock (Neeteson *et al.*, 2001; Langeveld *et*

al., 2002). Wijnands *et al.* (2001) elaborates on the history and the methodology of knowledge development in growers' networks. In 2003 the project entered its second phase and changed from a strong individual approach of farmers to a tactic with farmers in groups. From 2003 on networks were also organised for fruits and 'protected crops' (glasshouse vegetables and ornamentals), with the focus on crop protection (Dik, 2004). Although there is no difference in the basic idea and approach between the networks in the unprotected and protected crops, there are differences in organisation and operation of the networks justifying the differences between these sectors. Here, we focus on networks for glasshouse crops.

4.2.2. The growers in the network

The heart of the growers' networks is formed by a group of 6 to 8 growers who meet several times a year. These groups are lead by researchers (crop protection specialists), trained in managing processes of change. At the moment there are five crop-related networks: one for cucumber, one for tomato, one for rose, one for chrysanthemum and one for potted-plants. Each group consists of different types of entrepreneurs, *i.e.* growers with different attitudes towards biocontrol and choice of crop protection strategy, but with a common awareness of the need to change to IPM. The growers are from different regions of the country and are an authority within their crop, although not only trendsetters are chosen. Within the group discussions about (new) control measures and strategies are stimulated giving special attention to biocontrol and natural pesticides. In this way growers learn from each other and also get acquainted with new strategies. The flow of information is not directed in one way, *i.e.* to the grower, only. Questions and information on obstacles for 'best practices' (see paragraph 3) etc., flow back to research institutions, thus stimulating new research and demonstration projects.

Before the start of the crop (or a year) the grower, assisted by his regular crop protection consultant and using input of the latest knowledge from the researcher, designs a crop protection plan. The crop-protection strategy and corresponding plan remain the choice of the grower and will therefore differ between growers. At the end of the cropping season (or a year) the plans are evaluated individually and within the group. To help the evaluation of the chosen strategy, growers register the input of chemical and natural pesticides, natural enemies, and also costs involved (in time and money), as well as output, *i.e.* yield. Using these figures the researcher calculates the environmental impact and the economic results. For the following year, a new plan is made, based on the experiences of the previous year and with new input from research and consultants, thus accomplishing a stepwise implementation of 'best practices' (see paragraph 3).

4.2.3. Reaching growers outside the network

Next to coaching the individual growers and the networks, much effort is put into the dissemination of results to other growers and convincing them to also implement the strategies that prove to be feasible. For this purpose co-operation (in communication) is sought with stakeholders surrounding the growers, thus creating a solid basis for the implementation of new knowledge. Communication focuses on distribution of technical information as well as on increasing acceptance.

Communication with growers outside the networks occurs in numerous ways and often in co-operation with the extension division of the National Sector Organisation 'LTO', which started a communication project called 'Strategist' (in Dutch: Strateeg) for IPM in glasshouse

ornamental crops (see also paragraph 4.3.4). Communication involves leaflets with information about the major pests and diseases for each crop, publications and interviews in growers' magazines, presentations at meetings organised by growers' association, and nursery excursions to participating growers. There is also an Internet-site, www.telenmettoekomst.nl, where all leaflets and other relevant information like reports of the network-meetings can be found (in Dutch).

As addressed in the Introduction, the innovation process of biocontrol is complicated. Straightforward facts, like the efficacy of a (microbial) pesticide, are picked up easily by growers and find their way quickly via study groups and other contacts with and between growers. Knowledge about natural enemies, and more particularly IPM strategies, are never straightforward and require guidance when implemented. In the first place, this means that stakeholders surrounding the growers, in particular the advisers should acquire knowledge. For the large group of 'followers' amongst the chrysanthemum growers, crop advisers are even the main knowledge providers in crop protection and play an important role in the crop-protection strategy the grower chooses. The advisers may be independent (*e.g.*, the privatised extension service 'DLV'), but more often they represent a crop-protection supplier. These companies vary in state of knowledge and have their own - more or less sophisticated - IPM strategies. A complicating factor is that the natural aim of these companies is to sell as many products (biological or chemical) as possible to as many customers as possible.

Participation of crop-protection suppliers in this innovation process is sought in several ways. Advisors from different companies advice the growers within the network. These advisors are directly involved in the compilation and evaluation of the crop-protection plan of 'their' grower (see 4.2.2). Also, bilateral meetings of research and crop-protection suppliers and other companies involved in advising growers are organised to discuss strategies and research results. The advantage of this one-to-one approach is that the companies then discuss their strategy with the researchers more openly than when competitive companies are present. Awareness of these important stakeholders of the necessity and feasibility of IPM enhances the adoption of biocontrol and a custom-made IPM strategy.

4.2.4. Communication with policymakers and societal stakeholders

Policymakers and societal stakeholders also play an important role in the changeover to a more sustainable crop protection because they can stimulate the changeover, set the goals and determine the framework in which it should take place (institutional factors). In a country full of water like The Netherlands, regional water boards, drinking water companies and environmental organisations highly influence the present regional and national policy. Policy officials and politicians are also influenced by discussions with growers' organisations and organisations of biocontrol producers, chemical industries and suppliers, for instance as in the Agreement on Crop Protection.

The project 'Farming with Future' aims to provide policymakers and societal stakeholders a realistic view of the present and future (im) possibilities of biocontrol and IPM and to stimulate discussion among the stakeholders. For this purpose policymakers and societal stakeholders regularly receive a newsletter and also bilateral meetings as well as round-table discussions are organised.

4.3. Socio-technical networks

4.3.1. Definition and aim

A Socio-technical network (STN) is defined as: 'a set of direct and indirect social relations, centred around given persons, which are instrumental to the achievements of the goals of these persons, and to the communication of their expectations, demands, needs and aspirations' (Van der Ploeg, 2001). In this paper, the STN is elaborated as a tool to achieve sustainable plant production, which includes the innovation of IPM and implementation of biocontrol.

A Socio-technical network is another method to speed up an innovation process by collaboration of stakeholders. The aim of an STN is 1) to intelligently use the forces of People, Planet and Profit for speeding-up the innovation process to sustainable plant production, and 2) better utilise 'surrounding partners' to induce entrepreneurs. The 'technical part' of a STN consists of one or more specific innovations in the field of technical, knowledge, (consumer-) product or sector development. In addition to Profit, the innovations should improve the aspects of Planet and People.

A STN is primarily based on the capacity of entrepreneurs to innovate. Growers and stakeholders can be activated by meeting their interests, strategies and visions. The participants formulate a common vision on sustainable development of the sector and the problems that they want to work on themselves. They decide on an innovation agenda for sustainable development, without a specific focus beforehand. Hence, in a STN, the development (for instance of knowledge) is driven by demand.

Secondly, a STN aims at a consensus within the intermediate groups, such as producers' organisations, NGO's and government. Without consensus of intermediates from the start, there is an evident risk that the development and the dissemination of the innovation will become frustrated.

4.3.2. Method for setting up Socio-Technical Networks

Funded by the Dutch Ministry of Agriculture, Nature and Food Quality, a methodology has been developed to create a STN (Buurma *et al.* 2003; De Buck & Buurma, 2004). A STN requires participation of supporters of values that are related 1) with market (to generate Profit), 2) with society (to care for People and Planet) and 3) with human resource (to induce entrepreneurship and innovative power). A value triangle (Figure 2) is a tool to identify the mutual positions of the stakeholders. Firstly, stakeholders professionally involved in the innovation are identified for each part of the value triangle.

These stakeholders are interviewed in-depth, focussing on four items: the values of the respondents (see Table 6), their position in the professional environment, their vision on strategic development and the relevance for themselves and the barriers that hamper its implementation. In a second step, the results of each interview are summarised and visualised in a belief system (see Figure 3).

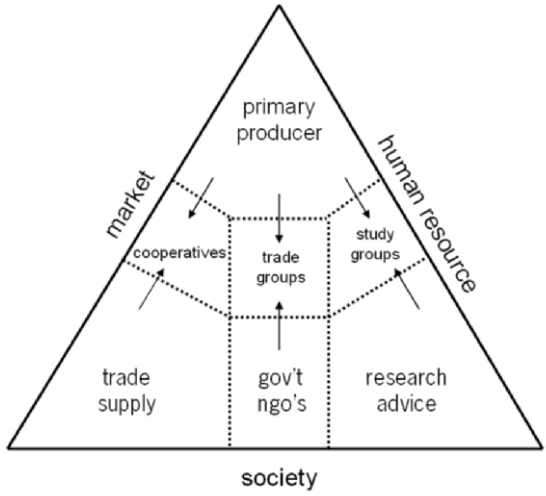

Figure 2: Value triangle: the position of actor groups in the agricultural sector between values that are related with market, human resource and society

Table 6: Three groups of values

Human Resource	**Market**	**Society**
Motivation	Food security	Care for the earth
Entrepreneurship	Transparency	Care for people
Flexibility	Food quality	Liveable countryside
Innovation	Internationalisation	Regional diversity
Knowledge	Production efficiency	Valuation
Spirituality	Economics of scale	Co-operation
	Uniformity	
	Competition	

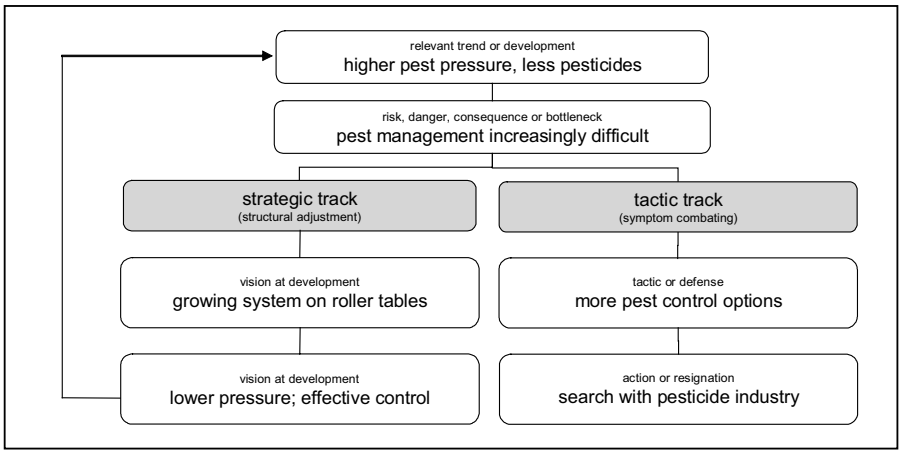

Figure 3: Example of a belief system of an individual stakeholder, visualisation of problem perception, tactic of symptom combating and strategy of structural assessment of the problem

Based on the interviews, the next step is the identification of potential coalitions in the mind landscape (Figure 4). Some conditions for a successful coalition are: compatibility of individual strategic solutions, innovative power and a balanced set of individuals' values. The coalition is formed around a central person (*cf.* the formation of a cabinet, fronted by a Prime Minister) with authority, goodwill, having the willingness and the ability to co-operate with mandate of intermediate groups. In the final step a collaboration agreement is composed, reflecting the intentions and commitment of the participants in this Socio-Technical Network to implement a specific innovation pathway. An appropriate action for this is a workshop with all interviewed stakeholders. This innovation is connected with the transition to a sustainable sector in the longer term. Methods of back-casting are used as a tool to set up this pathway (Grin & Grunwald, 2000). The back-casting methodology offers an approach to define future images of a certain subject. Next, a transition trajectory is designed, necessary to reach one or more of these desired future images. As an example, such back-casting exercises were used for setting an R&D agenda and planning and timing of activities on biocontrol in chrysanthemum growing.

4.3.3. Results of stakeholder interviews on IPM in Chrysanthemum

An example of the formation of a STN is the development of IPM in the chrysanthemum sector in The Netherlands. From the interviews of stakeholders within the cut-chrysanthemum sector, four pathways for transformation towards sustainability appeared in the mind landscape.

Adherents of pathway 1 (Figure 4) urged on the transition from chemical pest control to biocontrol and IPM. Pest control practices need to be revised, as organisms increasingly become resistant, due to abundant use of a limited number of pesticides. The decrease in the number of registered pesticides is a result of severe government regulations with respect to environmental protection, combined with the relatively small market demand for pesticides in Dutch glasshouse horticulture as a whole.

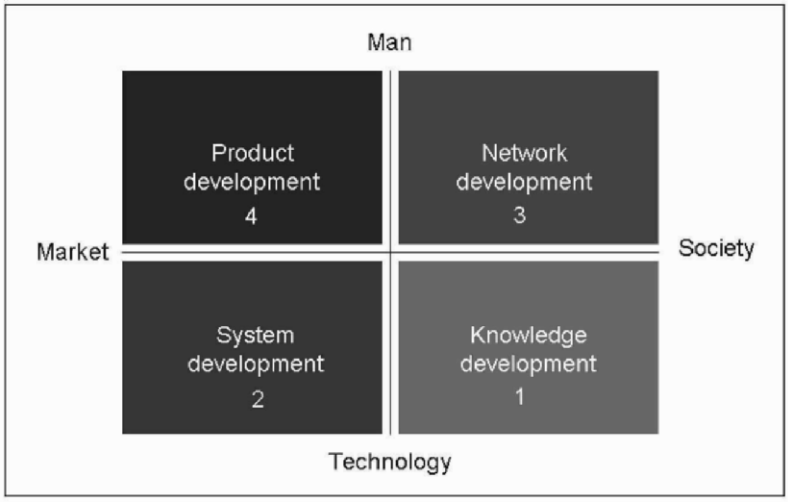

Figure 4: Mind landscape: the four innovation pathways for system innovation

The interviews do not just focus on a specific theme, *i.e.* IPM, but address the inter-relationships with other important issues as well. Hence, another group believed that cropping systems on mobile benches in artificial substrate are indispensable for a sustainable chrysanthemum sector (pathway 2; see also the Belief system of one participant in Figure 3). Firstly, the new system increases production efficiency and secondly the use of artificial substrate would eliminate problems with soil-borne pests (*e.g.*, *Scuttigerela* and nematodes) and diseases (*e.g.*, *Pythium*, *Verticillium*). The use of mobile benches offers possibilities for pest management and product development (small, separately manageable units). Results (a better productivity) should be available on the short term, as economic continuity of the chrysanthemum sector is at stake.

Adherents of pathway 3 believe that the market position of chrysanthemum needs to be improved. The negative image of chrysanthemum as a 'poisonous flower' and its character of cheap mass produce hamper this.

Some stakeholders urge the necessity of more collaboration in the knowledge system: the private companies, research and extension organisations and sector organisations need each other to develop and disseminate IPM in the chrysanthemum sector. This point of view can be considered as institution development (pathway 4).

4.3.4. Experiences on a socio-technical network on sustainable development in the Chrysanthemum sector

Changing over to a cropping system in artificial substrate on mobile benches looked promising for development towards profitability and ecological sustainability. Representatives of this development pathway operated with confidence had innovative power and found a link with

IPM (pathway 1) evident. Therefore, a Socio-Technical Network around technology development (pathway 2) and not directly around knowledge development on IPM was initiated (De Buck & Buurma, 2004). Moreover, there was already a serious research effort on development of an IPM strategy including testing biocontrol agents for Dutch cut-chrysanthemum production (collaboration between Applied Plant Research and the extension service DLV) (*e.g.,* Beerling & Boertjes, 2002; Beerling & Van den Berg, 2003a, 2003b; Van der Gaag & Pijnakker, 2003). Also a crop-protection producer, Syngenta, and its distributor, Van Iperen started an implementation project with their IPM strategy.

The chairman of the National Crop Committee (in Dutch: Landelijke Gewascommissie Chrysant, NGO), a chrysanthemum grower himself, was appointed as the central person or Prime Minister of the Socio-Technical Network. Through his position as chairman and grower, he was able to create support for the innovation by the sector. As a first activity of the STN, the researchers organised - on behalf of the central person - a meeting with all leaders of IPM initiatives in cut-chrysanthemum. Four projects were represented: 1) 'Strategist' (a communication project of the extension division of the National Sector Organisation, LTO), 2) 'Farming with Future', that at that time intended to start a growers' network (see paragraph 4.2), 3) the aforementioned implementation project of Syngenta and Van Iperen, and 4) the research project concerned with testing and developing IPM strategies (see above). This meeting has contributed to a close collaboration between all current projects on IPM in the chrysanthemum sector. In fact, this initiative can be considered as a first step in institutional development (pathway 4).

A second step in institutional development and the next product to facilitate the Socio-Technical Network was the drafting of a strategic document on sector development on behalf of the National Sector Organisation for Horticulture (De Buck & Buurma, 2004). This document elaborates sustainable development as a combined development of the four pathways (as mentioned in this paragraph). For the approval and funding of R&D proposals in a specific sector in horticulture the National Crop Committee (representing the sector; LTO) advises the National Sector Organisation for Horticulture (in Dutch: Productschap Tuinbouw, an NGO). Both organisations require support from the sector for their decisions. The sector will support those decisions that lead to sustainable sector development in terms of Profit as well as People and Planet.

Once the Socio-Technical Network had initiated an experiment on a new cropping system on artificial substrate, a first point of concern arose. The initiators were attracted by the economic benefits of the new cropping system and this forced the opportunities of sustainable crop protection to the background. The STN researchers facilitated consultation between the researcher of the chrysanthemum-growers' network and the central person of the STN, resulting in an agreement on co-operation. In this Socio-Technical Network, the link had therefore been restored between the development of a new cropping system (pathway 2), and the development of a new crop protection system (pathway 1).

As a conclusive step, a workshop was held for the stakeholders who had been interviewed. In this workshop, most participants recognised their own belief system and agreed upon the four pathways, required for sustainable development. There was full support for the fact that IPM should be incorporated in the development of the new production system as soon as possible. The participants were aware of the need for support from the whole sector for such extensive changes (system innovation) in cut-chrysanthemum production. Furthermore, the participants concluded that better craftsmanship in pest control is necessary; a few

demonstration objects are not sufficient to convince a substantial percentage of growers in the sector. It was also acknowledged that this fact was covered by recent initiatives, *i.e.* the projects 'Strategist' and 'Farming with Future'. Finally, the transition to a new production system and IPM should be used to enhance product and market development of chrysanthemum (pathway 3).

Some of the STN-participants felt the need to speed up the development to improve craftsmanship in pest control and did not want to wait for results coming from the strategic lines set out by the co-operating projects 'Strategist' and 'Farming with Future'. Therefore, a workshop for crop-protection advisers was organised in which the activities of the projects 'Farming with Future', 'Strategist' and the implementation project of one of the crop-protection advisers (Van Iperen & Syngenta) were presented and discussed. Although this is an efficient way to reach all advisers in crop protection at once, a drawback to this kind of workshops is that there is not an open and critical discussion about IPM strategies because of the presence of highly competing companies. A more critical discussion is to be expected from the bilateral communication approach of 'Farming with Future', as is agreed on by most crop-protection companies.

The present situation is that a project of chrysanthemum production on artificial substrate is approved by the National Sector Organisation for Horticulture. After the first year, the project will collaborate with existing initiatives on IPM.

5. Closing remarks

A Socio-Technical Network (STN) appears to be a useful tool and an appropriate method for stakeholders to decide on an innovation agenda for system innovation, such as the implementation of biocontrol and IPM. It is activated by the innovative capacity and common interests, strategies and visions of entrepreneurs.

The traditional 'trendsetter model' is not helpful in the diffusion of complicated innovations without a clear value to growers, such as biocontrol and IPM. Implementation of biocontrol and IPM will only take place when external and internal motives of different categories of growers are met. The Growers' network – for example those of the project 'Farming with future' - is an appropriate method for participative and stepwise learning, and enables the implementation of complicated knowledge about IPM and biocontrol.

STNs and Growers' networks mobilise all decisive stakeholders for the implementation of sustainable horticulture and biocontrol. The interrelationship between the two types of networks on a specific crop is evident. In the case of the cut-chrysanthemum sector, the Growers' network on IPM stands for the dimension of knowledge development of the STN on sustainable sector development. The Growers' network enhances the STN as it is driven by stakeholders rather than by researchers. Hence, these networks contribute to a new knowledge system as a successor for the traditional triptych of Research, Extension and Education in the Dutch agricultural sector. Briefly, in a modern knowledge system based on these networks, the focus has shifted from critical success *factors* to critical success *actors*.

Education is a fully recognized element of the knowledge system. As a next step in the construction of a new knowledge system, in a project, Growers' networks will be used as a learning environment in Agricultural Education. In order to initiate more fundamental changes on Agricultural Education, it is worth trying to set up a STN with all stakeholders involved.

Acknowledgements

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CHAPTER 7

BIOCONTROL IN PROTECTED CROPS: IS LACK OF BIODIVERSITY A LIMITING FACTOR?

Annie Enkegaard and Henrik F. Brødsgaard

1. Introduction

Protected crops are a dives entity, ranging from crops grown under very simple plastic or mesh construction to very high-tech glasshouse structures, which have a very high degree of automatisation of e.g. climate control, internal logistics, and robots for plant handling. But in general greenhouse crops are grown under very artificial conditions, where not even soil may be present but the plants are grown in e.g. rock wool or mats of coconut fibres. This makes protected crops very simple ecosystems with very poor biodiversity. On the other hand, once a pest species establish in such systems, it finds itself in an environment of unlimited food availability, a pleasant more or less constant climate that may prevail year round, and no enemies. Basically, biological control aims at provide the protected environment with natural enemies of the pests and thereby increase the biodiversity in the crops in a controlled manner. As implementation of biological control programs becomes widespread, the use of broad-spectrum pesticides decrease, and the global trade in plant material increase, the need for more different biological control agents will continue to increase. So, will the research community and commercial insectaries be able to supply this increasing demand for beneficial organisms for the fast growing industry of protected crops?

In this chapter we review the history of biocontrol in greenhouses illustrating the driving forces behind implementation of this plant protection method, providing examples of how new beneficials have been discovered and discussing factors limiting to an increased use of biocontrol. The chapter deals with biological control of arthropod pests, primarily with the use of macroorganisms. Figs. 1-12 show examples of some major pests, as well as some main biological control organisms.

2. Early history of biocontrol in greenhouses

2.1. The use of biocontrol before 1960's

The first record of consistent successful biological control of pests in protected crops by means of natural enemies is from Speyer (1927). He reported that *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) parasitised and controlled the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), on a tomato crop in England. During the subsequent years Speyer developed a mass rearing system and distributed *E. formosa* not only to local growers but to growers and colleagues in several countries (McCleod, 1938). The mass

J. Eilenberg and H.M.T. Hokkanen (eds.), An Ecological and Societal Approach to Biological Control, 91–122.

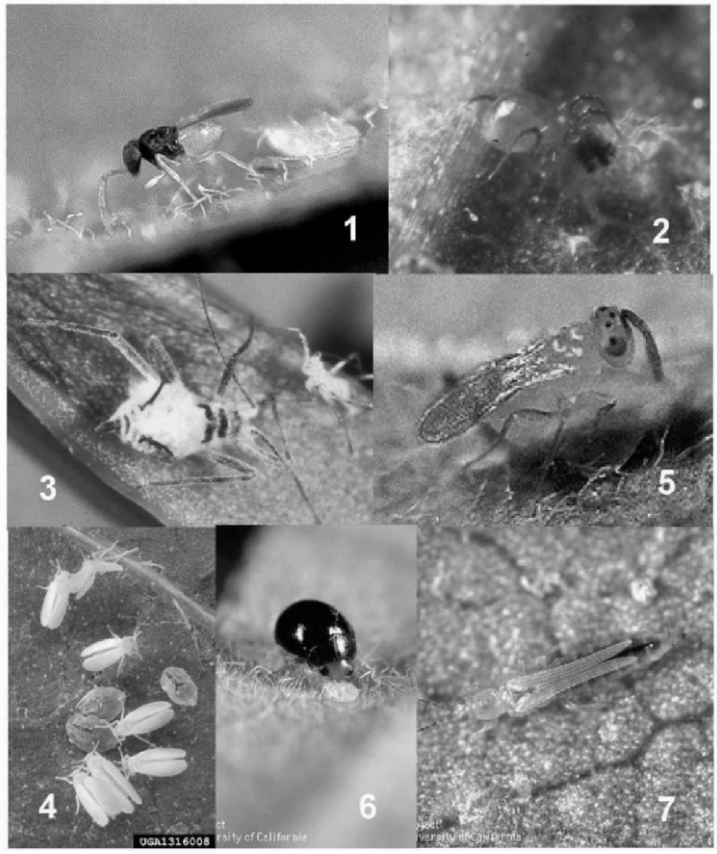

Figure1: Encarsia formosa – *a parasitoid of whiteflies. Photo F. Lind. Danish Institute of Agricultural Sciences.*
Figure2: Phytoseiulus persimilis *attacks a spider mite. Photo F. Lind. Danish Institute of Agricultural Sciences.*
Figure 3: Aphid killed by the fungus Verticillium lecanii*. Photo: Leif S. Jensen, KVL, Department of Ecology.*
Figure 4: Bemisia argentifolii*. Photo: Scott Bauer, USDA ARS Image Gallery, http://www.forestryimages.org.*
Figure 5: Eretmocerus eremicus – *a parasitoid of* Bemisia*. Photo: BioPol, NL. http://www.biopol.nl/UK/Whiteflies.html.*
Figure 6: The ladybird beetle Delphastus catalinae *(*D. pusillus*) feeding on a whitefly nymph. Photo: Jack Kelly Clark, University of California, http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html.*
Figure 7: Adult Western flower thrips, Frankliniella occidentalis*. Photo: Jack Kelly Clark, University of California, http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html.*

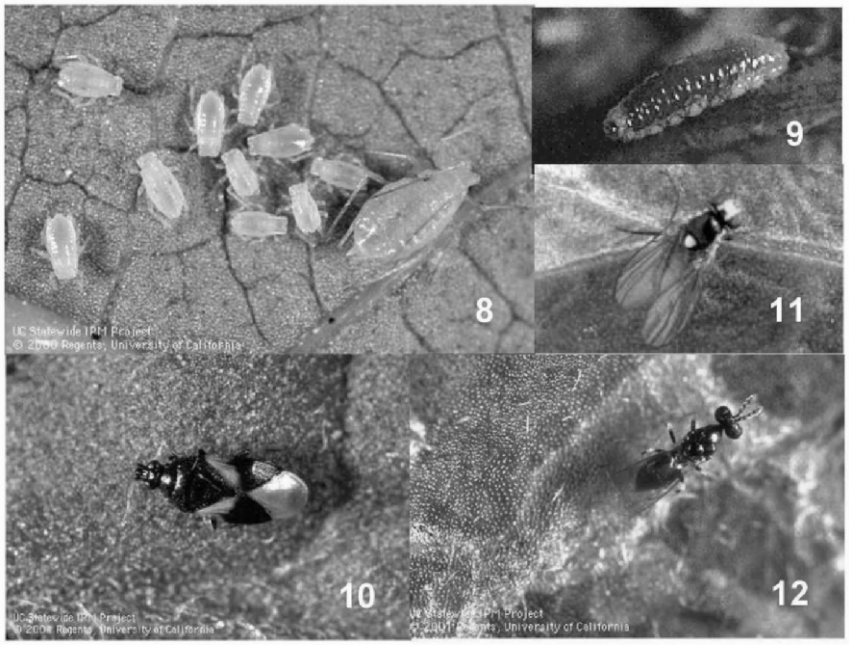

Figure 8: Peach-potato aphids, Myzus persicae*. Photo: Jack Kelly Clark, University of California,*
http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html.
Figure 9: Larva of the aphid gallmidge Aphidoletes aphidimyza*. Photo: J. Ogrodnick, 5th January 2005, "Biological Control: A Guide to Natural Enemies in North America, Aphidoletes aphidimyza", Weeden, Shelton, Li & Hoffmann (editors), Cornell University*
http://www.nysaes.cornell.edu/ent/biocontrol/predators/aphidoletes.html.
Figure 10: A minute pirate bug, Orius *sp. – a polyphagous predator of e.g. thrips. Photo: Jack Kelly Clark, University of California,*
http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html.
Figure 11: A leafminer, Liriomyza *sp. Photo: Garta.*
*Figure 12: Adult female serpentine leafminer parasite. (*Diglyphus begini*). Photo: Jack Kelly Clark, University of California,*
http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html

rearing and augmentation of *E. formosa* continued until 1949 when growers worldwide turned to the new synthetic pesticides such as DDT and discontinued the use of *E. formosa* (Hussey, 1985).

2.2. Renewed interest in biocontrol in the 1960's

Up through the 1950s growers of protected crops relied exclusively on pesticides for control of pests. Though resistance to DDT quickly was developed in a series of important pests, new groups of pesticides continued to be developed and enabled the growers to overcome resistance problems by shifts and rotation among different pesticide groups. However, by the late 1950s, pesticide resistance in the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), had become so severe that even very frequent pesticide applications did not control the pest. In 1960, Dosse (Bravenboer & Dosse, 1962) found an effective spider mite predator, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), on a crop of orchids imported from Chile to Germany. The predatory mite proved to be very effective and mass rearing systems were quickly developed. Several research stations and smaller commercial insectaries started mass producing *P. persimilis*, and the vegetable growers in Western Europe and Canada soon found the cost/benefit of the predatory mite so good that many turned to biological control of spider mites within a few years. By 1970, most cucumber growers used *P. persimilis* as their first choice of spider mite control and, by 1980, hardly any of the major cucumber growers in these areas used chemical spider mite control.

By the end of the 1960's, chemical control of the greenhouse whitefly became increasingly difficult due to build-up of insecticide resistance. Therefore, a British research station collected *E. formosa* from a botanical garden and started a culture. In 1972, a commercial production was re-established and, in the mid 1970, the use of biological control of whiteflies in tomato crops was widely used in Western Europe and Canada (Hussey, 1985). The rapid uptake of this re-discovered beneficial was due, not only to the effectiveness of *E. formosa*, but also to the fact that tomato crops have a rather simple pest species complex. In addition, the product development, where pupae of the parasitoids are glued to cardboard cards, makes *E. formosa* an easy manageable product with a relatively long shelf life. So by 1980, like with the spider mite control in cucumber crops, the greenhouse whitefly in tomato crops was more or less exclusively controlled by biological means in Northern Europe and Canada (van Lenteren *et al.*, 1992).

2.3. Development of biocontrol methods against secondary pests

The widespread use of biological control of spider mites and whiteflies in cucumber and tomato crops, respectively, and hence the termination of the use of broad-spectrum pesticides generated increased problems with former secondary pests. In cucumber crops the onion thrips, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), and the melon aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), are such examples and in tomato crops, problems with leaf miners, *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae), increased.

The first line of action to overcome these "new" severe pests and at the same time preserved the use of biocontrol was to implement IPM-programs incorporating the use of *P. persimilis* and *E. formosa* with the least harmful of the available pesticides, assisted by extensive side-effect evaluations of pesticides (e.g. Franz *et al*., 1980; Hassan *et al*., 1983, 1987, 1988). In

some cases integrating the use of pesticides with biocontrol could be eased by application of deliberately selected strains of organophosphorous pesticide resistant *P. persimilis* (e.g. Croft & Morse, 1979; Schulten, 1980). Attempts also to select similar strains of *E. formosa* failed (e.g. Walker & Thurling, 1984).

Concurrent, with the search for pesticide resistant *P. persimilis* and *E. formosa*, researchers throughout Northern Europe looked for new biological control agents to control the secondary pests. This strategy proved to be much more viable, and up through the 1980s a range of new beneficial arthropods was developed and marketed. By the end of 1980s, full biological control programs for glasshouse vegetable crops were developed using e.g. predatory mites (*Amblyseius* spp., *Neoseiulus* spp. (Acari: Phytoseiidae)) and bugs (*Orius* spp. (Heteroptera: Anthocoridae)) against *Thrips tabaci* (e.g. Shipp & Ramakers, 2004), parasitoids (*Aphidius* spp. (Hymenoptera: Braconidae)) and predatory gall midges (*Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae)) against aphids (e.g. Blümel, 2004), and parasitoids against leaf miners (*Dacnusa sibirica* Telenga (Hymenoptera: Braconidae), *Diglyhus isaea* (Walker) (Hymenoptera: Eulophidae)) (e.g. van der Linden, 2004).

The general method for release was to apply beneficials early in the growing season as soon as the first pests were observed. Sometimes this method did not result in control of the target pest because the pest population had increased too much at the time pest observation and the following application of beneficials. New introduction strategies were therefore invented: pest-in-first, preventive introductions (dribble method) and banker plants. In the first method pests are established in low numbers in the culture before release of beneficials to provide an optimal timing of introduction and a more stable foundation for the subsequent build-up of the natural enemies (e.g. Gould *et al*., 1975). However, the practical use of this method has been limited due the growers' understandable reluctance to introduce pests into their crops. In the dribble method beneficials are released already at the time of planting of a new culture in anticipation of later pest infestations (e.g. Parr *et al*., 1976). Banker plants are open rearing systems of beneficials established in the culture on an alternative prey host, e.g. establishment of aphid parasitoids on aphids incapable of attacking the crop reared on a suitable host plant (e.g. Bennison, 1992). Both dribble applications and banker plants are now widely used.

Biological control was initiated in UK and the Netherlands and from there the use gradually spread first to other North European countries and Canada (van Lenteren & Woets, 1978), and subsequently to more southern regions in Europe, e.g. France, Israel, and Italy (e.g. Woets & van Lenteren, 1983; Nucifora & Calabretta, 1985, van Lenteren, 1985), and eventually to other regions of the world e.g. USA, New Zealand and Australia (e.g. Woets & van Lenteren, 1984; van Lenteren, 1985; Martin, 1987; Spooner-Hart, 1989).

It should be noted that there is a noticeable difference between greenhouses of northern cooler climates (glasshouses) and those of warmer Mediterranean climates (plastic greenhouses, screenhouses, plastic tunnels). Glasshouses are rather closed units largely isolated from the outside environment whereas plastic greenhouses are more openly structured creating a constant interchange of pests and beneficials between the greenhouse crops and the neighbouring outdoor crops and weeds (e.g. Avilla *et al*., 2004). In these regions pests therefore constantly re-colonise greenhouse crops via infestation from the outside and released beneficials are more likely to escape from the greenhouses. On the other hand native natural enemies migrate into the greenhouses to a much larger extent than in cooler climates. Therefore they have a major role to play in biological control programs, which emphasise not only releases of beneficials in the greenhouses but also attempts to conserve the local native

population of beneficials in the surroundings (e.g. Gabarra & Besri, 1999). This exploitation of the native fauna in warmer climates have through the years lead to the discovery of a number of natural enemies that subsequently have been mass produced, first with the aim to augment the local populations through releases, but later also for application in northern glasshouses. Examples of such additions to the commercially available arsenal of beneficials for use in greenhouses from this Mediterranean climate reservoir of biodiversity are *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) and *Dicyphus tamaninii* Wagner (Heteroptera: Miridae).

3. Dissemination of biocontrol from vegetables to ornamentals

3.1. Initiation of use of biocontrol in ornamentals

Practical implementation of biological control in ornamentals via IPM programs structured around application of *P. persimilis*, *E. formosa* and/or the fungus *Verticillium lecanii* (Zimm.) Viegas (Deuteromycotina: Hyphomycetes) started already in the late 1970's and early 1980's on a very limited area in UK (Wardlow, 1979), Norway (Stenseth, 1979), Poland (Pruszynski, 1979) and the Netherlands (Woets & van Lenteren, 1982). The area of ornamentals under IPM did, however, not increase noticeably (van Lenteren & Woets, 1979, 1980; Woets & van Lenteren, 1981, 1982). Thus, during the 1970's and early 1980's the notion among researchers and practitioners was that implementation of biocontrol in ornamental cultures, especially pot plants, on a larger scale was unrealistic (van Lenteren & Woets, 1988) primarily because of the low damage threshold of these cultures.

However, like previously in vegetables, ornamental growers started to experience increasing difficulties in controlling pests chemically (Scopes, 1979; van Lenteren, 1988; van Lenteren & Wardlow, 1989) and in the mid 1980's a breakthrough occurred with increasing applications of biocontrol in North European countries in cultures like Chrysanthemum (Gould, 1984), roses (van Lenteren, 1985), Gerbera (van Lenteren, 1985) and Poinsettia, (Wardlow, 1989) initiating a new epoch in the history of biological pest control.

Since then, the use of biocontrol in ornamentals has increased stimulated by the availability of an ever increasing number of beneficial species (Figure 13, Table 1); the usefulness of *V. lecanii* for cleaning cuttings rooting under high humidity conditions (Sopp & Palmer, 1990); the adoption of new strategies for beneficial application (keep-down-strategy (Brødsgaard, 1995)), i.e. inundative releases (see Chapter 1); and increased use of preventive introductions. The uptake of biocontrol among ornamental growers has, however, been slower than among vegetable growers due to factors such as the low damage threshold of ornamentals; zero-tolerance for export items; the great diversity of plant species grown as ornamentals (more than 400 species in Europe alone (van Lenteren, 2000)); the frequently more complex production process of ornamentals; the lack of safety periods; and recent marketing of pesticides for which resistance among pest species has not yet evolved. In many cases it is therefore easier for ornamental growers to stick to effective pesticides, when available, as a plant protection measure or to revert to chemical control when new pesticides are marketed.

Despite these limitations implementation of biocontrol in ornamentals, especially in temperate climate regions, in some countries now amounts to up to 10-35% of the area (Enkegaard, 2003). For examples of IPM programs for various ornamental crops see Gullino & Wardlow (1999).

Table 1: List of commercially available beneficials used (or potentially usable) worldwide for biocontrol of pests on plants in protected crops, interior plant scapes etc. Endemic/exotic is in relation to Western Europe. A ? indicates that the origin of the beneficial species is uncertain

Natural enemy	Endemic	Exotic	Main target pest
Microorganisms			
Bacteria			
Bacillus thuringiensis	+		Lepidoptera, sciarids, Diptera
Fungi			
Beauveria bassiana	+		Whiteflies, aphids, thrips, sciarids, mites
Paecilomyces fumosoroseus	+		Whiteflies
Verticillium lecanii	+		Aphids, whiteflies
Vira			
Spodoptera NPV virus	+		Beet armyworm (*Spodoptera exigua*)
Parasitoids			
Parasitoids of eggs			
Anagrus atomus	+		Leafhoppers
Anaphes iole		+	Lygus bugs
Trichogramma brassicae	+		Lepidoptera
Trichogramma cacoeciae	+		Lepidoptera
Trichogramma dendrolimi	+		Lepidoptera
Trichogramma evanescens	+		Lepidoptera
Trichogramma maidis		+	Lepidoptera
Trichogramma pretiosum		+	Lepidoptera
Parasitoids of larvae/pupae			
Anagyrus fusciventris		+	Mealybugs
Anagyrus pseudococci	+		Mealybugs
Aphelinus abdominalis	+		Aphids
Aphidius colemani		+	Aphids
Aphidius ervi	+		Aphids
Aphidius matricaria	+		Aphids
Aphytis diaspidis		+	Scales
Aphytis holoxanthus		+	Scales
Aphytis lingnanensis		+	Scales
Aphytis melinus		+	Scales
Cales noacki		+	Whiteflies
Coccophagus lycimnia	+		Scales
Coccophagus rusti	+		Scales
Coccophagus scutellaris	+		Scales
Comperiella bifasciata		+	Scales
Cotesia marginiventris		+	Lepidoptera
Dacnusa sibirica	+		Leafminers
Diglyphus isaea	+		Leafminers
Encarsia citrina	+		Scales
Encarsia formosa		+	Whiteflies
Encarsia tricolor	+		Whiteflies

Table 1: Continued

Natural enemy	Endemic	Exotic	Main target pest
Encyrtus infelix		+	Scales
Encyrtus lecaniorum	+		Scales
Eretmocerus eremicus		+	Whiteflies
(E. californicus)			
Eretmocerus mundus	+		Whiteflies
Gyranusoidea litura		+	Mealybugs
Hungariella peregrina		+	Mealybugs
Hungariella pretiosa ?		+	Mealybugs
Leptomastidea abnormis	+		Mealybugs
Leptomastix dactylopii		+	Mealybugs
Leptomastix epona	+		Mealybugs
Lysiphlebus fabarum	+		Aphids
Lysiphlebus testaceipes		+	Aphids
Metaphycus bartletti		+	Scales
Metaphycus flavus		+	Scales
Metaphycus helvolus		+	Scales
Metaphycus lounsburyi		+	Scales
Metaphycus swirskii		+	Scales
Microterys flavus		+	Scales
Opius pallipes	+		Leafminers
Praon volucre	+		Aphids
Pseudaphycus angelicus		+	Mealybugs
Pseudaphycus flavidulus	+		Mealybugs
Pseudaphycus maculipennis	+		Mealybugs
Thripobius semiluteus		+	Thrips
Parasitoids of adults			
Scutellista cyanea		+	Scales
(S. caerulea)			
Predators			
Hemipteran predators			
Anthocoris nemorum	+		Aphids, thrips
Dicyphus hesperus		+	Whiteflies, spider mites, thrips
Dicyphus tamaninii	+		Whiteflies, thrips
Geocoris punctipes		+	Aphids, mites, thrips, whiteflies,
Macrolophus caliginosus	+		Whiteflies
Macrolophus pygmaeus	+		Whiteflies
Orius albidipennis	+		Thrips
Orius insidiosus		+	Thrips
Orius laevigatus	+		Thrips
Orius majusculus	+		Thrips
Orius minutes	+		Thrips
Orius strigicollis		+	Thrips
Orius tristicolor		+	Thrips
Picromerus bidens	+		Lepidoptera
Podisus maculiventris		+	Lepidoptera
Gallmidges			
Aphidoletes aphidimyza	+		Aphids
Feltiella acarisuga	+		Mites

Table 1: Continued

Natural enemy	Endemic	Exotic	Main target pest
Hoverflies			
Episyrphus balteatus	+		Aphids
Hunter flies			
Coenosia attenuate	+		Diptera, sciarids, leafminers, whiteflies
Lacewings			
Ceraeochrysa cubana		+	Whiteflies, aphids
Chrysoperla carnea	+		Aphids
Chrysoperla rufilabris		+	Aphids
Mallada signata		+	Aphids, moths, scales, whiteflies
Sympherobius sp	+		Mealybugs
Ladybeetles			
Adalia bipunctata	+		Aphids
Chilocorus baileyi		+	Scales
Chilocorus bipustulatus	+		Scales
Chilocorus circumdatus		+	Scales
Chilocorus nigritus		+	Scales
Clitostethus arcuatus	+		Whiteflies
Coccinella septempunctata	+		Aphids
Coleomegilla maculata		+	Aphids, mites, Lepidoptera
Cryptolaemus montrouzieri		+	Mealybugs, scales, aphids
Cybocephalus nipponicus		+	Scales
Delphastus catalinae		+	Whiteflies
Exochomus quadripustulatus	+		Scales
Harmonia axyridis		+	Aphids
Hippodamia convergens		+	Aphids
Hippodamia variegata	+		Aphids
Rhyzobius (Lindorus) lophanthae		+	Scales
Rodolia cardinalis		+	Cottony cushion scales (*Icerya purchasi*)
Scymnus (Nephus) reunioni		+	Mealybugs
Scymnus rubromaculatus	+		Aphids
Stethorus punctillum	+		Mites
Other beetles			
Atheta coriaria		+	Sciarids, thrips
Predatory thrips			
Franklinothrips megalops		+	Thrips
Franklinothrips vespiformis		+	Thrips
Karnyothrips melaleucus		+	Thrips
Scolothrips sexmaculatus	+		Mites, thrips

Table 1: Continued

Natural enemy	Endemic	Exotic	Main target pest
Predatory mites			
Amblyseius barkeri	+		Thrips
Amblyseius fallacies		+	Mites
Hypoaspis aculeifer	+		Sciarids, thrips
Hypoaspis (Stratiolaelaps) miles	+		Sciarids, thrips
Iphiseius degenerans		+	Thrips
Mesoseiulus longipes		+	Mites
Metaseiulus occidentalis.		+	Mites
Neoseiulus (Amblyseius) californicus		+	Mites
Neoseiulus (Amblyseius) cucumeris	+		Thrips
Phytoseiulus persimilis		+	Mites
Typhlodromips montdorensis		+	Thrips
Typhlodromips swirskii	+		Thrips, whiteflies
Typhlodromus doreenae		+	Mites
Snails			
Rumina decollata	+		Snails
Nematodes			
Heterorhabditis bacteriophora	+		Weevils
Heterorhabditis megidis	+		Weevils
Phasmarhabditis hermaphrodita	+		Slugs
Steinernema carpocapsae	+		Weevils, sciarids, soil borne insects
Steinernema feltiae	+		Sciarids

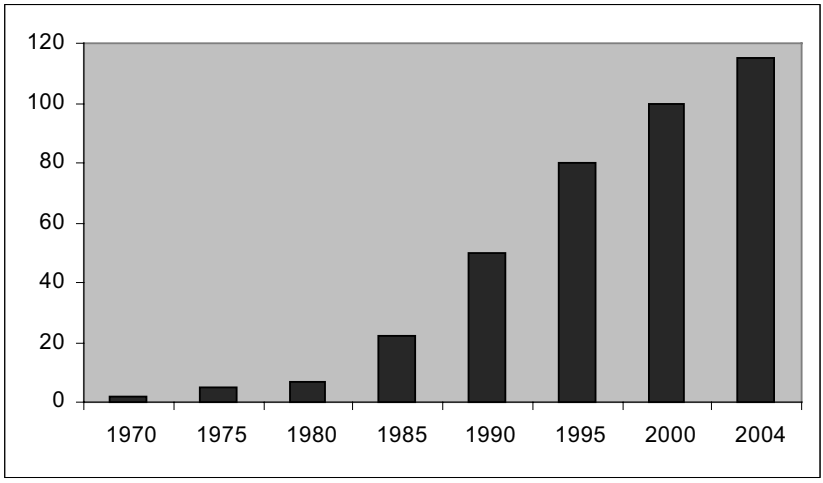

Figure 13: Development in number of commercially available beneficial arthropods. Adapted from van Lenteren & Nicoli (2004)

4. Threats to biocontrol in the 1990's

The major threats against the implementation of biological pest control programs have not only been developments of new effective pesticides against the primary pests or development of uncontrolled secondary pests, as mentioned earlier. Accidental introductions of new severe pest species for which there are no biological control agents developed also pose a thread to existing biocontrol programs. So-called zero-tolerance pest species are not tolerated within designated areas and eradication programs will be initiated should such pests be introduced (e.g. EPPO 2004). These eradication programs will almost always be based on applications of broad-spectrum pesticides that most certainly will destroy biological control programs already in action. Examples of this are the introductions of the American leafminers, *L. trifolii* (Burgess) and *L. sativa* Blanchard (Hymenoptera: Agromyzidae) into European glasshouse crops (Minkenberg, 1988). The eradication programs of some of these introduced species have not been successful and the pests have established in new areas. Two of these introduced pests that recently have managed to establish themselves as severe pests in protected crops almost worldwide are the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and the cotton whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae).

4.1. The western flower thrips Frankliniella occidentalis

The western flower thrips, *F. occidentalis*, is originally distributed in U.S.A. west of Rocky Mountains, where it for long has been a pest in the cotton agro-ecosystem. However, pesticide resistant populations build up and during 1980s insecticide resistant western flower thrips spread to protected crops worldwide (Brødsgaard, 1989a). In the areas where biological control programs were in function, *F. occidentalis* was a major obstacle to biocontrol because it could only, and with great difficulty, be controlled by broad-spectrum pesticides. This was a two-edged sword. Some growers simply gave up biocontrol while others, who experienced the difficulties in chemical control of this thrips, saw and hoped that biocontrol agents might be able to control *F. occidentalis*. Hence, research efforts in Western Europe and Canada were in the late 1980s and early 1990s put into developing biocontrol against *F. occidentalis*. First, the biocontrol agent, *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae), already used against *T. tabaci* in sweet pepper and cucumber crops were tested and used on *F. occidentalis*. However, due to the differences between the biology of the two thrips species such as *F. occidentalis* having a much broader host plant range, a much higher fecundity in flowering crops, and in part different pupation sites compared to *T. tabaci*, the control of *F. occidentalis* by biological means proved to be more difficult than of *T. tabaci*. As with *E. formosa* and *P. persimilis*, *N. cucumeris* was found more or less by chance in a glasshouse crop (Ramakers 1978) and this kick-started biological control of the onion thrips. However, in the case of the western flower thrips coordinated research programs were conducted in many countries on predatory mites and bugs, parasitoids, nematodes, and insect pathogenic fungi (Levis, 1997).

Within the predatory mites new species were investigated and, in addition, *N. cucumeris* as a biocontrol product was improved. Many of the "new" beneficial species were well known thrips predators but emphasis was put into quantifying their predation potential of *F. occidentalis* and their efficacy potential under growing conditions where *F. occidentalis* is a pest. Focus was on the performance of the mites under dry conditions and with availability of

pollen (Sabelis & van Rijn, 1997). The phytoseiid *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae) was found to be a promising candidate (van Houten & Stratum, 1995) and has been in commercial mass production since then. However, also mites not previously associated with thrips predation were discovered as biocontrol agents of *F. occidentalis*, e.g. the soil dwelling *Hypoaspis miles* Berlese (Acari: Hypoaspididae) that was developed by a Canadian research team and now is an implemented mass-produced thrips control agent in Canada and Europe (Gillespie & Quiring, 1990). But also the well known *N. cucumeris* was greatly improved as a biocontrol product in that a non-diapausing strain was selected from a strain originating from New Zealand (van Houten et al., 1995) and with the development of a slow release system for crops not producing pollen as alternative food for the mites (e.g. parthenocarp cucumbers) (Ramarkers, 1990; Shipp & Wang, 2003).

Minute pirate bugs of the genus *Orius*, known to be predatory on *F. occidentalis* in cotton, soybean, and strawberry crops in USA, had since the 1970s been investigated in relation to biological pest control in outdoor crops (e.g. Isenhour & Yeargan, 1981). With the spread of *F. occidentalis* to glasshouse crops, interest in *Orius* spp. increased and several research programs were initiated to develop *Orius* species into commercial biocontrol agents for *F. occidentalis* in protected crops. This has been a success and there are presently a handful different species of *Orius* commercially available for biological thrips control in Europe, Canada, and U.S.A. (Sabelis & van Rijn, 1997) (Table 1).

In many areas where commercial biocontrol agents are used in protected crops, the beneficial arthropods are not endemic to the local fauna. In these areas registration procedures are either lacking or the beneficials are approved based on the assumption that the alien biocontrol agents will not be able to establish permanent populations outside the protected crops due to unfavourable climatic conditions. However, in Australia no non-endemic arthropods are allowed to be imported and, hence, none of the already commercially available biocontrol agents against thrips could be used by the Australian greenhouse growers, when *F. occidentalis* was accidentally introduced in 1993 and thereafter spread throughout the continent. Therefore, to be able to control the highly pesticide resistant *F. occidentalis* biologically, the Australian authorities launched a research program with the aim of finding promising candidates for thrips control within the Australian fauna and developing one or more of these into commercially available biocontrol agents (Goodwin & Steiner, 1996). This quest resulted in hundreds of candidates collected and eventually, after extensive evaluations, two were picked out for mass release experiments (Steiner & Goodwin, 2002). One of these, the phytoseiid *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae), is now in commercial production and available in Australia and Europe (Steiner et al., 2003). Furthermore, a permit for its release in Canada is also currently being sought (Goodwin & Steiner, 2002).

Driven by the wish to find a selective biological control agent with a high searching efficiency against *F. occidentalis*, a Dutch research program, supported by the European Community, was conducted on parasitoids on thrips. Besides building on earlier Japanese results, the Dutch program was, like the Australian mentioned above, a "full" search for a biocontrol agent starting with a more or less global collection of parasitised thrips. Having collected a range of different parasitoid species and strains, a selection procedure was initiated based on studies of basic bionomics, laboratory experiments, glasshouse evaluations, and then mass production. Based on the results of the basic bionomics and laboratory experiments, a strain of *Ceranisus menes* was selected for the glasshouse and mass rearing experiments. Unfortunately, the parasitoid failed to provide adequate thrips control and mass rearing potential (Loomans, 2003), and, unlike the Australian program, the program was stopped.

4.2. The cotton whitefly Bemisia tabaci

In the mid 1980's a new pest appeared in greenhouses in North America and Europe – the B-biotype of cotton whitefly *B. tabaci* also known as the silverleaf whitefly *B. argentifolii* Bellows & Perring (Bellows *et al*., 1994). For a review of the *Bemisia* species-complex see Perring (2001).

This highly adaptable, polyphagous subtropical-tropical species is thought to have originated in Asia or Africa (Brown *et al*., 1995; Campbell *et al*., 1996). The species had formerly been recorded as a pest of especially field crops like cotton, sweet-potato, tomato, cassava, and cowpea (Greathead, 1986) but now the B-biotype began an expansion of its geographical range, attacking new crop species and quickly attaining status as a serious economic pest (e.g. Coudriet *et al*., 1985; Dittrich *et al*., 1986; Gill, 1992; Brown, 1994; Wisler et al., 1998). A range of characteristics accounts for the seriousness of *B. tabaci* as a pest, including its high potential to develop resistance to many pesticides (e.g. Prabhaker et al., 1985; Cahill *et al*., 1996; Horowitz *et al*., 1998, 2002; El-Kady & Devine, 2003); its ability to transmit a multitude of plant pathogenic viruses (e.g. Brown, 1994; de Barro, 1995; Jones, 2003) or induce plant physiological disorders (e.g. Paris, 1993; Baufeld & Unger, 1994; Brown, 1994); and its broad host range (Greathead, 1986; Cock, 1993) that allows it to survive and reproduce – and subsequently disperse between – many crop and weed species both in the field and in greenhouses. In the course of the geographical expansion of the species cross-infestation from field crops to greenhouse crops like Poinsettia occurred and paved the way for a further spread of the species via international trading of greenhouse plants between the continents.

As a consequence, *B. tabaci* soon became a serious pest in greenhouse crops (e.g. Nedstam, 1988; Baranowski *et al*., 1992; Maisonneuve, 1992). In northern temperate greenhouses infestations occurred primarily in ornamentals like Poinsettia, Begonia, Gerbera and Hibiscus (e.g. Anon., 1989; Broadbent *et al*., 1989; Baker & Cheek, 1993; Fransen, 1994). In southern temperate to subtropical regions also vegetables like tomato, cucumber and pepper were attacked (e.g. Al-Samariee *et al*., 1987; Kring *et al*., 1991; Desbiez *et al*., 2003; Lozano *et al*., 2004; Stansley *et al*., 2004). The reason for this difference presumably lies in the fact that *B. tabaci* in more warm climates established on outdoor crops and weeds from which it easily could penetrate the loose-structured greenhouses dominated by production of vegetables. In cooler climates this cross-infestation pathway was not available due to the lack of outdoor establishment and the spread of *B. tabaci* into and between these regions therefore hinged on international trade of growing plants where ornamentals constitute the major part.

Already in the beginning of its geographical expansion *B. tabaci* vectored viral diseases in greenhouse vegetables, for instance Tomato Yellow Leaf Curl Virus (TYLCV) (e.g. Sharaf & Allawi, 1981; Berlinger *et al*., 1983; El-Serwiy *et al*., 1987) in e.g. the Middle East – a fact potentially threatening to greenhouse production of vegetables in other regions. Also the prospective for *B. tabaci* to vector diseases potentially infective to greenhouse ornamentals was a cause for serious concern worldwide (e.g. Giustina *et al*., 1989). In the past decades the worst fears has indeed come through with regard to expansion of the range of viral infections in vegetables vectored by *B. tabaci* – TYLCV has broadened it geographical range (e.g. Louro *et al*., 1996; Moriones & Navas-Castillo, 2000), and new viruses have appeared in formerly uninfested regions, for instance Cucurbit Yellow Stunting Disorder Virus (CYSDV) in greenhouse cucurbits in Spain and France (Berdiales et al., 1999; Desbiez *et al*., 2003), Tomato Chlorosis Virus (ToCV) in greenhouse pepper in Spain (Lozano *et al*., 2004) and Lettuce Infectious Yellow Virus (LIYV) in greenhouse lettuce in Pennsylvania (Brown & Stanghellini, 1988). However, no incidences of transmission of viral diseases in greenhouse ornamentals have yet been reported.

Bemisia tabaci has by now established itself permanently as a greenhouse pest in regions like North Africa, Southern Europe, North America, South America, Australia and Asia (Sukhoruchenko *et al*., 1995; Demichelis *et al*., 2000; Hanafi, 2000; Kajita, 2000; Oliveira et al., 2001; Stansly *et al*., 2004, V.H.P. Bueno, UFLA, Brazil, pers. comm.; M. Steiner, NSW Agriculture, Australia, pers. comm.). In more northern regions for instance in Scandinavia and UK permanent establishment has not occurred but outbreaks of *B. tabaci* occurs annually in greenhouse ornamentals as a result of import of infested plant material (S. Cheek, CSL, UK, pers. comm.; N. S. Johansen, Planteforsk Plantevernet, Norway, pers. comm.).

When *B. tabaci* made its appearance in greenhouses it soon became clear that it was difficult to control with chemicals (e.g. Hamon & Salguero, 1987; Parrella *et al*., 1992) and frequent repeated sprayings became necessary. The use of selective pesticides to avoid side effects on beneficials was not an option and the presence of *B. tabaci* therefore became a serious threat to the recently initiated biocontrol in northern greenhouse ornamentals (Wardlow, 1988; Brødsgaard, 1989b; van Lenteren & Wardlow, 1989). Motivated by the need to effectively control this new whitefly and to some extent also by the wish to preserve the possibility for continued use of biocontrol of other pests, attempts to develop biological control strategies for *B. tabaci* were made.

Since the problems with control of *B. tabaci* was urgent and since no commercial beneficials at that time was targeted directly against *B. tabaci* attention first focused on beneficials available against the greenhouse whitefly, *T. vaporariorum*, i.e. the familiar *E. formosa* (e.g. Albert & Sautter, 1989; Krebs, 1989; Stenseth, 1990; Parrella *et al*., 1991). However, control of *B. tabaci* with this parasitoid was not satisfactory in many cases (e.g. Parrella *et al.,* 1991; Hoddle & van Driesche, 1999 a, b) and other natural enemies needed investigation. As a consequence the research on *B. tabaci* and on the possibilities for biological control increased in the decades to come as illustrated in Figure 14.

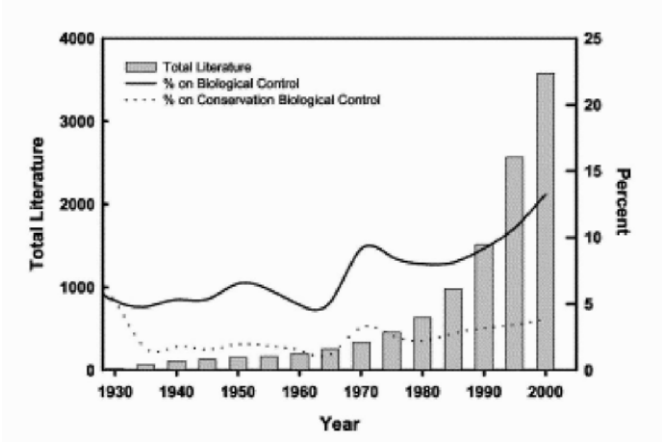

Figure 14: Historical summary of research on B. tabaci/argentifolii and the proportional effort on biological control in both greenhouse and outdoor crops. From Naranjo, (2001)

A number of natural enemies of *B. tabaci* was already known in the 1980's (e.g. Mound & Halsey, 1978; Gerling, 1986; López-Avila, 1986; Cock, 1993). Researchers began investigating some of these for their biocontrol potential (e.g. Gerling, 1987a, b; Kapadia & Puri, 1990) and, in addition, smaller and larger national and international research programmes were launched for worldwide surveys for yet undescribed beneficial species for control of *B. tabaci* (e.g. Faust, 1992; Polaszek, et. al., 1992; Hoelmer, 1996; Henneberry *et al*., 1997, 1998, 1999, 2000; Goolsby *et al*., 2000; Oliveira *et al*., 2001; Nomikou *et al*., 2002). These efforts focused on control of *B. tabaci* with all categories of biocontrol strategies (classic, conservation, inundative, inoculative; see Chapter 1) both in field crops and greenhouses and considerable research efforts have been (and is) undertaken providing information on new beneficial species, their basic biology and behaviour, their interaction with *B. tabaci* and their potential for control. The species of natural enemies investigated includes both extant and imported species. A vast number of natural enemies have been surveyed, and subsequently evaluated in laboratory and greenhouse studies and through release test (e.g. Lacey *et al*., 1993; Goolsby *et al*., 1998; van Lenteren & Martin, 1999, Hoelmer & Goolsby, 2002; Nomikou et al., 2002). As an interesting fact many indigenous parasitoids in the new geographical areas of the expanding *B. tabaci* have been able to attack the pest and to follow with its expansion (Gerling *et al*., 2001) supporting the notion that efficient natural enemies for biological control can indeed be found outside the original geographical source of the pest (e.g. Hokkanen & Pimentel, 1989; Gerling, 1996; van Lenteren & Manzaroli, 1999; van Lenteren & Tommasini, 1999).

By now the list of known natural enemies of *B. tabaci* encompass 114 predators with species of predatory mites (Phytoseiidae), lady beetles (Coccinellidae), lace wings (Chrysopidae) and mirid bugs (Miridae) dominating (Gerling *et al*., 2001); 54 species parasitoids with the genera *Encarsia* and *Eretmocerus* dominating (Gerling et al., 2001); and 11

species of fungi (Hyphomycetes, Entomophthorales) (Faria & Wright, 2001). Of the known species 21 predators and 3 parasitoids are now commercially available for use in greenhouses. The predators are, however, not necessarily developed or recommended for use against *B. tabaci* (Gerling *et al*., 2001). In addition, 3 of the fungi (*Beauveria bassiana* (Balsamo) Vuill (Deuteromycotina: Hyphomycetes)*., V. lecanii, Paecilomyces fumosoroseus* (Wize) Brown & Smith (Deuteromycotina: Hyphomycetes)) with control efficacy towards whiteflies are on the market (Faria & Wright, 2001). This list will, of course, expand in years to come as a result of continued research, including recently initiated research in geographical areas that are a recent addition to the geographical range of *B. tabaci* e.g. South America and Australia (de Barro et al., 2000; Gerling et al., 2001; V.P.B. Bueno, UFLA, pers. comm.). Provided that sufficient research funding is available it is therefore likely that new potentially important beneficials will be discovered and that these are eventually marketed for use in greenhouses, hereby adding to the existing arsenal.

Satisfactory control of *B. tabaci* in greenhouse crops can now in some instances be achieved with *E. formosa, Eretmocerus eremicus* (Rose and Zolnerowich) (Hymenoptera: Aphelinidae), *E. mundus* Mercet (Hymenoptera: Aphelinidae), *M. caliginosus*, *Delphastus catalinae* (Hom) (Coleoptera: Coccinellidae) (previously *D. pusillus* LeConte (Hoelmer & Pickett, 2003)), *Chrysoperla rufilabris* (Brumeister) (Neuroptera: Chrysopidae), *V. lecanii* and *P. fumosoroseus* (e.g. Breene *et al.,* 1992; Stenseth, 1993; Osborne & Landa, 1994; Hoddle *et al*., 1997, 1998; Hoddle & van Driesche, 1999a, b; van Driesche *et al*., 1999: van Lenteren & Martin, 1999; Alomar et al., 2003; Richter et al., 2003; Stansly et al., 2004). However, the impetus to apply biocontrol of *B. tabaci* in practice is limited presently due to availability of pesticides still able to provide adequate control (e.g. Ishaaya *et al*., 2002; Otoidobiga *et al*., 2003; Elzen, 2004; Liu, 2004). In addition, biocontrol of *B. tabaci* still remains difficult in many places and crops and further research and development of new additional beneficials and strategies for use is needed (e.g. Hoelmer, 1996; Gabarra & Besri, 1999; van Lenteren & Martin, 1999; Hoddle, 2004).

4.3. Present status of biocontrol

The overview of the history of biocontrol in greenhouses illustrates that the lack of efficient pesticides has been a major driving force in selection, development and implementation of beneficials for pest control in these crops. It is estimated that biocontrol is used on 15,000 ha of the 300,000 ha with greenhouses worldwide (van Lenteren, 2000). This evolution has resulted in about 115 species of beneficials now being commercially available for biocontrol of pest on the many different plant species grown as vegetable and ornamental crops in greenhouses (Table 1). Growers have therefore become increasingly equipped to cope with the many different pest species in their crops.

However, status quo is not a term that apply to the greenhouse industry. Especially ornamental growers are innovative, constantly trying to adapt to a market craving for new types of products and new plant varieties and species. As a consequence international trade of ornamental plants continues to escalate and markets in new geographical areas like South America, Asia and Africa are developed hereby increasing the risk of introduction of new pest species to areas formerly beyond their natural range (van Driesche, 2002). This threat to the greenhouse industry will continue to exist or may even increase in the future, since phytosanitary measures may prevent establishment of some introduced pests but not all. Thus new pests establish in new regions at rates of e.g. 0.6 (Australia), 1 (the Netherlands), 4 (Japan)

or 20 (Hawaii) every year (van Lenteren & Loomans, 2000). A characteristic of invasive arthropod species is their generally high resistance to pesticides (or perhaps herbivore species become invasive *because* they are highly resistant). This creates situations in which growers have to resort to existing biological solutions which may be insufficient towards new pest species, in which case the call goes to the scientific community for rapidly finding of new efficient natural enemies.

5. Factors limiting to bringing new beneficials in use

5.1. Biodiversity – a limiting factor?

The above examples from the history of biocontrol in greenhouses have illustrated that it through time has been possible to find natural enemies of various pest species and to implement their use in practice. That useful natural enemies of pests are available for such exploitation is further illustrated through the numerous examples of successful biocontrol (both classic and otherwise) of both pests and weeds in outdoor crops and landscapes.

No matter the origin of a herbivore species that enters a new geographical area and establish itself as a pest in greenhouses, a number of natural enemies exist that may eventually be adapted as a biocontrol product or in other ways made available for growers for seasonal inoculative or inundative releases in greenhouses.

Previously the notion that exotic pests could only, or at least most efficiently, be controlled by natural enemies of the same geographical origin prevailed (e.g. DeBach, 1964; Huffaker & Messenger, 1976), this notion presumably originating from the many well known examples of classic biological control of pest introduced e.g. to North America from Europe by releases of European natural enemies. However, there are no scientific arguments to support that this notion is an inescapable truth. On the contrary many examples have shown that exotic pests can just as well be controlled by indigenous natural enemies and vice versa (e.g. Hokkanen & Pimentel, 1989; Gerling, 1996; van Lenteren & Manzaroli, 1999; van Lenteren & Tommasini, 1999).

Thus, the biodiversity pool from which natural enemies of a new exotic pest are to be found is not limited to its original geographical area of distribution. The scientific community may look for natural enemies in the local fauna or perhaps even in the fauna of yet another geographical area. The number of insects and mites – which so far have been the most common choice for biocontrol of pests in greenhouses – worldwide is enormous and the proportion of predaceous or parasitic species is proportionally enormous. Add to this a worldwide flora of bacterial and fungal insect pathogenic species together with an equally diverse fauna of entomopathogenic nematodes and it becomes clear that it is not the natural availability of potential beneficials that in any way limits future development of new biocontrol agent. Rather, other factors play a crucial role.

5.2. Finding promising candidates

The above mentioned examples of how new beneficials have been found through times illustrates that the process of finding new promising candidates for biocontrol can take any shape between the two extremes – the empiric approach where a new biocontrol agents are

discovered mainly by chance and the painstaking, yearlong systematic search for and collection of candidates from different geographical regions of the world. No matter the approach research funding is crucial – naturally with no funding, no new natural enemies can be developed and implemented; and equally logic: the more funding the greater the opportunity to scrutinise the biodiversity pool in depth.

5.3. Evaluating and choosing between candidates

Once one or more natural enemies with a potential for controlling the pest in question has been found, a process of evaluation of these candidates sets into motion. This evaluation naturally aims at judging the candidates characteristics as biocontrol agents but assessment of possible unwanted qualities (i.e. potential harm to humans or livestock, polyphagy, hyperparasitism, etc.) and their magnitude and mass production potential are also needed.

Through the history of biocontrol in greenhouses this selection procedure has varied from rather simplistic and superficial tests of biocontrol efficacy to more elaborate and theoretically founded studies of various biological characteristics (rate of population increase, rate of prey kill, influence of climate, etc.) (e.g. van Lenteren, 1986a, 1986b; van Lenteren & Woets, 1988; van Lenteren & Loomans, 2000). The latter approach was developed to counterbalance the empirical procedure aiming at more optimised and efficient evaluation processes. The biological characteristics wanted in a good natural enemy (selection criteria) vary, of course, with the intended introduction strategy – in inoculative strategies focus will be on the synchronisation of the natural enemy with the pest, searching efficiency and reproductive capacity, whereas these aspects are of lesser importance when inundative strategies are used (van Lenteren & Woets, 1988). In the analytical approach several natural enemies are compared with respect to various characteristics in an attempt to time-savingly predict their efficiency (e.g. Drost et al., 1996). It should, however, be kept in mind that the range of enemies tested and compared still inherently is just a more or less random subset of all existing natural enemies of the pest aimed to be controlled.

Selection criteria should serve as guidelines for wanted and unwanted qualities in a potential beneficial, not as lists that should be followed dogmatically. Thus, it has often been claimed that exotic polyphagous predators should be disregarded as biocontrol agent out of the notion that this characteristic increases the risk that unintentional interactions with other beneficials in the cropping system or with the local fauna (Pimm, 1989; van Lenteren & Loomans, 2000). However, polyphagy might be accepted in cases where the predator in question can clearly be demonstrated to be unable to survive outside the greenhouse environment during unfavourable seasons – herewith establishment and subsequent negative impacts on the local fauna will be negligible (van Lenteren & Loomans, 2000). Interactions with other beneficials in the greenhouse system may still occur (e.g. Rosenheim *et al*., 1995) but if the predator is efficient towards the target pest this may be tolerated and/or managed. In addition, the polyphagous predator may in fact contribute to the control of other pests and through its polyphagous nature sustain itself when target pest populations are low in density (Brødsgaard & Enkegaard, 1997). Several examples of polyphagous predators among the arsenal of beneficials used in greenhouses exist (Table 1), e.g. *Orius* species successfully used for control of thrips and other pests.

Likewise a natural reaction is to disregard facultative phytophagous species as suitable candidates for biocontrol since these inherently possess the ability to damage the crops in which

they are to function. However, a trait of facultative phytophagy should be evaluated in conjunction with other characteristics and potentials of the species in question before it is deemed useless. *M. caliginosus* is an example of such a facultative phytophagous predator, known indeed to be able to inflict damage to certain crops, e.g. certain tomato varieties and Gerbera (e.g. van Schelt *et al*., 1996; Sampson & Jacobson, 1999). However, *M. caliginosus* is an efficient predator of especially whiteflies used successfully in many countries, often supplementing biocontrol by parasitoids (Lenfant et al., 1998; Muhlberger & Maignet, 1999). The fact that this predator is able to sustain its populations on a diet of plant sap alone (van Schelt *et al*., 1996) is in some instances beneficiary because it allows it to establish early when pest densities are low.

Other qualities in a potential beneficial that at first seem disqualifying might likewise be circumvented or managed in ways to make implementation of the species in question possible. The use of personal protection equipment for greenhouse workers might for instance facilitate the use of a new predatory mite that has been shown to provoke allergic reactions in humans.

A point to be noted with respect to selection of candidates is to keep in mind that successful biocontrol of a certain pest now a days often is based upon the use of more than just one natural enemy. Instead combinations of beneficials are used either in succession (e.g. the introduction of aphid parasitoids followed by later application of gallmidges) or simultaneously but aimed at different niches within the habitat of a greenhouse crop (e.g. the use of soil-dwelling predatory *Hypoaspis* mites for control of thrips pupae in addition to predatory mites and minute pirate bugs for control of nymphal and adult thrips on the above-ground plant parts).

Finally the theoretically based selection procedure may not be especially appealing to commercial producers wishing, as a competitive strategy, to be able to launch a new suitable beneficial without to much delay after it has been discovered and found efficient.

5.3.1. Registration

In addition to the evaluation of natural enemies with the aim of identifying the most suitable candidate for biocontrol of a specific pest species, other evaluations are becoming increasingly important as more and more countries implement regulation procedures for import and release of natural enemies. The aim is to try to ensure that the use of natural enemies for biocontrol does not have any negative impacts on the environment and the local fauna (see e.g. Hokkanen & Lynch, 1995; Haynes & Lockwood, 1997; van Lenteren *et al*., 2003). Statutory registration of microorganisms has already existed for a number of years in many countries and will not be dealt with further in this chapter (see e.g. Hall & Menn, 1999 for additional information).

However, many countries also apply some form of regulation concerning macroorganisms. As no harmonised system exists yet, requirements for registration of a macroorganism differ between countries – some require documentation that an alien macroorganism is unable to establish itself in nature or at least do not have any harmful impact on the local fauna (e.g. Norway, Nina S. Johansen, Planteforsk Plantevernet, Norway, pers. comm..) while others in addition also require documentation for efficacy in specified crops not only of alien but also of indigenous species (e.g. Switzerland, Serge Fischer, Station Federale de Recherches en Production Vegetale de Changins, Switzerland; pers. comm.).

The procedures of registration have impeded the continued development of new beneficials for biocontrol in the countries in question either by making it unattractive for companies to apply for approval due to the costs involved compared to the anticipated return income, or by

the delayed registration merely due to the bureaucratic evaluation procedure. This is illustrated by the fact that the assortment of commercially available macroorganisms for biocontrol in greenhouses in countries where macroorganism registration is required is much lower (20-25 species (Nina S. Johansen, Planteforsk Plantevernet, Norway; Sylvia Blümel, Austrian Agency for Health and Food Safety; Barbro Nedstam, Swedish Board of Agriculture; Serge Fischer, Station Federale de Recherches en Production Vegetale de Changins, Switzerland; pers. comm.)) than in countries without this legislation (more than 100 species, Table 1).

Attempts to develop a harmonised and relatively simple system of regulation regarding import and release of biocontrol agents is presently underway for Europe (see van Lenteren, 2005). The future will show if the intended simplicity can be achieved herewith pursuing the goal of stimulating the use of biological control.

5.4. Producing and selling the chosen candidate

Once a potential beneficial has been identified an economical method for mass production needs to be developed, either for implementation at a commercial producer or for establishment of local rearings at the growers or cooperatives. The list of presently available beneficials (Table 1) shows that it has indeed been possible to design mass production methods for numerous and very different types of organisms. However, in some instances mass production may not be feasible either because it is too time consuming or too expensive in terms of the material needed to sustain production. A potential candidate that has passed unhindered through the various selection steps might end up being discarded for commercial marketing on grounds of being e.g. too cannibalistic which for rearing would require time consuming efforts to keep this internal mortality factor at a minimum.

Another aspect related to production is quality – an otherwise suitable candidate might be abandoned because it is difficult to produce it in an appropriate quality or to formulate a product with an acceptable shelf life.

For a commercial company to commit itself to production of a new beneficial the company must judge that the beneficial can be sold with an acceptable profit. This means that potential candidates may be disregarded for production if the market is very limited, e.g. because the target pest of the beneficial is of limited importance or because the beneficial has a very limited host range. This necessity for profit making in some cases tends to promote marketing of beneficials with a more broad host range and/or beneficials that can be applied in many different greenhouse crops.

5.5. Making growers use the chosen candidate

That a new beneficial has been made available to the growers does not necessarily imply that it will be applied as a biocontrol agents. Several factors influence the uptake of biocontrol in general by growers, including the status of grower education; the availability of advisory systems; the quality of beneficials; the perceived complexity of applying biocontrol instead of chemical control; the costs; the possibility for overpricing the product (e.g. being organically grown); and – importantly – the availability of pesticides. These matters will not be discussed further, please refer to e.g. Bolckmans (1999), van Lenteren (2003), Bennison (2004).

6. The future

Even though the motivation for the increased use of biocontrol encompasses such factors as idealism among growers, concern for the working environment among greenhouse workers and a wish to avoid phytotoxic effects on plants, the overriding factor influencing the attitude to and willingness to use biocontrol still relates to pesticides issues: growers resort to biocontrol mainly when pesticides are lacking or low in availability (due to legislative regulations and/or limited marketing of new pesticides for the rather small horticultural market) or when existing pesticides are inefficient due to resistance development. A very illustrative example of this is from the tomato industry. In order to produce fruits, the tomato flowers need to be pollinated. This was previously done by hand and as such very time consuming and, thus, expensive. However, after a huge research effort in Belgium and The Netherlands, year-round rearing of bumblebees was developed. Bumblebees are excellent pollinators of tomatoes and when commercial production of bumblebee colonies became available, tomato growers switched away from hand pollination over-night. Besides adding to the biodiversity in tomato crops, bumblebee pollination more or less put a stop for the growers' possibilities to use insecticides on their crops. The result has been that all growers of greenhouse tomatoes in Northern Europe and Canada uses biological pest control.

On the other hand, the present interest among e.g. Danish ornamental growers for using biocontrol, for supporting continued development and innovation of existing and new methods – and for their integration with other plant protection measures – is limited compared with the 1990s due to the recent marketing of e.g. imidachloprid and spinosad for control of phloem suckers and thrips and leaf miners, respectively. Unfortunately, it does not take long for the majority of growers to abandon biocontrol application and revert to chemical control with little or any thought for longer-term resistance-management strategies.

In spite of the fact that the use of biocontrol in greenhouses has been and still mainly is driven by pesticide related motivation it is our belief that biocontrol is here to stay and that biocontrol, possibly in combination with other non-chemical measures, in the long run will be the most sustainable plant protection measure in greenhouses. Biocontrol is a truly sustainable means of control. Once a system is implemented it will be functioning as long as the plant production practices remain unchanged.

Therefore, the need for improved biocontrol and for finding and developing new beneficial agents will continue to exist to allow us to be able to combat not only those pests already harbouring our greenhouse crops but also those that in the future are bound to appear in these crops as a consequence of the incessantly increasing trade of plants and plant parts in a more and more globalised world.

7. Conclusion

Biodiversity is not a limiting factor for a continued expansion of the arsenal of beneficial species used for biocontrol of pests in greenhouses worldwide. New potential candidates can always be found in the local fauna in the geographical origin of the pest, in the area to which the pest has been introduced or in yet other geographical regions *provided* that 1) research

funding for search for and evaluation of natural enemies in terms of their biology, efficacy and mass production possibilities is available; 2) releases of the beneficial in question can be permitted in greenhouses; and 3) the species can be profitably mass produced and sold.

Unfortunately these conditions, especially 1 and 2, are far from fulfilled in most cases: research funding is presently decreasing in many countries and seldom allow thorough exploration and/or evaluations and new beneficials are still in many cases discovered by chance; and registration requirements are costly and many commercial producers may refrain from trying to obtain permits for beneficials, however much wanted, if the intended market is unprofitable.

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CHAPTER 8

THE SOIL AS A RESERVOIR FOR ANTAGONISTS TO PLANT DISEASES

Claude Alabouvette and Christian Steinberg

1. Introduction

The soil is often considered the milieu providing support for plant roots, water and nutrients for plant growth. But it is also considered a hostile environment harbouring plant pathogenic nematodes, bacteria and fungi. The most common attitude is to try to eliminate the plant pathogenic organisms by biocidal treatments such as methyl bromide fumigation, which are dangerous for man and the environment. Beside this pathogen eradication strategy, another approach to control soil-borne plant diseases consists in studying the plant-pathogen interactions at the cellular and molecular level to create new resistant cultivars or to develop new plant protection products based on elicitation of plant defence reactions. This field of research only focuses on plant pathogen interactions, not taking into account the environment in which they take place.

Although a plant disease results from the intimate interaction between a plant and a pathogen, the importance of these direct interactions should not hide the role of environmental factors which influence disease severity. These indirect interactions are particularly important in the case of diseases induced by soil borne pathogens. Indeed, the pathogens are not freely interacting with the plant; they are included in the soil matrix and thus can not escape to the soil environment. Both their inoculum density and infectious capacities are controlled by the soil. Evidence of these interactions is given by the existence of soils that suppress diseases. In suppressive soils disease incidence or severity remains low in spite of the presence of the pathogen, a susceptible host plant and climatic conditions favourable for disease development. These suppressive soils provide examples of biotic and abiotic factors affecting the pathogen, the plant or the interaction between plant and pathogen. In other words, suppressive soils provide examples where biological control, similar to conservation biological control, is active in nature.

Therefore many studies have been devoted to the understanding of soil suppressiveness in order to use suppressive mechanisms in biological control strategies. Since in many cases, antagonistic micro-organisms play a role in soil suppressiveness, the soil has been seen as a reservoir of potential biological control agents. For the two or three last decades the main approach was to identify effective antagonists in soil and try developing them as biological control agents. Most of the biological control agents on the market, even when aerial diseases are targeted, have been isolated from soil. But in order to control soil-borne diseases, one must admit that this strategy has not been as successful as expected. Indeed, even if the soil harbours effective antagonists, soil suppressiveness is due to an association of mechanisms and micro-

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organisms, and a single antagonist is never as efficient as the soil itself. Thus another more ecological approach consists in enhancing natural suppressiveness that exists in every soil. Some cultural practices might modify the microbial balance in a way that soil inoculum potential will be decreased, and/or the soil suppressiveness increased.

In this chapter, we will present the concepts of soil inoculum potential and soil receptivity to diseases, review the mechanisms by which soil suppresses some diseases, give examples of antagonistic micro-organisms selected from the soil microflora and developed as biological control agents, then indicate some alternative approaches such as the use of soil amendments, biodisinfestation and other cultural practices having a beneficial effect on soil quality and soil health

2. Soil receptivity to diseases and soil inoculum potential

Soil suppressive to diseases induced by the most important soil-borne pathogens have been described; they include fungal and bacterial pathogens but also nematodes (Schneider, 1982; Cook and Baker, 1983; Schippers, 1992, Westphal and Becker 2001). These soils control root rot and wilt diseases induced by: *Aphanomyces euteiches*, *Cylindrocladium* sp., several formae speciales of *Fusarium oxysporum, Gaeumannomyces graminis, Pythium spp., Phytophthora spp., Rhizoctonia solani, Ralstonia solanacearum, Streptomyces scabies, Verticillium dahliae, Thielaviopsis basicola (Chalara elegans)*. This large diversity of pathogens controlled by suppressive soils shows that soil suppressivenes is not a rare phenomenon. On the contrary every soil has some potential of disease suppression, leading to the concept of soil receptivity to diseases.

The receptivity of a soil to microbial populations is its capacity to control more or less the activity of the populations present in this soil; in case of plant pathogens, it is the capacity to control the pathogenic activity.

The soil is not a neutral milieu where pathogenic micro-organisms interact freely with the roots of the host plant; on the contrary the soil interferes in several ways with the relationships between and among micro-organisms, pathogens and plants, and it can modify the interactions among micro-organisms themselves. Soil receptivity (or soil suppressiveness) is a continuum going from highly conducive soils in which disease incidence is very high to strongly suppressive soils (Alabouvette *et al*., 1982; Linderman *et al*., 1983).

This concept of soil receptivity was already evoked in the definition of "inoculum potential" proposed by Garett (1956, 1970) as "the energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected". One of the most important words in this definition is "energy" of growth. It clearly states that the presence of the inoculum although necessary is not sufficient to explain the disease. Among the factors that affect the "energy of growth" of the inoculum, Garett (1970) pointed to "the collective effect of environmental conditions", and indicated that "the endogenous nutrients of the inoculum might be augmented by exogenous nutrients from the environment".

Applied to soil-borne pathogens, this concept of inoculum potential led to that of "soil inoculum potential" which was at the origin of both theoretical and practical studies. Baker (1968) gave a definition of inoculum potential as the product of inoculum density by capacity. Louvet (1973) proposed to define inoculum capacity as the product of inate inoculum energy by the effects of the environment on this inoculum. Thus in this definition, the effects of the

environment on the inoculum corresponds to what we have defined above as the soil receptivity to diseases.

Later, the soil inoculum potential was defined by Bouhot (1979) as the pathogenic energy present in a soil. This inoculum potential depends on three main factors: the inoculum density, the pathogenic capacity of this inoculum and the soil factors which influence both the inoculum density and capacity. These factors again correspond to the soil receptivity as defined above.

Thus, whatever the definition all these authors acknowledge that the soil plays a major role in influencing the interactions between a susceptible host plant and its specific pathogens present in soil. It is therefore very important to take into consideration both the inoculum potential of a naturally infested soil and its level of suppressiveness, when elaborating control strategies.

3. Mechanisms of disease suppression

In nature, suppressive soils can be detected by the observation that disease severity in a crop remains low despite the presence of a susceptible host plant, climatic conditions favourable to disease expression and ample opportunity for the pathogen to be present. It is quite easy to experimentally demonstrate that a soil is suppressive to a given disease. The pathogen has to be produced in the laboratory and introduced into the soil at increasing inoculum densities. A susceptible host plant is sown and cultivated under standardized conditions favourable to disease expression. Observations of symptom appearance enable disease progress curves to be drawn with respect to time and inoculum concentrations. Area under the disease progress curve (AUDPC) is the most common method to evaluate disease incidence or disease severity. Appropriate statistical methods (Baker *et al*., 1967; Höper *et al*., 1995, Jeger, 2004) enable these curves to be compared with those obtained from another soil known to be conducive to the disease. All experimental conditions being similar, differences in disease incidence must be attributed to differences in the soil environment, i.e. differences in the level of soil receptivity.

3.1. Nature of soil suppressiveness

Disease suppression does not necessarily imply suppression of the pathogen. In most cases the inoculum is still present but does not provoke the disease. Therefore, Cook and Baker (1983) distinguished: (i) pathogen-suppressive soils, where the pathogen does not survive, from (ii) disease-suppressive soils where inoculum is present but does not induce the disease. Only studies of the mechanisms of suppression enable the distinction between the two types of suppressiveness to be made.

From a theoretical point of view, both the abiotic characteristics of a soil and its biological properties can be responsible for disease suppression. However in most cases, suppressiveness is fundamentally microbial in nature. Disease suppression results from more or less complex interactions between the pathogen, and all or a part of the soil microbiota. Indeed, the suppressive effect disappears upon destruction of organisms by biocidal treatments such as steam or methyl bromide, and can be restored by mixing a small quantity of suppressive soil into the previously disinfested soil (Alabouvette, 1986). Suppressiveness can also be restored in the steamed disinfested soil by re-introduction of a mixture of micro-organisms previously isolated from the suppressive soil (Alabouvette, 1986).

This demonstration of the essential role of the saprophytic microflora does not establish that soil physical and chemical properties do not play any role in the mechanisms of suppressiveness. On the contrary, early studies on Fusarium suppressive soils established correlation between soil type, presence of smectite clays and soil suppressiveness to Fusarium wilt in Central America (Stover, 1962; Stotzky and Martin, 1963). In the case of Swiss soils suppressive to black root rot of tobacco, Stutz et al. (1989) showed that only soils derived from moraine and containing vermiculitic clay minerals were suppressive to *Thielaviopsis basicola*. Abiotic soil characteristics also play a major role in soil suppressiveness to *Aphanomyces euteiches* (Oyarzun *et al*., 1998, Persson *et al*., 1999) and *Rhizoctonia solani* (Steinberg *et al*., 2004).

3.2. Mechanisms of soil suppressiveness

There exist several types of soil suppressiveness and Cook and Baker (1983) proposed three criteria to characterize disease suppressiveness in soils: "the pathogen does not establish; it establishes but fails to produce disease; or it establishes and causes disease at first but then disease severity diminishes with continued growing of the same crop".
The well-known and widespread phenomenon of take-all decline is the best example of soils becoming suppressive with continuous cropping of the susceptible host plant. The disease increases in severity during the first years of wheat cropping, then decreases to an economically acceptable threshold (Hornby, 1998).

Fusarium wilt suppressive soils provide a good example of soils where the pathogen is present in the soil but fails to produce the disease (Scher and Baker, 1982; Alabouvette, 1986). It was established that the dynamics of a marked inoculum of *F. oxysporum* f.sp. *melonis* were similar in a conducive soil and in a suppressive soil from Châteaurenard; thus the difference in disease incidence had to be attributed to a reduced activity of the pathogen in the suppressive soil. Indeed, the percentage of germinating chlamydospores is always extremely low in the suppressive soil. This limited germination of chlamydospores was attributed to the general phenomenon of soil fungistasis (Lockwood, 1977), which is related to competition for nutrients. Addition of increasing concentrations of available carbon, in the form of glucose, resulted in increasing percentages of germinating chlamydospores in both conducive and suppressive soils. (Sneh *et al.,* 1984; Alabouvette, 1986). These results suggest that competition for nutrients, and fungistasis, are much more intense in suppressive than in conducive soils and contribute to reducing the activity of the fungal pathogens. Indeed, glucose amendments that induced chlamydospore germination of the pathogen also induce disease in the suppressive soils. Competition for nutrients, especially competition for energy among heterotrophic micro-organisms, is due to the communities of soil micro-organisms active at any given time and therefore should be linked to the activity of the microbial biomass of the soil.

The microbial biomass, measured by Jenkinson's method (Jenkinson and Powlson, 1976) is always greater in the Châteaurenard suppressive soil than in a conducive control soil. Studies on the kinetics of soil microbial respiration after glucose amendment (Alabouvette 1986; Amir and Alabouvette, 1993) showed further that the soil microflora of the suppressive soil is more responsive to carbon than that of the conducive soil. Consequently, carbon is utilized more quickly and the development of any given organism is stopped more rapidly after glucose amendment in the suppressive than in the conducive soil.

This type of phenomenon corresponds to the "general suppression" described by Cook and Baker (1983) as the inhibition of the pathogen in soil in relation to the total amount of the microbial activity acting as a nutrient sink. A high microbial biomass combined with a very intense competition is responsible for a permanent state of starvation leading to fungistasis inhibiting the growth of the pathogen. This general suppression was already proposed by Gerlach (1968) as an explanation for take-all decline of wheat in polders.

Competition for nutrients other than carbon, especially nitrogen and iron, has been involved in the limitation of germination of fungal propagules in the soil (Cook and Snyder, 1965; Benson and Baker, 1970; Scher and Baker, 1982). Consequently, the population of pathogens faces general competition resulting from the activity of the microbial biomass but also competition exerted by a specific population. For instance, the siderophore-iron competition achieved by fluorescent pseudomonads is responsible for the reduced growth of *Fusarium* spp. in vitro and in suppressive soils (Sneh et al., 1984; Elad and Baker, 1985). Addition of ethylenediaminedi-*o*-hydroxyphenyl-acetic acid (EDDHA), which limits the concentration of iron available for *Fusarium*, results in a lower percentage of diseased plants in a conducive soil. In contrast, addition of Fe-ethylenediaminetetraacetic acid (FeEDTA), which provides iron available for *Fusarium*, results in a higher percentage of diseased plants in the suppressive soils (Lemanceau, 1989). General competition occurs simultaneously for both carbon and iron, in the suppressive soil from Châteaurenard.

Competition for nutrients is not the only mechanism by which antagonistic populations interact with pathogens in soil. Today, antibiosis has been shown to be involved in the inhibition of the pathogen activity in suppressive soils. Indeed, Raaijmaker and Weller (1998) were able to correlate the suppressiveness of soils to take-all with the density of the population of *Pseudomonas fluorescens* producing 2-4 diacetyl phloroglucinol. But it must be underlined that this "specific suppression" always operates on a background of general suppression as stated by Cook and Baker (1983). The high intensity of general competition enhances or increases the significance of specific interactions, either competition or antibiosis, between pathogens and antagonists sharing the same ecological niches in the soil and the rhizosphere. The choice of focusing on specific populations of antagonists is justified by the objective of developing biological control agents.

4. Inoculation and inundation biological control

As stated above, suppressive soils were seen as a source of potential biological control agents. Rather than selecting antagonists at random, selecting them among the micro-organisms isolated from suppressive soils might increase the probability of success.

4.1. Screening of biological control agents

The first step in developing a biological control method is the screening of an effective strain of biological control agent. Two different approaches can be followed.

The first approach, the traditional one, is based on a random screening among many strains owing to a standardised method where the antagonist is confronted with the pathogen, in the soil environment and in the presence of the host plant. Several levels of bioassays are conducted, enabling to progressively decrease the number of strains tested. At the beginning, with the largest number of strains, the bioassay is conducted under artificial conditions,

sometimes in vitro, most often in a sterile substratum such as sand or peat to grow the plant. At the end of the process a very limited number of strains are evaluated for their biological control capacity under conditions similar to that of their application in the targeted crop (Hökeberg *et al*., 1997). This approach does not need any pre-existing knowledge of the modes of action of the antagonists that are most often chosen at random. This approach is space and time consuming, but enables to detect biological control agents fitting with the environment where they will be applied.

On the contrary, the second approach is based on the pre-existing knowledge that a given function, for example antibiotic production, plays a major role in the antagonism expressed by a microbial species against the pathogen. Then, the strategy consists in screening for this function owing to *in vitro* assays. In fact, when the genes coding for this function are known, it is possible to base the screening procedure on the tagging of these genes among a large population of micro-organisms. For example, in the case of the fluorescent *Pseudomonas* spp., most of the genes coding for antibiotic production, such as phenazine or 2-4 diacetylphloroglucinol, are characterised. Therefore it is possible to screen among a large collection of bacteria for the presence of these genes. But, then it is necessary to study the expression of these genes, since the presence of the genomic sequence does not necessary implies the production of the given metabolite in the environment where the biological control agent will be used.

Scientists in favour of the first approach argue that to be effective a biological control agent must not only possess the required modes of action but be also well adapted to the environment where they have to express theses functions (rhizosphere, spermosphere). And until now, only a few teams have been involved in the study of the genes coding for the "ecological fitness" of the biological control agents. Therefore there is a risk of selecting potentially very active antagonists that will not be able to survive or to express their beneficial properties in the soil environment. Scientist in favour of the second approach argue that knowing the most important functions will enable the manipulation of the biological control agents in order to add several modes of action in a single strain or to deregulate the production of an important metabolite in order to have it produced in greater quantity or at the right time.

4.2. Modes of action of biological control agents

Antagonistic effects responsible for disease suppression results either from microbial interactions directed against the pathogen, mainly during its saprophytic phase, or from an indirect action through induced resistance of the host-plant.

Microbial antagonism implies direct interactions between two micro-organisms sharing the same ecological niche. Three main types of direct interactions may be characterised: parasitism, competition for nutrients and antibiosis.

Parasitism of a plant pathogen by other micro-organisms including viruses is a well-distributed phenomenon. The parasitic activity of strains of *Trichoderma* spp. towards pathogens such as *Rhizoctonia solani* has been extensively studied (Chet and Baker, 1981) and other mycoparasites such as *Coniothyrium minitans* and *Sporidesmium sclerotivorum* are efficient in controlling diseases caused by *Sclerotinia* spp. and other sclerotia forming fungi (Adams and Fravel, 1993; Whipps and Lewis, 1980).

Competition for nutrients is a general phenomenon regulating population dynamics of micro-organisms sharing the same ecological niche and having the same physiological

requirements. Competition for carbon in soil is considered as responsible for the well-know phenomenon of fungistasis (Lockwood, 1977) describing the inhibition of fungal spore germination in soil. Energy deprivation in soil is also partly responsible for "general suppression of a pathogen which is directly related to the total amount of microbial activity at a time critical to the pathogen " (Cook and Baker, 1983). This general suppression has been demonstrated to play a role in the determinism of the suppressiveness of soils to fusarium wilts, where it controls competition for carbon between pathogenic and non-pathogenic *Fusarium oxysporum* (Alabouvette *et al*., 1986). Some strains of nonpathogenic *F. oxyxporum* are more competitive than other and should be selected for biological control (Couteaudier and Alabouvette, 1990). Competition for minor elements also frequently occurs in soil, and for example competition for iron is one of the modes of action by which fluorescent pseudomonads limit the growth of pathogenic fungi and reduce disease incidence or severity (Schippers *et al*., 1987; Bakker *et al*., 1991; Lemanceau and Alabouvette, 1993).

Antibiosis is the antagonism resulting from the production by one micro-organism of secondary metabolites toxic for other micro-organisms. Antibiosis is a very common phenomenon responsible for the biological control activity of many biological control agents such as *Bacillus* spp., *Streptomyces* spp., *Trichoderma* spp or fluorescent *Pseudomonas* spp. A large diversity of antibiotics, bacteriocines, enzymes and volatile compounds have been described and their role in suppression of several plant pathogens has been documented (Loper and Lindow, 1993; Thomashow and Weller, 1996). A given strain of a biological control agent may produce several types of antifungal compounds effective against certain species of fungal pathogens. For example, the strain CHAO of *Pseudomonas fluorescens* is producing siderophores, phenazines, 2.4-diacetylphloroglucinol and cyanide, a different combination of these metabolites being responsible of the antagonism expressed against *Gaeumannomyces graminis* var. *tritici* and *Chalara elegans* (Défago and Haas, 1990). It is important to emphasise that a single antifungal metabolite generally does not account for all the antagonistic activity of a biological control agent.

Induced systemic resistance classically occurs when an inducing agent pathogenic or not is applied prior to challenge inoculation with a pathogen, resulting in reduced disease in comparison to the non-induced control. More and more studies are devoted to induced resistance of the host plant after application of biological control agents. Kuc (1987) reported the first evidence of systemic protection of cucumber against *Colletotricum orbiculare* after pre-inoculation of the cotyledons of the plant with the same pathogen. It is also well established that the pre-inoculation of a host-plant with an incompatible *forma specialis* of *F. oxysporum* results in reduced disease severity when the plant is inoculated with the compatible pathogen (Biles and Martyn, 1989). The fluorescent pseudomonads selected for their plant growth promoting capacity or for their biological control activity have been shown to induce systemic resistance in the plant (Kloepper *et al*., 1996; Van Loon *et al*., 1998). Since induced systemic resistance is a general phenomenon that can protect the plant against several pathogens and can be induced by many biological control agents it retains more attention today than any other modes of action of biological control agents. However, it must be said that induced systemic resistance is not exclusive from other modes of actions and might, most often, only exert a complementary effect to microbial antagonism.

More generally, consistency of biological control needs the association of several modes of action, acting simultaneously or successively. As stated above, it is proposed to associate several modes of action in a single antagonistic strain, by genetic manipulation and the first

improved strains of *Pseudomonas fluorescens* producing phenazine and phloroglucinol have been evaluated for their improved biological control activity (Thomashow and Weller, 1996). Another approach consists in associating several strains of biological control agents in the same product. It has been well established that association of certain strains of *Pseudomonas fluorescens* with nonpathogenic *Fusarium oxysporum* always improves the control of fusarium diseases. Obviously these associations have to be based on the knowledge of the compatibility of the strains and of the modes of action in order to create a synergetic effect (Alabouvette *et al*., 2001, Olivain *et al*., 2003).

4.3. Production, formulation and application of biological control agents

Production and formulation of the biological control agents, the two last steps before application probably constitute one bottleneck for the development of biological control strategies. Indeed, too often these steps are not carefully considered by the academic research laboratories, which consider that these technological problems have to be solved by the industry. However, producing and formulating an efficient biomass at a low cost need a scientific approach based on the knowledge of the physiology of the micro-organisms. The aim of the fermentation is to produce and harvest a viable biomass that will have to express its beneficial properties after some time of storage and application to the crop. Moreover, this biomass must be pure, without contaminants. To achieve this goal, it is absolutely needed to study the physiology of the micro-organism to determine the fermentation parameters that will enable to obtain an effective biomass at an affordable cost. Several review papers have addressed these questions of how to produce and formulate an active biomass (Lewis *et al*., 1991; Lumsden *et al*., 1995). In most examples the micro-organisms are grown in liquid fermentation and the propagules after harvest are either mixed with a solid substratum, such as clay talc or peat, or embedded in alginate pellets (Fravel *et al*., 1985; Lumsden and Lewis, 1989). The final product must be easy to handle, to store and then to apply.

An alternative to liquid fermentation is the solid state fermentation, where the biological control agent is directly produced on solid material that provides nutrients and a substratum that can help to solve the formulation problem. The inoculum being stored in the substratum on which it has grown usually presents a better survival (Olivain *et al*., 2003). Moreover solid state fermentation enables to utilise different types of agricultural waste products that are cheap and can be found on the local market especially in developing countries.

The final step in developing a biological control method is to choose a method of application that will enable to deliver the biological control agent, at the right time and at the right place, where it has to be active. Depending on the target pathogen, the antagonist will be delivered with the seed or in the potting mixture to let it colonise the young roots of the plant. Obviously seed coating is the best approach to introduce a biological control agent in the rhizosphere of open field crops and this is the technique used to apply *Pseudomonas chlororaphis* to wheat seeds in Northern Europe (Hökeberg *et al*., 1997).

In any case, it is always necessary to study the compatibility of the biological product with the chemical pesticides used in the same crop. Indeed, the biological control agent is most often targeting a single type of pathogens; therefore it has to be integrated into the pest management programme. Much more research is needed to determine the exact use of biological strategies in disease and pest management.

4.4. Registration of biological control agents

As other plant protection products, biological control agents are subjected to the European directive 91-414 CCE, which lists all the plant protection products allowed to be on the market. It means that a full dossier giving all information related to the characterization of the micro-organisms, its biology, its toxicity for man and the environment will be reviewed at the European level to decide whether or not release of this micro-organism will pose an unacceptable risk for the applicator, the consumer or the environment. Obviously, the risks resulting from the application of a living organism are not the same as the risks posed by chemical molecules. Thus the directive 91-414 has been adapted to the specific case of micro-organisms in the directive 2001-36. It is not useful here to describe in details all the requirements necessary to characterize the dangers and evaluate the risks, but it must be stressed that this procedure is both expensive and time consuming. It represents a bottleneck for development of biological plant protection products. Experts are just afraid that the biological control agents could proliferate in the environment and threaten the ecological balance. But as presented above, all these micro-organisms have been isolated from the natural environment, mostly from soil, where they will be applied and where they will be submitted to various types of constraints (competition, antibiosis, UV radiation etc) limiting their growth and preventing their proliferation. Indeed, there is no example today of an uncontrolled proliferation of a biological control agent.

To promote biological control, the procedure of registration should be less expensive. Indeed, most of the time, these biological control agents are targeting niche markets which will never pay back the actual cost of registration. Obviously, one can not claim that being natural biological control agents present no danger. Thus, since registration is compulsory, one should find a more realistic approach to identify the dangers and estimate the risks.

4.5. Inoculation biological control versus inundation biological control

When applying a micro-organism isolated from a suppressive soil to a conducive soil, the expectation is to succeed in the establishment of the biological control agent in the soil and consequently transform the conducive soil into a suppressive soil. This corresponds to inoculation biological control, which, according to the definition given by Eilenberg et al., (2001), means that the biological control agent will multiply and control the pest for an extended period of time. Unfortunately, the introduction of a given antagonist in a soil is not sufficient to make the conducive soil suppressive even if it can control the disease efficiently for one season. Indeed, as underlined in paragraph 3 the mechanisms of soil suppressiveness are always complex and involved several populations of micro-organisms. Therefore, introduction of a single biological control agent to soil refers to inundation rather than to inoculation biological control.

5. Conservation biological control

Another ecological approach towards biological control of soil-borne plant pathogens consists in the management of the biotic and abiotic properties of a soil to reach a quality promoting beneficial microbial and physico-chemical interactions and thus limiting the pathogenic activity below a tolerable level of expression. Adaptation of cultural practices has been proposed in order to decrease the soil inoculum potential or increase the level of soil suppressiveness to diseases. Indeed, disease suppressive soils were developed through crop rotation (Cook *et al*., 2002), intercropping (Schneider *et al*., 2003), residue destruction (Baird *et al*., 2003), organic amendments (Tilston *et al*., 2002), tillage management practices (Sturtz *et al.,* 1997, Pankhurst *et al*., 2002) and combination of those regimes (Hagn *et al*., 2003; Peters *et al*., 2003; Garbeva *et al*., 2004). In the second part of this chapter we will review some of the practices which are developed or already in use to control diseases in a sustainable way.

5.1. Pathogen eradication versus microbial management

Forty years ago, in intensive vegetable cultivation in greenhouses, the use of heat-treatment by soil steaming was a common practice. Most of the pathogens are highly susceptible to heat, the lethal temperatures for pathogenic fungi being reached between 55 and 65°C for 15 to 30 minutes (Bollen, 1969). With the oil crisis, the cost of soil steaming became too expensive and the growers moved to application of chemical biocides which are dangerous for man and the environment. These molecules being biocide they kill not only the pathogens but also most of the beneficial soil micro-organisms, leading to an unbalanced equilibrium in soil. The use of the most dangerous product, methyl bromide will be banned at the end of year 2004. But most of the chemical products still in use produce ephemeral results including un-controlled side effects on both existing and forthcoming microbial communities leading to the infernal circle of applying repeatedly the same treatments to the soil. Fortunately less drastic techniques of pathogen eradication have been proposed, they have in common that they do not kill every micro-organisms but modify the microbial balance in a positive direction for pest control and plant growth.

5.1.1. Solarisation

Solarisation or solar heating is a method that uses the solar energy to enhance the soil temperature and reach levels at which many plant pathogens will be killed or sufficiently weakened to obtain significant control of the diseases. Solarisation does not destroy all the soil micro-organisms, but modify the microbial balance in favour of the beneficial micro-organisms. Indeed, many papers report situations where the efficacy of soil solarisation is not only due to a decrease of the pathogenic populations but also to an increase of the density and activity of populations of micro-organisms such as *Bacillus* spp., *Pseudomonas* sp. and *Thalaromyces flavus* antagonistic to the pathogens. Several review papers are available that describe both the technology of solar heating and the mechanisms involved in the control of pests, pathogens and weeds by soil-solarisation (DeVay *et al.*, 1991; Katan and DeVay, 1991; DeVay, 1995; Katan, 1996).

Solarisation is a hydrothermal process; its effectiveness is not only related to the temperature but also to the soil moisture. Indeed, temperature maxima are obtained when the

soil water content is about 70% of the field capacity in the upper layers and the soil should be moist to a depth of 60 cm. Various kinds of plastic films have been used. Polyethylene film, as thin as possible (25 to 50 μm) is recommended because it is transparent to most solar radiations and less transparent than some other plastic to terrestrial radiation. The duration of solarisation is an important factor determining the effectiveness of the treatment. The longer the mulching period, the greater the depth of effective activity, the higher the pathogen killing rates. Usually, in Mediterranean areas, four weeks are required to achieve control of the diseases. As stated above, disease control results both from the reduction of inoculum density and from increased activity of some antagonistic micro-organisms. Depending on the target pathogen one or the other mechanism is predominant.

An important characteristic of soil solarisation is its very large spectrum of activity. This method controls fungi, nematodes, bacteria, weeds, arthropod pests and some unidentified agents. Indeed, solarisation often results in increased yield when applied to monoculture soils, where specific pathogens have not been identified. In this case, solarisation probably controls the weak pathogens or deleterious micro-organisms responsible for "soil sickness". All the pathogens do not present the same susceptibility to solar heating, if most of the fungi are well controlled some failures have been reported. Another interesting property of solarisation is its long-term effect. Disease control and yield increase have been reported two and sometimes three years after solarisation. This long time effect is probably due to both the reduction of the inoculum density and some induced level of suppressiveness of the soil (Katan *et al*., 1983). Obviously, solarisation is effective in warm and sunny areas in the world and particularly under the Mediterranean climate. However some interesting data have been reported from cooler regions of the world where solarisation may be applied under plastic frames or in greenhouses.

5.1.2. Biofumigation or biodisinfection

Better adapted to cooler regions of the world, biological soil disinfection is based on plastic mulching of the soil after incorporation of fresh organic matter (Blok *et al*., 2000). The mechanisms involved by this newly developed technique are not totally understood.

Two main mechanisms contribute to the efficacy of the biodisinfection: the fermentation of organic matters in soil under plastic results in the production of toxic metabolites and in anaerobic conditions which both contribute to the inactivation or destruction of the pathogenic fungi.

Many species of *Brassicaceae* (*Cruciferae*) produce glucosinolates, a class of organic molecules, which may represent a viable source of allelochemic control for various soil-borne plant pathogens (Kirkegaard and Sarwar, 1998). Toxicity is not attributed to glucosinolates but to products such as isothiocyanates, organic cyanides or ionic thiocyanates resulting from their enzymatic degradations achieved by a group of similar-acting enzymes called myrosinase. Myrosinase and glucosinolates are separated from each other in intact plant tissues. When the *Brassicaceae* (cabbage, mustard, horseradish), grown as intermediate crop are buried into the soil as green manure, the disruption of cellular tissues allows mixing of glucosinolates and myrosinase resulting in the rapid release of glucosinolates degradation products. The hydrolysis products have a broad biocidal activity towards nematodes, insects and fungi as well as putative phytotoxic effects. They act either as selective fungicides or as fungistatic compounds limiting therefore the development and activity of fungal populations, some of them being putative pathogenic agents for the forthcoming crop (Sarwar *et al*., 1998). For that purpose new

cultivars of *Brassicaceae* have been selected for their high content in glucosilonates, they are now available on the market

Other plant families are able to release other types of toxic compounds. This is the case of *Alliacae*. Degradation of garlic, onion, and leek tissues is releasing sulfur volatiles such as thiosulfinates and zwiebelanes which are converted into disulfides having biocidal activities against fungi, nematodes and arthropods (Arnault *et al*., 2004). Based on the type of mechanisms involved, two definitions have been proposed by Lamers *et al.* (2004). Biofumigation corresponds to the use of specific plant species containing identified toxic molecules, biodisinfection refers to the use of high quantities of organic matter which after soil tarping results in anaerobic conditions mainly responsible for the destruction of the pathogens.

5.2. Crop rotation versus mono-cropping

In general, continuous cropping with a susceptible host causes the build up of populations of specific plant pathogens resulting in increasing soil-borne disease occurrence or severity. On the contrary, rotation including non-host plants or plant less susceptible to the pathogenic agents will limit the build-up of the population of the pathogens and in some cases will even lead to a decrease of the inoculum density. Indeed, some non host-plants are able to trigger the germination of the conservation structures (sclerotia, chlamydospores, oospores). But in the absence of a susceptible host, some pathogens are not able to survive saprophytically in soil. Therefore the cropping of such a non-host plant will result in a decrease of the inoculum potential of the soil. Moreover, crops in a rotation scheme may also stimulate some microbial populations resulting in the development of a suppressive effect towards the pathogens. For example Mazzola (1999) showed that growing wheat in orchard soil prior to planting apple reduced infection by elements of a fungal complex including: *Cylindrocarpon destructans*, *Phytophthora* spp., *Pythium* spp. and *Rhizoctonia solani*. This beneficial effect was correlated with the increased population of specific antagonistic populations of fluorescent pseudomonads making the soil suppressive towards *Rhizoctonia solani*.

On the contrary the case of take-all decline of wheat illustrates the benefit obtained through long term monocropping. Indeed, monoculture will first favour disease which in return will favour the antagonists of the pathogens. Therefore, the take-all disease of wheat caused by *Gaeumannomyces graminis* var *tritici* can be naturally controlled by monocropping of the cereal providing that monoculture lasted for more than 4 years (Dulout *et al*., 1997). This feature was related to the development of populations of fluorescent pseudomonads within the root and straw fragments remaining post harvest which make the soil suppressive to the disease. These bacterial populations produce antibiotic compounds (phenazine, 2,4-diacetylphloroglucinol) which are deleterious to the pathogen (Thomashow and Weller 1988; Raaijmaker and Weller 1998). However, several consecutive crops of wheat enduring take-all are necessary to ensure an effective threshold of disease suppression and the best yields following take-all decline are rarely equal to those achieved with crop rotation. Therefore, although wheat monoculture does induce take all decline, short crop rotation based systems are preferred (Cook, 2003).

5.3. Residues management

As in the case of take-all decline of wheat, plant residues left on or near the soil surface may contribute to an increase of soil suppressiveness to disease through the promotion of the general microbial activity which is involved in the mechanisms of disease suppression. The incidence and severity of Fusarium wilt of cotton increased when levels of plant residue in the soil were increased by the incorporation of whole cotton plants into the soil (Wang *et al*., 1999). Indeed, the debris not only promote the microbial activity but also help to preserve the pathogens, preventing a decrease of the inoculum density. This is the case for *Macrophomina phaseolina* causing charcoal rot in soybean (Baird *et al*., 2003), *Fusarium* sp. causing root and crown rot on maize (Cotten and Munkwold 1998), *Rhizoctonia solani* causing crown and root rot on sugar beet (Guillemaut 2003). Some practices used by growers to kill living plants at crop termination (foliar application of herbicide and mechanical destruction of the vines) could be counterproductive with respect to disease management. Indeed, such putatively preventive strategies might enhance the fungal reproduction and increase the soil inoculum as it was shown in the case of the root-infecting fungus *Monosporascus cannonballus* causing vine decline of melons. In such cases, destruction of infected roots prior to pathogen reproduction would be a method of preventing inoculum build-up in soil (Stanghellini *et al*., 2004).

Therefore, attention should be paid to residue management by burial through tillage practices or promotion of rapid decomposition (Toresani *et al*., 1998). When residues are buried, the pathogens are displaced from their niche to deeper layers in the soil thus their ability to survive is severely decreased. Repeated incorporations of crop residues can affect a change in the activity of the residue-borne microorganisms that in turn influence the decomposition of crop residues. Carbon released from this decomposition contributes to a more general increasing soil microbial activity and so increases the likelihood of competition effects in the soil, resulting in enhancement of general suppression. Developing disease suppressive soils by introducing organic amendments and crop residue management takes time, but the benefits accumulate across successive years improving soil health and structure (Bailey and Lazarovits, 2003).

5.4. Soil tillage

It is difficult to assess the role of tillage on disease suppression as its evaluation is often combined with the effects of other agricultural practices such as organic amendments and green manure burial, residue management or crop rotations (Bailey and Lazarovits, 2003, Peters *et al*., 2003). Therefore tillage appears as giving conflicting effects on disease suppression. Conventional tillage results in considerable disturbance of the soil but removes residue from the surface. Tillage also disrupts hyphae altering for instance the ability to survive of *R. solani*, (Roget *et al*., 1996, Bailey and Lazarovits, 2003). On the contrary, reduced tillage can favour pathogens by protecting the pathogen's refuge in the residue from microbial degradation, lowering soil temperature, increasing soil moisture, and leaving soil undisturbed (Bockus and Shroyer 1998). Reduced tillage systems change the availability of nutrients in the soil increasing microbial biomass, microbial activity and subsequent competition effects. Total soil nitrogen, organic matter and denitrification processes are increased but mineralization and nitrification processes are reduced. Soil inoculum potential and disease incidence might be differently altered according to the pathogens considered. Indeed, the impact of tillage

practices depends on specific pathogen-soil-crop-environment interactions, environment being sometimes, the most important factor limiting the severity of disease regardless of tillage or crop rotation practices (Bailey *et al*., 2000).

5.5. Organic amendments

In the sixties and seventies, organic amendments have been proposed to control soilborne diseases (Lumsden et al., 1983). Although their effects were not studied in relation to induction of suppressiveness in soil, many papers reported a beneficial effect of organic amendment on the reduction of disease incidence or severity. In one case, the beneficial effect was clearly linked to induction of suppressiveness in the soil. Indeed, the Ashburner system to control *Phytophthora* root rot of avocado in Australia is based on the incorporation of large amounts of organic matter to reproduce the environment of naturally suppressive soils that exist in the rain forest (Baker, 1978). Since that time, addition of organic amendments to control soil borne pathogens has been extensively studied. Hoitink (1980) has developed a growth medium based on composted bark to grow rhododendron and azaleas. This substrate is suppressive towards root rots induced by several species of *Pythium* and *Phytophthora*. After the peak heating that creates a biological vacuum, the compost can be colonized by a great diversity of micro-organisms some being antagonist of the pathogens. The level of disease control obtained depends on many factors such as the chemical properties of the parent material, the composting process and obviously the type of micro-organisms present. This is probably why such contrasted data have been published regarding the efficacy of disease control obtained by organic amendments of soil. Under the frame of a European project (Compost Management in Horticulture QLRT-2000-01442: http://www.agro.nl/appliedresearch/compost) 18 composts from different origins were evaluated for their capacity to suppress 7 different diseases. It appeared that there is no general rule, some compost controlled some diseases but not others, and the only exception is Fusarium wilt which is controlled by almost all the composts

To enhance the suppressive potential of composts and thus to improve the efficacy of disease control it has been proposed to inoculate these composts after peak heating with specific strains of biological control agents. Although promising, this strategy has not yet been successfully applied. Indeed, as every soil, every compost possesses a certain level of suppressiveness towards introduced micro-organisms. Thus it is not easy to establish some biological control agents in composts even after peak heating.

Despite these difficulties, compost amendment has been successfully used to increase soil suppressiveness to diseases including nematode diseases (Erhart *et al*., 1999; Lumsden *et al*., 1983; Oryazum *et al*., 1998; Serra-Wittling *et al*., 1996; Steinberg *et al*., 2004; Windmer *et al*., 2002) as well as disease suppression in farm truck and horticultural crops (Tilston *et al*., 2002, Cotxarrera *et al*., 2002; Hoïtink and Boehm, 1999). The mechanisms involved in these examples of successful disease suppression are diverse and not clearly understood. In a recent study (Perez *et al*., 2005) the effects of three composts added to two different soils were carefully addressed. Assessing the density and the activity as well as the physiological and genetic structure of the soil microflora revealed that the phytosanitary state of the soil might be governed by the repercussions of the organic amendments at the functional level but no general rule could be stated. The impact of organic matter on the soil biota differed with the nature of the compost and the soil types. The structures of the bacterial and fungal communities were perturbed in different ways according to the soil-compost mixtures. More generally, looking

through all the already published data, there has been no definitive work linking narrowly biological control in soil to applications of organic amendments. This is probably due to the large diversity in the chemical composition of the composts, manures and other organic matters that does not fit in a suppressive way with the large biodiversity and ecological requirements of the pathogens.

Composts can also act as a non-host plant: an interesting example is provided by the incorporation of composted onion wastes into the soil to control Allium white rot due to *Sclerotium cepivorum*. This fungus is an obligatory parasite which can survive as dormant sclerotia in the soil for many years but can only germinate in the presence of the host plants. The stimulus for germination is the exudation of alk(en)yl cysteine sulphoxides by the roots of Allium species. Properly composted, onion wastes contained some sulphoxides (di-n-propyl disulphide) which trigger the dormant sclerotia to germinate in absence of the root while these germinated sclerotia are unable to survive without the living host, what contributes to the decrease in the primary inoculum faced by the next onion crop (Coventry *et al*., 2002).

6. Conclusion

In this chapter, we presented two approaches towards biological control of soil-borne plant pathogens. The first one consists in the selection of an antagonistic micro-organism which will be developed as a plant protection product; the other consists in a modification of the soil management practices to increase the level of soil suppressiveness to diseases. These two approaches are not novel; both have already been proposed during the first congress on "ecology of soil-borne plant pathogens, prelude to biological control" hold in Berkeley in 1965. Most of the ideas presented above were already discussed, and one may wonder why so little progress has been made during these last 40 years.

The first reason is linked to the high complexity of the soil ecosystem. The few examples presented in this chapter show how complex are the interactions between soil abiotic characteristics, soil microbiota and soil suppressiveness to diseases and pathosystems. It is therefore clear that one single population is unlikely to be responsible for the whole functioning of the soil. On the contrary, all the microbial populations including bacteria, fungi, protozoa and microfauna are involved in this functioning but with the constraints of the environment. One must admit that we still are at the descriptive stage, and that we have difficulties addressing the question of soil health following a holistic approach. At least we made progress understanding that disease incidence or disease severity does not rely only on the inoculum density. When reviewing some of the papers published in the seventies and dealing with organic amendments to control soil borne diseases, one must admit that scientists were always trying to explain disease control by a direct effect of the soil environment on the inoculum density. Today we know that the interactions are much more complex and that the effects of a given organic matter depend on the soil environment to which it will be added. The objective is no necessarily to induce a *decrease* of the inoculum density but to *increase* soil suppressiveness to diseases. Many agricultural practices may result in an increased level of soil suppressiveness but in order to advise farmers one need to better understand the effects of management practices on the diverse components of soil health and to determine when and what kind of management is necessary to increase soil suppressiveness to diseases.

The fast development of molecular and physiological tools is enabling the characterization of the structure of the microbiota as a whole. Until today, we were obliged to focus on a very

limited number of microbial populations, either pathogens or antagonists, but were unable to detect changes affecting the soil microbiota without having, a priori, a specific hypothesis. Thus the development of new techniques enabling the evaluation of biodiversity in soil microbiota is totally changing our views of the microbial balance (Mazzola, 2004). If, as expected, these new methods can be run automatically, they will make possible the characterization of many samples and thus enable comparison of the microbial communities in different soils under different cropping systems or in a same soil submitted to different practices. These techniques will be useful for correlating changes affecting the level of soil suppressiveness with shifts affecting microbial communities. We will be able, for example, to detect and characterize shifts in the microbial communities following application of any treatment to the soil (e.g. fertiliser, pesticide, biological control agents, and organic matter) and to correlate these changes with variations in the level of soil suppressiveness to a set of diseases. Moreover, the molecular techniques should enable by consulting a gene data bank, to determine which populations are affected by the treatments and then to study their role or their function, in the ecosystem. It will be possible to determine if these populations are really involved in mechanisms controlling soil health or if they are only indicators (markers) of soil health. But, it is obvious that several, or probably many, indicators will be needed to characterize soil health. Therefore mathematical modelling will be necessary to organize all these data and to follow the dynamics of the measured parameters either biotic or abiotic. The resulting and evolving models will allow us to propose management techniques useful for farmers and for the preservation of the environment. Solutions proposed to farmers will be more complex to achieve that the traditional chemical control applied as insurance. Thus it will demand the active participation of farmers with the support of the consumers which should understand what the benefits will be for the society.

The second approach which was favoured during the 20 last years consists in developing plant protection products based on micro-organisms. The discovery of soil naturally suppressive to diseases, which should have promoted research on ecology of soil micro-organism, paradoxically stimulated the development of bio-control agents isolated from these suppressive soils. As already stated, the soil is a reservoir of beneficial micro-organisms, not only for plant but also for human disease control, and it seemed easier to solve all the questions related to the development and application of a biological control agent than to understand all the conditions that make a soil suppressive. But it was a mistake, because to be successful inoculation biological control requires a full understanding of the ecology of the biological control agent. In fact, at that time people were thinking at developing a biological control agent as a chemical pesticide, with the same requirements for formulation, shelf life, and efficacy. This way of thinking partly explains the failure of this strategy since only a very few of the antagonists studied in the laboratories are actually on the market. Being living organisms, biological control agents have special requirements that both the producer and the applicator must take into account. Moreover, most of the antagonistic micro-organisms have narrow host specificity; they are able to control a single disease, when the farmer has to deal with several soil-borne pathogens. Consequently, biological control has to be integrated in a strategy of disease management. Several approaches have been proposed such as the use of an association of several antagonists or the application of antagonists after solarisation or mixed with organic amendments.

The difficulties encountered to apply biological control agents invite us to think in a different manner and as presented above consider biological control application as part of the agricultural practices which have to be chosen to promote soil suppressiveness to diseases.

Developing disease suppressive soils by introducing organic amendments and or biological control agents, crop residue management, crop rotations and adapted tillage practices will probably not provide immediate return compared to the use of fumigants or pesticides, but the benefits accumulate across successive years and improve soil health and structure. Farmers should not rely exclusively on a single management practice but a combination of practices should be integrated to develop a consistent long term strategy for disease management that is suited to their production system and location.

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CHAPTER 9

THE SOIL AS A RESERVOIR FOR NATURAL ENEMIES OF PEST INSECTS AND MITES WITH EMPHASIS ON FUNGI AND NEMATODES

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1. Introduction

The soil is the home of innumerable forms of plants, animals and microbes, and life in the soil is highly diverse, ranging from microscopic single-celled organisms to large burrowing animals. As in above ground environments, there are well-defined food chains and competition for survival in the soil environment (Foth & Turk, 1990). Biotic and abiotic interactions in soil ecosystems may enhance or reduce populations of pest arthropods (defined here as insects and mites). Ninety percent of arthropod pest species spend at least part of their life cycle in soil (Gaugler, 1988; Villani & Wright, 1990; Kaya & Gaugler, 1993). Soil dwelling pest arthropods have natural enemies among soil organisms, but also pests that occasionally come into contact with soil might be consumed by predators or become infected with pathogenic propagules (Sunderland 1975; Purvis & Curry, 1984, Tanada & Kaya 1993; Hajek, 1997; Eilenberg & Meadow, 2002).

Soil ecologists often work with single groups of minute organisms in the cryptic soil environment. In this cryptic environment it is not easy to conduct studies that reveal the effect of specific factors on natural enemies of pest arthropods. "Acts" in what can be called the "ecological theatre" are played out on various scales of space and time. To understand the drama, it must be viewed in the appropriate scale (Wiens, 1989). In soil ecological studies it is therefore important to define the scale of the organism and ecosystem. The scale of a soil ecosystem might vary between a few cubic mm of soil to an entire landscape unit extending for several hundred km^2 (Coleman, 1986). To use the appropriate scale there is a need for knowledge about the size, fragmentation and duration of organism's habitat. Moore *et al*. (1988) also emphasise the importance of using the functional scale to identify the mechanisms controlling the ecosystem. They suggest that the use of groups based solely on taxonomy, such as nematodes or microarthropods, is misleading because function rather than taxon should be the focus of ecosystem research.

In this chapter we will try to give an overview of different organisms, physical soil factors and management systems that might be important to natural enemies of pest insects and mites. We will focus on insect and mite pathogenic fungi and insect parasitic nematodes, but other pathogens and arthropod natural enemies are mentioned briefly. At the end of the chapter we present a few examples of successful use of the soil as a reservoir for these natural enemies.

J. Eilenberg and H.M.T. Hokkanen (eds.),An Ecological and Societal Approach to Biological Control, 145–211.

2. Epizootiology of insect and mite pathogenic fungi and insect parasitic nematodes in the soil ecosystem

Epizootiology is defined as the science of causes and forms of mass phenomena of diseases at all levels of intensity in an animal host population. The study of insect epizootiology, linked to the broader science of ecology, includes diseases caused by non-infectious (amicrobial) and infectious (microbial) agents (Tanada & Kaya, 1993). For a more thorough coverage of different aspects of epizootiology we refer the reader to many excellent studies conducted within this field (Bovien, 1937; Dutky, 1959; Poinar, 1975; Fuxa & Tanada, 1987; Keller & Zimmermann, 1989; Tanada & Kaya, 1993; Hajek, 1997; Pell *et al.* 2001). In this section we will briefly mention some of the most important factors influencing the epidemic development of insect pathogens and insect parasitic nematodes in the soil ecosystem.

The development of a disease in an insect or mite population involves a complex interaction of factors associated with the pathogen, host, environment, and time. Humans also occupy a special position with respect to these systems by affecting and managing the ecosystem in which these interactions occur. Plant pathologists have long recognized this five-way interaction, and it has been illustrated as the disease tetrahedron, which is also used to understand insect and mite disease epizootics (Agrios, 1997) (Fig.1). The practical use of insect pathology for the control of pest arthropods demands a full understanding of the interactions described by the disease tetrahedron (Carruthers & Soper, 1987; Hajek & Leger, 1994). To study epizootic development, it is critical to study the habitat in which the arthropod pathogen interactions take place. It is microenvironmental rather than ambient conditions that influence disease dynamics, however, spatial aspects of epizootic development have rarely been addressed (Hajek, 1997). In the soil, the microenvironment is the scale most pertinent to the survival and activity of individual microorganisms such as insect and mite pathogens and insect parasitic nematodes, because ultimately it is at this scale the microbes interact with their environment (Buckley & Schmidt, 2002). The scale of relevance to the study of the epizootic development of a pathogen in a larger soil dwelling insect might, however, be very different, all depending on the question asked. In the cryptic soil environment it may be difficult to define exactly the scale of the system one would like to study since the different processes are hidden within the soil matrix. The time scale of an epizootic study is also of importance, and long-term investigations over numerous host generations are needed (Keller & Zimmermann 1989). Such investigations are rare, especially on naturally occurring pathogens in the soil. The different factors and interactions influencing patterns of insect and mite diseases over place and time are complex and differ between pathogens. Fungi, protozoa, and nematodes require close contact for their transmission, but viruses can cause epizootics in less dense populations (Weiser, 1987).

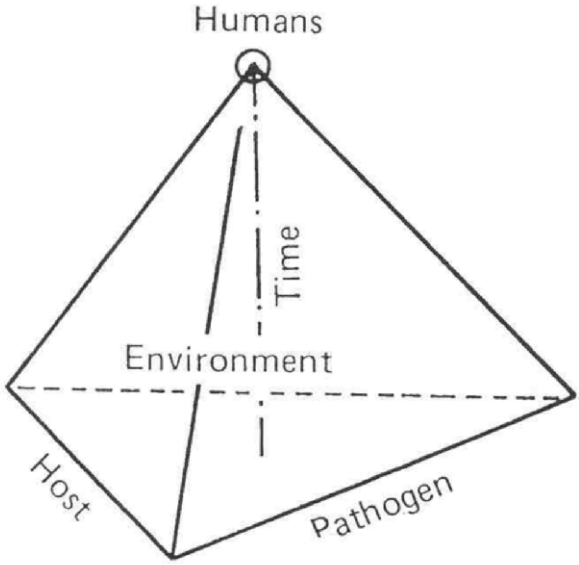

Figure 1: Agrios´ (1997) schematic diagram of the interrelationships of factors involved in plant disease epidemics is also representative for insect and mite disease development

3. Natural enemies of pest insects and mites and some soil organisms important to them

All ecosystems have two types of organisms based on carbon sources, namely autotrophs (the producers), that use inorganic carbon (principally CO^2) and heterotrophs (the consumers and decomposers) that use organic carbon (Foth & Turk, 1990). Plants belong to the autotrophs and can affect pest arthropods and their natural enemies in many ways in the soil. Among the heterotrophs belonging to the soil ecosystem, both microorganisms and soil animals affect pest arthropods and their natural enemies. Among the soil organisms; the host population, host plants of target insects or mites, predators and antagonists of the natural enemies and alternate hosts all influence natural enemies in soil (Barbercheck, 1992). To exploit the natural populations of insect parasitic nematodes and arthropod pathogenic fungi for controlling pest populations, further knowledge is required to understand their ecology. In this section we will give a short presentation of plants, microorganisms and soil animals that are present in the soil ecosystem, and how these might affect pest arthropods and their natural enemies.

3.1. Plants

Plants belong to the autotrophs and constitute the principal biochemical motive force for all subsequent activities of heterotrophs in soils. The inputs come from two directions: (1) from aboveground onto the soil surface as litter and (2) from belowground, as roots, which constitute exudates and exfoliated cells while the root is alive, and root litter when the root dies. The root-fungus mutualistic association, mycorrhiza, is equally important to the above mentioned inputs. This symbiotic association has a significant effect on soil microbes and fauna (Coleman *et al*., 2004). The rhizosphere is the area immediately surrounded and influenced by plant roots (Foth & Turk, 1990), and the great majority of organisms in the rhizosphere are microorganisms, including the major groups: bacteria, fungi and protozoa. It is also well known that nematodes and mites are found in higher concentrations in the rhizosphere than in root-free soil (Lynch, 1990).

Plants may inhibit or stimulate soil organisms in many different ways, for example through the release of plant root exudates. The main part of root exudates consists of carbohydrates. Free amino acids and organic acids are also commonly reported root exudates. Numerous other substances found include nucleotides, phenolic compounds and vitamins (Sundin, 1990). Root exudates release important host signals for soil dwelling plant pathogens, nematodes and herbivorous insects and mites. Among the cyst forming plant-parasitic nematodes, *Globodera rostochiensis* and *G. pallida*, show sophisticated hatching mechanisms that ensure host invasion. Root exudates from the host plant stimulate hatching of the cysts. This reliance on root exudates to stimulate hatching favours persistence of the nematode in the soil (in cysts) in the absence of host plants. Large numbers of infective juveniles from the cysts may therefore be present to invade when host plants are introduced (Perry, 2002). Van Tol *et al*. (2001) showed that the roots of a conifer plant, attacked by vine weevil larvae, release chemicals that attracted the entomopathogenic nematode *Heterorhabditis megidis*. Root exudates have also been suggested as the cause of enhanced germination and survival of the insect pathogenic fungi *Metarhizium anisopliae* in the soil around plant roots (Klingen *et al*., 2002b).

Secondary plant compounds are released in root exudates or upon wounding of plant roots. Brassica plants for example produce isothiocyanates, a group of secondary plant compounds, upon wounding of roots (e.g. pest insect attack). Isothiocyanates are used by pest insects specializing on Brassica plants to localize the plant. This has been shown for the soil dwelling larvae of the dipteran *Delia floralis* which is a Brassica specialist (Ross & Anderson, 1992). It is also known that isothiocyanates affect insect pathogenic fungi, and several laboratory studies not including soil have shown that isothiocyanates may inhibit insect pathogenic fungal species in the class Hyphomycetes (Vega *et al.*, 1997, Inyang *et al.*, 1999, Klingen *et al*., 2002b). No such effects were, however, observed in a more realistic fungus/plant/soil microcosm study (Klingen *et al.*, 2002b).

3.2. Heterotrophic microorganisms

Fungi, bacteria, viruses and protozoa may be beneficial to pest arthropods or they may be pathogenic and hence behave as natural enemies. They may also be pathogenic to other natural enemies such as predators and parasitoids of pest arthropods (Steenberg *et al.*, 1995; Lacey *et al.* 1997; Howarth, 2000; Vestergaard *et al.*, 2003). Soil is a natural reservoir for many insect pathogens, and many arthropod species are hosts to a wide number of pathogens. Jackson *et al.* (2000) report that at least 30 different pathogen species belonging to fungi, bacteria, viruses or protozoa are commonly associated with soil-dwelling insects. Scarab beetles appear to be host

to the widest numbers of pathogens. The soil can be inoculated with insect and mite pathogens either by an infected insect or mite entering into the soil and subsequently dying, or by deposition of pathogenic propagules on the soil surface. For some pathogens, the soil environment also provides a medium for growth and potential dispersal (Hajek 1997). Since most pest arthropod populations come into contact with the soil at some point in their life cycle, the soil is important for the introduction of pathogens into pest arthropod populations. Despite a wide range of known pathogens for soil-dwelling insects, natural epizootics of disease are not frequently observed. This is due, in part, to the difficulty of observing diseased insects within the soil and the rapid decomposition of cadavers. It may, however, also reflect natural resistance to pathogens. Moreover, microbial competition is intense and the presence of other soil microbes may limit the efficacy of pathogens against pest arthropods (e.g. Popowska-Nowak *et al*., 2003). Soilborne pathogens such as nematophagous fungi and bacteria may have quite a significant effect on nematode populations and has been reviewed by Timper & Davies (2004) for nematodes in general and by Kaya (2002) for entomopathogenic nematodes. Timper & Davies (2004) describe four types of interactions where other organisms harm nematodes: predation, parasitism, amensalism and competition. A comprehensive review by Stirling (1991) on the range of antagonists involved is recommended reading.

3.2.1. Fungi

Traditionally, living organisms have been divided into two Kingdoms: Plantae and Animalia, and fungi have been placed in the Kingdom Plantae. However, many biologists now recognize five Kingdoms: Procaryotae, Protoctista, Fungi, Plantae and Animalia. The fungi are placed in the separate Kingdom Fungi, primarily on the basis of their simple eukaryotic thallus with heterothrophic and absorptive nutrition. They are divided in two groups; the Myxomycota in which the vegetative phase lacks a cell wall, and the Eumycota that are typically filamentous or unicellular with a well-defined cell wall (Tsuneda, 1983; Ingold & Hudson, 1993). Assessing the total number of fungal species worldwide is problematic, but three different arguments have led to an estimate of 1.5 million species. The arguments are: (1) only about 5% of the fungi on earth have been identified, (2) there are around six times as many fungi as vascular plants; and (3) the fungi are the largest major group of organisms apart from arthropods (Hawksworth, 1991). The estimate of fungal species is, however, constantly under revision, ranging from 500 000 to 9.9 million species (Hawksworth, 2001). Fungi play many roles in different ecosystems, but the most significant of these is decomposition of organic matter (Cannon, 1996). Probably around two thirds of all fungi on earth are associated with soil or leaf litter for at least part of their life cycles (Cannon & Kinsey, 1996). Fungi can be divided in ecological terms into those that complete their life cycles within the soil, or those with a more complex system involving infection of aerial parts of plants, or animals. Fungi that do not complete their life cycle in soil may either exist as dormant propagules, or live saprobically on decaying host matter (Cannon, 1996). Fungi are food sources for a wide variety of vertebrates such as mice and squirrels that use fungal fruit bodies as a significant part of their diet. Fungi are also a major food source for soil invertebrates such as collembolans and nematodes. However, fungi themselves can exploit insects, mites, nematodes, rotifers etc. as a food source (Cannon & Kinsey, 1996).

Insect pathogenic fungi are natural enemies of pest insects and mites. The most important groups are, Deuteromycetes and Entomophthorales and the soil is the main reservoir of

infective propagules of many species within these groups. Deuteromycetous fungi is well known to grow and disperse in or in very close connection with the soil, and this fungal group causes natural epizootics in soil dwelling pest insects. Fungi in the order Entomophthorales cause epizootics mainly in foliar insects and mites (Pell *et al.*, 2001), but some species are also found to cause epizootics in soil dwelling arthropods. For some examples of fungi causing epizootics in pest arthropods that spend some time on or in the soil see table 1. Even though the soil is not the most common habitat for epizootics caused by Entomophthorales, the soil is an important reservoir for resting stages of fungi in this order. Insects or mites infected with Entomophthorales produce cadavers with resting propagules under unfavourable conditions. These drop down onto the soil where they contribute to the soil reservoir of insect pathogenic fungi. Several studies have shown that Entomophthoralean fungi can survive long periods at low temperatures and still be infective (Klubberttanz *et al.,* 1991; Odour *et al.,* 1995; Hajek & Humber, 1997; Nielsen *et al.,* 2003; Hajek *et al.,* 2004). One example is the aphid pathogenic fungus *Pandora neoaphidis* where the fungal inoculum retains the ability to initiate infections in aphids after storage on the soil for at least 95 days at 5^{o} C (Nielsen *et al.*, 2003). Also the fungus *Entomophaga maimaiga* that is pathogenic to the gypsy moth *(Lymantria dispar*) retains the ability to initiate infections up to 8 months after storage at 4^{o} C (Hajek *et al.,* 2004).

Nematophagous fungi are well known parasites of nematodes e.g. fungi in the genera *Arthrobotrys*, *Dactylella*, *Duddingtonia* and *Monocrosporium* (Timper & Davies, 2004). Nematode trapping fungi and entomopathogenic nematodes occur naturally in many soils, and observations in the laboratory have shown that these fungi trap entomopathogenic nematodes on agar (e.g. Koppenhofer *et al*., 1996). Observations on their interactions in soil is rather limited, however Koppenhofer *et al*. (1997) conducted a study where it was found that the fungus *Arthrobotrys oligospora* competes well against other nematode trapping fungi.

Table 1: Reports on epizootics caused by insect pathogenic fungi on pest insects that spend some time on or in the soil

Scientific name of host insect or mite (Order: Family)	**Fungal species (Hyphomycetes/ Entomophthorales)**	**Host and fungi in the soil ecosystem**	**References**
Costelytra zealandica (Coleoptera: Scarabaeidae)	*Beauveria bassiana* *Beauveria brongniartii* (Hyphomycetes)	*B. bassiana* caused an epizootic with prevalence reaching up to 99% in *C. zealandica* larvae sampled from soil. *B. brongniartii* caused an epizootic with prevalence reaching up to 30% in *C. zealandica* larvae sampled from soil.	Townsend *et al.,* 1995
Tipula paludosa (Diptera: Tipuloidea: Tipulidae)	*Conidiobolus osmodes* (Entomophthorales)	The fungus caused an epizootic with prevalences reaching about 40% in *T. paludosa* larvae extracted from the soil. Several mummified larvae were also found on the soil surface.	Gökce & Er, 2003
Ostrinia nubilalis (Lepidoptera: Pyralidae)	*B. bassiana* (Hyphomycetes)	The fungus caused up to 84% mortality in overwintering larvae of *O. nubilalis* in corn residues. Corn residues were laying or standing on the soil surface.	Bing & Lewis, 1993
Cydia pomonella= Laspeyresia pomonella (Lepidoptera: Tortricoidea: Tortricidae)	*B. bassiana* *Paecilomyces farinosus* (Hyphomycetes)	*B. bassiana* and *P. farinosus* caused 34.4% and 29.5% mortality respectively, in *C. pomonella* larvae overwintering beneath the bark at the base of apple trees. The larvae come in contact with soil after emerging from apples, dropping on the ground, before crawling up a tree trunk.	Subinprasert, 1987

Scientific name of host insect or mite (Order: Family)	**Fungal species (Hyphomycetes/ Entomophthorales)**	**Host and fungi in the soil ecosystem**	**References**
Agrotis segetum (Lepidoptera: Noctuidae)	*Tolypocladium cylindrosporum* (Hyphomycetes)	The fungus was found to severely reduce populations of *A. segetum* larvae hibernating in the soil.	Steenberg & Øgaard, 2000
Pseudoplusia includens Anticarsia gemmatalis (Lepidoptera: Noctuidae)	*Nomuraea riley* (Hyphomycetes)	This fungus often causes natural epizootics in populations of noctuids. *N. riley* overwinter in the soil and the level of overwintering inoculum is probably one of the key factors in the development of epizootics.	Carruthers & Soper, 1987
Pemphigus penax (Homoptera: Aphidoidea: Pemphigidae)	*Erynia (Pandora) neoaphidis Conidiobolus coronatus* (Entomophthorales)	These fungi cause about 70% mortality in nymphs and adults on carrots in the soil. *E. neoaphidis* being the most prevalent.	Pers. obs.

3.2.2. Bacteria

Bacteria are numerous in the soil, and a gram of soil may contain over one billion bacteria (Foth & Turk 1990). In adequately aerated soils, both bacteria and fungi dominate, whereas bacteria alone account for almost all the biological and chemical changes in environments containing little or no oxygen. Bacteria isolated from soil can be placed into two broad divisions: the indigenous species that are true residents, and the invaders. Indigenous bacteria may have resistant stages and endure long periods without being active metabolically, but under favourable conditions they become active. Invaders, however, do not participate in a significant way in community activities. They enter the soil with precipitation, diseased tissues, animal manure or sewage sludge, and they may persist for some time in a resting form and sometimes even grow for short periods (Alexander, 1977).

Several soil dwelling bacteria are pathogenic to arthropods. Some of these are obligate pathogens, but the majority are facultative and a few are potential pathogens that may show a certain degree of pathogenicity. Under conditions of stress, non-pathogenic bacteria present in the digestive track of organisms (e.g. insects, nematodes) may exhibit pathogenicity (Tanada & Kaya, 1993). Other bacteria have a close association with insects, but are not pathogenic. One such example is soil dwelling insects such as *Delia* spp. that have a close association with plant soft-rot bacteria (*Erwinia* spp.). The *Delia* larvae transmit decay-causing bacteria to healthy plant tissues, aiding in the development and spread of the plant rot. The association of the larvae and the bacteria is coincidental and not obligatory (Coaker & Finch, 1971). *Delia* larvae are known to have a very low susceptibility to insect pathogenic fungi (Vänninen *et al*., 1999a;

Vänninen *et al*., 1999b; Klingen *et al*., 2002c), which has led to speculations that the bacteria present on *Delia* compete with insect pathogenic fungi. Other factors such as plant metabolites seem, also, to affect the fungal infection of *Delia* spp. (see section 3.1). Volatiles emitted by some bacteria, e.g. *Bacillus subtilis*, *B. pumilus* and *Pseudomonas aurantiaca,* are also known to have a fungistatic effect on insect pathogenic fungi important in the soil ecosystem (Popowska-Nowak *et al.*, 2003), and bacteria are also known to lyse fungi (Ekesi *et al*., 2003).

Enright *et al*. (2003, pers. comm.) found endospore-forming bacteria in the genus *Paenibacillus* associated with entomopathogenic nematodes. These bacteria were found to inhibit nematode movement, thus contributing to the regulation of nematode populations. For details on effects of bacteria on nematodes in general we refer to the excellent reviews by Stirling (1991) and Hominick & Kerry (2002). A unique association between bacteria and nematodes, in which bacteria (*Xenorhabdus* and *Photorhabdus*) require nematodes to gain entry into host insects is mentioned later in section 3.3.3.

3.2.3. Viruses

Viruses are of considerable economic and medical importance because they cause diseases of plants, animals and humans. Each viral particle requires the presence of a viable metabolic organism for its reproduction. In the absence of the host, little activity and no reproduction or duplication is possible. Many viruses are limited in their host range and are often species specific (Alexander, 1977). Exceptions do exist, for example the family Reoviridae comprise viruses that infect vertebrates, invertebrates and plants (Hunter-Fujita *et al.*, 1998; Hull, 2002). The classification of viruses is without a natural base, primarily because there is no time-related information on their evolution and on relationships between virus species and genera. An effective system for classifying viruses has been developed by Hull (2002). Insect viruses belong to at least 13 families, some of which occur exclusively in arthropods and some of which include vertebrates and/or plants. Occlusion is a feature of many arthropod viruses, which does not occur in plant or vertebrate viruses. Occlusion means that the virons are embedded within a proteinaceous body. Occlusion bodies (OBs) vary in size but are visible under the light microscope (Hunter-Fujita *et al.*, 1998).

Viral diseases are among the most widely investigated infections in insects, and there are several examples of viral diseases causing death in pest arthropod populations living in, on or in close contact with the soil. An example is the *Wiseana* spp (Lepidoptera: Hepalidae), which are important pests in pastures in New Zealand. Larvae in this genus live on or in the soil and become infected with Nuclear Polyhedrosis virus (NPV) as young larvae by ingesting viral occlusions present on the soil surface, on the underside of grass leaves, or in pasture debris. Infected larvae usually die outside their burrows, where they are consumed by birds or become part of the soil reservoir (Tanada & Kaya, 1993). The ultimate deposition for viruses, particularly the occluded viruses, is the soil, which can protect the inoculum for many years. Viable viruses will remain close to the surface, provided that the soil is undisturbed (Evans, 2000). The high occurrence of viruses in the soil reservoir increases the competition with other soil natural enemies for susceptible arthropod hosts. Many viruses are, as mentioned earlier, quite host specific and will therefore not compete for a wide range of arthropod hosts. This applies for example for the family Baculoviridae that is also widely used in microbial control (Hunter-Fujita *et al.,* 1998).

3.3. Animals

Animals, the other group of major heterotrophs in soil systems, exist in elaborate food webs containing several trophic levels. Animal members of the soil biota are numerous and diverse, and are often divided into the microfauna, the mesofauna and the macrofauna based on their size, and the method for collection of these animals. The micro, meso and macrofauna are linked to each other through food webs. The animals, especially the small ones, are also linked to soil microorganisms through food webs. Representatives of the microfauna are protozoans (Flagellates, Naked Amoeba, Testacea, Ciliates). The mesofauna is represented by Rotifera, Nematoda, Tardigrada and microarthropods such as Collembola, Mites, Protura, Diplura, Microcoryphia, Pseudoscorpionidae, Symphyla and Pauropoda. Representatives of the macrofauna are Isopoda, Diplopoda, Chilopoda, Scorpionidae, Areanae, Insects, Spiders, Gastropoda and Earthworms (Coleman *et al*., 2004). Only the animal groups most numerous or relevant to the subject discussed in this chapter will be mentioned below. They will be presented according to their systematic position, and not according to their size as indicated above.

3.3.1. Protozoa

Protozoa are single-celled organisms and are the smallest of the soil animals. They live in the films of water surrounding soil particles and are in a sense aquatic animals. Soil protozoa are largely predators, feeding on soil bacteria. Some also feed on fungi, algae or dead organic matter (Foth & Turk, 1990). Most of the insect pathogenic protozoa occur in the phyla Apicomplexa and Microspora. The microsporidia (Microspora) are the most important protozoan pathogens of insects, and they are the most promising candidates for use in microbial control. Insects in nearly all taxonomic orders are susceptible to microsporidia but more than half of the hosts are registered in two orders, Lepidoptera and Diptera (Tanada & Kaja, 1993). Very few reports show that microsporidia have been isolated from nematodes, and it is possible that many infections are missed (Kaya, 2002).

3.3.2. Rotifers and tardigrades

Soil rotifers are considered to be aquatic organisms and more than 90% are in the order Bdelloidea, or wormlike rotifers. The importance of these organisms is largely unknown, and is often not listed in major compendia of soil biota even though they might be very numerous in soil (Coleman *et al.*, 2004). Tardigrades are essentially aquatic and these interesting animals, also called “water bears”, range in size from 50 µm to 1200 µm, rarely exceeding 500 µm. Soil inhabiting tardigrades are found in the upper porous strata where oxygen concentration is high. The degree of compaction of the soil is probably one of the most important factors affecting their distribution. Soil tardigrades feed on algae, fungi, bacteria, protozoa, rotifers, nematodes, organic debris, and other tardigrades (Nelson & Higgins, 1990; Coleman *et al.*, 2004).

3.3.3. Nematodes

Nematodes, or roundworms, are among the most numerous of the multicellular organisms in ecosystems, and have adapted to almost every environment wherever there is moisture available (Wallace, 1963; Freckman & Baldwin, 1990; De Ley, 2000; Coleman *et al.*, 2004; Lee, 2002). The soil offers an excellent site for insect-nematode interactions. Previous and current work on the ecology of nematodes in soil related to plant and soil health can give valuable information for further studies on the ecology of insect parasitic nematodes.

De Man is considered as one of the pioneers of nematode ecology based on his studies in the late 1800's (Filipjev & Schuurmans-Stekhoven, 1941). He divided the soil nematodes into 5 groups: (1) the ubiquitous species, (2) the meadow and forest forms which live in a soil rich in humus, (3) the nematode fauna of sandy soil and dunes, (4) species living in soil, soaked in brackish water and (5) fresh-water species. A number of reviews concerning aspects on the ecology of nematodes have been published since that time, (Overgaard Nielsen, 1949; Goodey, 1951; Winslow, 1960; Overgaard Nielsen, 1967; Wallace, 1973; Norton, 1978; Yeates, 1971; 1979, 1981, 2004; Kaya, 1990; Norton & Niblack, 1991; Ferris, 1993; Lewis, 2002,). As with other soil fauna, taxonomy, sampling and extraction procedures and the difficulty of *in vivo* observations, are some of the limitations imposed on the study of nematode ecology. Nevertheless research into nematode ecology has progressed increasingly in the past couple of decades. The recognition of different feeding groups, i.e. the functional role of soil nematodes, forms a basis for ecological classification. It distinguished, rather broadly at first, between plant feeders, predators, fungivores, microbial-feeders and omnivores (Yeates, 1971). Yeates *et al*., (1993) published the first comprehensive overview of nematode feeding habits presenting 8 essential feeding types (table 2).

Much work has been done on studying differences between species at the molecular level. It is becoming clear that there is a need to develop molecular methods for classifying whole nematode communities in soil (Adams & Nguyen, 2000; De Ley & Blaxter, 2002). The application of molecular techniques for studying animal communities in soil will greatly improve our knowledge regarding many aspects of their life in soil.

Table 2: Ecological classification of soil nematodes based on feeding types, adapted from Yeates et al., (1993)

Feeding type	**Nematode orders**	**Description of feeding group**
1. Plant feeding	Dorylaimida Tylenchida	Most of these are plant parasitic and many are quite well studied. Presence of a stylet (spear). Sub-divided further into 6 groups: Sedentary parasites, migratory endoparasites, semi endoparasites, ectoparasites. Plant feeders may be polyphagous or show host specificity. Epidermal cell and root feeders, algal, lichen or moss feeders.
2. Hyphal feeding	Dorylaimida Tylenchida	Penetration of fungal hyphae using a stylet (spear). Includes alternate cycles of some invertebrate parasites. Not known whether the same nematode species can feed on both saprophytic and mycorrhizal fungi.
3. Bacterial feeding	Araeolaimida Chromadorida Diplogasterida Enoplida Isolaimida Monhysterida Rhabditida	Includes species that feed on a prokaryote food source, through a narrow or broad mouth part. The soil stages of certain nematode parasites of vertebrates and invertebrates that feed on bacteria should be included. Some may use insects as a phoretic host.
4. Substrate ingester	Diplogasterida Monhysterida	More than one pure food source is ingested, but it is unknown whether nematodes can digest complex organic substrates.
5. Predatory	Chromadorida Diplogasterida Dorylaimida Monhysterida Mononchida Tylenchida	Nematode species in this group may feed on protozoa, other nematodes, rotifers and/or enchytraeids either as “ingesters” or “piercers”.
6. Unicellular eukaryote feeding	Chromadorida Diplogasterida Enoplida Monhysterida Tylenchida	Reported to feed on algae, but difficult to prove, includes ingestion of fungal spores and whole yeast cells.
7. Dispersal stage or infective stage of animal parasites	Rhabditida Stichosomida Tylenchida	The entomogenous species included here, life cycle with stages in the soil.
8. Omnivore	Dorylaimida Enoplida	Restricted to certain groups, but when possible nematodes should be classified in types 1-7.

Bongers (1990) proposed an ecological measure based on nematode species composition defined as the maturity index (MI). This index weighs nematode species mean abundance by a colonizer-persister (c-p) scale, related to r and K life strategies, and reflects the maturation of communities. The MI index, or faunal nematode analysis, has been enhanced and refined by Ferris *et al*. (2001). Faunal nematode analysis may be employed for investigating the effect of entomopathogenic nematodes to the soil nematode community, although few studies have been conducted so far. In one study, the application of entomopathogenic nematodes significantly reduced the number of genera and abundance of plant-parasitic, but not free-living, nematodes (Somasekhar *et al*., 2002).

Insect parasitic nematodes comprise several different groups of nematodes and it is beyond the scope of this chapter to give a detailed review on all of them. The main emphasis will be on Steinernematidae and Heterorhabditidae (Gaugler, 2002), but the terrestrial Mermithidae and Sphaerularioid nematodes will also be mentioned at the end of this section. Nematodes in the families Steinernematidae and Heterorhabditidae, commonly known as entomopathogenic nematodes, are the most studied nematodes for biological control of insects, and currently comprise the genera *Steinernema*, *Neosteinernema* and *Heterorhabditis* (Gaugler & Kaya, 1990; Bedding *et al*., 1993; Gaugler, 2002). Entomopathogenic nematodes are characterized by having a unique mutualistic relationship with bacteria (*Xenorhabdus* and *Photorhabdus*). The infective stage of the nematodes (also known as the dauer stage) provides protection and transportation for their bacterial symbionts, this is the only stage in the life cycle of these nematodes that can disperse and survive outside the host. The bacterial symbionts contribute to the relationship by killing the insect host, establishing and maintaining suitable conditions for nematode reproduction, and providing nutrients and antimicrobial substances that inhibit growth of a wide range of microorganisms. Understanding these multitrophic interactions among the nematodes their symbiotic bacteria, and insect hosts is of fundamental importance for nematode infectivity, survival and use in biocontrol. Some species are produced commercially, and much research has gone into production and formulation of these nematodes (Ehlers, 1996; Grewal, 2002; Gaugler & Han, 2002).

Entomopathogenic nematode species exhibit differences in habitat preferences, host range, infectivity, environmental tolerances and suitability for commercial production. For example Sturhan (1999) revealed that some species like *Steinernema affine* is a species characteristic of grasslands, whereas *S. kraussei* appears to be characteristic of woodlands in lowland parts of Europe (Spiridinov *et al*., 2004). *S. carpocapsae* has shown to be relatively tolerant to desiccation (Womersley 1990). The great diversity of habitats exploited by entomopathogenic nematodes is demonstrated in the numerous isolation records published (Kaya & Gaugler, 1993; Hominick 2002). The genus *Steinernema* is the most intensively studied of the entomopathogenic nematodes. Spiridinov *et al*. (2004) have recently published a comprehensive study on the phylogenetic relationships within the genus, including ecological patterns. The patterns reveal possible habitat preferences for *Steinernema* species, as mentioned above. The major factors determining these habitat preferences are likely to involve both soil physical factors and availability of hosts, although further studies are required to reveal this. To increase our dearth of knowledge on the ecology of entomopathogenic nematodes, Koppenhofer & Kaya (1999) presented a number of simple experiments that can be conducted on any new nematode species that is described, which could serve as a model for ecological outlines of entomopathogenic nematodes.

Few studies have been conducted on the population dynamics of naturally occurring entomopathogenic nematodes (Kaya, 1990; Lewis *et al*., 1998), although some interesting models have been developed recently (Dugaw *et al*., 2004; Fenton & Sands, 2004). A review on the population dynamics of nematodes has recently been published by Boag & Yeates (2004). They show that no long-term studies have been conducted on soil nematodes, except for some economically important plant-parasitic nematodes. Epizootic outbreaks have been reported for entomopathogenic nematodes for example in bibionids (Bovien, 1937; Mráčzek & Sturhan, 2000), scarabs (Akhurst *et al*., 1991), and sawflies (Mráčzek & Bečvář, 2000). Mráčzek (1982) investigated the horizontal distribution of *Steinernema kraussei* in two localities with an outbreak of the sawfly *Cephalcia abietis*; he found that 24-27% of the pest (diapausing larvae) was killed by *S. kraussei* annually. Peters (1996, pers. comm.) has collected useful data of known natural occurrence of entomopathogenic nematodes in insects, (Table 3).

Table 3: Reports of naturally occurring infections of insects with entomopathogenic nematodes (adapted from Peters, 1996 and pers. comm.; Adams & Nguyen, 2002.)

Nematode species	**Host Insect order**	**Host species**	**Reference**
Steinernema affine	Diptera	*Bibio* sp.	Peters, 1996
		Helina duplicata	Peters, 1996
	Coleoptera	*Cantharis* sp.	Nielsen & Philipsen, 2003
		Phyllopertha horticola	Nielsen & Philipsen, 2003
		Pterostichus nigrita	Nielsen & Philipsen, 2003
S. arenarium	Coleoptera	*Anomala dubia*	Peters, 1996
		Melolontha hippocastani	Poinar, 1992
S. bicornutum	Coleoptera	Curculionidae (Carabidae) *Harpalus* sp.	Gradinarov, 2003
S. carpocapsae	Coleoptera	*Agriotes lineatus*	Peters, 1996
		Cleonus mendicus	Peters, 1996
		Diaprepes abbreviatus	Peters, pers. comm.
		Graphognathus leucoloma	Peters, 1996
		Hylobius pales	Peters, 1996
		Otiorhynchus sulcatus	Peters, 1996
		Popillia japonica	Peters, 1996
	Hymenoptera	*Cephalcia arvensis*	Peters, pers. comm.
		C. lariciphila	Peters, 1996
		Vespula sp.	Ehlers *et al*., 1991
	Diptera	*Rhagoletis pomonella*	Peters, 1996
	Lepidoptera	*Cydia pomonella*	Peters, 1996
		Heliothis armigera	Peters, 1996
		Mamestra brassicae	Peters, pers. comm.
		Pieris brassicae	Peters, 1996

Nematode species	**Host Insect order**	**Host species**	**Reference**
S. carpocapsae (continued)	Lepidoptera	*Scotia segetum* *Semiothisa pumila* *Vitacea polistiformis*	Peters, 1996 Peters, pers. comm. Peters, 1996
S. feltiae	Coleoptera	*Amphimallon solstitiale* *Bothynoderes punctiventris* *Capnodis tenebrionis* Curculionidae *Graphognathus leucoloma* *Hylobius abietis* *Onitis alexis* *Otiorhynchus sulcatus* *O. ovatus* *O. dubius* *Pentodon algerinum* *Phyllobius urticae* *Phyllopertha horticola* *Pytho depressus* *Rhagium inquisitor* *Selatosomus melancholicus*	Peters, 1996 Peters, 1996 Peters, 1996 Nielsen & Philipsen, 2003 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Nielsen & Philipsen, 2003 Peters, 1996 Peters, 1996 Peters, 1996
	Diptera	*Bibio hortulans* *B. ferruginatus* *Delia radicum* *Dilophus vulgaris* *Mycetophila fungorum*	Bovien, 1937 Peters, pers. comm. Nielsen & Philipsen, 2003 Peters, pers. comm. Poinar, 1992
	Lepidoptera	*Agrotinae* gen.sp. *Agrotis ipsilon* *A. lineatus* *Crambus simplex* *Heliothis armigera* *Hepialus lupulinus* *Leucania acontistis* *Scotia segetum*	Peters, 1996 Peters, 1996 Nielsen & Philipsen, 2003 Peters, 1996 Peters, 1996 Nielsen & Philipsen, 2003 Peters, pers. comm. Peters, 1996
S. glaseri	Coleoptera	*Anomala flavipennis* *Migdolus fryanus* *Popillia japonica* *Strigoderma arboricola*	Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996
S. intermedium	Coleoptera	*Cantharis* sp.	Nielsen & Philipsen, 2003,

Nematode species	**Host Insect order**	**Host species**	**Reference**
S. intermedium (continued)	Diptera	*Bibio marci*	Gradinarov *et al*., 2000 Mráčzek & Sturhan, 2000
S. kraussei	Hymenoptera Coleoptera	*Cephalcia abietis* *C. falleni* Curculionidae	Peters, 1996 Peters, 1996 Gradinarov, 2003
S. kushidai	Coleoptera	*Anomala cupre*	Peters, 1996
S. rarum	Lepidoptera	*Heliothis sp.*	Peters, 1996
S. riobravis	Lepidoptera	*Helicoverpa zea* *Spodoptera frugiperda*	Peters, 1996 Peters, 1996
S. scapterisci	Saltatoria	*Scapteriscus* *S. borelli* *S. vicinus*	Peters, 1996 Peters, 1996 Peters, 1996
S. scarabaei	Coleoptera	*Anomala(=Exomala) orientalis* *Popillia japonica*	Koppenhofer & Fuzy, 2003 Stock & Koppenhofer, 2003
S. neocurtillae	Saltatoria	*Neocurtilla hexadactyla*	Peters, 1996
Steinernema sp.	Coleoptera Lepidoptera Diptera	*Acantholyda nemoralis* *Adoryphorus couloni* *Amphimallon solstitiale* *Melolontha hippocastani* *M. afflicta* *Phyllopertha horticola* *Scitala sericans* *Graphognathus* sp. *Agrotis ipsilon* *Scotia segetum* *Sesamia nonagrioides* Asilidae	Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Gradinarov, 2003
Neosteinernema longicurvicaudum	Isoptera	*Reticulitermes flavipes*	Peters, 1996
Heterorhabditis bacteriophora	Coleoptera	*Amphimallon solstitiale* *Curculio caryae* *Cyclocephala hirta* *Diabrotica balteata* *Diaprepes abbreviatus* *Drasterius bimaculatus* *Hoplia philanthus* *Popillia japonica* *Phyllophaga* sp.	Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Gradinarov, 2003 Ansari *et al*., 2003 Peters, 1996 Peters, 1996

Nematode species	Host Insect order	Host species	Reference
Heterorhabditis bacteriophora (continued)	Lepidoptera	*Diatrea grandiosella* *Heliothis punctigera* *Helicoverpa zea*	Peters, 1996 Peters, 1996 Peters, 1996
H. indica	Lepidoptera	*Scirpophaga excerptalis*	Poinar *et al*., 1992
H. megidis	Coleoptera	*Amphimallon solstitiale* *Otiorhynchus sulcatus* *Phyllopertha horticola* *Popillia japonica*	Peters, 1996 Peters, 1996 Klingen *et al*., 2002d Peters, 1996
H. marelata	Lepidoptera	*Hepialus lupulinus*	Strong *et al*., 1995
H. zealandica	Coleoptera	*Heteronychus arator*	Peters, 1996
Heterorhabditis sp.	Coleoptera	*Agriotes ponticus* *Antitrogus consanguineus* *Cylas formicarius* *Graphognathus leucoloma* *Lepidiota crinita* *L. negatoria* *L. picticollis* *Pachneus litus* *Phyllopertha horticola*	Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996 Peters, 1996

The effect of entomopathogenic nematodes on non-target hosts when used in biological control has been investigated, but few long-term studies have been conducted. Most of the early work involved laboratory tests with a wide range of animal species (Georgis *et al*., 1991). Bathon (1996) has conducted an excellent review and field study on the impact of entomopathogenic nematodes on non-target hosts. The release of entomopathogenic nematodes can cause mortality to non-target arthropod populations but it was found that the effect was spatially restricted and temporary only affecting part of the population. It is important to monitor entomopathogenic nematode populations and their effect on non-target organisms in the field after their release. This should become an important recommendation in experimental and practical work with entomopathogenic nematodes.

Predatory nematodes may have a negative effect on entomopathogenic nematodes, although this is not well documented (Kaya, 2002). Duncan *et al*. (2003) showed the apparent importance of competitors such as free-living bactivorous nematodes as potential significant regulators of entomopathogenic nematodes

In sections, 4.2., 5.2, and some parts of section 6, we refer to nematodes in general and more specifically to entomopathogenic nematodes (Steinernematidae and Heterorhabditidae), the other two important groups of nematodes parasitic in insects are briefly described below.

Terrestrial mermithids include species ranging from a few millimetres to 405 mm, where most are between 50 and 150mm long. Kaiser (1991) gives an excellent review on the terrestrial and semiterrestrial Mermithidae, which is briefly summarized here. Reports on infections with mermithids are found for virtually all insect orders, and Poinar (1975) has compiled an extensive host list. Many of the mermithids reported in insect hosts are not identified to species, because these parasitic stages lack distinguishing characters for identification. Three phases of parasitic development are described, (1) penetration into the

host, only slight growth, and important changes in the cuticle take place for uptake of food, (2) the growth phase, the nematode grows rapidly in length almost filling the hemocoel, the cuticle is still a thin membrane that can burst easily, (3) the end of the growth phase is signalled by the increasing thickness of the cuticle, and this stage bores its way out of the host, enters the soil for further free-living development. The diversity of mermithids depends on the host diversity and on the nature and moisture of the soil. The most studied insect order with respect to mermithids is grasshoppers in which the evidence for moisture dependence has been shown. Mermithids are considered common parasitoids of agricultural pests, and they have a significant impact on for example regulating the population dynamics of grasshoppers; however their potential as biological control agents has yet to be realized.

Nematodes in the superfamily Sphaerularioidea, and the Allantonematidae represent the basic type of Sphaerularioidea. The complex host-parasite relationships of Sphaerularioid nematodes are not well known (Remillet & Laumond, 1991). In brief, survival and reproduction is ensured by annual parasitism, the host's fecundity reduction, dissemination of juvenile nematodes by living adult insects, adaption of the length of the free-living period of infective females, and the synchronization of the host larval development. Free-living or plant parasitic generations allow the survival of the nematodes in the absence of hosts. These highly specialized adaptions lead to a high degree of specificity between the nematode and insect species. This specificity and the complex balance maintained between hosts and parasites are limiting factors in the use of Sphaerularioids in biological control. Anderson & Skorping (1991) found that levels of parasitism by *Heterotylenchus autumnalis* (Allantonematidae) to carabid beetles was significantly enhanced in certain protected microhabitats (silty, more or less vegetated, often shady sites) compared to more open microhabitats. This difference was not attributed to the differences in micro-climate but to the differences in soil type and location. The open sites were close to a river, with a coarser soil type and were subject to flooding and erosion.

3.3.4. Earthworms

Earthworms are the most familiar, and with respect to soil processes often the most important group of soil fauna. They play an important role in influencing soil structure and in the breakdown of organic matter in soil (Coleman *et al*., 2004). Soil fungi are considered to be an important food source for earthworms (Bonkowski *et al*., 2000); however, fungi and bacteria are also known to be pathogenic to earthworms. Many soil animals such as protozoa, rotifers, platyhelminths, mites, dipterous larvae, beetles and centipedes prey on earthworms (Wallwork, 1970; Grewal & Grewal, 2003; Shah *et al.*, 2003). Nematodes belonging to the genera *Rhabditis* and *Cephalobus* have been found to naturally infect between 7 and 13% of earthworm cocoons (Kraglund & Ekelund, 2002). None of these nematode genera are, however, used in biological control of insects or slugs. Studies show that biological control agents such as entomopathogenic nematodes and insect pathogenic fungi do not appear to have negative effects on earthworms (Capinera *et al.*, 1982; Iglesias *et al.*, 2003; De Nardo *et al.,* 2004; Hozzank *et al.*, 2003a). The ecology and host range of *Phasmarhabditis*, a nematode parasite of slugs, needs to be better understood before it can be claimed completely safe for earthworms, even though laboratory studies so far indicate that there is no negative effect (Grewal & Grewal, 2003; Morand *et al*., 2004).

It has been suggested that earthworms might work as a vector of insect pathogenic fungi in the soil (Milner *et al.*, 2003). Shapiro *et al*. (1995) reported that upward dispersal of two species

of entomopathogenic nematodes increased in the presence of earthworms, they also suggested that nematodes may have a phoretic association with earthworms.

3.3.5. Arthropods

Many arthropods have one or several stages of their life cycle associated with the soil environment. Some are permanent soil inhabitants, where all life stages are found in or on the soil. Immature stages of other species are soil dwellers whereas the adult stages live and feed in aboveground food chains (see Fig. 2). A high proportion of soil animals are arthropods, and the most abundant are collembolans (springtails) and mites (Coleman *et al.*, 2004). Many soil dwelling arthropods are pests of plants, but several of them, such as predators and parasites, are also important natural enemies of pest arthropods. Centipedes, mites, spiders, beetles, and wasps are all common predators in or on the soil. Predatory mites in the orders Mesostigmata and Prostigmata feed on a variety of soil animals such as Collembola, Protura, Pauropoda, nematodes, enchytraeids and eggs, larvae and pupae of insects. The predatory mites *Hypoaspis aculeifer* and *H. miles* (Mesostigmata) are used in inundative biological control against thrips, fungus gnats and bulb mites in greenhouses (Walter & Proctor, 1999). Spiders are another familiar group of carnivores. Many species are found in above ground habitats, but some are cryptozoans in litter and on the soil surface (Coleman *et al*., 2004). Even though many spiders are not true soil-dwellers the families Lycosidae, Linyphiidae, Gnaphosidae, Tetragnathidae, Clubionidae, Thereidiidae and Agelenidae can establish a close association with the soil community and prey on other arthropods (Wallwork, 1970; Coleman *et al*., 2004). Two of the most widespread beetle families in soil are carnivorous Carabidae and the Staphylinidae, which includes both predatory and saprophagous forms (Wallwork, 1970). Recent studies with monoclonal antibodies have revealed the importance of earthworms and slugs as prey sources for ground beetles (Shah *et al.*, 2003). Some Dipteran larvae such as the Brachycera may prey on other insect larvae, small molluscs and annelids, and nematodes. Several Brachycera species in the families Tachinidae, Phoridae and Calliphoridae are parasites of earthworms, molluscs, and soil-inhabiting arthropods (Wallwork, 1970). *Ormia depleata* (Tachinidae), for example, is well known as a classical biological control agent against mole crickets, *Scapteriscus* in USA (Parkman *et al.*, 1996). Many Hymenoptera in the families Mutilidae, Scoliidae, Chalcididae, Proctotrupidae, Tiphiidae and Sphecidae parasitize soil-dwelling insect larvae. *Larra bicolor* (Sphecidae) is known as a classical biological control agent against mole crickets in USA (Wallwork, 1970; Frank *et al.*, 1995). Parasitoids from other families and even other orders are also known as parasites of soil dwelling pupae of pest insects. Pupae of the soil dwelling pests *Delia radicum* and *D. floralis,* for example, are parasitized by the following: *Trybliographa rapae* (=*Cothonaspis rapae*) (Eucolidae: Hymenoptera), *Aleochara bilineata, A. sufussa* (Staphylinidae: Coleoptera) and *Phygadeuon trichops* (Ichneumonidae: Hymenopthera) (Sundby & Taksdal, 1969; Jonasson *et al*., 1995). High levels of parasitism have been observed, and *T. rapae* has been shown to parasitize up to 50% of *D. radicum* and *D. floralis* pupae in Norway (Sundby & Taksdal, 1969). The soil environment also functions as a reservoir for insect parasitoids that attack insect pests above ground since many of these parasitoids spend their diapausing or over wintering stage in the litter or the upper layer of the soil (Stary, 1988).

Predators and parasites in the soil environment can interact antagonistically with insect and mite pathogens and insect parasitic nematodes by decreasing host density and by competing for

hosts and vice versa (Bathon, 1996). Insect and mite pathogens and insect parasitic nematodes might also directly decrease soil arthropod natural enemy populations. Steenberg *et al.* (1995) and Vestergaard *et al.* (2003) for example report that insect pathogenic fungi can infect soil dwelling arthropod natural enemies. Several studies of epigeal systems show that arthropod natural enemies change their behaviour and often avoid hosts that are infected with a pathogen (Hajek, 1997; Pell *et al.*, 2001). Behavioural studies are difficult to conduct in a soil ecosystem and to our knowledge no studies on avoidance by predators and parasitoids to infected hosts have been conducted. Competition between pathogens and parasitoids inside an insect or mite host after infection and parasitation is also known to occur, and most pathogens kill the host faster than a coidiobiont parasitoid. Parasitation therefore affects the pathogen development only when the host is parasitized before it is infected (Hajek, 1997; Pell *et al.*, 2001; Lacey *et al*., 2003). Natural enemies of pest arthropods and other non-target arthropods can also interact synergistically with insect and mite pathogens and insect parasitic nematodes, by for example enhancing transmission and dispersal. Studies conducted with predators and parasitoids in epigeal systems show that the presence and activity of these natural enemies resulted in a substantial increase of pathogen transmission, both because the natural enemy vectors the pathogen and because it increases the movement of the host (e.g. Roy & Pell, 2000). Evans (2000) also shows that predators and parasitoids have a role to play in dispersal of insect pathogenic viruses from the soil inoculum to the host. Microbes can be disseminated by soil microarthropods, where microarthropods can passively transport bacteria, fungi, and protozoa in the gut or on the cuticle across regions of soil that are impenetrable to the microbiota. Microphytophages such as collembolans are well known to feed on fungi (Moore *et al.*, 1988), and they are non-susceptible to insect pathogens (Broza *et al.*, 2001). Considerable amounts of viable conidia of insect pathogenic fungi can be carried on the cuticle and in the gut of collembolans (Broza *et al.,* 2001; Dromph, 2001). Dromph (2003) also showed that insect pathogenic fungi like *Beauveria bassiana*, *B. brongniartii* and *Metarhizium anisopliae* can be vectored by collembolans and as a result cause mortality in susceptible host insects in the soil. Little work has been done on dispersal of entomopathogenic nematodes by arthropods, (Kaya, 1990), although phoretic relationships between other nematodes and insects is well known. Hosts that have become infected with entomopathogenic nematodes may disperse nematodes in the soil before they die.

Insects and mites are hosts of arthropod pathogens and insect parasitic nematodes, and the presence of a host affects the persistence and abundance of arthropod pathogens and insect parasitic nematodes in the soil. A soil ecosystem with a high density of host arthropods will therefore also support a high abundance of insect and mite pathogens and insect parasitic nematodes. Although saprophytic growth of some arthropod pathogens are known (Hajek, 1997), the growth is often limited and primarily restricted to host insects or mites in native soils (Kessler *et al*., 2004). Entomopathogenic nematodes are obligate pathogens of insects, and in order to persist they need to reproduce (recycle) within a host (Kaya 1990). Kowalska (2000) reported on the presence of an alternative host, the curculionid *Strophosoma faber* that could enhance the effect of entomopathogenic nematodes against the turf pest *Amphimallon solstitiale* (Scarabaeidae). An interesting study investigating the recycling of entomopathogenic nematodes in cruciferous crops showed that relatively small and abundant insects that only pupate in the soil can contribute to maintaining entomopathogenic nematode populations in soil (Nielsen & Philipsen, 2004).

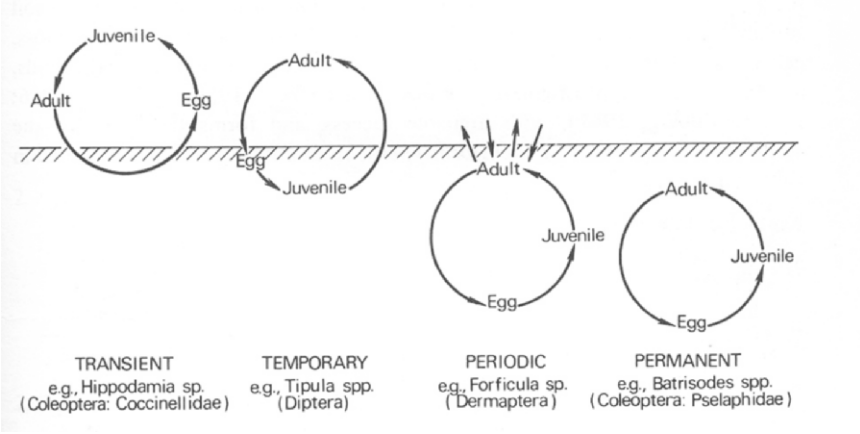

Figure 2: Categories of soil animals defined according to degree of presence in the soil, as illustrated by some insect groups (from Wallwork, 1970)

3.3.6. Slugs and snails

Terrestrial gastropods (snails and slugs) are important herbivores and several species are important pests in agroecosystems (Barker, 2002). The majority of species, however, feed on decaying tissue as well as numerous Basidomycetes, facilitating decomposition on soils and return of plant litter to the soil (Dallinger *et al.*, 2001; Coleman *et al.*, 2004). There is a wide range of natural enemies of slugs and snails, including predators, parasites and diseases, recently reviewed by Barker (2004). Important predators are vertebrates such as birds and mammals (Allen 2004). Among the predatory arthropods, Coleoptera are important, especially carabid beetles. Sciomyzid fly larvae (Marsh flies) are also well studied predators of slugs and snails (Symondson, 2004; Barker *et al.,* 2004). The trombidiform "slug mite" *Riccardoella limacum* is an ectoparasite of slugs, Fain (2004) gives an update on predaceous and parasitic mites. Nematodes have been recorded as parasites of slugs and snails on a number of occasions (Grewal *et al.*, 2003), but are not well studied. Morand *et al.* (2004) have listed 8 families and 27 described species of nematodes parasitic in terrestrial gastropods, it is likely that there are several more nematode species that have yet to be discovered. In recent years one particular nematode, the rhabditid *Phasmarhabditis hermaphrodita*, has been developed as a biological control agent of slugs, (Wilson *et al.*, 1993; Morand *et al*., 2004).

3.3.7. Vertebrates

Vertebrates have a great influence on the soil community through an impressing diversity of interactions. It is difficult, however, to make a rigid definition of the vertebrate soil fauna, and several species may be mentioned that influence the soil. Animals that burrow or make nests in the soil, animals that feed on other soil animals (moles, rodents and birds) and animals that graze and deposit dung on the soil surface all affect the soil community in one way or the other. One example is the mole that can consume between 18 and 36 kg earthworms and insects each year over an area of 0.1 acre (Wallwork, 1970). It is also known that birds or grazing sheep can disperse a NPV virus pathogenic to the lepidopteran pasture pest complex *Wiseana* spp (Tanada & Kaya, 1993).

4. Soil physical factors important to natural enemies of pest arthropods

Several physical soil factors are important to natural enemies of pest arthropods, and in this section we will review some of them. Soil texture (the relative proportions of sand, silt and clay particles) and soil structure (the combination and arrangement of primary soil particles into secondary particles, aggregates) has a strong impact on the accessibility of food, shelter, water, oxygen and nutrients to the soil biota (Coleman, 1986; Foth & Turk, 1990). Different sized organisms have different amounts of space available to them depending on soil texture. Smaller particle size and finer soil texture results in reduced pore size and increased tortuosity that can impede the movement of soil organisms. Structure is strongly affected by climate, biological activity, density and continuity of surface cover, and soil management practices. Most research on effects on biological control, however, has been concerned with texture (Barbercheck, 1992). Soil pH can have some impact on insect and mite pathogens and insect parasitic nematodes (Smith, 1999; Kessler *et al.*, 2003). Soil climatic conditions such as temperature, gases, water status and humidity are also important factors (Barbercheck, 1992). Soil temperature will vary depending on the geographical location, aspect and gradient of surface slopes, exposure, soil colour, soil cover and the nature and density of plant cover (Keller & Zimmermann, 1989). Water status and humidity are dependent on soil texture, structure, organic matter and the climatic conditions (Foth & Turk, 1990). At the surface, moisture is frequently in equilibrium with the atmosphere, and under dry climatic conditions, growth of many soil organisms might be restricted or inhibited. In the deeper soil layers and in temperate climatic zones the moisture content is higher. Rainfall influences the vertical movement of soil organisms (Keller & Zimmermann 1989; Inglis *et al.*, 2001).

4.1. Pathogens of insects and mites

Soil can provide favourable physical conditions for survival of insect and mite pathogens. In comparison to the epigeal environment, pathogens in soil are not subject to destruction by solar radiation, and humidity is relatively high and stable (Barbercheck, 1992).

4.1.1. Soil texture, structure and organic matter

The activity and location of the host insect or mite are important for the contact between the pathogen and the host. Contact between soilborne pathogens and their hosts in the soil are also determined largely by soil factors affecting passive percolation into the soil profile (texture, structure and organic matter) (Storey & Gardner, 1987; Barbercheck, 1992). Several studies have been conducted on the distribution, abundance, persistence and percolation of arthropod pathogens in the soil (e.g. Rath *et al*., 1992). Many studies suggest that a high clay content of soil enhances the abundance and persistence of many insect pathogenic fungi because clay particles adsorb conidia (Kessler *et al.*, 2003; Vänninen *et al.,* 1989; Studdert *et al*., 1990). The mechanisms responsible for the high retention of conidia in clay soils are unknown, but may be related to their high cation exchange capacity (the capacity of soils to adsorb ions) and/or its reduced pore size (Inglis *et al*., 2001). Ignoffo *et al.* (1977) further hypothesize that electrical differentials between conidia and clay particles might be responsible. Conidia adsorbed in this way in clay are retained where they were originally produced (from a host cadaver) or where they were artificially applied (as a microbial control agent), and not washed away by rainwater. This could be an advantage or a disadvantage depending on where the host is located or whether other soil organisms or water are able to spread the fungal propagules to the sites where the host is located. The movement of soil during cultivation (ploughing, harrowing and hoeing) can also disperse microorganisms within 20-30 cm of the plough depth and several meters horizontally. Ploughing and harrowing increases porosity of the soil, but heavy traffic during cultivation causes compaction of the soil destroying macropores. The former thus aids dispersal and the latter hinders it (Dighton *et al.*, 1997).

Soil with high organic matter content can affect arthropod pathogenic fungi. Whether the net effect is positive or negative for their occurrence and persistence is not clear. Several authors suggest that arthropod pathogenic fungi have low persistence in soil high in organic matter (Studdert *et al.*, 1990; Vänninen *et al.*, 2000; Kessler *et al.*, 2003). They explain this by the high biological activity and presence of numerous antagonistic organisms. On the other hand, soil high in organic matter has a greater diversity and density of arthropods, which are possible pathogen hosts. It has been suggested that soil low in organic matter tends to retain fewer fungal propagules than soils high in organic matter, explained by the fact that the latter has a higher cation exchange capacity, that helps adsorb fungal conidia (Ignoffo *et al.*, 1977; Inglis *et al*., 2001). This means that although it is suggested that soils high in organic matter adsorb conidia of several insect pathogenic fungi, the conidia that are present in the soil are probably killed or degraded faster. An increase in new fungal propagules produced in soil high in organic matter, due to the high density of arthropod hosts, should also be taken into account.

Differing water content and temperature of the soil studied may confuse the results obtained. In several of the soil type studies, water content and temperature were not measured and hence the differences observed could be due to these other factors rather than the properties of the soil. Studdert *et al*. (1990) report for example that conidia half-lifes were significantly longer in Yolo fine sand loam (<1% organic matter) than in peat (62% organic matter) in the middle range of water potentials (-0,3 to –15 bars) and at temperatures up to 20^{o}C. At the more extreme water potentials and at the higher temperatures, these differences were no longer significant. According to Keller & Zimmermann (1989), it also appears that the structure of the

fungal spores, their formulation and probably the addition of a wetting agent may interfere with how easily fungal propagules percolate through the soil. Some of the soil type studies are conducted with formulated fungi and some with clean spores. This may confuse what the actual effect of a specific soil type is. For example in a study conducted by Storey & Gardner (1987) they were not able to show that high clay composition in soil restricted vertical movement of formulated *B. bassiana* conidia even though studies with clean spores show restricted movement in clay soils.

4.1.2. Temperature

Differences in the geographical distribution of insect and mite pathogenic fungi may partly be explained by their average temperature requirements. Vänninen *et al.* (1989) and Vänninen (1995) found that *M. anisopliae* and *Paecilomyces fumosoroseus* were more prevalent in the south of Finland than for example *P. farinosus* and *B. bassiana* which were more prevalent in northern locations. Vänninen (1995) also suggest that the frequency of insect pathogenic fungi in general appears to decline northwards in Europe. In Norwegian studies, *M. anisopliae* has been found further north ($67^{o}16^{l}$N, $14^{o}27^{l}$E) than in the Finnish study, but the location was close to the coast where the temperatures are generally higher than inland (Klingen *et al.* 2002a). Several studies confirm that *P. farinosus* and *B. bassiana* can tolerate a wider range of climatic conditions and that *M. anisopliae* is more thermophilic. Laboratory studies conducted by Mietkiewski *et al.* (1994) and Tkaczuk *et* al. (2000) for example show that *M. anisopliae* was the most thermophilic of the fungi tested while *P. farinosus* show best growth at the lowest temperature (5^{o} C). It is important to mention, however, that in a study conducted by De Croos & Bidochka (1999), *M. anisopliae* isolates have also been deemed cold-active (grow at 8^{o} C). In this study, all the cold-active isolates were isolated from the more northern sites, and no isolate originating below 43.5^{o} N showed cold activity. Both *B. bassiana* and *P. farinosus* are known to tolerate a wide range of climatic conditions and *B. bassiana* has been found as far north as 75^{o} N in Canada (Widden & Parkinson, 1979). The insect pathogenic fungi *Tolypocladium cylindrosporum* is also known from northern locations and has been found in Norway at $69^{o}20^{l}$N, $19^{o}19^{l}$E (Klingen *et al.*, 2002a).

Several authors have focused on finding cold-active strains of insect pathogenic fungi for use as microbial pesticides, and as suggested above the influence of temperature on the activity of these fungi has shown to be linked to the provenance of the isolates. Indigenous strains are therefore often regarded as the best candidates for biocontrol agents. Considerable intraspecific variation with respect to temperature tolerance among isolates or strains originating from the same geographical location does exist, however, and sometimes isolates originating from warm areas outperform more northern isolates, even under cool conditions (Vänninen, 1999). It is also suggested that the habitat type decides the temperature requirements for an isolate and Bidochka *et al.* (2001) found that fungal isolates collected from forested areas show an ability for cold-active growth (at 8^{o} C), while fungal isolates from agricultural areas showed ability for growth at high temperature (37^{o} C).

As mentioned in the introduction to this section, the soil temperature might be modified by other factors than geographic location, soil cover being one of them. Hummel *et al.* (2002) observed that insect pathogenic fungi were negatively affected when soil temperatures were artificially raised due to the presence of black plastic mulch or bare ground. They suggest that these fungi are adapted to lower temperature ranges and that the increase in soil temperature

reduce their survival. The negative effects of high temperature on insect pathogenic fungi have been shown by several authors (e.g. Mietkiewski *et al*., 1994).

4.1.3. Water potential and moisture

Water potential is the primary factor determining the availability of soil water to plants and animals. In general nematodes, protozoans and bacteria often require a water film for activity, whereas fungi do not. It is known that free water can adversely affect fungal propagules (Barbercheck, 1992). There might be several explanations for this. One is the lack of oxygen and hence the production of carbon dioxide that harms fungal propagules in water saturated soil (Keller & Zimmermann 1989). The other is that bacterial activity and movement is positively related to soil moisture. Active bacteria lyse fungi and reduce the number of fungal propagules under humid or wet soil conditions (Ekesi *et al.*, 2003). Drier soil has been suggested to benefit fungi for the opposite reasons. Fungi are known to survive as resting propagules under very dry conditions (Keller & Zimmermann, 1989). Little is known about optimal field moisture conditions for entomopathogens, but several studies have identified critical parameters in the laboratory (Barbercheck, 1992). One of these studies shows that *B. bassiana* conidia half-lives were longest in non-sterile soil at –15 bars, and decreased as soil became moister or drier (Studdert *et al.*, 1990). Another microcosm study demonstrated that both soil temperature and moisture influence the survival and infectivity of *M. anisopliae* to four fruit fly species. It also showed that the effect of soil moisture is dependent on temperature. At 20-30^{o} C, fungal induced mortality in puparia of the fruit fly, *Ceratitis capitata*, was highest at water potential of -0.1 and -0.001 mega Pascal (Mpa) and lowest at water potential of –0.0055 and –0.0035 Mpa, but infection across all soil moisture levels was similar at 15^{o} C (Ekesi *et al*., 2003).

Water in the form of rain might influence the vertical movement of insect and mite pathogens. It is shown that conidia of insect and mite pathogenic fungi deposited on the surface of soil become washed into the soil at varying degrees depending on soil type (Hajek, 1997). Soil texture and organic matter appear to be the most important factors determining vertical movement of fungal propagules in water. The ratio of polar to neutral lipids in the fungal conidia also determines the relative miscibility of the conidia in water and thus influences their vertical percolation in soil (Storey & Gardner, 1987). Sandy-textured soil low in organic matter tend to retain fewer propagules than clayey and organic soils (Inglis *et al.*, 2001). Many studies show that fungal propagules tend to remain very close to the soil surface (Hajek, 1997), although some surveys show that insect pathogenic fungi can be found at depths down to 30 cm (Mietkiewski *et al.*, 1995). The occurrence of insect pathogenic fungi in deeper soil layers may be due to the vertical saprophytic growth of the fungi. For pathogens that are able to grow as saprophytes, fungal growth can extend far beyond cadavers in the soil environment (Hajek, 1997). To our knowledge, however, no studies have been conducted on how deep naturally occurring fungal infected cadavers can be found. Soil dwelling insect or mite hosts are known to move down to 45 cm undercertain conditions (Colemanet *al.,* 2004). It should be expected that insect pathogens inhabiting these hosts could be found at these soil depths as well. Infected insects or mites might, however, alter their behaviour and move to abnormal soil depths. This has been shown for the common armyworm *Pseudaletia separata* infected with either the fungus *Entomophaga aulicae* or the virus PsNPV. Healthy larvae exhibited a daily rhythmic pattern of movement, feeding on plants above ground during the night and burrowing into the soil during the day. When infected with either *E. aulicae* or PsNPV the pattern of move-

ment was disturbed: larvae crawled out of soil during the day and died near the top of the plant (Ohbayashi & Iwabuchi, 1991). The third-instar larvae of the masked chafer grub (Scarabaeidae) parasitized by the fungus *Tiphia pygidialis* is another similar example, where infected grubs burrowed to depths of 12-16 cm whereas healthy grubs remained in the upper 4 cm soil (Rogers *et al.*, 2003).

4.1.4. pH

The soil microflora is highly influenced by the soil pH. In general, high acidity decreases the growth of bacteria and increases that of soil fungi (Keller & Zimmermann, 1989). Fungi are important in all soils, and their tolerance of acidity makes them particularly important in acid forest soils (Foth, 1984). The influence of soil pH and ionic conductivity is not well understood (Inglis *et al.*, 2001). This might be due to the fact that in most studies, the average pH of bulk soil is used, which may vary considerably from the pH of the microenvironment (Barbercheck 1992). Since the microenvironment is the scale most pertinent to the survival and activity of individual microorganisms (Buckley & Schmidt, 2002), studies at this level might clarify the effect of pH on insect and mite pathogens further. A number of studies using the average pH of bulk soil have demonstrated, however, no or minimal effects of soil pH on the distribution and abundance of insect and mite pathogenic fungi (e.g. Rath *et al*., 1992; Kessler *et al.*, 2003). Laboratory studies also show that 29 different isolates of *B. bassiana* tolerated quite a wide range of pH from 5 - 13, but that pH 3 was toxic to all isolates, and the pH optimum varied between isolates (Padmavati *et al.*, 2003). Rath *et al*. (1992) also found that a specific isolate of *M. anisopliae* was able to grow across a wide range of pH (from 4.0 - 7.8). To our knowledge, little is known about the mechanisms of aluminium toxicity to insect pathogens in soil, even though aluminium may be a major factor limiting microbial growth and activity in acid soils. Some insect pathogenic fungi, like for example *P. fumosoroseus* are frequently found in natural habitats, particularly in hedges and forest soils (Vänninen, 1995; Chandler *et al.*, 1997). There is as yet no good explanation for this, but it might be that *P. fumosoroseus* thrives in more acid forest soil or is more tolerant of aluminium than e.g. *M. anisopliae*.

4.2. Insect parasitic nematodes

Several studies have investigated the physical factors in soil that affect nematodes in general; (Wallace, 1971; Jones, 1978; Norton, 1978; Norton, 1989; Kaya, 1990; Baur & Kaya, 2001). Nematode behavioural response to environmental factors (physical, chemical, mechanical and energy) has recently been reviewed by Barbercheck & Duncan (2004). Several decades ago Wallace (1968) stated that the principal soil factors affecting nematodes are pore size (soil texture), water (moisture and water potential), aeration, temperature and the chemistry of the soil solution, which still holds true today, although some more knowledge has been acquired (Kaya, 1990; Barbercheck, 1992). With respect to entomopathogenic nematodes, studies on physical factors have been conducted with emphasis on trying to understand their efficacy as biological control agents in the field (Gaugler & Kaya, 1990; Gaugler, 2002). Entomopathogenic nematodes require an insect host to complete their life cycle, hence during periods when hosts are scares or unavailable they must possess mechanisms that enable them to persist for long periods in the soil. Some studies on soil physical factors that affect entomopathogenic nematodes are presented in table 4. Most of these studies are controlled laboratory experiments.

Table 4: Selected reports on soil physical factors affecting entomopathogenic nematodes

Nematode species	**Abiotic factor studied**	**Brief comments**	**References**
Heterorhabditis bacteriophora *Steinernema feltiae* *S. carpocapsae* *S. glaseri* *S. kraussei* *Steinernema* sp.	Temperature	Detailed study on behaviour and infectivity at different temperatures	Molyneux; 1986
S. carpocapsae *S. glaseri*	Soil type	Persistence in different soil types	Kung & Gaugler, 1990
S. carpocapsae *S. glaseri*	Soil pH and oxygen	Persistence at different pH and oxygen levels	Kung *et al*., 1990
S. carpocapsae *S. glaseri*	Soil temperature and moisture	Persistence and infectivity at different temperatures and moisture	Kung & Gaugler, 1991
H. bacteriophora *S. carpocapsae*	Soil texture	Host finding and soil texture	Barbercheck & Kaya, 1991
H. bacteriophora *H. megidis* *H. zealandica* *Heterorhabditis* sp *S. feltiae* *S. carpocapsae*	Soil temperature	Reproduction at 10^{o}C	Wright, 1992
S. carpocapsae *S. glaseri*	Soil moisture and depth	Infectivity at different soil depths and moisture	Koppenhofer *et al.,* 1995
S. carpocapsae *S. glaseri* *S. riobravis*	Temperature	Survival under freezing conditions	Brown & Gaugler, 1998
S. kraussei	Soil temperature	Rate of infection at 10^{o}C	Mráčzek *et al*., 1999
S. riobravis	Soil depth and moisture	Distribution at different moisture levels and depths	Gouge *et al*., 2000
S. feltiae *S. kraussei* *H. megidis*	Soil temperature	Infectivity at low temperatures	Long *et al*., 2000

Nematode species	Abiotic factor studied	Brief comments	References
S. riobravis	Soil moisture	Persistence and infectivity in the root zone under dry conditions	Duncan & McCoy, 2001
S. arenarium *S. carpocapsae* *S. feltiae* *H. bacteriophora* *H. megidis*	Soil temperature	Infectivity against *Delia radicum* at different temperatures	Chen *et al*., 2003
S. carpocapsae *S. feltiae* *S. glaseri* *H. bacteriophora*	Soil moisture	Effect on virulence under fluctuating moisture conditions	Grant & Villani, 2003a,b

4.2.1. Soil texture, structure and organic matter

The efficiency with which nematodes can explore their physical environment is important for their ability to locate a host, mate and avoid predators. Wallace (1968) gives an extensive account on nematode movement in soil, describing how they predominately propel themselves through the soil using the surface tension in the water films surrounding soil grains. The movement of nematodes is significantly affected by many factors like chemical gradients in soil, temperature (see 4.2.3.) and the size of the nematode, but to enable movement through soil, soil texture, soil structure and soil moisture (see 4.2.2.) are critical. Soil pore space is related to particle size (soil texture); an increase in particle size gives an increase in width of pores and pore necks. The elongate cylindrical shape of nematodes appears to be an adaption for migration through narrow spaces. Wallace (1968, 1971) describes the importance of nematode size with respect to pore size and moisture, as the length and diameter of the nematode increase, the optimum pore and particle size also increase. Most studies focus on soil texture rather that soil structure, where structural pore space is determined by size and arrangement of aggregates and affects movement of water, air, chemicals and organisms. Soil compaction greatly impedes movement in fine-textured soils, but has little effect in sandy soils. Models for nematode movement in soil have been conducted where it was found that slower movement in fine textured soils would be expected to increase isolation among local populations, and increase the number of species that can co-exist in a given area. (Hunt *et al.*, 2001).

Portillo-Aguilar *et al*. (1999) showed the importance of soil structure for entomopathogenic nematodes by examining the influence of bulk density, (degree of soil compaction), on survival and movement of *H. bacteriophora*, *S. glaseri* and *S. carpocapsae*. The data indicated that the relative compaction of a sandy loam soil strongly affected the survival of the 3 species, but that the effects differed among the species. High bulk densities reduced survival in *H. bacteriophora* whereas *S. glaseri* survived well. It was suggested that the larger nematode *S. glaseri* (diameter 45μm) was restricted in movement thus conserving metabolic reserves, whereas the smaller *H. bacteriophora* (diameter 25 μm) was not restricted in movement resulting in a depletion of energy reserves.

There is evidence of differences in the active dispersal behaviour among entomopathogenic nematodes (Lewis, 2002). Understanding dispersal abilities has practical importance for biological control of pest species. As indicated by several authors (Kung & Gaugler, 1990; Portillo-Aguilar *et al*., 1999), soil texture and structure influences survival and pathogenicity of nematodes. The non-feeding infective juveniles of *Steinernema* and *Heterorhabditis* must rely on their stored reserves for survival and pathogenicity and soil texture can affect nematode energy reserves indirectly by regulating their movement.

Organic matter is an essential component of all soils and its influence on the general microbial population of the soil has been well studied. Less is known about specific relationships between nematodes and organic matter. The effect of organic amendments on plant parasitic nematodes has been studied mostly with respect to reducing crop damage. Soil organic matter contains predaceous fungi and other potential agents for the biological control of nematodes (Duddington, 1965). However ecological studies also show that the bacterial feeding nematodes increase with the content of organic matter. For entomopathogenic nematodes it can be expected that soils high in organic matter might be detrimental due to the presence of predators and pathogens, on the other hand, the increased abundance of possible arthropod hosts in organic soils may sustain or increase entomopathogenic nematode populations (Kaya 1990). Bednarek & Gaugler (1997) found that increased organic matter (organic manure) appeared to encourage nematode establishment and recycling. With regard to nematode movement, Barbercheck & Kaya (1991) found that *H. bacteriophora* was more motile in organic soil than *S. carpocapsae*. In Scotland and Ireland entomopathogenic nematodes (*S. carpocapsae*, *H. downesii* respectively) have been tested in the field against large pine weevil larvae (*Hylobius abietis*), with promising results (Kenis *et al*., 2004). In this case, the nematodes have to move through soil with high organic matter content to reach the pine weevil larvae. Dillon (2003) reported that *S. carpocapsae*, *S. feltiae*, *H. downesii* and *H. megidis* were capable of infecting *H. abietis* larvae at least 40 cm from the zone of application when nematodes were applied to pine stumps. Nematodes migrated further under natural conditions than in containerised peat (Aiofe Dillon pers. comm.). These studies support the hypothesis that the presence of roots plays an important role in the migration of nematodes through soil (van Tol *et al*., 1998, see also 4.2.4.).

Torr *et al*. (2004) demonstrated for the first time that entomopathogenic nematodes (*S. carpocapsae*, *S. feltiae* and *H. megidis*) responded positively to seismic vibrations in peat soil, hypothetically responding to noises made by host larvae feeding on roots.

4.2.2. Water potential and moisture

Soil moisture is one of the main factors affecting nematode activity in soil (Wallace, 1968, 1971). Moisture is critical for movement because nematodes need a water film in the interstitial spaces of soil for effective propulsion. The moisture content, (grams water per 100g dry soil), for different soil types gives little indication of the percentage of pores that contain water or air (moisture characteristic), for example sandy soils have large pore spaces but less total pore space than clay soils. When the soil becomes dry, nematode movement is inhibited because there is no water film available. Oxygen becomes the limiting factor for nematodes in clay soils, water saturated soils, or soil with high organic content. Temperature is also affected by moisture, since solar heat penetrates deeper in wet soil but produces a smaller rise in temperature than in dry soil (Kaya, 1990).

It has been shown that some nematodes are able to survive extremely low moisture levels and enter into a state of anhydrobiosis in which metabolism comes reversibly to a standstill (Womersley, 1987; Wharton, 2002; McSorely, 2003). Inactivity caused by abiotic factors, such as dehydration, that induce these physiological changes increases nematode persistence and often reflects the habitat and life cycle of the nematode. The plant parasitic nematodes *Ditylenchus dipsaci* ("stem nematode") and *Anguina tritici* ("wheat nematode") are well known to be capable of anhydrobiosis (Norton, 1978; Sturhan & Brzeski, 1991; Krall, 1991).

Effects of soil moisture on entomopathogenic nematodes have been studied in relation to behavioural strategies, virulence and survival (Koppenhofer *et al*., 1995; Grant & Villani, 2003a; Grant & Villani, 2003b). Koppenhofer *et al*. (1995) hypothesized that differences in nematode establishment (numbers of nematodes entering a host) observed between *S. glaseri* and *S. carpocapsae* at different moisture levels was due in part to the size difference between the two nematodes. *S. glaseri* the larger of the two, requires a thicker film of water (ie. higher soil moisture) for optimal movement compared to *S. carpocapsae*. In wet soil, however, *S. carpocapsae* will not find enough surface tension to enable movement, and will be affected earlier than *S. glaseri*. Thus nematode species, soil texture and moisture interact to affect the nematodes ability to infect a host. The possibility for nematode infection is better over a wide range of water potentials in a sandy soil containing some silt and clay than in clay soil (Kaya, 1990). It has also been shown that the optimum moisture level required for survival is much lower than the optimum required for infection of a host (Womersley, 1990; Gouge *et al*., 2000).

The effect of desiccation on entomopathogenic nematodes has been reviewed by Womersley (1990). There are essentially two basic groups of anhydrobiotes, slow-dehydration and fast-dehydration strategists. This realization has helped to explain why different nematode species appear to require completely different conditions to induce anhydrobiosis (Womersley, 1987). Studies on entomopathogenic nematodes so far show that they require slow dehydration and that they cannot become fully anhydrobiotic, but enter a quiescent phase. These studies have mainly focussed on the commercial aspects of entomopathogenic nematodes with the aim to improve long-term storage of the infective stages. Womersley (1990) presumes that it is highly unlikely that for example *Steinernema* spp. have evolved strategies for tolerating rapid dehydration stress, as their natural habitat is in the upper soil profile where they are subjected to slow rates of evaporation.

Until now we have discussed the physical factors that can affect the infective stages of entomopathogenic nematodes directly in soil, however factors affecting the host, especially after infection, are also important to consider. Studies on the effect of host desiccation on entomopathogenic nematodes have been conducted (Koppenhofer *et al*., 1997; Serwe-Rodriques *et* al., 2004). In both studies implications for nematode survival and infectivity in desiccated hosts are discussed. Interestingly, an increased infectivity of emerging infective juveniles was observed in the latter study. It appears that for *S. carpocapsae*, originating from Wisconsin, the "in host desiccation process" selects for populations that enhance survival under environmental conditions native to the United States mid-west (e.g. Wisconsin).

4.2.3. Temperature

Responses to temperature extremes may be inactivity (quiescence), or behavioural. Variations in temperature affect nematode development, reproduction and the length of the life cycle (Freckman & Baldwin, 1990; Wharton, 2002; Barbercheck & Duncan, 2004). There will be an

optimum temperature at which nematode life cycles can proceed at their fastest rate. As temperatures increase or decrease from the optimum, these rates will decrease until normal processes are disrupted. The optimum temperature and rates of decrease in activity or development will vary from species to species and is likely to occur over a range of temperatures (Wharton, 2002).

Entomopathogenic nematodes have been isolated from many different habitats including temperate (cold climate) areas, indicating that they are adapted to low temperatures, as well as hot arid regions indicating their tolerance to high temperatures (Hominick *et al.*, 1996; Hominick, 2002). Several studies, mostly laboratory experiments, have investigated the effect of temperature on entomopathogenic nematodes. Temperature is one of the important factors limiting the success of entomopathogenic nematodes. Low temperature restricts use of some species in temperate regions of the world, and similarly, high temperatures are a constraint for their use in tropical countries. Exposure to extremes of temperature is damaging for nematodes, but the extent of damage depends on the duration of the exposure and on the nematode strain (Griffin, 1993). New *Heterorhabditis* isolates from arid regions or tropical climates have been shown to be heat tolerant the tolerance involving the presence of Heat-shock proteins (Glaser, 2002).

Thermal preferences were investigated by Grewal *et al*. (1994) for several species and strains of entomopathogenic nematodes at a range of temperatures between 8 ^{o}C and 39 ^{o}C (Table 5).

Table 5: Thermal preferences for some entomopathogenic nematodes adapted from Grewal et al. (1994)

Species	**Thermal niche breadths for different development stages in the life cycle**		
	Infection (mortality)	Establishment (number of nematodes entering a host)	Reproduction (nematode reproduction within a host)
Steinernema riobravis	10 – 39 ^{o}C	12 – 37 ^{o}C	20 – 35 ^{o}C
S. feltiae	8 – 30 ^{o}C	8 – 30 ^{o}C	10 – 25 ^{o}C
S. glaseri	10 – 37 ^{o}C	10 – 37 ^{o}C	12 – 32 ^{o}C
S. carpocapsae	10 – 32 ^{o}C	12 – 32 ^{o}C	20 – 30 ^{o}C
S. anomaly (arenaria)	10 – 35 ^{o}C	10 –32 ^{o}C	12 – 32 ^{o}C
S. scapterisci	10 – 35 ^{o}C	20 – 32 ^{o}C	20 – 32 ^{o}C
Heterorhabditis bacteriophora	10 – 32 ^{o}C	15 – 32 ^{o}C	15 – 30 ^{o}C
H. megidis	10 – 35 ^{o}C	12 – 35 ^{o}C	20 – 32 ^{o}C

Nematodes which survive low temperatures in their natural habitat are said to be cold tolerant. In cold habitats the free-living stages of nematodes are exposed to, and must be able to survive, sub-zero temperatures for shorter or longer periods. Cold tolerance strategies for nematodes in general are discussed by Wharton (2002) and for entomopathogenic nematodes by Brown & Gaugler (1996). Brown *et al*. (2002) discuss the possibilities of latent infection in hosts as a strategy for overwintering, and suggests it is a rare event but of great advantage to those nematodes that successfully overwinter in their host. Sturhan & Reuss (1999) isolated

Steinernema sp."E" from subarctic heath soil in Sweden (68°20'N, 51'E). Surveys in Norway and Finland have also revealed the presence of *Steinernema* spp. in the northernmost areas (Vänninen *et al*., 1989; Haukeland, 1993; Salinas, 1996; Klingen *et al*., 2002d). In the Norwegian survey, *Heterorhabditis* sp. was isolated for the first time in the coastal southern part of the country, and a *Steinernema* sp. was isolated far north of the arctic circle, near the Russian border, at 69°27'N, 30°02'E (Haukeland pers. obs.). *S. kraussei* is commercially sold as a cold-active nematode, although there are few published reports on the biology and thermal preferences of this nematode (Willmott *et al*., 2002). *S. feltiae* is also considered to be a cold-active nematode (Grewal *et al*., 1994; Hazir *et al*., 2001).

4.2.4. Soil solution

Soil nematodes are affected by a wide range of chemicals in soil, and soil water acts as a medium for transport of, for example, host exudates that can trigger specific responses. A model has been reported for nematode migration through soil in response to a chemical gradient (Feltham *et al*., 2002).

The importance of plant roots for host-finding by entomopathogenic nematodes has been shown by several authors (Lei *et al*., 1992; van Tol *et al*., 1998; van Tol *et al*., 2001; Boff *et al*., 2002; Cutler & Webster, 2003). These nematodes are highly dependant on finding a suitable host and have shown to be attracted to host related chemicals such as root exudates, host faeces, and CO2 gradients (Schmidt & All, 1978, 1979; Gaugler *et al*., 1980; Pye & Burman, 1981; Kaya, 1990; O'Halloran & Burnell, 2003) Torr *et al*. (2004) suggest that with increasing content of soil organic matter, the utility of host chemical cues will decline, necessitating alternative host cues.

In most soils pH ranges from 4 to 8 and probably has little effect on nematode activity. Studies have shown that pH above or below this range can have negative effects on nematode survival (Kaya, 1990; Kung *et al*., 1990).

5. The effect of agroecosystems on the diversity and abundance of natural enemies

As mentioned in sections 3 and 4 both soil organisms and soil physical factors influence natural enemies such as arthropod pathogens and insect parasitic nematodes in soil. The action of man and activities such as frequency and type of pesticide application, the use of inorganic fertilizer or manure, the plant species grown, cultural practices and tilling also affect the diversity and abundance of different natural enemies in the soil. Management practices aimed at improving soil health frequently enhance or stimulate the natural enemies of plant pests. Magdoff (2001) discusses strategies for improving soil health in which the addition of soil organic matter, use of cover crops and reduced tillage, are some of the suggested strategies. Field boundaries, and the more diverse ecosystem they represent, are also known to influence the survival and propagation of natural enemies. More studies have been conducted on the effect of cropping systems and cultural practices on predatory and parasitoid arthropods than studies on arthropod pathogens and insect parasitic nematodes (e.g. Dritschilo & Wanner, 1980; Purvis & Curry, 1984; Hokkanen & Holopainen, 1986; Andersen, 1997; Fadl *et al*., 1996; Andersen, 1999; Wardle *et al*., 1999; Andersen & Eltun, 2000; Hummel *et al*., 2002; Shah *et al*., 2003; Andersen *et al*., 2004). Predators and parasitoids will not be treated thoroughly in this section, but will

only be mentioned in connection with the implications they have on insect and mite pathogens and insect parasitic nematodes.

5.1. Insect and mite pathogens

A positive relationship between the presence of insect pathogenic fungi and organically farmed fields has been shown in some studies (Kleespies *et al.,* 1989; Klingen *et al.*, 2002a; Hozzank *et al*., 2003b). This could be explained by the absence of synthetic pesticides in organically farmed soil, and the use of organic instead of mineral fertilizers.

The absence of pesticides, especially fungicides could to have a positive effect on the natural occurrence of arthropod pathogenic fungi. Numerous papers have been published on the effect of pesticides to arthropod pathogenic fungi (see Table 6), and very different conclusions are reached. A pattern appears to exist however. There is a strong tendency for insecticides not to be very harmful and herbicides to be moderately harmful, mostly affecting vegetative growth. The fungicides are most harmful but vary greatly depending on their active ingredient and fungal species. This is probably explained by the fungicides mode of action and the biology and response of each fungal species.

In a field experiment conducted by Hummel *et al.* (2002) it was found that several pesticides significantly reduced the presence of naturally occurring insect and mite pathogenic fungi in the soil. Laboratory studies also confirm the negative effect of pesticides (Vänninen & Hokkanen, 1988; Majchrowicz & Poprawski, 1993; Poprawski & Majchrowicz, 1995; Todorva *et al*., 1998; Li *et al.* 2004). Results from laboratory experiments and field conditions might differ, however Mietkiewski *et al.* (1997) examined the effect of several pesticides on naturally occurring insect and mite pathogenic fungi in field and laboratory experiments. In some cases the results obtained in the field were confirmed in the corresponding laboratory experiment, in other cases not (see table 6 for details). They suggested that several factors may be responsible for these results; including biotic and abiotic factors, the uneven distribution, different concentration and degradation rate of pesticides in a dynamic soil micro-environment. Keller *et al.* (1993) suggested that the non-target effect of chemical pesticides to arthropod pathogenic fungi applied as a microbial control agent might not be significant under practical conditions. For the use of *B. brongniartii* in orchards, for example, the fungus is applied at a soil depth of some centimetres so that direct contact with fungicides is avoided thereby avoiding adverse effects. For naturally occurring arthropod pathogenic fungi, the effect of fungicides will also depend on where they are located, which again depends on the movement of infected hosts in the soil profile (see section 4.1.3.). Studies also show that although several fungicides seem to be incompatible with the use of arthropod pathogenic fungi as microbial agents, the proper evaluation and timing of application can increase compatibility (Anderson & Roberts, 1983; Kouassi *et al.*, 2003). Several studies also suggest that some fungal species are more tolerant to pesticides than others, and *M. anisopliae* for example is considered to be tolerant to pesticides. It is shown, however, that the tolerance to pesticides varies between isolates within one single species (Mietkiewski *et al.*, 1997). Another complicating factor is that the application of insecticides in crop management systems, that causes insect host mortality, will indirectly reduce host density and fungal inoculum in the soil.

The use of organic fertilizers, could possibly provide arthropod pathogenic fungi with favourable conditions in organically farmed soil. This has been found to be the case in field studies of insect pathogenic nematodes, where organic manure resulted in increased densities

of a native population of *S. feltiae* while inorganic fertilizers suppressed nematode densities (Bednarek & Gaugler, 1997). Many of the mechanisms found in their study, such as the positive response of soil inhabiting insects to manure, could also be relevant to insect pathogenic fungi, because soil inhabiting insects are potential hosts and contribute to their spread and survival (Keller & Zimmermann, 1989).

In the few studies where the occurrence of insect and mite pathogenic fungi from organic and conventionally managed soil are compared there appears to be a weak dominance for fungal species other than *M. anisopliae* in organically managed soil (Klingen *et al*., 2002a; Hozzank *et al*., 2003b). *B. bassiana* is known to be associated with undisturbed habitats high in organic matter (Mietkiewski *et al.*, 1997). Soil high in organic matter is typical for organically managed soil and one could therefore possibly expect increased prevalence of *B. bassiana* in these soils compared to conventionally managed soils. There is a tendency for this in one study conducted by Klingen *et al*. (2002a). More studies are, however, needed to confirm this.

Minimal tillage can benefit the accumulation of pathogens and it has been shown that maximum benefit is obtained from arthropod pathogens by maintaining and restoring older pastures rather than engaging in regular cultivation for pasture renewal (Jackson *et al.*, 2000). Hummel *et al.* (2002) found that arthropod pathogenic fungi (*B. bassiana* and *M. anisopliae*) were more abundant in conservation tillage compared to conventional tillage systems. Keller *et al.* (2003) also found that meadows contained higher densities of *M. anisopliae* than in adjacent arable land, probably due to the scarcity of hosts as a result of control measures, soil cultivation and the application of fungicides. Sosa-Gomez & Moscardi (1994) showed that the density of entomopathogenic fungi were higher in no-tillage soy bean crops compared to tilled crops. In a study conducted by Bing & Lewis (1993), the greatest number of *B. bassiana* Colony Forming Units (CFUs) were observed in no-till systems. They also found that numbers of CFUs from soils varied greatly depending on the sample date, and suggested that *B. bassiana* inoculum in soil is probably influenced more by environmental conditions than by tillage practices. They do not specify, however, what they mean by environmental conditions. The success and survival of insect pathogens in soil is strongly dependent on stable environmental conditions, including the continuous, or at least frequent, presence of host insects. Vänninen *et al.* (1989) therefore suggested that the high occurrence of insect pathogenic fungi found under rowan trees was partly due to the continuous presence of larvae or pupae of the apple fruit moth (*Argyresthia conjugella*) and the absence of pesticides. Similar studies have also found an increased abundance of insect pathogenic fungi in more permanent habitats compared to arable fields (Chandler *et al.*, 1997).

Table 6: Reports on the effect of pesticides on insect- and mite pathogenic fungi that are known from the soil environment

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
Propiconazole Thiram Vinclozolin	Fungicides	*Metarhizium anisopliae* *Beauveria bassiana* *Paecilomyces fumosoroseus* *Paecilomyces farinosus*	Propiconazole, thiram and vinclozolin: Inhibited the linear growth of the fungi in petri dishes. Propiconazol and thiram: Also inhibited sporulation. (Laboratory study, *in vitro*).	Vänninen & Hokkanen, 1988
Benomyl Metalaxyl+ mancozeb	Fungicides	*M. anisopliae* *B. bassiana* *P. fumosoroseus* *P. farinosus*	Benomyl and metalaxyl+ mancozeb: No effect on *B. bassiana*, but the other species was inhibited up to three days after treatment. Metalaxyl+ Mancozeb: *P. fumosoroseus* was sensitive. (Laboratory study, *in vitro*).	Vänninen & Hokkanen, 1988
Captan+ Penconazole (Topas) Captan +Pyrifenox (Rondo) Copper-oxychloride (Recop) Triforine (Funginex)	Fungicides	*Beauveria brongniartii*	All fungicides completely inhibited mycelial growth *in vitro*. "Semi field" study on growth of fungus on barley kernels (commercial preparation) on soils sprayed with fungicides showed smaller differences. (Laboratory, *in vitro* and "semi field" study).	Keller *et al.,* 1993

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
Copper oxychloride (Miedizan 50) Hymexazol (Tachigaren) Mancozeb (Dithane M-45) Metalaxyl (Ridomil) Sulfur (Siarkol Extra) Sulfur +nitrothal-isopropyl (Siarkol N) Triadimefon (Bayleton) Zineb +copper oxychloride (Cynkomedzian)	Fungicides	*B. bassiana* *Conidiobolus coronatus* *Conidiobolus thromboides* *M. anisopliae* *P. farinosus* *P. fumosoroseus* *Scopulariopsis brevicaulis* *Verticillium lecanii*	Zineb+ copper oxychloride and mancozeb: Completely inhibited germination of *C. coronatus, C. thromboides, B. bassiana, P. farinosus, M. anisopliae* and *V. lecanii in vitro.* Triadimefon, copper oxychloride, metalaxyl, Sulfur, sulphur +nitrothal-isopropyl and hymexazol: Exhibited various effects on the fungi *in vitro*. Generally, adverse effects were much greater against the Entomophthorales than against the Hyphomycetes *in vitro*. (Laboratory study, *in vitro*)	Majchrowicz & Poprawski, 1993
Carbendazim (Bavistin) Flusilazole (Nustar) Prochloraz (Sportak) Propiconazole (Cane sett treatment)	Fungicides	*M. anisopliae*	Carbendazim, flusilazole, prochloraz and propiconazole: Inhibited mycelial growth and sporulation. (Laboratory study, *in vitro*).	Li & Holdom, 1994
Anilazine Benomyl Chinomethionat Copper hydroxide Dithianon	Fungicides	*V. lecanii* (*Trialeurodes vaporariorum*)	Chinomethionat, dithianon, triflumizole and zineb: Highly toxic to conidial germination *in vitro*. From some to no inhibition of the fungal killing	Saito & Yabuta, 1996

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
(study continued) Mepronil Polyoxin Procymidone Sulfur Triflumizole Zineb			capacity of *T. vaporariorum* larvae when treated (39%, 54%, 72% and 100% *T. vaporariorum* mortality respectively). Copper hydroxide, dithianon, mepronil, polyoxin, procymidone, sulphur and zineb: No negative effect on mycelial growth. Anilazine, benomyl, chinomethionat and triflumizole: Inhibited mycelial growth. (Laboratory study: *in vitro* and insect bioassay).	
Benomyl Triadimefon	Fungicides	*B. bassiana* (*Galleria mellonella*, bait insect)	Benomyl: Significantly fewer *G. mellonella* became infected in field soil treated with benomyl. Confirmed in *in vitro* experiments. Triadimefon: Significantly more *G. mellonella* became infected in field soil treated with Triadimefon. *In vitro* experiments showed inhibition rather than stimulation. (Laboratory study *in vitro* and field studies with *G. mellonella* as bait insect).	Mietkiewski *et al*., 1997

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
Chlorothalonil Mancozeb Maneb Metalaxyl +mancozeb Thiophanate-methyl Zineb	Fungicides	*B. bassiana*	Chlorothalonil, mancozeb, maneb, metalaxyl +mancozeb, thiophanate-methyl, zineb: Inhibition of mycelial growth and sporulation *in vitro*. (Laboratory study, *in vitro*).	Todorova *et al.,* 1998
Copper oxide Mancozeb Metalaxyl	Fungicides	*B. bassiana*	Copper oxide, mancozeb, metalaxyl: Strongly fungistatic and inhibited the fungal radial growth *in vitro.* (Laboratory study, *in vitro*).	Kouassi *et al*., 2003
Penycuron Quintozene	Fungicides	*B. bassiana*	Quintozene: Affected the *B. bassiana* conidial germination negatively *in vitro*. It also significantly impacted vegetative growth and sporulation of *B. bassiana in vitro.* Penycuron: Compatible with *B. bassiana.* (Laboratory study, *in vitro*).	Andalo *et al.,* 2004
Trifluralin	Herbicide	*M. anisopliae* *B. bassiana* *P. fumosoroseus* *P. farinosus*	Trifluralin: Inhibited the linear growth of the fungi in Petri dishes (Laboratory study, *in vitro*).	Vänninen & Hokkanen, 1988
Glyphosate MCPA	Herbicides	*M. anisopliae* *B. bassiana*	Glyphosate and MCPA: Only *B. bassiana* was sensitive.	Vänninen & Hokkanen, 1988

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
(study continued) Simazine		*P. fumosoroseus* *P. farinosus*	Simazine: Affected only the two *Paecilomyces* species. (Laboratory study, *in vitro*).	
Atrazine (Atrazine) 2-4-D amine (Amicide) Diuron (Diuron) Glyphosate (Glyphose) Paraquat dichloride (Gramoxone) Pendimethalin (Stomp) Trifluralin (Treflan)	Herbicides	*M. anisopliae*	Atrazine, 2-4-D amine, diuron, glyphosate, paraquat dichloride, pendimethalin and trifluralin: The negative effect of mycelial growth and sporulation were not very pronounced, but it varied between isolates. As the concentrations became lower the negative effect was less pronounced or not pronounced at all. Diuron, paraquat dichloride and pendimethalin seemed to have the most pronounced negative effect. (Laboratory study, *in vitro*).	Li & Holdom, 1994
Chloridazon Lenacil Metolachlor Phenmedipham +desmedipham	Herbicides	*C. thromboides* *C. coronatus* *B. bassiana* *M. anisopliae* *P. farinosus* *V. lecanii*	All herbicides except from lenacil had pronounced adverse effects on all fungi. Lenacil temporary stimulated *C. thromboides* at low concentrations (0.1X). (Laboratory study, *in vitro*).	Poprawski & Majchrowicz, 1995
Glyphosate	Herbicide	*B. bassiana*	Glyphosate: No significant difference compared to control for	Mietkiewski *et al*., 1997

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
(study continued)			infection of *G. mellonella. In vitro* experiments showed inhibition of fungal growth at increasing glyphosate concentrations. (Laboratory study: *in vitro* and insect bioassay)	
Acetochlor Azafenidin 2,4-D Dimethylurea Glyphosate Oxyfluorfen Simazine +Ametryne	Herbicides	*B. bassiana*	Acetochlor, azafenidin, 2,4-D, oxyfluorfen and simazine +ametryne: Affected the conidial germination negatively. Acetochlor, azafenidin, 2,4-D, dimethylurea, glyphosate, oxyfluorfen and simazine +ametryne: Significantly impacted vegetative growth and sporulation of *B. bassiana* negatively. (Laboratory study, *in vitro*).	Andalo *et al.,* 2004
Azinphos-methyl (Gusathion 50WP & 2F) Carbaryl (Servin 50WP) Carbofuran (Furadan 4F) Diflubenzuron (Dimilin 25WP) Endosulfan (Thiodan 3EC & 50WP) Fenvalerate (Pydrin 2EC)	Insecticides	*B. bassiana*	The formulation of the insecticide was often more significant than the active ingredient on *B. bassiana* inhibition. Permethrin and fenvalerat caused significant inhibition of *B. bassiana (in vitro).* Separate application of *B. bassiana* and insecticides greatly mitigated *B. bassiana* inhibition (microcosm study).	Andersen & Roberts, 1983

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
(study continued) Permethrin (Ambush 2EC & Ectiban 25WP) Piperonyl butoxide (PBO 8EC, Prentox) Oxamyl (Vydate 2EC)			(Laboratory: *in vitro* and microcosm studies).	
Diazinon Cypermethrin Pirimicarb	Insecticides	*M. anisopliae* *B. bassiana* *P. fumosoroseus* *P. farinosus*	Cypermethrin, diazinon and pirimicarb: No effect on growth and sporulation. (Laboratory study).	Vänninen & Hokkanen, 1988
Abamectin Thuringiensin Triflumuron	Insecticides	*B. bassiana* *(Leptinotarsa decemlineata)*	Abamectin, thuringiensin and triflumuron: No significant inhibition of *B. bassiana in vitro.* In field cage test: Extremely variable, but *B. bassiana* +insecticide caused generally higher *L. decemlineata* mortality than one agent alone. (Laboratory *in vitro* and semi field studies).	Anderson *et al.,* 1989
Aldicarb (Temik) Carbofuran (Furadan) Chlorpyrifos (Lorsban) Ethoprophos (Mocap) Fenamiphos (Nemacur)	Insecticides	*M. anisopliae*	Aldicarb, carbofuran, chlorpyrifos, ethoprophos and fenamiphos: The negative effect of mycelial growth and sporulation were very weak, but it was some variation between isolates. (Laboratory study).	Li & Holdom, 1994

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
Aldicarb Chlorfenvinphos	Insecticides	*B. bassiana* (*G. mellonella*, bait insect)	Clorfenvinfos: fewer *G. mellonella* (but not significantly) became infected in field soil treated with clorfenvinfos Confirmed in *in vitro* experiments. Aldicarb: No significant difference compared to control for infection of *G. mellonella* in field soil. *In vitro* experiments showed stimulation of fungal growth. (Laboratory, *in vitro* and field studies with G. *mellonella* as bait insect).	Mietkiewski *et al*., 1997
Triflumuron (Alsystin)	Insecticide/ acaricide	*B. bassiana* *(Tetranychus urticae)*	Triflumuron: Reduced mycelial growth, but not conidial germination of *B. bassiana* (*in vitro*). When *B. bassiana* and triflumuron were sprayed in combination against *T. urticae* the effect was less than with *B. bassiana* alone (bioassay). (Laboratory study: *In vitro* and mite bioassay).	Irigaray *et al.,* 2003
Abamectin (Vertimec 18) Acrinathrin (Rufast 50) Amitraz (Parsec) Chlorfenapyr (Citrex) Cyhexatin (Sipcatin 500)	Acaricides/ insecticides	*B. bassiana*	All acaricides/ insecticides tested caused different levels of inhibition on germination, vegetative growth and sporulation on *B. bassiana*.	De Olivera & Neves, 2004

Active ingredient/common name (Trade name)	**Pesticide group**	**Fungal species (host species if relevant)**	**Effect on insect and mite pathogenic fungi (type of study)**	**References**
(study continued) Dimethoate (Dimetoato) Fenbutatin oxide (Partner) Fenpyroximate (Kendo) Hexythiazox (Savey) Pyridaphethion (Ofunack 400) Pyridine (Sanmite)			The acaricides: Amitraz, clorfenapyr, cyhexatin, dimethoate, fenbutatin oxide, fenpyroximate, hexythiazox, pyridaphethion, and pyridine belonging to the organophosphate and organostatic groups drastically affected conidial germination as well as vegetative growth and sporulation. Abamectin and acrinathrin belonging to the Avermectins and pyrethroids were more compatible with *B. bassiana.* (Laboratory study, *in vitro).*	
Imidacloprid	Insecticide	*B. bassiana*	Imidacloprid was compatible with *B. bassiana.* (Laboratory study, *in vitro*).	Andalo *et al.,* 2004
Carbofuran Thiamethoxam	Nematicide	*B. bassiana*	Thiamethoxam, and carbofuran were compatible with *B. bassiana.* (Laboratory study, *in vitro*).	Andalo *et al.,* 2004
Endosulfan Esfenvalerat Imidacloprid	Insecticides	*B. bassiana* *M. anisopliae*	Detection of *B. bassiana* and *M. anisopliae* was significantly lower in plots treated with chemical versus biological pesticides. Synthetic herbicides, fungicides	Hummel *et al.,* 2002

Active ingredient/common name (Trade name)	Pesticide group	Fungal species (host species if relevant)	Effect on insect and mite pathogenic fungi (type of study)	References
(study continued) Chlorothalonil Copper hydroxide Atrazine Ethalfluralin Metolachlor Metribuzin Napropamide Paraquat	Fungicides Herbicides		and insecticides were used as recommended for the area and the crop. (Field study).	

5.2. Insect parasitic nematodes

Bardgett & Cook (1998) reviewed extensively the factors influencing the abundance of important soil animals, including naturally occurring nematodes in grassland, and concluded that organically managed low-input farming systems are optimal for an increase in soil biotic diversity. They did however stress that future studies are necessary to prove that soil biodiversity is positively associated with stability and productivity of the ecosystem. There have been several short-term studies of effects of various crop management systems on plant and soil nematodes. The economically important cyst nematodes (*Globodera rostochiensis* and *G. pallida*) are the most studied in long-term experiments (Whitehead, 1997). Yeates *et al*. (1999) studied soil nematode communities over a 7 year period in different agroecosystems: an annual and a perennial crop using three weed management practices (cultivation, herbicide application and mulching). The greatest long-term effects were from sawdust mulching, where total nematode populations initially increased, but subsequently declined, co-inciding with an increase in predatory nematodes. Herbicide use did not result in any consistent effects on the nematode communities. Their work revealed that some of these effects were only apparent after 3 years, underlining the importance of long-term studies. Nematode faunal analyses have been conducted with respect to changes in the soil, mainly for bioindicator purposes (Neher, 2001; Yeates & Bongers, 1999; Yeates, 2004).

Entomopathogenic nematodes are mostly used as biopesticides, applied as a drench to the soil surface to target the susceptible insect pest in the soil. With some exceptions, entomopathogenic nematodes are generally applied against insect pests in high value crops such as ornamentals and strawberries. There are numerous reports on the application of nematodes against different insect pests (Gaugler & Kaya, 1990; Bedding *et al.*, 1993; Ehlers, 1996; Gaugler, 2002). The effect of different agroecosystems regarding entomopathogenic nematodes has not been well studied until fairly recently. In a review by Lewis *et al*. (1998), it is stated that the requirements and limitations for field use of entomopathogenic nematodes are quite well understood, whereas the requirements for population level survival are poorly known. Millar & Barbercheck (2002) reported on the effect of tillage practices in no-till and conventional-till maize (corn) on entomopathogenic nematodes. The study involved two endemic nematode species (*S. carpocapsae* and *H. bacteriophora*) and one inundatively applied nematode (*S. riobravis*), where the objective was to evaluate the effect of tillage on all three nematodes. Interestingly the study suggested that the three nematode species had different sensitivities to the conditions created by tillage. *H. bacteriophora* did not appear to be affected by tillage, *S. carpocapsae* appeared to be negatively affected by tillage, and in contrast the inundatively applied *S. riobravis* was favoured by tillage. The effect of tillage on abiotic and biotic factors could have contributed to these effects, as well as the differences in dispersal behaviour of the nematodes themselves.

The compatibility of entomopathogenic nematodes with agrochemicals has been reviewed by Grewal (2002). Entomopathogenic nematodes are tolerant of short exposures to many agrochemicals. Some pesticides can reduce nematode activity, but it has also been shown that low rates of insecticides combined with entomopathogenic nematodes can give strong synergistic effects against target pests (Koppenhofer & Kaya, 1998; Nishimatsu & Jackson, 1998). In general Heterorhabditidae tend to be more sensitive to pesticides than the Steinernematidae. In a laboratory study, Bednarek & Gaugler (1997) also found that heterorhabditids were more sensitive to inorganic fertilizers.

6. Successful use of the soil as a reservoir of natural enemies

A few examples of the successful use of soil as a reservoir in classical, inoculation, inundation and conservation microbial control as defined by Eilenberg *et al*. (2001) will be given in this section. For practical purposes the term microbial control includes entomopathogenic nematodes in this section. The ultimate indicator of successful microbial control is a reduction in crop damage to an acceptable level. Success also depends on avoiding adverse effects on health and environment. Aspects of health and environment in microbial control have been covered thoroughly elsewhere by Howarth (2000), Strasser *et al*. (2000), Wajnberg *et al*. (2001), Goettel *et al*. (2001) and Hokkanen & Hajek (2003).

Soil-dwelling pests have always been difficult to manage, and inexpensive persistent chemicals, applied prophylactically, were long considered as ideal for their control. These chemicals no longer meet environmental standards and have been withdrawn from most markets. The challenge for microbial control is to fill this niche. Many microbial control agents can persist in soil and provide long-term pest control so that their costs may be spread over several years. Production and application costs of most microbial control agents are often too high for control of soil-dwelling pests in extensive agricultural systems. Thus it is no surprise that many applications are made through inoculation, baiting and strategic application methods (Jackson *et al*., 2000). In our examples we will therefore focus on these strategies.

6.1. Classical biological control

Classical biological control is considered successful when the non-indigenous biological control agent controls and becomes established in the targeted host pest population. In addition the control agent should not have any detrimental long or short-term effects on non-target organisms. It is in the nature of this definition that we will never know for certain whether a classical biological control agent is truly successful. It is not possible to monitor every single organism in space and time that may be affected by the introduction of an exotic biological control agent. To enable evaluation of possible non-target impacts it is important to develop methods for identifying the introduced pathogen or nematode (Hajek *et al.*, 2003). The introduction of parasitoids and predators is most common in classical biological control and out of 5500 programs, less than 50 involve the introduction of exotic insect and mite pathogens (Hajek *et al*., 2000). Among these, only a few have been on soil dwelling insects or mites, but as the soil is an important environment in most organisms' life cyc1e, the soil plays an important role in the establishment of several exotic agents. According to a model developed by Fenton *et al*. (2001) entomopathogenic nematodes were shown not to be particularly suitable for classical biological control. Among the relatively few programs conducted only some have been successful. See table 7 for examples.

Table 7: Examples of classical biological control where the soil is an important reservoir for the natural enemy

Exotic agent introduced (pathogen or nematode)	**Host species (common name)**	**Type of introduction**	**Role of the soil environment (reference)**
Entomophaga maimaiga (fungi)	*Lymantria dispar* (Gypsy moth)	Accidental introduction and subsequent redistribution.	The soil is an important reservoir of resting spores for this insect pathogenic fungus, and hence is important for the survival and establishment of this exotic control agent (Hajek *et al*., 2003).
Oryctes virus, OrV (virus)	*Oryctes rhinoceros* (Rhinoceros beetle)	Introduction.	*O. rhinoceros* larva develop in separate breeding sites witch includes compost heaps, rubbish pits and decaying logs. In these sites the OrV inoculum can be available to many larvae (Hunter-Fujita *et al*., 1998).
Steinernema scapterisci (nematode)	*Scapteriscus* spp. (Mole cricket)	Initial introduction into a limited area. Has been isolated as far as 23 km from the original release site.	Mole crickets spend nearly all their life cycle underground. Eggs are deposited in underground chambers. Nymphs tunnel to the surface and feed in the upper soil layer and litter. Juveniles and adults make and occupy extensive gallery and tunnel systems (Adjei *et al.*, 2003). *S. scapterisci* is applied to pasture or incorporated into sand below mole cricket traps (Jackson *et al.* 2000).

6.2. Inoculation and inundation biological control

In inoculation biological control the long-term effect of the beneficial organism released into the environment is essential for the control efficacy of the target pest arthropod. The long-term effect may be considered a problem when it comes to the environmental risk from the released organism due to possible non-target effects. This risk is much reduced if the natural enemy is highly host specific. Long-term effects of insect pathogenic fungi in soil have been studied by several authors (Enkerli *et al.*, 2004; Vänninen *et al.*, 2000; Keller *et al*., 2003; Kessler *et al.*, 2004). Persistence of up to 40 years for *B. brongniartii* is reported (Keller *et al*., 2003). In another study, a field trial with inoculation of different *B. brongniartii* strains showed that all strains were detected at all test sites up to 14 years after the application (Enkerli *et al.*, 2004). This is the first time that applied fungal strains have successfully been re-isolated after such a long time in the field. *M. anisopliae* is also known to persist for at least three years post-application. After three years fungal propagule levels caused up to 80% infection in the bait insect *Tenebrio molitor* (Vänninen *et al.*, 2000). High persistence of *B. brongniartii* is considered desirable because of its narrow host range. In contrast *M. anisopliae* has a much wider host range, and persistence is not desirable. It is however, important to remember that the host range is much more restricted for a specific isolate (Vestergaard *et al*., 2003). *B. brongniartii* is considered a successful inoculative control agent for the long term control of cockchafer *M. melolontha* and *M. hippocastani* due to the narrow host range and long persistence. A method based on sterilized barley kernels colonized by *B. brongniartii* is used to apply the fungi (Keller, 1992). By the use of an adapted seed-drilling machine, the fungus colonized barley kernels are directly applied into soil of *M. melolontha* infested sites. Based on this technique, a successful commercial product (*Beauveria* Scweizer, Eric Schweizer Seeds Ltd., Switzerland) has been available in Switzerland since 1991 (Enkerli *et al.*, 2004). *B. brongniartii* was also registered as the product Melocont©-Pilzgerste in Austria in 2000 (Bipesco Midterm Report, Interim-Report 3). No other EU countries have at present registered *B. brongniartii* as an active ingredient of any product (http://europa.eu.int/comm/food/plant/protection/evaluation/stat_active_subs_3010_en.xls). *B. brongniartii* is, however, registered as the active ingredient of products in several non-European countries.

Entomopathogenic nematodes have been commercially available in several countries in Europe, USA and Australia for a number of years. As far as we are aware there are no reports to date of successful inoculation biological control for entomopathogenic nematodes and most studies indicate poor long-term resistance. Apart from the fact that long-term studies are rare, there is growing evidence that under certain conditions, such as the presence of suitable hosts, persistence can be improved. A major limitation with the inoculative approach in microbial control is the time taken for the pathogen or nematode to spread from the site of application to other sites of the pest population. This limitation can be overcome with an inundative release where the organism is applied to the whole population within a defined area. Selection for an appropriate species, biotype or strain of the control organism is a key factor for inundation biological control of soil-dwelling pests. Appropriate biological properties, pathogenicity and environmental competence, however, are not enough to ensure success. The agent must also be easy and cheap to mass-produce and distribute. Friedman (1990) provides an excellent early account on the techniques and factors involved for mass-production of entomopathogenic nematodes. For a more recent update on production technology, a review is published by Gaugler & Han (2002). Microbial control products are often applied with propagule densities sufficient to initiate an epizootic of disease. This usually mimics the level found during natural

epizootics (Jackson *et al*., 2000). Many pest managers focus on the deposition of large quantities of virulent propagules on to the target host. This approach has often resulted in inadequate suppression of insect and mite pests, since the inoculum threshold is not static and is influenced by many aspects of the disease tetrahedron described in section 2. A thorough understanding of the epizootiology of the specific host pathogen or host nematode combination is therefore required to be able to develop an agent that may be used successfully (Inglis *et al*., 2001). Competition with other soil organisms is one factor that affects the epidemic development of a microbial control product applied to the soil (section 3.2.). Persistence of applied microbial control agents is therefore a major challenge. Microbial control agents are also susceptible to desiccation and ultra violet (UV) radiation and avoidance of these conditions during application has been a major hurdle. Since soil is an environment that may protect the applied microbial product from desiccation and UV radiation, subsurface applications have often been used to overcome these problems.

Unlike leaf and stem feeding pests, where generalist strains of insect pathogens, such as B.t. var *kurstaki*, have been used to control a wide range of target insects, there are few agents or products that have proven successful against more than one species of soil-dwelling insect. It has been reported that at least 13 different microorganisms or nematodes are used as biological control agents against at least 16 different soil dwelling pest insect species. Some of them are used for inundative biological control and some for inoculation biological control (Jackson *et al.*, 2000).

6.3. Conservation biological control

Eilenberg *et al*. (2001) proposed that conservation biological control is distinguished from other strategies in that natural enemies are not released. Whereas Fuxa (1998) has suggested that research on conservation biological control falls into two categories: (1) to enhance natural epizootics, (2) in conjunction with, but not simultaneous with, releases of the biocontrol agent. In this section we will only discuss enhancement of natural epizootics and not enhancement of released or applied agents. As viewed by Gurr *et al.* (2000), conservation biological control is based on a two-stage strategy: (1) reduced pesticide induced natural enemy mortality, (2) habitat manipulation to provide key ecological recourses. There has been a growing level of international research on conservation biological control in the last 10 years, but there are few documented applications of this biological control strategy (Gurr *et al.* 2000; Pell *et al.* 2001; Eilenberg *et al.*, 2001). The conservation approach has until recently been dominated by entomologists aiming to control arthropod pests by enhancing activity of arthropod agents. Recently, however, some attention has focused on conservation of entomopathogens (Gurr *et al*., 2000). The research has largely focused on viruses and fungi, probably because these groups have the best ability to produce disease epizootics with a high case fatality rate. There have also been attempts, however, at conservation approaches with entomopathogenic nematodes. Lewis *et al.* (1998) suggests three conditions that should be met to enhance or sustain biological control by entomopathogenic nematodes with special reference to turf: (1) moderately susceptible pests should be present throughout most of the year, (2) pests should have a high economic threshold level, and (3) soil conditions should be favourable for nematode survival.

Research on environmental manipulation of insect and mite pathogens and insect parasitic nematodes has mainly focused on four areas: (1) improved transport of the pathogen from the

reservoir, usually the soil, to a site where the insect or mite host can come into contact with the pathogen or the nematode, (2) improvement in persistence of the pathogen or the nematode at the site where it contacts the insect or mite host, (3) overall growth of the pathogen or nematode population, (4) activation of latent infections (especially for viruses) (Fuxa 1998). The success of conservation biological control is very difficult to measure since it is based on a hierarchy of different criteria involving several trophic levels. It is apparent that reduced pesticide-induced natural enemy mortality has been successful in making a contribution to IPM (e.g. Steinkraus *et al.*, 1996). According to Gurr *et al.* (2000), evidence for the success of conservation biological control through habitat manipulation is less clear-cut than the effect of pesticides.

In section 5.1. we have mentioned several effects of pesticides, and other human manipulation of the agro-ecosystem on natural enemies belonging to insect and mite pathogenic fungi. Most of these studies have been conducted in the laboratory or in semi-field trials, and few practical solutions for arthropod fungal pathogen systems have to our knowledge been achieved. Carruthers (1981) and Carruthers *et al*. (1985) developed a model where a combination of adapted pesticide use and habitat manipulation was used to enhance the prevalence of *Entomophthora muscae* in an onion maggot (*Delia antiqua*) population. This conservation approach affects the prevalence of *E. muscae* in adult (not soil dwelling) onion maggot flies by reducing the negative effect of pesticides to the beneficial fungi. The model also suggests enhancing spore germination and host infection by grassy boarder areas and strip planting onion with other crops. *E. muscae* attacks the adult fly stage and not the soil dwelling larval and pupal stage of the onion maggot. The soil is probably an important reservoir for the resting spores of this fungus and hence also for the initial infection in the spring. In the onion maggot/*E. muscae* system it was revealed that primary infection in the spring was much higher in adults emerging from pupae in the border areas than in adults emerging in the field. Due to the comparable biology of the cabbage root fly and the turnip root fly (*D. radicum* and D. *floralis*) with the onion maggot there are reasons to believe that this also applies for the *D. radicum*/ *D. floralis*/*E. muscae* host pathogen system (Klingen, 2000). Systems have also been suggested to enhance the natural occurrence of the more typical arthropod pathogenic soil fungi. Bing & Lewis (1993) for example, suggested that epizootics of *B. bassiana*, in overwintering larvae of the European corn borer (*Ostrinia nubilalis*) in maize residues, could be enhanced by modifying agronomic practices, such as no-till or reduced tillage systems. Similar suggestions were also made by Hummel *et al*. (2002) who conducted a field experiment on effects of different production practices on soil born entomopathogens in vegetable systems.

There are quite a few examples of enhancement of insect pathogenic viruses by the use of conservation control strategies. One fascinating example involving soil or soil litter is the blowing of NPV contaminated forest litter up into trees for the initiation of a viral epizootic in larvae of *Lymantria dispar* (Fuxa, 1998).

According to Fuxa (1998) there has been only one attempt to enhance natural epizootics of nematodes. In that study tillage, weed management, and irrigation were investigated for enhancement of *Heterorhabditis bacteriophora* (*heliothidis*) in *Diabrotica undecimpunctata howardi* infesting maize. No-till and the presence of weeds significantly increased the numbers of nematodes in soil bioassays, but irrigation had no effect (Fuxa, 1998). Lewis *et al*. (1998) discuss a conservation approach to using entomopathogenic nematodes, and emphasise the need to understand the requirements and structure of natural populations before this approach can be recommended for practical use.

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CHAPTER 10

DEGENERATION OF ENTOMOGENOUS FUNGI

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1. Introduction

Fungi are notorious for losing virulence and changing their morphology when successively sub-cultured on artificial media. Various terms have been used to describe this phenomenon including phenotypic degeneration, phenotypic instability, phenotypic deterioration, dual phenomenon, saltation and attenuation (Butt, 2002; Kawakami, 1960; Nagaich, 1973; Ibrahim *et al*., 2002; Ryan *et al*., 2001). Morphological changes include a change in colour, and growth form as well as reduced sporulation. In this article, the term degenerate is used to cover both attenuation of virulence and phenotypic degeneration. Degenerate cultures are a major concern to manufacturers of fungal biocontrol agents (BCAs) since batches that are inconsistent in spore yield or virulence will make the product commercially unviable. Growers naturally would be reluctant to use any product, which was potentially unstable.

Degenerate cultures have been reported in a wide range of insect-pathogenic fungi (Table 1). However, very little is known about why the cultures degenerate. What is clear is that strains differ in their stability when maintained on artificial media with some strains clearly degenerating more rapidly than others irrespective of whether the parent culture was derived from a single spore or multi-spore colony. Examination of the single spore isolates of the unstable cultures reveals that these generally produce more phenotypic variants than stable cultures (Butt, unpublished observations). The pattern of degeneration also varies between isolates; some will produce sectors while others will decline in spore production and/or virulence. This chapter will examine two attributes of degenerate cultures: attenuation of virulence and phenotypic degeneration and briefly review some of the factors that could explain these phenomena. Since attenuation of virulence is widely reported in human and plant pathogenic fungi, comparisons will be made with these organisms.

2. Attenuation

Attenuation of virulence has been observed in nearly all the major taxa of entomogenous fungi (Table 1). Strains differ in the rate at which they decline in virulence. Some strains decline in virulence after a single-subculture (Butt, unpublished observations). Nagaich (1964) reported a loss of virulence in *Verticillium lecanii* after 2 or 3 subcultures. Some strains need to be successively subcultured 10-12 times before a significant decline in virulence is observed (Morrow *et al.,* 1982; Hajek *et al.,* 1990). In contrast, several workers noted no decline in virulence after >12 transfers (Brownbridge *et al*., 2001; Hall, 1980; Ignoffo *et al*., 1982; Vandenberg & Cantone, 2004). The most extreme case of stable virulence was reported for an

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isolate of *Culicinomyces clavisporus,* which maintained its virulence against mosquitoes even after 8 years of continuous subculturing on nutrient agar (Sweeney, 1981). Studies by Hajek *et al*., (1990) suggest that the rate of subculturing has an impact on virulence while the absolute length of time in axenic culture does not influence virulence. For some unknown reason, virulence may be temporarily restored in some subcultures but the overall trend is a decline.

Exactly what components of the invasive and developmental processes of fungi are affected is unclear but it is probably a combination of inter-connected factors. Figure 1 briefly outlines some components that may be affected when a pathogen becomes attenuated. Conidia of attenuated strains may differ in their adhesive properties; some strains may increase in adhesiveness while others may adhere poorly to the host cuticle (Butt, unpublished observations). Since mortality is dose-related, a decline in spores adhering naturally will increase the LT_{50} value (Inglis *et al*., 2001). However, some strains show no decline in virulence but are still attenuated because far more spores are adhering which normally would lower the LT_{50} value. Morrow *et al*. (1989) noted differences in the attachment patterns of attenuated and virulent conidia of *Nomuraea rileyi* to the surface of the host cuticle. Studies by Ibrahim *et al.* (2002) show that nutrition can influence the sugar composition at the surface of *Metarhizium anisopliae* conidia but no clear pattern was established between carbohydrate groups and adhesiveness. Besides the number of conidia adhering, attenuation may also influence the speed of germination. Faster germination has been correlated with higher virulence in *M. anisopliae* and *Paecilomyces fumosoroseus* (Altre *et al*., 1999; Inglis *et al.,* 2001).

Table 1: Stability of entomogenous fungi: influence of subculturing on artificial media or passaging through insect host

Pathogen	**Stability *in vitro***	**Passaging through insect host**	**References**
Beauveria bassiana		Increased virulence by passaging through pea aphid	Aizawa 1972
Beauveria bassiana	Loss of virulence		Samsinankova *et al.*, 1981
Beauveria bassiana	No decline in virulence after 15 successive subcultures		Brownbridge *et al*., 2001
Beauveria bassiana	No decline in virulence	No increase in pathogenicity after 3 passages through larvae of *Oryctes rhinoceros.*	Latch, 1976

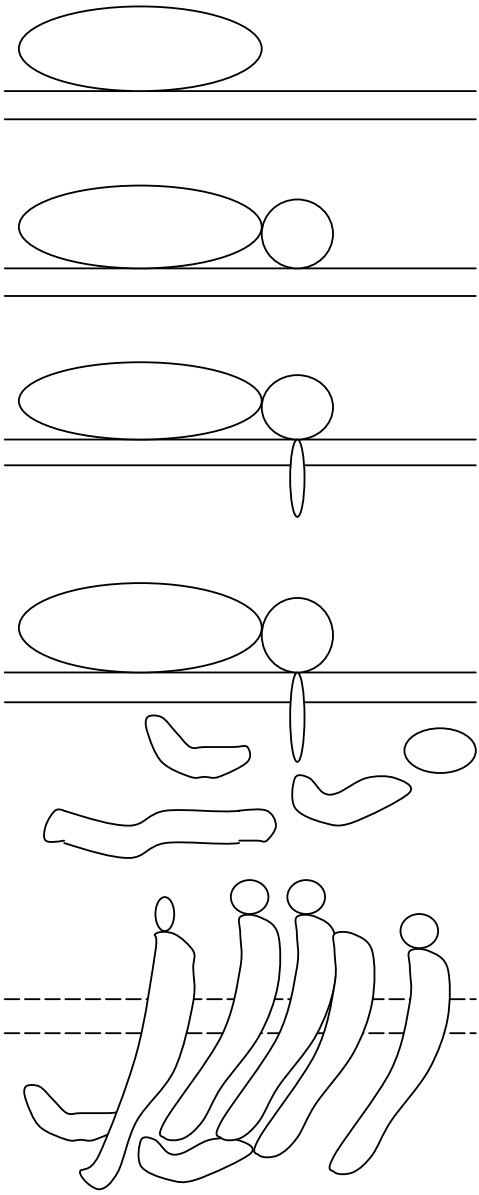
Table 1: continued

Beauveria bassiana		Increased virulence for larvae of lesser mealworm (*Alphitobius diaperinus)* after single passage through mealworm larvae. In comparison, conidia from original host i.e. the house fly (*Musca domestica*) were less virulent to mealworm larvae.	Steinkraus *et al*., 1991
Beauveria bassiana	Loss of virulence after 16 successive subcultures against *Leptinotarsa decemlineata* (Colorado Potato Beetle)		Schaerffenberg 1964
Beauveria bassiana		Virulence for *Porthetria dispar* larvae increased by two succesSsive passages through *Galleria mellonella* larvae	Wasti & Hartman, 1975
Conidiobolus coronatus		Virulence for *P. dispar* larvae increased by three passages through *G. mellonella* larvae	Hartman & Wasti, 1974
Culicinomyces clavisporus		*In vivo* conidia more pathogenic than conidia produced *in vitro*	Cooper & Sweeney, 1986
Entomophaga maimaiga	Loss of virulence after 15 *in vitro* passages. Attenuated cultures produced aberrant protoplasts *in vitro* (large, spherical) and resting spores *in vivo* but no conidia *in vivo*.	Virulence restored following *in vivo* passage (i.e. through larvae of gypsy moth host)	Hajek *et al*., 1990
Erynia (Pandora) neoaphidis	Loss of virulence		Butt & Wilding (unpublished observations)
Erynia (Pandora) neoaphidis	Loss of virulence		Rockwood, 1950

Table 1: Continued

Lagenidium giganteum	Loss of virulence, poor sporulation (unable to produce oospores and zoospores)	Virulence and sporulation restored following 15 passages through host mosquito larvae	Lord & Roberts, 1986
Metarhizium anisopliae	Loss of virulence		Al-Aidroos & Seifert, 1980
Metarhizium anisopliae	Loss of virulence	No increase in pathogenicity after 3 or 5 passages through larvae of *Oryctes rhinoceros*	Latch, 1965
Metarhizium anisopliae	Loss of virulence, white or pale yellow-green, decline in sporulation, loss of small chromosome	Virulence not restored of mutant lacking small disposable chromosome	Wang *et al.,* 2003
Metarhizium anisopliae	Loss of virulence, white or pale yellow-green conidia, decline in sporulation	Virulence restored by passaging through susceptible host (e.g. larvae of *Tenebrio molitor* and *Galleria mellonella*)	Shah *et al*., 2005
Metarhizium anisopliae	dsRNA mycovirus free cultures generally more infective	Isolates taken from infected ticks were more pathogenic than *in vitro* culture	Frazzon *et al.,* 2000
Nomuraea rileyi	Decline in virulence after 10^{th} transfer. Progeny conidia from 16^{th} transfer were avirulent to larvae of *Anticarsia gemmatalis*. Changes in morphology noted.	Attenuated cultures initially infected larvae of *Anticarsia gemmatalis* but failed to sporulate on cadavers. Finally, they failed to infect.	Morrow *et al*., 1989
Nomuraea rileyi	No change after 12 successive subcultures in morphology, sporulation or virulence for *Trichoplusia ni*	No change in virulence after passaging through *Trichoplusia ni*	Ignoffo, *et al*., 1982
Nomuraea rileyi	Decline in virulence for silkworm		Kawakami, 1960

Paecilomyces farinosus	Virulence restored by repeated subculturing on agar medium containing *Sitobion avenae* (grain aphid) cuticle. Virulence un-changed after subculturing (15 times) on yeast extract-peptone-dextrose agar.	Virulence restored after single passage and continued following consecutive passaging through aphid host (*S. avenae)*	Hayden *et al*., 1992
Paecilomyces farinosus	Loss of virulence, sparse mycelial growth, decline in sporulation.	Virulence restored after passaging through insect host.	Kawakami, 1960
Paecilomyces farinosus		Virulence restored after several passages through sawfly (*Cephalcia abietis*) eonymphs	Prenerova 1994
Paecilomyces fumosoroseus	Virulence unaffected following 30 successive serial transfers *in vitro*	Virulence unaffected if passaged through Russian wheat aphid (*Diuraphis noxia*) or Diamondback moth (*Plutella xylostella*). Two strains lost specificity for *D. noxia* when passaged 15 times through *P. xylostella*.	Vandenberg & Cantone, 2004
Tolypocladium cylindrosporum		Virulence not enhanced after 18 passages through mosquito larvae	Goettel, 1987
Verticillum lecanii	Loss of virulence after 2 or 3 subcultures		Nagaich. 1973
Verticillum lecanii	No decline in virulence even after 98 subcultures against *Macrosi-phoniella sanborni.* Changes in colony morphology and de-cline in colony growth rate.	No change in virulence after single passage through host	Hall, 1980
Zoophthora radicans	Loss of virulence	Virulence unaffected after passaging through host	Dumas & Papierok, 1989



Adhesion – conidia fail to stick. Presumably due to changes in surface properties (e.g. reduced hydrophobicity) or inability to secrete mucilage

Germination – slow to germinate and differentiate appressoria. Presumably due to inability to secrete appropriate enzymes or poor signalling (i.e. lacks receptors to respond to cuticular cues)

Penetration – slow to penetrate. Presumably due to the inability to produce the right set of cuticle-degrading enzymes and or inability to generate sufficient mechanical force to penetrate cuticle.

Colonisation – slow development in haemocoel. Presumably due to pathogen being unable to adapt to environment in haemocoel. Entomophthoralean protoplasts are usually swollen, spherical and vacuolated. Hyphomycete hyphal bodies may possess a cell wall/form that triggers an immune response by the host, which subsequently impedes development of the pathogen.

Conidia and conidiophore differentiation –few conidia and conidiophores produced. This is presumably due to changes in physiology and/or inability to respond to the appropriate cues. Attenuated cultures take more time to complete this cycle. However, virulence is restored after passagging through a suitable host.

Figure 1: Components of the invasive and development processes that may be affected when cultures of entomogenous fungi become attenuated

Attenuated conidia may germinate and infect their hosts marginally slower than non-attenuated strains. This may partly be due to the attenuated conidia lacking the right set of enzymes to facilitate host penetration. Indeed, conidia produced on insect cadavers have higher levels of Pr1, an important cuticle-degrading protease, bound to the spore wall than conidia produced *in vitro* (St. Leger *et al.*, 1991; Shah *et al.,* 2005). Inability of attenuated strains to adapt to the host environment would not only delay development but also trigger a defence response that would further retard colonisation (Butt *et al*., 1996). Degenerate cultures may not differentiate into the form adapted for the haemocoel (see next section) or they may not produce the metabolites that normally suppress the host defences. For example, Wang *et al.* (2003) found that attenuated cultures did not produce destruxins, the secondary metabolites harmful to the host's immune system (Vey *et al.,* 2001). A comparison of the different stages of the invasive and developmental processes of attenuated and virulent strains of entomopathogens may reveal only slight differences but the sum of these subtle differences could explain why attenuated strains have higher LT_{50} values than virulent strains.

Virulence of entomopathogens is restored when passaged through a suitable host. This is an important point to consider when comparing strains in bioassays since differences detected in their relative pathogenicity for the target pest can be attributed to intra-strain variation rather than a function of culture conditions (Butt & Goettel, 2000; Brownbridge *et al*., 2001). Some workers believe passaging enhances virulence but it is not clear if they are simply restoring the pathogens original insecticidal activity. Virulence is restored or enhanced usually after a single passage through a suitable host but some workers report that virulence is increased after two or more successive passages (Table 1). There are several reports that passaging does not enhance virulence presumably because the strains are at their full insecticidal potential or the strains have undergone irreversible physiological changes. Interestingly, researchers that reported no enhancement of virulence *in vivo* also noted that these strains did not become attenuated *in vitro* clearly demonstrating that some strains are more stable than others (Brownbridge *et al*., 2001; Hall 1980; Latch 1976; Vandenberg & Cantone, 2004; Ignoffo *et al*., 1982).

Few studies have been undertaken to determine to what extent the host influences restoration or enhancement of virulence in attenuated cultures. Most workers use the highly susceptible larvae of the waxmoth (*Galleria mellonella*) or flour beetle (*Tenebrio molitor*) to isolate or passage pathogens (Butt & Goettel, 2000; Hartman & Wasti, 1974; Wasti & Hartman, 1975). Kawakami (1960) restored virulence of entomopathogenic fungi after passing through silkworm larvae. However, our own work shows that virulence of *M. anisopliae* can be restored irrespective of whether it is passed through *Galleria* or *Tenebrio* (Shah *et al*., 2005). Conidia from *C. clavisporus*, produced in *Culex quinquefasciatus* were highly pathogenic to *Aedes aegyptii* (Cooper & Sweeney, 1986). In contrast, Vandenberg & Cantone (2004) noted that strains of *P. fumosoroseus* lost specificity for the Russian wheat aphid (*Diuraphis noxia*) when passaged 15 times through the diamondback moth (*Plutella xylostella*). Steinkraus *et al.* (1991) noted passaging of a strain of *Beauveria bassiana* through the lesser mealworm (*Alphitobius diaperinus*) enhanced its virulence for this pest. However, conidia from the original host for this isolate (i.e. *Musca domestica*) were less virulent for the mealworm. These observations suggest that the pathogen adapts to its host and retains those specificity determinants. Conversely, some arthropod hosts appear to enhance virulence and influence specificity whereas some hosts enhance virulence irrespective of the pathogens host range.

The question that remains unanswered is what attributes of the pathogen change when it loses or declines in virulence? No one has yet fully elucidated the underlying mechanisms for attenuation in insect-pathogenic fungi.

3. Phenotypic degeneration

Whatever the mechanism for attenuation, the fact remains that loss of virulence is often accompanied with or preceded by phenotypic changes suggesting the two are intractably interlinked. There are a few exceptions. For example, Hall (1980) and Vandenberg & Cantone (2004) noted that successive subculturing resulted in morphological changes in cultures of *V. lecanii* and *P. fumosoroseus*, respectively, but no noticeable decline in virulence.

The phenotypic changes associated with culture degeneration typically include changes in colour, growth rate and form, production of sectors and a decline or loss in spore production and/or selected metabolites (Table 1). Lord and Roberts (1986) found that 2 isolates of *Lagenidium giganteum* progressively lost the ability to form oospores and zoospores and to infect *Aedes aegyptii* larvae, after prolonged culture on sterol-free PYG (peptone, yeast extract, glucose) agar medium. The authors suggested that loss of vigour was due to the unavailability of sterols. Interestingly, *L. giganteum* requires exogenous sterols for zoosporogenesis. The sterols available from the host are those that maximally induce sporulation, implying strong adaptation of the pathogen to its host. In contrast, Hajek *et al*. (1990) noted that virulent cultures of *Entomophaga maimaiga* produced conidia or both conidia and resting spores in cadavers whereas attenuated cultures produced only resting spores. Furthermore, the *in vivo* protoplasts of attenuated and non-attenuated cultures differed in their phenotype and pattern of development. Morrow *et al*. (1989) noted that attenuated cultures of *N. rileyi* failed to produce yeast-like hyphal bodies or sporulate on infected cadavers. Earlier studies had shown that the mycelial phase, unlike the hyphal body stage, was non-infectious when injected into a larval host and triggered a rapid cellular defence response by the host (Morrow & Boucias, 1988). These observations suggest that attenuated cultures have "faulty" developmental processes that interfere with their adaptation to their respective hosts. It would also partially explain why high LT_{50} values are obtained in bioassays with attenuated strains. It should be noted that phenotypic switching with associated changes in virulence is well documented in human pathogenic fungi (see Fries *et al* 1999 and references therein).

Fungal colonies may spontaneously give rise to morphologically distinct degenerate sectors. Exactly why sections of a culture degenerate and the remainder remains intact is unclear. Some studies suggest that sectors arise as a result of cultural degeneration caused either by the age of culture, method of propagation, or nature of the culture medium. Our studies show that independent of strain, more sectors were produced on carbon rich (>4% glucose) media or high osmolarity media (Shah & Butt, 2005). Furthermore, most sectors were sterile or sporulated poorly. Media can influence phenotype but where the mycelial phenotype of the sector persists upon subculturing, regardless of the media type suggests a heritable, genetic component to phenotypic degeneration.

Several genetic mechanisms may explain instability in fungi including: alteration of gene expression due to transposable element activity or DNA methylation, influences of dsRNA viruses, and chromosome polymorphism. These mechanisms are briefly discussed below and where possible we draw attention to the link between phenotypic degeneration and attenuation of virulence.

Transposable elements or transposons are widespread and mobile genes flanked by terminal repeat DNA sequences that can cause spontaneous phenotypic changes in fungi. The *hAT*, *Fot1*/*pogo*, and Tc1/*mariner*superfamilies have been identified in filamentous fungi including plant pathogens and saprophytes but are poorly documented in insect pathogenic fungi (Kempken & Kuck, 1998,2000). Transposons may affect gene/chromosome structure and function including gene expression and gene inactivation Insertion into genes involved with metabolism and/or signalling may directly or indirectly influence development/differentiation and importantly the regulation of virulence genes. For example, in *Tolypocladium inflatum* ,the transposon *restless*was shown to be active by its spontaneous insertion into *tnir*, a gene involved in nitrogen metabolism (Kempken and Kuck 2000). The transposon *Scooter-2* spontaneously tag *thn1*, a gene encoding a putative regulator of G protein signalling resulting in faster growing and fluffier colonies (Fowler & Mitton, 2000). As yet, there is no clear evidence that transposons are inserted into virulence genes but they may directly or indirectly influence fungal virulence. For example, the transposon *Palm* is present in pathogenic strains of *Fusarium oxysporum* but is absent from non-pathogenic strains of this fungus (Daboussi & Langin, 1994).

Double-stranded RNA (ds RNA) viruses are common in most fungal taxa and have been implicated in degeneration of cultures. Mycovirus-associated degeneration has been reported in many plant pathogenic fungi including: *Cryphonectria parasitica* (Zhang *et al.,* 1993), *Botrytis cinerea* (Castro *et al*., 2003), *Nectria radicicola* (Ahn & Lee, 2001) and *Fusarium graminearum* (Chu *et al*., 2002*)*. The mycoviruses were responsible for hypovirulence, reduced sporulation and a decrease in the production of certain enzymes (e.g. laccases) and metabolites (e.g. trichothecenes). Curing the fungus of the mycovirus restored the attributes of virulent or wild type strains (Ahn & Lee, 2001). Conversely, virulence declined if the virus free strain was infected with the dsRNA virus from the hypovirulent strain using either purified virus particles or hyphal fusion (Castro *et al*., 2003; Chu *et al*., 2002). Mycoviruses have been reported in entomogenous fungi including *M. anisopliae* (Leal *et al*., 1994) and *Paecilomyces* spp (Inglis & Valaderes-Inglis, 1997) but little is known about their influence on fungal growth and virulence. Gimenez-Pecci *et al*. (2002) reported a mycovirus infected strain of *M. anisopliae* that spontaneously lost some of the dsRNA components and concomitantly altered in colony morphology and spore production, suggesting that viral genes interfere with fungal phenotype. Furthermore, the virus-free isolates secreted more endo-chitinase than strains infected with the virus. Frazzon *et al.* (2000) found that virus free isolates *M. anisopliae* were generally more infective for ticks than isolates infected with dsRNA viruses.

"Switching off" or silencing of genes through DNA methylation is not widely reported in fungi but could potentially account for phenotypic degeneration or attenuation of virulence. Increased methylation is associated with low transcriptional activity in *Neurospora crassa* (Selker & Garrett, 1988). Wang *et al*. (2005) and Shah & Butt (2005) observed reduced transcripts of Pr1 and other pathogenicity related genes of *M. anisopliae* in degenerate cultures including sectors but it was not clear if this was due to methylation or some other transcriptional control mechanism. Furthermore, Api-Zym revealed that the enzyme profiles of sectors differed from that of the parent cultures and also from other sectors. Sectors of *M. anisopliae* also produced less destruxin than the parent cultures independent of the strain (Shah & Butt, 2005).

Alterations in karyotype (= chromosome polymorphism) consisting of variation in chromosome size and/or number may also account for phenotypic degeneration and attenuation of virulence.

Karyotype instability has been observed in human pathogenic fungi like *Cryptococcus neoformans* (Franzot *et al*., 1998; Fries *et al*., 1999) and chromosome abnormalities such as chromosomal loss, length polymorphism, possible chromosomal translocations and changes in copy number of the ribosomal DNA repeat have been evident in association with strain degeneration of mushroom, *Agaricus bisporus* (Horgen *et al*., 1996). For most plant pathogens, they may lose the smallest chromosome (<2 Mb, usually 1.6 Mb) in their genome, which carry pathogenicity-related genes. This results in loss of virulence as well as morphological changes. These "mini" chromosomes have also been referred to as conditional dispensable chromosomes, supernumerary chromosomes and B chromosomes (Taga *et al.,* 1999; Hatta *et al*., 2002). A study of these chromosomes has helped to understand the role of the pathogenicity related genes in causing disease. For example, Akamatsu *et al.* (1999) observed that virulent strains but not avirulent strains of *Alternaria alternata* possessed mini-chromosomes (<1.7Mb). Toxin genes are usually located on these dispensable mini-chromosomes (e.g. Hatta *et al*., 2002). The dispensable chromosome of *Nectria haematococca* carries the *Pda6* and *Mak1* genes that increase virulence on host plants by detoxifying the phytoalexins pisatin and maackiain, respectively (Han *et al*., 2001). Dispensable mini-chromosomes have been reported in other plant pathogens including *Cochliobolus heterotrophus* (Tzeng *et al*., 1992), *C. carbonum* (Pitkin *et al*., 2000), *Colletotrichum gloeosporoides* (Masel *et al.,* 1993) and *A. alternata*(Johnson*et al*., 2001). Recently Wang *et al*. (2002, 2003) reported that disposal of the mini-chromosome of *M. anisopliae* resulted in a decline in virulence and altered phenotype. Furthermore, these authors established that the pathogenicity related genes encoding for an important cuticle degrading protease (Pr1) and a toxin (destruxin) responsible for suppressing the host's defences were located on this chromosome. The fact that chromosome polymorphism has also been observed in another entomopathogen, *Tolypocladium inflatum* (Stimberg *et al*., 1992), suggests that this is probably a more widespread phenomenon. It may also explain the different karyotypes reported between different strains of *M. anisopliae* (Shimazu *et al*., 1992) and within other species of entomopathogenic fungi (Shimazu *et al*., 1991; Pfeifer *et al*., 1993; Viaud *et al.,* 1996).

4. Conclusion

Entomogenous fungi will degenerate when continuously cultured on nutrient rich media. Phenotypic changes (alterations in growth rate, reduced sporulation, paler cultures, and formation of sectors) may precede or occur concomitantly with a decline in virulence. Although this is a widespread phenomenon, having been also reported in plant and human pathogenic fungi, little is known about the underlying mechanisms. Several disparate factors have been identified which influence culture phenotype and/or virulence including dsRNA mycoviruses, and conditional dispensable chromosomes. Why attenuated strains should "switch off" or lose pathogenicity determinants such as the cuticle-degrading enzyme Pr1 and the toxin destruxin is unclear. It could be argued that it is more energy efficient but it also means that the fungus is restricted to a saprophytic mode of nutrition since it is less effective in infecting arthropod hosts. One thing is clear, that virulence determinants e.g. Pr1 and destruxins could be used as markers to monitor the virulence of entomogenous fungi during mass production.

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CHAPTER 11

BIOLOGICAL CONTROL OF MOSQUITOES: MANAGEMENT OF THE UPPER RHINE MOSQUITO POPULATION AS A MODEL PROGRAMME

Norbert Becker

1. Introduction

Biological control is defined as the use of living organisms to reduce the target populations of pests. Biological control includes the use of predators, parasites and pathogens (Eilenberg et al, 2001). It aims to reduce the target population to an acceptable level and at the same time to avoid side-effects to the ecosystem. As far as mosquito control is concerned, biological control measures should integrate the protection of humans from mosquitoes with the conservation of biodiversity, whilst avoiding toxicological and eco-toxicological effects. As a result, the regulatory power of the ecosystem is maintained by protecting the existing community of mosquito predators.

The use of beneficial organisms for the control of mosquitoes was first recognized in the 19th century (Lamborn, 1890). However, the mass-breeding and successful introduction of predators such as hydra, flatworms, predacious insects or crustaceans, often brings a range of problems. Such problems occur to only a limited extent with the use of fish such as the mosquito fish, *Gambusia affinis*, or related species, which were successfully introduced into many countries to control mosquito larvae since the early 1900s (Legner, 1995).

With the discovery and large-scale use of synthetic insecticides in the 1940s and 1950s, the biological control of mosquitoes was no longer considered to be an important matter. However, the initial euphoria that greeted the success of synthetic insecticides rapidly dissipated as resistance subsequently developed within the target populations. Moreover, despite the beneficial effects of chemical insecticides, they also often have unwanted characteristics, such as their non-selectivity which frequently causes ecological damage. As public awareness of environmental issues increased, the regulations controlling the application of chemicals were tightened. As a result, a renaissance of the biological control of mosquitoes took place in the 1960s and 1970s. Today, the literature on mosquito antagonists is immense.

Predators are potentially a possibility for biological control of mosquitoes. However, predators have specific ecological requirements and can only be used where their preferred living conditions are met. The life-cycle of the predator is frequently not adapted to that of the target-organism so that it is unable on its own to bring about an effective reduction of the target-population. Mass-rearing and release of the predators or parasites is often expensive or even impossible. This limits their large-scale use in a number of specific habitats. Special attention has therefore been given to the search for microbial control agents.

J. Eilenberg and H.M.T. Hokkanen (eds.),An Ecological and Societal Approach to Biological Control, 227–245.

Over the past decades efforts on an international scale have led to the discovery of a great variety of pathogens, including entomopathogenic fungi, protozoa, bacteria and viruses (Weiser, 1991; Davidson and Becker, 1996; Becker *et al*., 2003).

2. Properties of mosquitocidal bacilli

The discovery of the gram-positive, endospore-forming bacterium *Bacillus thuringiensis subsp. israelensis* (Bti) in the Negev desert of Israel in 1976 (Goldberg and Margalit, 1977) and of potent strains of *B. sphaericus* in recent years have inaugurated a new chapter in the control of mosquitoes and black flies (Singer, 1973; Weiser, 1984; Becker and Margalit, 1993). The new subspecies of *B. thuringiensis* is highly toxic to larvae of most mosquito species and to blackly larvae. New strains of *B. sphaericus*, such as strain 2362 isolated from an adult blackly in Nigeria (Weiser, 1984), and strain 2297 isolated in Sri Lanka (Wickremesinghe *et al*., 1980) are much more potent than the first isolates and are particularly active against larvae of *Culex* and *Anopheles* species.

2.1. Bacillus thuringiensis israelensis (Bti)

During sporulation this *Bacillus* produces protein toxins that are concentrated in a parasporal body (PSB), the so-called protein crystal (Fig. 1). These proteins are highly toxic to mosquito and blackly larvae.

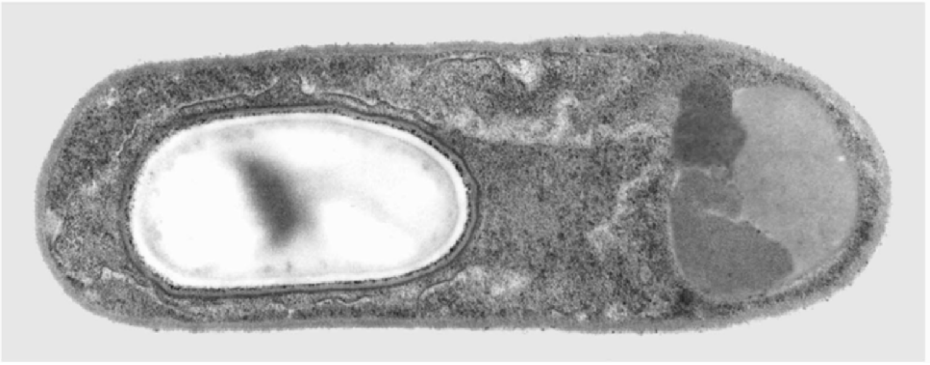

Figure 1: B. thuringiensis israelensis *with spore (left) and parasporal body, the so-called protein crystal (right) (Micrographs courtesy of J.-F. Charles, Pasteur Institute, Paris)*

The enormous selectivity of the *Bacillus* derives from a variety of factors:

1) The target insect has to ingest the protein crystal (inactive protoxins), this depends on its feeding habits.
2) The inactive pro-toxin has to be converted into biologically active toxins by proteases in the alkaline midgut milieu of the target insect.
3) The toxins must then bind to a cell surface receptor (glycoprotein) of the midgut epithelial cells of the target insect.

As a results of these processes the osmoregulatory mechanism of the cell membrane is disturbed, ions and water flow into the midgut cells, which swell and finally burst.

Non-target-organisms remain undamaged because of the lack of specific receptors on their intestinal cells or/and do not ingest or activate the pro-toxins into the toxin.

The insecticidal properties of Bti derive from the parasporal body (PSB), which is formed at the end of the sporulation and contains 4 major toxin proteins of different molecular weight, which are named as Cry4A (125 kDa), Cry4B (135 kDa), Cry10A (58 kDa) and Cry11A (68 kDa) (Delecluse *et al*., 1996). These toxins bind to specific glycoprotein receptors on the larval midgut brush border (Charles *et al*., 1996). A 5th toxin, called the CytA protein (27 kDa), binds to lipids and does not exhibit the specific binding mechanism which the Cry proteins do (Höfte and Whiteley, 1989; Federici *et al*., 1990; Priest, 1992). The high toxicity of the PSB is caused by the synergistic interaction of the 25 kDa protein (split from the 27 kDa protein) with one or more of the higher molecular weight proteins (Ibarra and Federici, 1986; Chilcott and Ellar, 1988; Chang *et al*., 1993). It is thought that the synergism in the mode of action among the proteins reduces the probability of resistance. Neither the spore nor the living bacilli appear to be involved in the insecticidal process.

2.2. Bacillus sphaericus

B. sphaericus has become increasingly important in recent years. The high potential of *B. sphaericus* as a bacterial control agent lies in its spectrum of efficacy (especially effective against *Anopheles* and *Culex* species) and its ability to recycle or to persist in nature under certain conditions, which means that long-term control can be achieved (Hertlein *et al*., 1979; Mulligan *et al*., 1980; Lacey, 1990; Ludwig *et al*., 1994). The time-span between retreatments can thus be extended and personnel costs reduced. This opens up the possibility for a successful and cost-effective control of e.g. *Cx. quinquefasciatus* which is the most important vector of lymphatic filariasis and is breeding primarily in highly polluted water-bodies in urban areas. Furthermore, anophelines such as *An. gambiae* s.l. is more sensitive to *B. sphaericus* as to Bti (Fillinger et al, 2003).

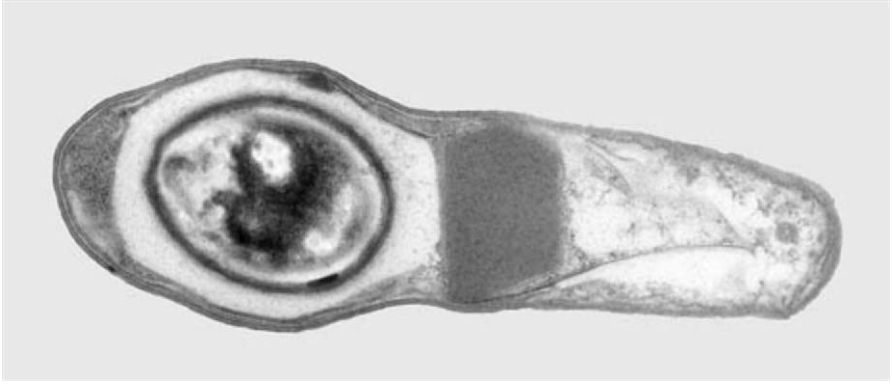

Figure 2: B. sphaericus *with round spore and the parasporal protein inclusion (dark structure on the right site of the spore) which is located in a coated "spore crystal complex". (Micrographs courtesy of J.-F. Charles, Pasteur Institute, Paris)*

B. sphaericus can easily be identified by its round spore located terminally in a swollen sporangium (Fig. 2). *B. sphaericus* only kills mosquito larvae and, when higher dosages are applied, larvae of Psychodidae. Certain mosquito species, such as *Cx. quinquefasciatus* and *An. gambiae*, are highly susceptible whereas *Ae. aegypti* larvae are more than 100 times less susceptible. Blackfly larvae as well as other insects, mammals, and other non-target-organisms are not susceptible to *B. sphaericus*.

Its efficacy, like that of Bti, is based on parasporal protein inclusions which are located in a coated "spore crystal complex". The toxin of *B. sphaericus* differs from that of Bti and has been shown to be a binary toxin (btx) consisting of proteins of two different molecular weights, 51.4 kDa and 41.9 kDa. Both are required for a high level of mosquitocidal activity (Broadwell *et al*., 1990; Baumann *et al*., 1991; Berry *et al*., 1991; Priest, 1992; Davidson and Becker, 1996). Some *B. sphaericus* isolates further produce protein toxins of a molecular weight of about 100 kDa, the mosquitocidal toxins (MTX), which are not homologous neither to the binary toxin nor to Bti toxins (Thanabalu *et al*., 1991; Priest, *et al*., 1997; Monnerat *et al*., 2004).

The mode of action of *B. sphaericus* toxins is based on binding of the toxins to specific receptors presented on the mid gut cell membrane.

Following extensive safety tests and environmental impact studies, these two microbial control agents were rapidly developed for the routine use. This rapid exploitation was aided by a series of useful properties of the bacterial control agents. In addition to the relative ease with which they can be mass-produced, bacterial control agents are highly efficient, environmentally safe, easy to handle, stable when stored, cost-effective and suitable for integrated control programmes based on community participation (Becker *et al*., 2003).

2.3. Formulations

A basic requirement for the successful use of bacterial control agents was the development of effective formulations suited to the biology and habitats of the target-organisms. Bti preparations can be obtained as water dispersable granules WDGs), wettable powders, fluid concentrates, corn cob, sand and ice granules, pellets, tablets or briquets (Becker *et al*., 2003)

A few hundred grams or even less of powder, half to two litres of liquid concentrate or a few kilograms of granules per hectare, are usually enough to kill all mosquito larvae. In some situations, a long-term effect can be achieved if larger amounts are used. With the production of tablet or briquet formulations, progress has been made towards achieving a long-term effect. Sustained-release floating granules are being developed.

The tablet formulations, based on Bti or *B. sphaericus* material sterilised by γ-radiation to prevent contamination of drinking water with spores, is successfully used for control of container breeding mosquitoes such as *Cx. pipiens* or *Ae. aegypti* (Becker *et al*., 1991; Kröger *et al*., 1996; Mahilum *et al*, 2005).

In addition to commercially available granules based on ground corn-cobs, sand granules can also serve as a carrier for wettable powder formulations: 50 kg fire-dried quartz sand (grain size 1-2 mm) with 0.8 - 1.4 litres of vegetable oil (as a binding material) and 1.8 kg of Bti powder (activity: 10.000 ITU/mg) should be mixed in a cement mixer. This mixture is sufficient to treat 2-3 hectares. Recently, more cost-effective granules have been developed in the form of ice-pellets (Becker, 2003). Ice granules can be easily produced when water suspensions containing the bacterial toxins are frozen into small ice cubes or pearls (3-5 mm) and kept in cold-storage rooms until used. The advantages of using ice granules are:

1) the toxins are bound in the ice pellet, and so any loss of active material by friction during application is avoided;
2) as the specific weight of ice is less than that of water, ice pellets remain in the upper water layer where they release the toxins into the feeding zone of the mosquito larvae as they melt;
3) ice pellets penetrate dense vegetation and do not stick to leaves even when it is raining.

The amount of active material per hectare can thus be significantly reduced when compared with granules based on sand, and ice granules can be more cost-effective than commercial granules.

One limitation to the use of Bti, for example against anophelines in rice fields, lies in the relatively brief duration of its activity. Expensive retreatments are frequently necessary. Formulations with a long-term effect, such as sustained-release floating granules, are thus needed.

A particularly attractive feature of *B. sphaericus* is its potential to persist and recycle under certain field conditions. Appropriate formulations have shown a significant residual activity against larvae of *Culex pipiens* and *Cx. quinquefasciatus* in highly polluted breeding habitats (Hertlein *et al*., 1979; Davidson *et al*., 1984; des Rochers *et al*., 1984; Lacey, 1990; Becker *et al*., 1995).

When appropriately stored, most preparations based on bacterial toxins can be kept for a long period of time without losing activity. Experience has shown that powder or granule formulations lose little of their activity even after many years in storage. On the other hand, the activity of fluid concentrates may be more labile. Preparations should therefore be retested in bioassays according to WHO guidelines (de Barjac, 1983) when they have been stored for more than a year.

2.4. Environmental safety

The exceptional environmental safety of bacterial control agents has been confirmed in numerous laboratory and field tests as well as in the course of world-wide application of Bti and *B. sphaericus* formulations by thousands of tons each year since more than two decades without any harm of the environment. The US Environmental Protection Agency (EPA) approved the use of Bti as early as 1981. In safety tests on representative aquatic organisms it was shown that in addition to plants and Mammalia none of the taxa tested such as Cnidaria, Turbellaria, Rotatoria, Mollusca, Annelida, Acari, Crustacea, Ephemeroptera, Odonata, Heteroptera, Coleoptera, Trichoptera, Pisces and Amphibia appeared to be affected when exposed in water containing large amounts of bacterial preparations (Becker and Margalit, 1993; Becker *et al*., 2003). The World Health Organization (WHO) states that Bti can also be used in drinking water reservoirs for the control of mosquitoes WHO, 1999). In Germany and tropical countries as Indonesia, Philippines or Thailand, Bti-tablets (Vectobac DT/Culinex) are already used against the larvae of *Cx. pipiens* and *Ae. aegypti* (Becker *et al*., 2003; Mahilum *et al*., 2005). All products used in drinking or potable water are sterilized by Gamma-radiation and don't contain living bacilli or spores (Becker, 2002).

Even within the Diptera, the toxicity of Bti is restricted to mosquitoes and a few nematocerous families (Colbo and Undeen, 1980; Miura *et al*., 1980; Ali, 1981; Garcia *et al*., 1981; Molloy and Jamnback, 1981; Margalit *et al*., 1985b; Mulla *et al*., 1982; Becker and Margalit, 1993). In addition to mosquito and blackfly larvae, only those of Psychodidae, Chironomidae, Dixidae, Sciaridae, and Tipulidae are sensitive to Bti, however, generally far less sensitive than those of mosquitoes or blackflies.

In contrast to Bti, the toxins of *B. sphaericus* are toxic to a much more restricted range of insects. Blackfly larvae as well as other insects (except for Psychodidae), mammals and other non-target-organisms are not susceptible to *B. sphaericus.*

Another important aspect is the widespread occurrence of both bacilli in the soil. They are natural components of the soil micro-ecosystem and not an artificial man-made product where toxic residues may remain after application against pest insects.

2.5. Handling and cost-effectiveness

No special equipment is required for the application of bacterial control agents. Generally, simple knapsack sprayers are adequate for accessible breeding sites. Standard ULV Micronairs, air blast or mist blower spray equipment may also be used. When dense vegetation or wide spread breeding sites occur aerial application should be preferred. Rotary seeders or pressurised air sprayers are suitable for the application of granules. Safety precautions as used with toxic chemicals do not have to be considered. Because of the rapid knock-down effect and the high level of efficiency, the success of the treatment can generally be monitored within a few hours after application.

Compared to conventional insecticides, the application of bacterial control agents can be cost-effective. For instance, the KABS mosquito abatement project in Germany effectively suppresses mosquitoes deriving from a catchment area of more than 600 km^2 involving on average 100 km^2 of actual breeding grounds per year. The total annual budget is 1.9 million Euros. More than 2.7 million residents of the area are protected from an intense nuisance. But environmental considerations, which cannot be expressed in monetary terms, should also be included in these economic calculations.

2.6. Lack of potential for resistance development

The development of resistance to chemical insecticides represents a serious problem. Bacterial control agents, however, appear less likely to provoke resistance because their mode of action is more complex (Davidson, 1990). However, the resistance of a stored grain pest, *Plodia interpunctella*, to the Lepidoptera-specific *B. thuringiensis kurstaki* has been demonstrated in the laboratory (McGaughey, 1985). Recent studies have shown that the commercial use of *B. thuringiensis* preparations in agriculture can lead to resistance within a few years. For example, the diamondback moth, *Plutella xylostella*, which was repeatedly treated with *B. thuringiensis* on farms in Hawaii, was found to be 41 times more resistant than populations that were only minimally exposed to *B. thuringiensis kurstaki* (Tabashnik *et al*., 1990). Such resistance phenomena have not yet been observed with Bti.

Resistance studies have been carried out by the KABS with populations of *Ae. vexans* which were constantly exposed to Bti over a period of 20 years and were therefore subjected to constant and intense selection pressure. These mosquitoes were compared with similar *Ae. vexans* taken from a remote location which had never been exposed to Bti and had never been under selection pressure. No reduction in the sensitivity of these mosquitoes to Bti could be detected (Becker and Ludwig, 1993). Similar results were obtained by Kurtak *et al*. (1989) and by Hougard and Back (1992). They found that after 10 years of the intensive application of Bti in West Africa the susceptibility of the blackly, *Simulium damnosum*, had not changed.

The complex mode of action of Bti partly explains the relative absence of resistance. The lethal changes in the midgut cells are induced only by the synergistic effects of the different toxin

proteins present in the parasporal body of Bti. This combination reduces the likelihood of resistance. On the other hand, when the gene encoding a single toxin protein was cloned into a microorganism and then fed to larval mosquitoes, resistance was induced within a few generations (Georghiou and Wirth, 1997).

Resistance is less likely when there is a variable gene pool of target populations. The large size of many mosquito and blackly populations thus inhibits the development of resistance because only a portion of the population is exposed to the toxicant. Floodwater mosquitoes and most blackflies undertake considerable migrations. This behaviour produces a substantial gene flow within their populations which should at least slow the onset of resistance.

However, resistance to *B. sphaericus* has been demonstrated in both the laboratory and the field. In southern France, a population of *Cx. pipiens* developed a level of resistance of more than 16 000 fold after 18 rounds of *B. sphaericus* treatment (Sinegre *et al*., 1994). Nielsen-LeRoux *et al*. (1995) could demonstrate in a laboratory population of *Cx. pipiens* that resistance at a level of 100 000 fold to *B. sphaericus* binary toxin can be caused due to a change in the receptor on midgut brush-border membranes. In other cases, binding to the receptor took place but there was no toxicity. In all cases resistance was shown to be recessive (Charles and Nielsen-LeRoux, 1996). It seems that the risk of resistance to bacterial toxins is inversely proportional to the complexity of the mode of action, which is definitely less complex with *B. sphaericus* than with Bti.

2.7. Suitability for integrated control programmes with community participation

Bacterial control agents are particularly well suited for use in integrated programmes. Because their toxic effect is selective, they do not affect predatory organisms. These can therefore be included as additional elements in an integrated control strategy. However, some factors influencing the efficacy of bacterial control agents should be considered (Becker *et al*., 1993; Ludwig *et al*., 1994).

The efficacy depends upon the developmental stage of the target organisms, their feeding behaviour, the organic content of the water, the filtration effect of target larvae as well as that of other non-target-organisms, photosensitivity and other abiotic factors such as water temperature and depth, the sedimentation rate as well as the shelf life of the Bti and *B. sphaericus* formulations (Mulla *et al*., 1990; Becker *et al*., 1992). The long-term effect is also strongly influenced by the recycling capacity of the agent (Aly, 1985; Becker *et al*., 1995).

It is important to understand the impact of these factors on routine treatment, particularly on the calculation of the optimal dosage, the selection of the right formulation in a particular environmental situation and the optimal timing for application against different mosquito species (Becker and Rettich, 1994).

3. The German Mosquito Control Association (KABS)

Since more than a decade Bti and *B. sphaericus* have been successfully used as biological control agents against mosquitoes in Germany. Over 2500 km ^{2} of breeding areas have been treated with Bti, resulting in a reduction of the mosquito population year by year by more than 90% and without evidence of any harmful impact on the environment.

The control of mosquitoes in Germany has a long history. In the 1920s and 1930s breeding sites were treated with petroleum oils. During the 1950s and 1960s adulticides were used. However, in the early 1970s, the mosquito population was extremely high because of frequent fluctuations of the water level of the Rhine. The outdoor attack rate on humans was more than

500 mosquito bites per minute, greatly restricting the time village residents could spend outside. As a reaction to this natural disaster, 44 towns and communities on both sides of the River Rhine merged their interests in the GMCA/KABS, a united mosquito control programme founded in 1976 under the leadership of Paul Schädler. Now 98 cities and municipalities along a 310 km stretch of the Upper Rhine River, with a total population of 2.5 million people, have joined forces to control the mosquitoes, [mainly *Aedes vexans* (Meig.)] over a breeding area of some 600 km^2 of the Rhine's flood plain. The budget of the programme is approximately 1,9 million Euro/year, which results in overall costs per person per year of less than 1 Euro. The overall goal of the KABS is to control mosquitoes while conserving biodiversity. This goal can be reached effectively only, when biological control methods are used.

The control of *Aedes* mosquitoes by GMCA/KABS is based mainly on the use of Bti products. Domestic mosquitoes [(*Culex pipiens* (L.)] are controlled mainly by the use of Vectobac DT/Culinex®- Bti-tablets in containers and septic tanks, as well as by the application of *B. sphaericus* to eutrophic ponds and ditches. The conservation and promotion of predators is also an important goal of our programme. Therefore, the microbial control methods are integrated with environmental management (eg. improving ditch systems for regulation of water levels and for provision of permanent habitats for aquatic predators such as fish).

3.1. A microbial mosquito-control strategy

For the successful use of microbial agents to control mosquitoes certain prior studies are necessary:

(1) Entomological studies of the biology and ecology of the native nuisance mosquito species (eg. species composition and population dynamics related to climatical conditions);
(2) Precise mapping and numbering of all major breeding sites; assessment of the minimum effective dosage in laboratory bioassays with field collected larvae (LC_{99}= minimum effective dosage);
(3) Assessment of the optimum effective dosage in small field tests conducted in dominating breeding types under various abiotic and biotic conditions;
(4) Adaptation of the application technique to the requirements in the field;
(5) Design of the control strategy based on the results obtained during the preparation phase;
(6) Training of field staff;
(7) Governmental application formalities for the use of microbial control agents.

Further details of some of these steps in the GMCA/KABS programme are discussed in more detail below.

3.2. Mapping of the breeding sites

Mapping and numbering of the breeding sites provides a reference on location and size of each site. Several other studies are also carried out during mapping: (1) assessment of the diversity and abundance of mosquito species in the control area; (2) characterization (typing) of the breeding sites according to their productivity (densities) and analysis of mosquito population

dynamics; and (3) assessment of the ecological conditions of the major breeding sites (e.g. plant associations or occurrence of predators or rare and sensitive organisms that indicate the frequency of floods.

GIS/GPS technology can greatly improve the survey, logistics and documentation of mosquito control operations. The possible applications range from direct digital site mapping using GPS assisted mobile devices, to timely aggregation of operational reports.

A spatially referenced database containing all features of interest, is the basis for all further data collection and analysis. This spatial element enables thematically related features (*e.g.* population densities of certain species, flooding areas, plant associations and vegetation type, zones of human nuisance or disease) to be organised in separate layers of information, which can then be analysed and displayed in a user defined context. Remote sensing data provide useful information about factors (e.g. elevation, soil moisture, vegetation type or flooding-extend) which were essential environmental variables that have direct or indirect influence on larval habitats.

The use of remote sensed data in the mapping process leads to a higher efficiency of field mapping and maintenance of breeding-site data. Based on the knowledge of the biology of target organisms and field experience dominant factors suitable for larval habitats have to be defined and located (e.g. soil moisture, vegetation-characteristics). Data of high spatial ground resolution (1-5m/Ikonos-QuickBird) are considered adequate to assess areas as potential breeding sites. Combination of these results with high resolution digital elevation models, hydrological data and limited GPS-assisted ground checks show promising results for mapping purposes (Klaus Hoffmann, personal communication).

3.3. Design of the control strategy

The control strategy for large-scale operation is elaborated according to several considerations.

(1) The migration behaviour of the target mosquitoes. The objective of the strategy is to keep mosquitoes away from human settlements, and so the migratory behaviour of the nuisance mosquitoes needs to be considered. Species like *Ae. vexans* that readily migrate need to be controlled even in breeding sites that are far away from settlements (*Ae. vexans* can migrate more than 15 km when population pressures are high). Domestic mosquitoes (*Cx. pipiens*) that migrate no more than a few hundred metres are controlled only within the settlements and within a radius of 500 m.
(2) The potential productivity of mosquitoes of a breeding site. This is a criterion for the relevance of a breeding site (assessment of the mosquito threshold for the control).
(3) The climatic conditions (changes of the water level, length of rainy and dry season), which influence the occurrence of the mosquitoes.
(4) The population dynamics of the target organisms. These determine the best timing of the treatment which causes the strongest negative impact on the target organisms.
(5) The residual effect of the microbial control agent, which can be relevant for the sequence of re-treatments.
(6) Adaptation of the control technique to the ecological conditions. According to ecological conditions, such as water level and vegetative growth, the application of Bti may be made on foot or by helicopter.

(7) Development of an integrated control strategy, including predators, environmental management, and community participation.

3.4. Routine treatments

The flood plains of the Rhine are usually inundated two to four times each summer. The extent of the flood water depends on the snow-melt in the Alps and on rainfall, and it is constantly necessary to monitor the water flow in the Rhine and in the flood plain. During flooding, mosquito larvae hatch within minutes or hours when the temperature exceeds 10°C. Before control measures are begun, the larval density and the larval stages are checked by means of ten sample scoops at representative breeding sites, in order to justify the action being undertaken and to establish the correct dosage and the best formulation to use. One day after application, spot sample scoops are taken at the reference breeding sites to check mosquito density and thereby establishing the efficacy of the treatment.

According to the extent of the flooding, 10-20% of the potential breeding areas of 600 km² has to be dealt with regularly by the 400 collaborators of the GMCA/KABS. For treating first and second larval instars, 250 g of powder formulation (3,000 ITU/mg) or 1/2 liter of liquid concentrate (1.200 ITU/mg) are dissolved in 9-10 litres of filtered pond water for each hectare treated and applied by a knapsack sprayer. For deeper sites or when later instars are present, the dosage is doubled. During the worst floods, a third of the area is treated with Bti granules dispensed from a helicopter (dosages: 10-20 kg/hectare). From 1981 to 2004, 70 tonnes of Bti powder, more than 1100 tonnes of Bti granules (ice or sand granules), and 45 tonnes of Bti liquid concentrates have been used, treating over 2500 km2 of breeding area.

Control of domestic mosquitoes is mainly carried out by householders. To assist with this, GMCA/KABS provides information on the biology of *Cx. pipiens molestus* and on appropriate control measures. Culinex® Bti/*B. sphaericus* tablets have been particularly successful. They kill *Culex* larvae in water containers over a period of several weeks. In drainage systems and large cesspools with eutrophic water bodies, *B. sphaericus* as a liquid or powder formulation (Vectolex WDG) is applied against *Culex* larvae. Each year about 1 million of Culinex-Bti/*B. sphaericus* tablets are successfully applied against *Culex pipiens* especially, in rainwater containers.

3.5. Monitoring the programme

Some 8% of the GMCA/KABS resources are invested in monitoring mosquito numbers, mosquito resistance and environmental impact. All the studies carried out to date show that the introduction of Bti and *B. sphaericus* has reduced the numbers of nuisance mosquitoes to a tolerable level while the ecosystem as a whole has not been damaged.

3.5.1. Monitoring mosquito numbers

To monitor mosquito abundance, 40 comparable sites throughout the entire inundation area are assessed. These are monitored twice a month from April to September, on each occasion for a whole night, and the mosquito density is sampled by means of carbondioxide-light traps. Catches in areas where no control measures have been undertaken serve as points of reference (100% of the mosquito population) for catches from areas being controlled, in order to determine the success of the measures (mortality rate in percent). It has been shown that since

the widespread application of Bti began in 1981, over 90% of the population of *Aedes vexans* has been killed each year and, despite extreme serious flooding in the past few years, mass occurrences of mosquitoes have been successfully averted. Naturally, these control measures have had an extremely positive reception among the local people.

3.5.2. Monitoring the environmental impact

It has been essential to document the environmental impact of Bti and *B. sphaericus* applications in order to provide a scientific basis for rebuting the arguments commonly brought against mosquito control by its opponents. Before large-scale application of microbial control agents was undertaken, the most important members of various aquatic groups (*Cnidaria* to *Amphibia*) were screened in the laboratory and in small-scale field trials for their susceptibility to microbial control agents. This work showed that in addition to mosquitoes and black flies, only a few species of midges were affected by Bti. For the most part these midges were much less susceptible to Bti than the target organisms. *B. sphaericus* is toxic to an even narrower range of insects: certain mosquito species, such as *Culex* species, are highly susceptible, *Aede/Ochlerotatus* species are much less susceptible, and black fly larvae as well as other insects (exception: *Psychodidae*) and nontarget organisms are not susceptible.

The development of insects in treated and untreated water is continuously monitored using emergence traps (photo eclectors). The occurrence of insects in treated areas is assessed by regular light trap catches. All investigations have shown that while the numbers of *Aedes/Ochlerotatus* mosquitoes are drastically reduced, all other insects continue to develop in the water and, as winged adults, provide a food resource for birds, amphibians and bats.

3.5.3. Monitoring Resistance

Mosquito populations are checked at regular intervals for the development of resistance. No resistance has been detected after twenty years of treatment with Bti. To prevent resistance to *B. sphaericus* developing in *Culex, B. sphaericus* and Bti are used alternately in the control management plan for this species.

In general, predators of the immature mosquito stages are more effective than predators of the adults. As a rule, mosquito larvae and pupae are concentrated at their breeding sites and are more easily available to predators than the widely dispersed adults. Moreover, adult mosquitoes evade many predators by their mostly nocturnal way of life. Mosquitoes have the characteristics of typical r-strategists which especially mean a high rate of reproduction, and relatively short life cycle. Predators are particularly effective if they have a similarly high rate of reproduction and/or a high rate of feeding, like fish.

4. The use of microbial control agents in other European countries

Croatia: The city of Osijek is located along the banks of the Drava and the natural protected area, the Kopacki rit. Both areas are extreme productive for floodwater mosquitoes, first of all *Aedes vexans* and *Ochlerotatus sticticus* (Merdić & Lovaković, 2001) and during long lasting floods *Culex modestus* and *Anopheles messeae* (Merdić & Sudarić 2003) Since 2004 Bti ice granules are successfully used in a wide-scale under the auspice of the Health public Institute

and Department of Biology, University of Osijek (J. Milas and E. Merdic, personal communication).

France: Organised mosquito control has a long history in France, which reflects the severe mosquito problems in some areas. At present five independent organizations are responsible for mosquito control: EID Méditerranée (Entente Interdepartementale pour la Demoustication) based in Montpellier which covers the French part of the Mediterranean basin, EID Ain Isère-Rhône-Savoie based in Chindrieux which covers the Rhone-Alps region, EID Atlantique based in St-Crépin which covers the Atlantic coast, one abatement district in Paris and one in Alsace which protects the human population on the French side of the Upper Rhine Valley.

Most effort is directed at larval control, either through physical methods, management of wetlands and taking into account the whole fluvial hydrosystem, or by use of larvicides that are effective against larval instars in specific habitats. From the mid 1980's, chemical insecticides were partly replaced with biological larvicides. By year 1997 some of the organizations, such as EID Rhone-Alpes and in the Alsace region, based their larval control exclusively on biological larvicides (*B. sphaericus* and Bti).

Greece: In the areas of Serres, Thessaloniki, and Athens abatement districts have been founded end of the 90ies. The major pest species are rice field mosquitoes *An. sacharovi*, *Oc. caspius* and *Cx. modestus*. In the cities *Cx. pipiens* is the nuisance species. Beside the use of Temephos more and more Bti is used especially in ecological sensitive areas. Especially during the Olympic games in 2004 several thousands of hectares close to Athens have been treated with Bti.

Italy: Large scale mosquito control operations started in Northern Italy in the middle of the 1980's. Several programmes were implemented mainly based on regional and local public financial support. One of the first programmes to be established in 1987 was in Bologna province, an irrigated agricultural area of about 900 km^2 in the Po plain. From the beginning major attention was devoted to larval control with occasional adult control support in defined areas. As a result of specific research, surveys, and monitoring programs, the control program has adopted larviciding as the only control technique. In the campaigns against the two major target species *Oc. caspius* and *Cx. pipiens,* 95% of the total larvicide products used are based on Bti., while the remaining 5% consist of Temephos which is still used in catch basins only.

Breeding habitats are regularly treated with Bti at intervals of 5 to 7 days depending on the water temperature. The mosquito season of the region usually requires 20-24 treatments when *Cx. pipiens* is controlled. The *Cx. pipiens* populations are regularly checked for Bti resistance. So far no signs of resistance could be measured.

In the region of Comacchio, a tourist resort in the Po delta and a natural protected area which includes 47 km of coast with an expected yearly presence of 500,000 tourists, the mosquito control program was started in 1991. The strategy is based on a combination of both larvicides and adulticides. The main target species of this region are *Oc. caspius*, *Cx. pipiens* (biotype *molestus*) *Oc. detritus*, and *Cx. modestus*.

By introducing a monitoring system for mosquito adults densities by using CO_2 traps and by fixing a threshold level for adult mosquitoes, the use of toxic chemicals could be reduced.

From 1996, several programmes were started in the Piedmont region, which rapidly became the most organised region in Italy. In this area approx 100,000 hectares of rice fields provide *Oc. caspius*, *An. maculipennis* and *Cx. modestus* ideal breeding conditions. The control of these species is based on an integrated control strategy using Bti. and predators (*Gambusia holbrooki*). The use of *Gambusia* is currently under evaluation, however, due to its exotic

origin a controversial discussion started regarding its possible impact on indigenous water organisms. Bti-water suspensions are applied by helicopters at a dosage of 1 litre of Vectobac 12AS per hectare at the beginning of the rice growing season. When the rice plants are grown up to 4 litres per hectare of Vectobac 12AS are used in July and August (Romeo Bellini, personal communication) Tests using Bti ice granules in rice fields by aerial application showed promising results even when less active ingredient was used. Critical agronomic practices such as periodic draining and flooding of the rice field support the development of *Oc.caspius*. Thus alternative cultural techniques are under evaluation in order to reduce the mosquito productivity of rice fields (Romeo Bellini, Andrea Mosca, Asghar Talbalaghi, personal communication).

Aedes albopictus, the Asian Tiger Mosquito, is rapidly spreading all over the country. In many urban areas the species is now the main noxious organism which requires specific control measures. The populations are carefully monitored in surveillance programmes using special ovitraps. This mosquito is controlled by environmental sanitation, source reduction campaigns and larval control. Vectobac DT tablets are also used by millions in Italy.

Serbia: The province of Vojvodina in the northernmost part of former Yugoslavia has been a subject to a continuous mosquito control program for the last 30 years. The floodplains of three large rivers, the Danube, Tisza and Sava, with a total length in Vojvodina of 597 km, provide ideal breeding sites for *Aedes*/*Ochlerotatus* mosquitoes (e.g. *Ae. vexans*, *Oc. sticticus*, *Ae. cinereus*, and *Ae. rossicus*) as well as for *An. maculipennis s.l.* larvae in billions.

Mosquito control in Vojvodina has been organized since 1976 under the umbrella of the Province government. The Faculty of Agriculture, (University of Novi Sad) was the founder and organizer of the control program until 1985. Since 1980, when Bti was first introduced as larvicide in parallel with organophosphates and IGR compounds, Bti has been a subject of continuous research and application programs at various scales. From 1993 on, the municipality of Novi Sad has started to support the “Environmentally friendly approach in mosquito control" project. The project’s main goal is to provide a base for rational biological mosquito control, mainly by use of Bti products. As a result of the project activities, since 1997 at least one generation of floodwater mosquitoes is controlled solely by Bti application. Other seasonal outbreaks of floodwater mosquitoes are suppressed by combined methods (Bti for larviciding and ULV pyrethroids/organophosphates for adulticiding) using air or ground equipment.

The use of Bti has additional expenditure to the Simulid breeding sites, which have been recognized as a severe nuisance of the region. At present, Bti treatments are of a minor scale, usually tens of hectares, which include mainly streams and the Danube tributaries. Recently, (2004) the local government has approved the project for an Integrated Mosquito Control in the Vojvodina Province (20.000 km^2) for the period of five years, which should start the implementation of the bioinsecticides commencing 2005 on a larger scale. The project is based on a gradual implementation of the Bti on the surface of 87.680 ha, mainly at the inundation area of the major rivers.

Slovenia: Since more than 10 years Vectobac 12 AS is used by ground application against floodwater mosquitoes in the flood plains of the Sava River close to Kranjska Gora.

Spain: In Spain, organized mosquito control started in the early 1900's following the discovery of mosquitoes as vectors of malaria. Nevertheless, interest in mosquito studies ceased as soon as the disease was eradicated in this country in 1963 (Pletsch, 1965).

Due to increasing quality of life, the public recognized mosquito nuisance as a limiting factor for the development of the country. This fact was especially important in towns and cities close to mosquito breeding places, and in areas where tourism was the most important industry.

The first current mosquito control service (MCS) was created in 1982, in Roses Bay and Ter River which lies between the Pyrenees and the Montgri massif, forming a bay with swamps, salt marshes and lagoons. The area covers about 34.000 ha of which 7.200 ha are natural mosquito breeding places, about 700 ha of rice fields and thousands of septic tanks, in one of the most important tourist area in Catalonia. *Ochlerotatus caspius*, *Oc. detritus* and *Ae. vexans* are the most important species in natural breeding places, while *An. atroparvus* is the major species of the rice fields. Swamps and salt marshes, including natural parks are exclusively treated by Bti while the most important *Cx. pipiens* breeding places (the septic tanks spread over the residential areas) are treated with Pyryproxifen.

In 1983 the MCS of the Baix Llobregat was created, followed by the MCS of Huelva in 1985 (Anonimous, 1989), and the MCS of the Ebro Delta in 1991. All the MCS operate on the basis of integrated pest management focused on larval control, have complete independence, and are related to different local public administrations. The goal of the MCS of the Baix Llobregat region east of Barcelona is to avoid mosquito nuisance along the river area where important tourist resorts exist. The control is about 25.000 ha, including 9.000 ha of the river delta.

The species causing the nuisance are mainly *Cx. pipiens* and *Oc. caspius*. *Cx. pipiens* is proliferating between May and November in the 290 km of polluted ditches in the district which are treated by Bti.

The MCS of the Ebro Delta, covering more than 32.000 ha of rice fields and swamps. Because of the intensive nuisance caused by mosquitoes in villages and touristic areas, the abatement district has been established in 1991. The major nuisance species are *Oc. caspius*, *Oc. detritus*, *An. atroparvus and Cx. modestus*. *Cx. pipiens* problems occur in ditches and septic tanks in all villages spread in the delta. The control operations in the swamps are based on Bti. For the control of rice field mosquitoes, a buffer zone around each village is treated with Temephos.

The MCS in the region of Huelva was established in 1985, and covers an area of more than 130.000 ha. This region is located on the southwest coast of Spain and partially extends along the tidal salt marshes between Portugal and the Lower Guadalquivir River marsh. More than 15.000 hectares of the tidal marsh, where *Oc. caspius* and *Oc. detritus* are the most important species, are regularly treated by both the conventional insecticide Temephos, and by Bti. *Cx. pipiens* larvae in ditches and gutter are controlled on a bi-weekly basis.

Sweden: The first Swedish abatement district (Biologisk Myggkontroll-Nedre Dalälven, BMK-ND) was created in September 2000, as a response to many years of complaints about mosquito nuisance by the local population of the lower portion of the Dalälven river (Nedre Dalälven) in Central Sweden. Approximately 10 km^2 of the Dalälven flood plain between the city of Avesta and the Sea of Bothnia provide temporary wetland areas as breeding sites. Some 24 different species of mosquitoes have been recorded in the Nedre Dalälven area, however, *Oc. sticticus* (80%) followed by *Ae. rossicus* (8%) and *Ae. cinereus* (7%) are the most important nuisance species. In spring, *Oc. communis, Oc. punctor* and *Oc. intrudens* can also be

found in high numbers in the wetlands. The control of *Ochlerotatus* and *Aedes* mosquitoes is solely based on the use of Bti products (Lundström, personal communication).

Switzerland: Switzerland has several wetland conservation areas at the foothills on both sides of the Alps. They are located at the end of valleys where the rivers have formed plains or deltas before flowing into lakes. These wetlands are flooded periodically during the spring and summer months by snowmelt and increasingly heavy precipitations along the rim of both sides of the Alps. Many of they are protected like the Bolle di Magadino in the south of Switzerland, which is located along one of the main route for migratory birds. Another area of interest, exhibiting an extraordinary dynamic it situated at the upper end of the Lac de la Gruyère. The floodplains of these areas are major breeding sites for floodwater mosquitoes such as *Ae. vexans* and *Oc. sticticus*.

Since the mosquito control program using exclusively products based on Bti, was so successful, a second project followed in 1995 at the Lac de la Gruyère (Lüthy, 2001). Aerial application of Vectobac corn cop granules by helicopter has proved to be the only efficient method to reach the larval breeding sites.

5. Conclusions

With the development of appropriate formulations based on Bti and *B. sphaericus* effective and economic control of mosquitoes and blackflies is possible without harming the environment. World-wide thousands of tons based on both microbial control agents are annually used against mosquitoes and blackfies. Microbial control agents are useful supplements to, or replacements for, broad-spectrum chemicals and are promising agents in the fight against dangerous mosquito and blackfly-borne diseases such as malaria, lymphatic filariasis, dengue or onchocerciasis. In the Onchocerciais Control Programme in West Africa, each year hundreds of tons of Bti had been successfully used against the larvae of *Simulium damnosum*, the vector of *Onchocerca volvulus*, the parasite causing onchocerciasis. Formulations like Bti tablets will support the fight against the dengue vector, *Ae. aegypti.*

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CHAPTER 12

BIOLOGICAL CONTROL OF SCARABS AND WEEVILS IN CHRISTMAS TREES AND GREENERY PLANTATIONS

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1. Introduction

In Danish forestry the economically most important insect problems and consequently the most intensive use of chemical insecticides occur in the production of Christmas trees and decoration green (Kirkeby-Thomsen and Ravn, 1997; Ravn, 2000). Nordmann fir (*Abies nordmanniana*) and noble fir (*Abies procera*) are the dominant tree species in this production today. *Abies nordmanniana* and *A. procera* cover approximately 22,600 ha and 9,200 ha, respectively. *A. nordmanniana* is mostly used for Christmas trees, while *A. procera* is mostly used for decoration green. Other conifer species to be used for the same purposes are only grown on a negligible scale.

Christmas trees and decoration green are both important for the home market and export. In 2003, more than 9 mio Christmas trees and 30,000 tonnes decoration green were exported to several countries in Central and Northern Europe with Germany as the main recipient. The prices obtained by the producers vary depending on quality, but can typically be around EUR 10 per tree of 200 cm height and EUR 1 per kg decoration greenery. The total export value for Denmark was thus above EUR 150 mio in 2003. The market demands a very high product quality, and no damage from feeding of insect pests is accepted. Only the highest quality with the required shiny, dark green needle colour of Christmas trees and decoration greenery without any signs of insect feeding on the needles are saleable at reasonable prices. It is therefore a prerequisite for the producers to ensure pest control.

Control of insect pests in the Danish production of Christmas trees and decoration green has historically been based on the use of chemical pesticides. However no chemical pest control have been permitted in state forestry since 2003 (The Danish Environmental Protection Agency, 1998), and this applies to the 4,600 ha production area for Christmas trees and decoration greenery. For privately owned forests there is a political wish from the state authorities to phase out chemical pesticides (Ravn, 2000). Further, there is an increasing desire from consumers to buy organically grown Christmas trees produced without chemical pesticides. The products are associated with strong emotions among consumers: Christmas trees and decoration green are linked to family traditions during the Christmas period, which in Denmark lasts about one month.

Biological control using natural enemies of the pest populations may thus provide an attractive alternative to conventional chemical treatment. This chapter provides a short review

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of the first major European experiments to implement biological control in the production of Christmas trees and decoration green.

2. *Melolontha melolontha* in Christmas tree plantations

The European cockchafer, *Melolontha melolontha* (Coleoptera: Scarabeidae) is a serious pest in the production of Christmas trees in Denmark (Harding, 1994). The larvae feed on the roots of especially young trees causing extensive and lethal damage in the plantations (Harding, 1994). The cockchafer has a four-year-life cycle and the larvae dwell in the soil for 3 growth seasons. Feeding by the small larvae has no major impact on the vitality of the trees, but the large third instar larvae may totally eradicate the root system. A single individual is capable of damaging several trees. The damage results in discoloration of the needles and in case of extensive feeding on the root system the trees eventually die. Plantations on land which were recently converted from agricultural fields into Christmas tree plantations are particularly subjected to damage with the result that intensive re-plantation is required in patches throughout the plantations after a few years. Chemical control of the soil-dwelling larvae of *M. melolontha* is not allowed in Danish forestry and mechanical control is not possible in these perennial crops. The growers have therefore no current options to control the scarab larvae and thus prevent attack.

The fungus *Beauveria brongniartii* is considered to be the most important natural enemy of *M. melolontha* and promising results of biological control have been obtained in orchards and pastures in Central Europe using barley kernels colonised by this fungus (Keller, 1992; Zelger, 1996; Keller *et al*., 1997; Enkerli *et al*., 2004). It was therefore sensible to test the efficacy and applicability of this control method in the Christmas tree plantations. The studies in Denmark were therefore based on European strains of *B. brongniartii* (BIPESCO 1 or 2). The first field experiments were carried out in the spring 2000, which happened to be a flight year of *M. melolontha* (Vestergaard *et al*., 2002). Our experiments were based on inoculation biocontrol strategy that relies on establishment of the fungus for at least one season after application (Eilenberg *et al*., 2001). In a Christmas tree plantation at Vallø, (Zealand) kernels with *B. brongniartii* were inoculated in the following two ways: 1) kernels were placed in holes of 10 cm depth around existing small trees, or 2) kernels were thoroughly mixed with the soil from the planting hole and placed around the new tree during re-plantation.

In order to assess the damage, we developed a score index of the health status of the trees, based on needle colour. Category 5 meant that the trees had no damage and that the needles were shiny and dark green. Trees in category 4 had a slight discoloration of their needles, indicating decreasing vitality. Trees in category 4 were in risk of ending in a lower category before harvest. Category 1-3 showed substantial discolouration and needle loss, category 1 referring to trees verging on death. Category 0 was used to characterize dead trees. Health scores were performed in autumn 2001 and 2002, more than one and two years, respectively, after treatments. In addition to assessing the health score we also surveyed the persistence of the fungus over time and the effect of fungal treatment on non-target insects.

The application of fungus via inoculation kernels in the soil during re-plantation resulted in statistically significant effects of the treatment. It is especially noteworthy that in the treated plots there were 30 % more trees in category 5 compared with untreated plots, which clearly indicates the advantage of the treatment. The density of *B. brongniartii* colony forming units (cfu) in the soil was approximately the same one year after application, as the density

immediately after application. The lowest density found was 10^3– 10^4 colony forming units (cfu's) per g soil while the highest density was 10^5 – 10^6 cfu's per g soil (Vestergaard *et al*., 2002). In spite of intensive sampling of insects from several orders we did not find any infected non-target insects in the plots. Thus, long-term control of cockchafers can be achieved using simple methods.

Based on these data a second experiment was initiated in Northern Jutland in spring 2002 in order to: 1) test the dosage of fungus needed for successful control of scarab larvae in the plantations and 2) apply the fungus by a simple and practically feasible method. The experiments were carried out in an area where severe and lethal damages on newly planted Christmas trees had occurred on several occasions at four-year intervals. In connection with an extensive replanting resulting from a major attack by *Melolontha* larvae two different dosages were tested: 1) 10 g barley kernels per tree and 2) 30 g barley kernels per tree. The colonised barley kernels were simply thrown directly into the planting hole before the small tree was inserted into the hole, thus avoiding the time consuming thorough mixing of kernels into the soil as done in the previous experiment.

After 6 months an effect of the biocontrol treatments was apparent: In the *B. brongniartii* treated plots 98% (low dosage) and 100 % (high dosage) of the trees were scored as category 4 and 5 compared to about 93% of the control trees. This effect increased significantly after 1 ½ year when almost 13% of the untreated trees had been killed and another 25% showed decreasing health (category 1-3). None of the treated trees died and only single trees showed discolouration. The benefits of the biocontrol agent were apparent more than two years after the application. There was no difference in effect between the low and the high dose of fungus applied.

Based on our finding we suggest an easily applicable system for long lasting control of *M. melolontha* in *A. nordmanniana* plantations: during plantation or re-plantation, kernels with the fungus *B. brongniartii* are simply placed in the planting hole.

3. Strophosoma spp. in decoration green plantations

In the Danish production of decoration green, weevils (Coleoptera: Curculionidae) are frequently occurring insect pests. In particular, two species from the genus *Strophosoma,* the nut leaf weevil *S. melanogrammum* and *S. capitatum* are economically important pests (Harding, 1993; Kirkeby-Thomsen and Ravn, 1997; Thorbek and Ravn, 1999; Ravn, 2000). The damage is caused by the adult weevils feeding on the needles. The weevils feed on current-year needles as well as older needles. Although weevil damage is observed in the whole canopy, the damage is most pronounced in the top of the crown, where also the needles of the leader are frequently heavily grazed upon. The damage may result in economically significant losses for the growers, since branches harvested and sold as decoration green must be completely without damage.

The two weevil species exhibit a fascinating life cycle, which was studied as part of the experiments by means of sticky traps, funnel traps, emergence cages, and soil samples. In spring, overwintering adults emerge from the soil and start feeding on the needles before oviposition, which takes place on the shoots in the tree canopy. In order to get to the top of the trees the weevils need to climb the stems. After egg hatch the first instar larvae drop from the canopy to the ground in early summer. The 'shower' of small larvae can be as high as almost 3000 larvae per m^2. The larvae enter the soil and spend the rest of their time as larvae in the upper soil layers feeding on small roots. The following year the larvae pupate and emerge into

the new adult generation in August-September. The new generation of adults start feeding on the needles again before they hibernate in the soil. Thus, the complete life cycle is approximately 15-18 months for both species.

Only very little is known about the naturally occurring enemies of *Strophosoma* spp. However, although no records existed of insect pathogens on the weevils, initial bioassay in the laboratory documented that fungal biocontrol agents could infect both adults and larvae of this pest insect. Under field conditions the adult weevils could potentially be targeted by a soil application in late spring upon emergence from the soil and before oviposition in the canopy and the larvae could be targeted by a soil application in mid summer. An application in mid summer would possibly also persist to infect the new generation of adult weevils emerging from the soil later in the season. We therefore decided to test if a biocontrol agent could successfully be applied in spring or summer.

As a biocontrol agent the fungus *Metarhizium anisopliae* (BIPESCO 5) was used. The fungus is not a natural occurring enemy of *Strophosoma* species in Denmark, and the fungus did no toccur naturally in the soil in the selected *A. procera* plots. *M. anisopliae* was chosen because of existing knowledge about the growth conditions *in vitro* in medium-large scale and thus its potential for industrial production. The fungus is available in several countries, including some European, as a product for insect control, including weevil species from other genera, for example the genus *Otiorhynchus,* but mostly in rather controlled environments: glasshouses or strawberry crops. It had, however, never been tested before against *Strophosoma* spp. in perennial cropping systems, and the application in a stand of *A. procera* gave additional challenges: grass, shrubs, wilted twigs and branches from earlier harvests covered parts of the area. Conidia of *M. anisopliae* were suspended in an aqueous suspension before application as an inundation biocontrol agent using a personal back-pack spray device.

The evaluation of the effect of the fungal treatment was based on estimations of population densities of the two *Strophosoma* species. This was measured by weekly counts of adult weevils emerging from the soil. In addition, we assessed the prevalence of *M. anisopliae* infections in adult weevils collected after the spring application. Finally the persistence of the fungus over time as well as infection of non-target arthropods was assessed.

Immediately after fungal application we detected up to 90 % *M. anisopliae* infection in the adult weevils in the treated plots, compared to only a single infected weevil found in the untreated control plots during the entire experimental period (Nielsen *et al*., 2004).

The summary results on population effects are shown in table 1. Both spring and summer applications resulted in a lower density of the target, but the effect was not apparent until one year after application. The highest reduction was 60 % compared with untreated plots measured in autumn 2001. In none of the cases, however, did we document a long-term effect, despite the presence of significant amounts of inoculum in the treated plots up to 419 days after treatment. Inoculum was still detectable in autumn 2004, more than 3 years after application. Among non-target invertebrates collected in treated plots we documented *M. anisopliae* on ticks, on coleopterans and on hemipterans, thus some non-target effects were present.

Table 1: Effects of Metarhizium anisopliae *applied as a biological control agent against weevils from the genus* Strophosoma *in a Danish stand of* A. procera*. The plots, which were treated in summer 2000, were treated again in summer 2001. A 'YES' means that a reduction in target weevils was obtained, a 'NO' means that this was not the case*

Treatment	Autumn 2000	Spring 2001	Autumn 2001	Spring 2002	Autumn 2002	Spring 2003	Autumn 2003
Summer 2000	NO	NO	**YES**	**YES**	**YES**	NO	NO
Spring 2001			NO	NO	**YES**	NO	**YES**

Based on these data we conclude that it is feasible to obtain a significant level of control by applying *M. anisopliae* on a regular basis. A range of questions arises, however, concerning biocontrol:

- What is the optimal time of application and is control solely possible by spraying using personal equipment?
- Is it ecologically sound to use a fungus, which was not a naturally occurring enemy of the target, did not occur naturally in the soil in the forest plots, and may infect certain non-target invertebrate species?
- Should other methods be tested, for example strips baited with fungus on tree stems to infect beetles climbing to the top?
- Is the public concerned about inundating high amounts of a fungus into a forest eco-system, which is regarded as natural (although the tree itself is an exotic species)?

4. Grower's attitude

We assume that growers will adopt to biological control options, provided that the methods work and that standard technology can be used or, that some cheap and easy new technology is available. The Christmas tree and greenery growers' attitude to new technologies like biocontrol is that they are willing to pay an additional cost of 10 – 25 % compared to the conventional control methods (K. Østergaard, pers. comm.). We therefore assume that a biocontrol product can be accepted even if it may cost more than a conventional product.

Our main method for application of *B. brongniartii* as a biocontrol agent to the soil of Christmas trees was based on low technology: applying kernels with fungus into the planting hole during plantation or re-plantation. The method is based on an existing *B. brongniartii* product, and an application of biocontrol will provide protection of a high value crop at a very low cost. The main obstacle will probably be convincing the growers that many of them would have to use the fungus as prophylaxis, despite the fact that only some areas will suffer from serious damage. We feel, however, that the implementation of an easy to use guideline will assist the growers in benefiting from the biocontrol.

The method for application of *M. anisopliae* as a biocontrol agent of weevils needs optimization before we can recommend it to the growers and prepare easy to use guidelines.

5. Public perception

It is normally assumed that many consumers prefer crops produced without chemical agents such as conventional pesticides. The present situation in Denmark with many organic products sold at a higher price than conventionally grown food products documents that consumers are in general willing to pay a higher price in order to avoid chemicals. The sales of organically grown Christmas trees and decoration green have increased in Denmark over the years and may point towards a future demand for such products. The question is how the public will accept biocontrol in Christmas trees and greenery plantations and how much more they are willing to pay for such products. Our studies were not accompanied by socio-economic studies, which could document the public attitude. It is, however, our impression that Christmas trees and decoration green provide an excellent example of crops, for which biocontrol programmes will immediately receive public acceptance for a number of reasons:

1) Christmas trees and decoration green are products with strong emotional aspects. They are used in the month of December as part of Christmas celebrations and, for many people, a range of important family events. In Denmark, Christmas time is the most significant family event during the year. The trees and branches of conifers to be used should reflect the importance of this emotional significance by being environmentally sound.
2) The trees are grown in forests and woods and especially around major cities, the public are frequent visitors using the production areas. The plantations and the pest control attempts are thus highly exposed to the public, and our studies received much public attention. The biocontrol studies of *M. melolontha* were subjected to a presentation in prime time news on the major national television channel, the first of our biocontrol experiments ever to receive such exposure. The story was presented very positively as an example of environmentally sound plant protection and research directed to public benefit. As part of our experiments to control weevils in the greenery plantation, we placed a poster in the forest plot with a short explanation of the purpose of the studies, our names and how to contact us. The plot was situated near a small road used by people biking or walking in the forest. Responses by people passing by and watching our sampling and other activities during the season were very positive and we regard the poster as an important element in the communication with the public. The local newspapers reported on the experiments and local people, who were curious to learn about the biocontrol experiments, frequently approached the people employed in the forest. We received *no* negative comments to our application of a fungus by inundation in the plots. Based on these personal experiences with communication with the public we conclude therefore that implementation of biocontrol in both Christmas trees and in decoration green would be very well received.

6. Perspectives

Concerning the development from an initial potentially good idea onto a marketable product and application methods for commercial use, an obvious question is, however: who pays? The growers' economic situation is often very sensitive to small changes in the market prices, and in some years the growers face an economically difficult situation. The grower's attitude is thus that they support biocontrol experiments on their sites without compensation, while they do not have funding for the development of biocontrol products. For companies involved in biocontrol products, the Christmas tree and decoration green markets are inferior, due to the overall small (yet increasing) number of hectares in Europe. Therefore, it is solely to be expected that companies will only contribute by using existing products with very few modifications for a new market. Public funding has so far financed the major parts of the studies presented here. Potentially additional funding for testing can be achieved but it is not to be expected that public funding can in itself guarantee product development.

We believe that biocontrol of pest insects in Christmas trees and decoration green has great potential (table 2).

Table 2: Overview of important parameters on our evaluation of the potential for biocontrol in the two crop-pest-fungus systems

1: Products based on Metarhizium anisopliae *exist but so far for other targets and/or other cropping systems*
2: Based on the assumption that inundation by simple spraying equipment will be tested further

Parameter	*A. nordmanniana* *M. melolontha* *B. brongniartii*	*A. procera* *Strophosoma* spp. *M. anisopliae*
High value of crop	YES	YES
Economic losses due to pest insect	YES	YES
Chemical control feasible	NO	NO
Biocontrol agent a natural enemy of target insect	YES	NO
Biocontrol agent commercially available	YES	NO[1]
Application system simple and cheap	YES	YES[2]
Biocontrol based on inoculation	YES	NO
Biocontrol based on inundation	NO	YES
Immediate effects on target	NO	NO
Effect of biocontrol after approximately one year	YES	YES
Lasting effects on target expected	YES	NO
Non-target effects in treated plots	NO	YES
Grower's acceptance	YES	YES
Public acceptance	YES	YES

Biocontrol in these crops will meet the consumer's demands and a successful biocontrol in crops with emotional significance has further value as models for teaching the public about biocontrol in general. It is therefore our hope that the obvious advantages of biocontrol combined with easily applicable technologies will allow the development towards practical biocontrol in Christmas trees and decoration green in Europe and elsewhere.

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CHAPTER 13

AN INTEGRATED APPROACH TO BIOLOGICAL CONTROL OF PLANT DISEASES AND WEEDS IN EUROPE

Maurizio Vurro and Jonathan Gressel

1. Introduction

Biocontrol has been ineffective against major agricultural pests in the field, and has not provided the tools to cost-effectively compete with chemical pesticides, despite the theoretical benefits. The ecological and evolutionary reasons for the lack of effectiveness have been examined in detail in a recent book (Vurro *et al.,* 2001a) along with suggestions on how to safely enhance their activity.

The advent of the first call of the 6th European Union[a] Framework Programme for Research and Technological Development requesting research projects looking for "safer and environmentally friendly production methods and technologies and healthier foodstuffs", with the specific challenge "to develop lower input farming systems based on systems such as integrated production and organic agriculture", provided the impetus to found a consortium to enhance biocontrol agents so that they might actually fill the gap.

Suitable targets were chosen and a team organized with the necessary expertise. Plans were advanced with reasonable objectives and the scientific activities integrated, and the project was funded. This integrated project is described below.

2. The targets: uncontrollable agricultural pests

Among all the living organisms that can attack crops causing qualitative and quantitative reduction of production, those living in the soil, such as plant pathogens and weeds are among the worst and the more difficult to control by traditional methods and strategies.

2.1 Soil borne plant pathogens

Soil borne plant pathogens are a major problem in many open field and greenhouse crops. Pathogens are often able to survive for several years in the soil as dormant, environmentally persistent resting structures, until a susceptible crop is introduced. The pathogens responsible for damping off, crown and root rots, as well as wilts are of utmost importance in vegetable crops. Various *Pythium, Rhizoctonia,* and *Phytophthora* spp. Damage the lower part of tomato, pepper, cucumber, and many other vegetables, both in soil and soil-less cultures. *Sclerotinia sclerotiorum* is an important soil-borne pathogen responsible for the rot disease of over 400 plant species, including economically important field and glasshouse crops (Boland & Hall, 1994), and survives between crops in the soil as sclerotia (Coley-Smith & Cooke, 1971;

J. Eilenberg and H.M.T. Hokkanen (eds.),An Ecological and Societal Approach to Biological Control, 257–274.

Merriman, 1976). These sclerotia may germinate, producing mycelia that then infect the plant directly or, more typically in glasshouse crops, produce large numbers of ascospores with the potential to infect plants over a wide area. The crown-rot and wilt-inducing strains of *Fusarium oxysporum* are responsible for severe damage to many important crops (tomato, cucumber, muskmelon, asparagus, radish, onion, flax, carnation, and cyclamen). *Fusarium* wilt pathogens have a high level of host specificity and are classified into more than 1200 *formae speciales* and races.

2.2. Parasitic and perennial weeds

The parasitic *Orobanche* species (broomrapes) attack nearly all vegetables, legumes, and sunflowers in southern Europe to the Balkans and Russia, the Middle East and North Africa. *O. ramosa* and *O. aegyptiaca* infest about 2.6 millions hectares planted in the Solanaceae and grain legume crops, particularly tobacco, potato, tomato, eggplant, chickpeas, peas, and faba beans (Sauerborn, 1991). *O. cumana* severely restricts and limits sunflower production in Spain and eastern Europe. The broomrapes interfere with water and mineral intake and by affecting photosynthate partitioning, and are responsible for both qualitative and quantitative damage to these high value crops.

These parasitic plants can produce 10^4 - 10^5 seeds per flower stalk, which can remain viable for many years. They germinate after stimulation by host root exudates, and produce a germ tube that can attach and develop a haustorium penetrating the root, forming a tubercle. This is followed by the most damaging phase, with the parasitic withdrawal of water, nutrients and photosynthates from the host. Due to the long underground tubercle phase, flower stalk emergence occurs only when most of the damage has already been produced.

Perennial weeds are among the most troublesome weeds to manage. *Cirsium arvense* is considered one of the world's worst weeds (Holm *et al*., 1977), and the third most important weed in Europe (Schroeder *et al*., 1993). The weediness of this species is largely attributed to its capacity for vegetative reproduction and regenerative growth by recruitment of shoots from adventitious buds on a creeping root system (Donald, 1994). *Sonchus arvensis* is another perennial species that presents a considerable weed control challenge, especially to organic farming. *Cyperus esculentus*, another top ten "Worlds Worst Weeds" (Holm 1977) is one of the most serious invasive alien species in southern Europe, both in crops and non crop areas.

3. Traditional solutions: usefulness and limits

For years, the most common approach to the control of the above pest problems was soil fumigation, before or after cropping. Many fumigants are health hazards, environmental pollutants, and even contribute to atmospheric ozone depletion. Increased environmental concern has triggered regulatory restrictions on treatments with soil fumigants, drastically reducing methyl bromide use, which had been the most widespread and effective soil fumigant. Fumigants such as 1,2-dibromochloropropane (DBCP) and ethylene dibromide (EDB) are generally less effective than methyl bromide in controlling pathogens and weeds. Their use has been discontinued or suspended in many countries. In many crops no real alternatives to methyl bromide have been found. Furthermore, soil fumigants (as well as solar heat treatment) reach pathogens and weed seeds in all physical and biological niches in the soil. As a result, their use often also leads to the eradication of beneficial organisms, and a negative shift in the biological

equilibrium. This creates a biological vacuum, which then leads to an increase in the population of pathogens, causing more damage than those originally targeted for control. Soils, especially those with low microbial populations are more vulnerable to reinvasion of pathogens following fumigation. Thus, non-chemical methods of selectively controlling soilborne diseases and weeds are needed.

Other control strategies such as soil solarization (allowable in organic agriculture) could be possible, but have environmental and temporal constraints, as warmer temperatures than normally found in Europe are needed and the fields must remain covered for much of a growing season. Seed treatments with conventional fungicides provide some initial protection to soil pathogens, but this is not effective for a sufficient duration in heavily infested soils. None of the old and environmentally unfriendly fungicides still allowable in organic agriculture (copper salts, sulfur) are very effective against soilborne pathogens.

Traditional control methods have been tried against parasitic weeds on different crops, but none have proved to be effective. *Orobanche* spp. usually cannot be managed by persistent selective herbicides, since herbicides are not able to differentiate between the crop and the parasite, except with herbicide-resistant transgenic crops (Joel *et al*., 1995; Aviv *et al*., 2002) or with mutant crops such as imidazolinone resistant sunflowers. Multiple applications of low rates of crop-degraded herbicides can provide a modicum of control and may be more useful when integrated with other methods, such as biological control (Hershenhorn *et al*., 1998). Furthermore, as these weeds attach to crop roots, they cannot be controlled mechanically, except by removing their flower stalks to reduce seed accumulation and dispersal. Recently, inexpensive seed treatments have been developed for a different parasitic weed – *Striga* in Africa, using 20 times less herbicide than would be sprayed on a field, yet providing season long control (Kanampiu *et al*., 2003).

Perennial weeds are difficult to control using traditional methods because they usually cannot be easily removed mechanically, due to their well developed root systems or subterranean organs, and because repetitive chemical treatments are often required, which are expensive in conventional agriculture. None of the few very old and environmentally unfriendly herbicides (sulfuric acid, perchlorate, soaps) allowable in organic agriculture actually control perennial or parasitic in weeds. Weed control is considered the major expense in, and major biotic limitation to, organic agriculture. Soil degrading and energy expensive mechanical cultivation as well as back-breading manual weeding are the major alternatives to herbicides, but are also ineffectual against perennial and parasitic weeds, suggesting the need for new paradigms to deal with an old problem.

4. Biological control with fungi: a solution with benefits and limits

Some effort during the last few decades has been dedicated to biological control of weeds and plant diseases, but it is a fraction of the efforts expended on developing new chemical pesticides.

Many potential microorganisms were found, but their use is still very limited. This is due to many evolutionary constraints, including: biological (virulence, stability, defence mechanisms of the target pest, interaction with other microorganisms); technological (poor sporulation, lost of aggressiveness, special growth requirements); environmental (need for extended dew periods for establishment, physical characteristics of the soil (physical and chemical barriers) and

commercial (limited market, registration problems including secondary toxicity and registration costs, costs of production).

All the host-specific organisms proposed for inundative biocontrol had evolved to be in equilibrium with their weed/pathogen hosts. The hypervirulence needed by the farmer could lead to mutual extinction of the biocontrol agent and its host. Thus, virulence of the organisms must be enhanced to overcome these evolutionary barriers to provide similar disease and weed control as conventional pesticides. Only a limited number of commercial products are available against a few of the greenhouses diseases, and no commercial bioherbicides are available in Europe. Cost has not been the major limiting factor for the adoption of biocontrol agents for the pest constraints discussed above. If effective, almost anything could compete with the cost of methyl bromide in high input agriculture, or with mechanical cultivation in low input agriculture. The major problems have been with consistency and lack of near complete control activity, when biocontrol agents are active. The systems used by the team are described below.

4.1. Coniothyrium minitans

Coniothyrium minitans is an obligate mycoparasite of ascomycetous sclerotium-forming fungi, including important plant pathogenic species of *Sclerotinia* and *Sclerotium,* such as *Sclerotinia sclerotiorum*, *S. minor*, *S. trifoliorum* and *Sclerotium cepivorum* (Whipps & Gerlagh, 1992). They act by infecting and reducing the viability of the sclerotia in soil.

Unlike other mycoparasites of sclerotia, such as certain *Trichoderma* species and *Sporidesmium sclerotivorum, Coniothyrium* does not grow through soil and initially would not appear to be a likely candidate for long-term successful biocontrol. Nevertheless, it survives well in soil, and can be recovered three years after application in the field (McQuilken *et al*., 1995). Other mechanisms of its spread are involved, such as water splash and aerosol dispersal, which allow sclerotia of *S. sclerotiorum* to be infected over 2 metres away from a source of *C. minitans*. It is also dispersed by slugs, collembolans, mites and sciarid larvae (Turner & Tribe, 1976; Williams *et al*., 1998 a, b; Whipps, 1993; Whipps & Budge, 1993).

The potential use of *C. minitans* as a biocontrol agent by soil incorporation of solid substrate has long been recognised. The organism has been successfully used in glasshouse and field experiments to control *Sclerotinia* diseases of a number of crop plants (Whipps & Lumsden, 2001). A commercial product has been registered in seven European countries, the USA, and Mexico. The major constraints of its wider use in agricultural practice in the field outside of glasshouses are the limited knowledge of its ecology, and the scanty information on its physiology and genetics, preventing attempts at strain improvement.

4.2. Trichoderma spp.

Trichoderma strains are among the more effective fungi applied against fungal diseases, both in conventional and organic farming. They are commercially produced and several patents protect their use (Harman et al., 1994, 1996). Biopesticides based on antagonistic *Trichoderma* strains are used for biocontrol of phytopathogenic fungi causing root and crown rot of vegetable seedlings damping off, vascular diseases, ‘take all’ of cereals, etc. (Harman & Björkman, 1998). *Trichoderma* strains are registered both in Europe and USA as the active principles of biopesticide formulations, and are allowed in organic farming.

Regardless of the obvious potential, there are some problems that limit the development and application of these biofungicides. In addition to the lack of strains for every disease, and of

very effective and optimally-formulated preparations, there is a limited availability of basic information for further product registration, including a sufficient knowledge of the mechanisms of action and interaction with other biocontrol agents. More efficacy tests are needed in each geographic area where the product has to be registered. Methods for monitoring the production of possibly mammalian toxic metabolites produced by some of these fungi are necessary to allow an evaluation of possible risks derived from large scale application.

4.3. *Antagonistic Fusarium spp.*

The concept of using non-pathogenic strains of *Fusarium oxysporum* to control *Fusarium* diseases came from the demonstration that the suppression of the disease in suppressive soils results from interactions between pathogenic and non-pathogenic strains. Therefore, non-pathogenic strains were developed as biocontrol agents (Lemanceau & Alabouvette, 1991). The non-pathogenic *F. oxysporum* strains have several modes of action contributing to their biocontrol capacity (Couteaudier & Alabouvette, 1990; Lemanceau *et al*., 1993). They are able to compete for nutrients in the soil, suppressing pathogen chlamydospore germination. They can also compete for infection sites on the root, and can trigger plant defence reactions, inducing systemic resistance (Fuchs *et al*., 1997). Several strains of non-pathogenic *F. oxysporum* have good efficacy in many trials, but as with other biocontrol agents, there is a lack of consistency.

4.4. Potential mycoherbicides

Despite isolation of many promising pathogenic organisms that could be useful for control of parasitic weeds, none has received continual widespread use. Two very promising strains of *F. arthrosporioides* and *F. oxysporum* were isolated in Israel from juvenile *O. aegyptiaca* plants. They also attacked *O. ramosa* and *O. cernua* (Amsellem *et al.*, 2001). Some very promising strains were also isolated in Italy, (Boari & Vurro, 2004). These species can be formulated as mycelia, reducing the dew period and the expense of spore production (Amsellem *et al.,* 1999). It was also possible to enhance their activity two fold by engineering in genes for overproduction of auxin (Cohen *et al*., 2002), but more than a doubling of virulence is needed.

Perennial weeds in arable farming are ideal targets for biological control, that could replace one or more herbicide treatments. In organic farming systems, biological control of perennials, especially *Cirsium arvense*, would reduce the number of time consuming, expensive, and soil degrading mechanical treatments that require large amounts of fossil fuel compared to biocontrol and to herbicides.

Phomopsis cirsii, Ramularia circii, and *Septoria cirsii* were chosen as promising candidates in systematic field surveys of diseased *C. arvense* carried out in Denmark (Leth & Andreasen, 1999) and Russia (Berestetski, 1997). Their necrotrophic nature makes them able to grow in liquid artificial media. *Sonchus arvensis* is another perennial species that is an ideal target for biological control. Several virulent pathogens have been isolated by the partners (Berestetski & Smolyaninoca, 1998), but their efficacy has to be fully evaluated and improved.

5. The team

The group has been gathered on the basis of the proven excellence of each partner in a field of research and its ability of carrying out innovative activities in biological control. Among the nine partners, coming from seven different countries (as shown in the map), there had been complementarities and interactivity through international projects and COST actions (http://cost.cordis.lu). Many team members participated in a workshop on enrich biocontrol agents (Vurro *et al*., 2001a). Each group has a long tradition in research on biological control, leading to important scientific, technological and applicable contributions in their respective complementary fields of interest.

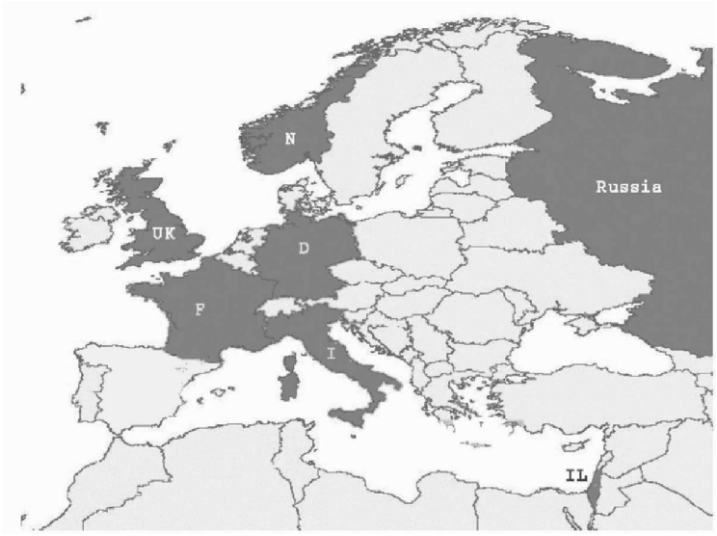

Figure 1: European countries involved in the project (in dark grey)

Many different microorganisms are considered into the project, and thus, many different types of biotechnological, molecular, physiological, and applicative expertise were needed. Even though each group works on the organisms on which it has already accumulated a high level of knowledge, this expertise will be made available for the enhancement of other's microorganisms. Each partner works in collaboration with several partners, in more than one task, and on more than one organism. For example, four partners are involved with *Coniothyrium* studies, five on *Trichoderma*, four on antagonistic *Fusarium*, six on perennial or parasitic plants.

Each working group includes experts in mycology, physiology, biotechnologies, molecular biology, chemistry, weed and crop science, allowing for multifaceted work-plans. Each partner has well suited laboratories for the project activities. A continuous flow and exchange of materials, strains, technologies and protocols has been created within sub-packages, which should allow attaining the planned objectives.

6. The project

The project has been divided in nine interactive and transversal work-packages, starting from the genetic and physiological enhancement of microorganisms to their application and the assessment of field efficacy, until the evaluation of food quality after their use, and the acceptability by consumers. Some of the main tasks of each work-package are briefly illustrated below.

6.1. Efficacy enhancement through the knowledge of genetic characters

Scientific risk assessment is used throughout the project to ascertain where risk may be of consequence, as well as to inform the public and allay not always rational fears, where appropriate, or to devise methods to minimize risk when there is one.

Within the project, the extensive use of genetic tools and techniques has the main aim to understand the modes of action of biocontrol agents and to manipulate gene expression to enable their better and safe use in the future. Thus, transgenics are considered to suppress the production of mammalian toxins by otherwise excellent "natural" biocontrol agents. A further important aim is to study new instruments to obtain more efficacious fungal strains, and to carefully ascertain the impact of their release.

One of the activities planned is to identify changes in enzyme production and gene expression by biocontrol agents during infection of the host. The spectrum of enzymes involved in biocontrol activity is known to include glucanases, chitinases, lipases and proteases but knowledge of their quality, regulation and characterisation is often poor (Lorito *et al*., 1993). Knowledge of these factors should help deployment of biocontrol agents under optimal environmental conditions for activity. Even though the involvement of cell-wall degrading enzymes in pathogenicity by fungal biocontrol agents is well established, the changes in genes controlling other pathogenicity traits are not well known. The project examines changes in gene expression during early stages of infection. Genes differentially expressed during various stages of infection will be identified and cloned using macro and microarray technologies and suppressive subtractive hybridisations using genomic and stage specific libraries (Yang *et al*., 1999). Transgenic overexpression of these same genes can later be considered to enhance virulence.

It is essential to identify the biocontrol agent following application and during ecological impact and risk assessment studies concerning the use of biocontrol agents in the glasshouse and field. One way of doing this is to introduce genetic markers that facilitate easy recovery and monitoring, and such genetically-marked strains of *Coniothyrium minitans* and *Fusarium oxysporum* are already available (Eparvier & Alabouvette, 1994) for use in the environmental impact studies. Nevertheless, other DNA based marker systems such as "biobarcoding" with pre-planned and easy to detect nonsense (non-coding) sequences (Gressel & Ehrlich, 2002) may prove valuable, potentially more sensitive and environmentally safer alternatives to those containing coding genetic inserts.

Several transformation-based techniques are beginning to appear to allow reproducible genetic modifications in fungi. It should be possible to knock out genes in the biocontrol agent as well as to transfer specific genes into the biocontrol agent, and then determine effects on pathogenicity. This will demonstrate the role of any gene in pathogenicity and, in the long term, will enable the development of more effective biocontrol agents.

The characterisation and utilisation of mating type genes in biocontrol strains to improve mycoherbicide activity is another objective. *Fusarium oxysporum* and several other *Fusarium* species that reproduce asexually harbour mating type genes, which were appropriately transcribed and processed (Yun *et al*., 2000). The presence of mating type genes in asexual species of *Fusarium* and the fact that they are fully functional (Moretti *et al*., 2002) is consistent with the hypothesis that asexual fungi may have a cryptic sexual cycle, even though sexual structures have never been identified in these fungal species. This will allow recognition of compatible strains that can be used in crossing experiments within each species to obtain sexual states. *In vitro* crosses to assess their sexual compatibility will be made based on this information. Strains will be selected for crossing that have high levels of pathogenicity and toxicity, to increase the chances of obtaining strains with higher levels of both traits among the progeny.

Fusarium spp. that have been engineered to control *Orobanche* spp. with genes for the over-production of auxins, provided a modicum of increased virulence, but only when the fungi were preloaded with tryptophan, a precursor for IAA biosynthesis (Cohen *et al*., 2002). Similarly a *Colletotrichum* sp. controlling *Abutilon* transformed with the same genes, was exceedingly hypervirulent, when the fungus was sprayed together with tryptophan. Mutants of many species have been selected for overproduction of anthranilate synthase by using tryptophan analogs as selectors, overproduce tryptophan (Romero *et al*., 1995), and may be useful here.

The production of asporogenic mutants of biocontrol agents would allow propagation and preclude off-target movement, as well as prevent their environmental persistence (Gressel, 2001; 2004). An important part of the task will be the study of hypervirulent and safe mycoherbicides.

6.2. Physiological enhancement of biocontrol activity

Different approaches are being used to increase the efficacy of biocontrol agents without using genetic or transgenic manipulation. New protocols will be tested with different species of biocontrol agents, including both mycoparasites and mycoherbicides, permitting the development of novel and fully integrated protocols to simultaneously enhance pathogen and weed control.

Molecular activation of specific genes occurs during the antagonist-pathogen and antagonist-plant interactions. The production of inducers of mycoparasitism released from the pathogen or the plant and "detected" by a fungal biocontrol agent has been recently demonstrated (Lorito *et al*., 2001). The project plans to identify and characterize both proteins and small molecules (as well as the genes specifically induced) produced during the complex interaction between antagonistic fungi, the plant and the pathogenic fungi, as well as in the presence of symbionts. The molecules identified will be tested for their capacity to induce physiological alterations in the plant that correlate with resistance (i.e. production of PR-proteins, accumulation of salicylic acid, accumulation of Ca^{2+}, oxidative burst and increased resistance to foliar pathogens) and control the antagonist-pathogen-plant interaction to improve biocontrol by *Trichoderma*.

The transgenically enhanced hypervirulence of a biocontrol agent has the advantage of constitutiveness: it is there, and there are no needs for additives. Conversely, if the same effect can be achieved physiologically by an additive, then there is the advantage that the organism is no different from the wild type after the additive has dissipated. For example, organisms can be mutated to supply tryptophan to an organism with the potential to be hypervirulent via over

production of auxin. Conversely, an organism could be engineered to overproduce oxalate (Gressel, 2002), to overcome calcium dependent weed defenses, or the biocontrol organism could be provided with exogenous oxalate to achieve the same hypervirulence (Gressel et al., 2002), yet the organism lacks hypervirulence when the oxalate is gone.

6.3. Ecological fitness

The biocontrol agents used in this project are expected to control soil-borne diseases or weeds, and therefore they will be directly or indirectly applied to soil. To be efficacious, biocontrol agents must establish, survive and be active in soil. Physical and chemical characteristics in soil influence the population dynamics of microorganisms. Factors such as the proportion of sand and clays, the nature of the clays, the organic matter content and the pH are very important in relation to survival and activity of microorganisms introduced in soil (Alabouvette & Steinberg, 1995; Höper *et al*., 1995). The ecological fitness (Butt *et al*., 2001) of the biocontrol agents selected during this project will be assessed, studying the ecological behaviour of the biocontrol agents in relation to soil type, climatic conditions, temperature and water potential, as well as crop species to be protected.

6.4. Environmental impact of biocontrol agents

The environmental impact of a variety of biocontrol agents will be assessed by tracking their movement, assaying non-target effects and any changes in host range (especially after genetic or physiological modifications), together with determining long term environmental persistence. All these together are part of risk assessment to evaluate whether risk mitigation is needed, developing the tools for this, where necessary.

A major effort is devoted to the identification of molecular markers to recognize strains of biocontrol agent after their release into the soil. The genetic diversity within species will be determined by using DNA molecular analysis such as sequencing of the nuclear ribosomal DNA, beta-tubulin gene, calmodulin gene and elongation factor gene, and AFLP. The sequence data obtained along with comparing sequence data available in the EMBL/GenBank databases, will allow screening for the determination of probes that would lead to the development of species-specific discriminatory primers. The AFLP polymorphisms will be used as tools for obtaining markers of fungal populations and to detect polymorphisms between the target and non-target species, to provide maximal flexibility for subsequent primer design. A real-time PCR assay will be set up using the primers designed for the detection of the biocontrol agents, as it provides both qualitative detection and quantitative determination of the fungal pathogen eliminating post-PCR processing.

The introduction of biocontrol agents into soil may pose a risk of unforeseen or detrimental activities on the soil microbial population. The EU directive 2001/36 clearly says that side effects on non target soil microorganisms should be addressed, but there are no validated methods available. Until recently, techniques for monitoring direct effects on microorganisms have been restricted to *in vitro* culture based methods that ignored 90% or more of the microbial population that could not grow on culture media in the laboratory. The study of the composition of the microbial communities will be based on the direct extraction of DNA from soils. Improvements or developments will be required to address the diversity of fungi and protozoa communities. Terminal restriction fragment length polymorphism analysis (T-RFLP) and ribosomal intergenic spacer analysis (RISA) will be adapted to the analysis of soil

communities. The molecular markers revealing shifts in the structure of the microbial communities will be cloned and sequenced for a subsequent comparison with data sets available in international databases. This will allow the identification of the microbial groups appearing as putative bio indicators of the transient and longer-term impact of biocontrol agents.

Biocontrol fungal strains, such as *Fusarium oxysporum* and *F. arthrosporioides,* transformed with innocuous markers *GUS* and *GFP* genes together with a hygromycin resistance gene are already available to follow their "natural" movement and persistence in the field. An alternative way to allow simple recognition of competing organisms in the same habitat is the insertion of non-coding biobarcode™ sequences (Gressel & Ehrlich, 2002) having universal primer pairs and variable generated sequences, using algorithms a group member developed for identifying organisms. The use of the biobarcode concept would require regulatory acquiescence that non-coding sequences are not genes, and thus the organisms bearing such sequences are not "genetically modified" in the legal sense.

6.5. Cost effective production of competitive biocontrol agents

Biological control of plant diseases, insect pests, and weeds will only successfully compete with chemical pesticides if the products are as effective as the chemical products and if they are not more expensive and complicated to use. Apart from the efficacy of the strain of the microorganism used, this is mainly dependent on how it was produced and formulated. The production technology used must ensure the highest possible yield of live propagules. The formulation must ensure an application of the propagules to the soil or to the plant, as easy or nearly as easy as the application of a chemical pesticide. One paradigm for development of biocontrol agents states that the formulation must improve or at least assist the effectiveness of the microorganism and must ensure a shelf life of the product of at least one year, better two or more years. A different paradigm states that the only element is cost-effectiveness that is competitive with current technologies. In these days of inventory control, shelf life is less important, and if the product is really good, yet requires special application techniques, custom applicators and farmers will invest in the equipment, just as they had for specific equipment for methyl bromide fumigation.

Suitable culture media for the production of the fungal propagules will be selected using an appropriate fermentation technology followed by: the evaluation of the most suitable growth conditions; the selection of the best technology to separate the propagules from the fermentation product; the evaluation of the most suitable methods and conditions for the formulation of the propagules produced; and the determination of the shelf life of the formulated products

6.6. Application methods

One of the main problems in releasing biocontrol agents is to find suitable methods of application that allow the agent to reach the target bio-constraint and to control it. Different approaches, such as the use of irrigation methods, application at the transplanting or seed coating will be developed using the different biocontrol agents, and their effect on efficacy and survival of agents can be considered.

Above or below ground, drip irrigation is often used for vegetable crops, with several advantages for the plants and the environment, such as saving of water, better management of

nutrients, and fewer weed problems. The development of the plant root systems is influenced by water, and roots tend to grow close to the water application systems. Roots and unwanted microorganisms tend to clog or foul drip irrigation systems. Microorganisms might be an ideal way to control weeds and soil pathogens, as they would be conveyed directly in proximity of the roots. There might be great benefits from such methods of application, in terms of efficacy, reduced amounts of inoculum, protection from sources of inactivation (wind, dry air, UV light), no off-target spread, and homogeneity of control.

The best methods of application of the biocontrol agents will be determined and, in particular: the compatibility of irrigation systems with the application of living microbial agents will be evaluated. Optimized application technologies of wild type and modified *Fusarium* mycelial formulations will be developed in laboratory and greenhouse for the control of *Orobanche*. Other mycoherbicides for control of *Cirsium* sp. will be developed. The ability of phytotoxins to prevent irrigator clog by weed roots will be evaluated.

6.7. Assessment of field efficacy

The targets of the biocontrol agents are plant diseases and weeds that represent problems of utmost importance for many vegetable crops throughout Europe, in all climatic and environmental conditions, both in open fields and in greenhouses. The evaluation of the efficacy of the studied biocontrol agents is of strategic importance for determining their market size. In fact, the greater the possibility to use the organisms in different environmental conditions, crops and soil conditions, the wider will be the possibility to use the same formulation everywhere.

The ultimate selection of strains or combinations of strains, formulation, application technology and timing of the most promising microbial control organisms will be evaluated in field trials with cabbage, carrots, or lettuce.

6.8. Integration of biocontrol agents with other biocontrol agents and bioactive fungal metabolites

The combination of different pathogenic biocontrol agents, and of biocontrol agents with bioactive natural compounds is another strategy to improve their efficacy. Therefore, significant integrated research is likely to produce readily applicable protocols for effective exploitation of various biocontrol agents.

Several cell wall degrading enzymes and antibiotics play a major role in the complex biological processes involving *Trichoderma* strains for biocontrol (Harman & Kubicek, 1998). Some of these are applicable, both as proteins or genes, for the development of new defense strategies, transgenic and not, against phytopathogenic fungi (Lorito *et al*., 1998). It appears especially promising to use of mixtures of these enzymes (chitinases and glucanases) capable of degrading the fungal cell walls, since they are active on a wide spectrum of fungi. They are produced in large amounts by *Trichoderma* spp., are stable at room temperature, are capable of reaching efficacy levels similar to that of chemical fungicides.

Phytotoxic metabolites can weaken defence mechanisms of plants, rendering them more susceptible to pathogen attack. Thus, the application of toxins jointly with the pathogens could strongly enhance their bioherbicidal properties (Vurro *et al*., 2001b).

The use of combinations of biocontrol agents may also synergize the efficacy and reliability of biocontrol. For example, the combination of non-pathogenic *Fusarium* spp. plus a

Pseudomonad was more effective in controlling Fusarium wilts than either organism used singly (Alabouvette *et al*., 1996). Combinations of microorganisms must be fully compatible, i.e., the components of the microbial inoculant mixture must express their antagonistic activity against the target organism but not against each other. The metabolites produced by a component of the mixture must not interfere with growth and activity of the other components and possibly act synergistically with metabolites produced by these latter. Such an interaction has been shown with *Pseudomonas* lipopeptides and fungal cell wall-degrading enzymes of *Trichoderma* (Fogliano *et al*., 2002). A combination of antagonistic *Trichoderma* and non-pathogenic *Fusarium oxysporum* would be highly desirable because it would achieve better control of soil-borne pathogens of vegetable crops than either *Trichoderma* or *F. oxysporum* alone. However, *Fusarium oxysporum* can produce metabolites with antifungal activity (e.g. enniatins, fusaric acid) that could inhibit antagonistic *Trichoderma* spp. Conversely, some *Trichoderma* spp. produce isonitrile and peptide antibiotics (peptaibols), which inhibit fungal and bacterial growth.

6.9. Assessment of crop quality

Besides the ability of microbial agents to control bio-constraints, some strains may improve plant growth and productivity by other effects on the crops. Some *Trichoderma* strains have improved tolerance to stress, better induced resistance, and some solubilise and sequester of inorganic nutrients and enhanced uptake of nutrients by plants (Altomare *et al*., 1999; Bailey & Lumsden, 1998). The increased availability of both macro- and micro-nutrients to plants due to biocontrol agent activity may not only result in better plant growth, but also in a change in the general physiological state of the plant, which in turn influences its health, yield and most likely also the product quality in terms of nutritional factors, shelf life or taste. For this, comparative evaluations of nutritional value of biocontrol agents treated tomatoes vs. conventional products will be carried out.

The evaluation of the olfactory features of the crops has a strategic importance for the food companies that have to position their products correctly on the market and check consumer preferences or deal with special consumer sectors. Olfactory evaluations are also needed to check the effects caused by modifications in the production processes or the raw material, and to identify the ideal profile of a product by eliminating the defects. Objective measurements by panels (Scanlan, 1977) are valuable tools for development of high quality products.

7. Objectives relevant to the food quality and safety priorities of the EU

The first consequence of the improvement of the efficacy of biocontrol agents should be their wider use and consequently a reduction of the use of chemicals in Europe. Many European consumers have increased their interest in products obtained by organic or low input farming systems. Conversely, it is not cost-effective, and sometimes near impossible to produce healthy fruits and vegetables without means of controlling weeds, pathogens, and insect pests. Partially diseased or insect damaged fruits and vegetables often contain toxins produced by the plants to ward off the pests, or they contain the mycotoxins produced by pathogens of the crops, and these ‘natural’ chemicals often have human toxicities.

The use of alternative and environmentally friendly biocontrol systems for weed, disease and insect control in food production must be compatible with accepted concepts of food

quality and safety. Management strategies to reduce chemicals are welcomed by both the public and by governments. For those reasons, the proposed project fulfils the primary objective of this EU Thematic Priority, that is to improve the health and well being of European citizens through a higher quality of food, with improved control of food production and of related environmental factors.

The quality and safety of food products is assured throughout a very long chain that begins in the field. The growth and the harvest of safe and high-quality crops is the first essential step in the production wholesome nourishment, and the use of technologies and strategies having the least possible inputs is of utmost importance to attain those quality products.

Despite the increased attention in biological control, the market of biocontrol microbes is presently still quite small. Some fungi are produced and sold by local companies, within a well defined niche, often without being registered as biocontrol agents, such as *Trichoderma* or bacteria species that are often registered and commercialized as bio-fertilizers or plant strengtheners. This is in part due to: the ease of registering microorganisms with vague 'growth promotion' activity compared to registration of a microbial pesticide; the lack of availability of efficacious agents; the lack of interest of the large companies in these products; the lack of knowledge of the potential of those microbes when used in different environments and crops; and, the lack of knowledge of the application methods. The availability of new and enhanced microorganisms and a well defined knowledge for their use can enlarge the market and can render those organisms more interesting as products for commercialization. One of the companies included in the project is a leader in the production and commercialization of biocontrol agents. It is actively involved in innovative aspects related to the technological properties of biological control agents that make a microorganisms suitable for use in the market. They have developed suitable media for growth and inoculum production, technologies to separate conidia, formulation, fitness, and microbial shelf life. This company can assist the group in the estimation of costs for production and formulation of microbial agents, together with the evaluation of registration procedures and the market size of those products. This will provide important support in developing technologies and opening markets for biocontrol agents.

The aim of the project fits well within the EU main objective of obtaining "safer and environmentally friendly production methods and technologies and healthier foodstuffs". In fact, the targets chosen (plant diseases and weeds) are among the worst bio-constraints of vegetable crops, acting at the soil level, and those are even more difficult to manage in low input and organic farming systems. The application of soil fumigants is one of the most efficient and widespread practices used to control soil bio-constraints' as discussed in the introduction. Most chemical control strategies are forbidden in organic agriculture, and the pesticides allowed in organic agriculture (sulphur, copper salts for fungi, pyrethrum, nicotine for insects, perchlorate, sulfuric acid, and soaps for weeds) are hardly benign to the environment, and are not cost-effective compared to the newest, more ecologically neutral synthetic pesticides. Interestingly, organic agriculture has not adopted the fermentation produced, natural herbicide, bialophos, which seems to meet all criteria for use as an organically produced material.

The European call required research to "harmonize methodologies for monitoring the effectiveness of the agents", and this request will be satisfied by the work-packages dealing with the "assessment of field efficacy" and with the "application methods", that will allow to

identify the best conditions for the use of microbial agents, and to harmonize methodologies for their application.

The programme guidelines require noteworthy attention for "risk assessment", and one work-package is specifically devoted to the evaluation of the possible undesired effects of the release of microbial agents, and systems to track the microorganisms in the soil after application or to mitigate the risk of their spread after the distribution will be developed. The influence of the introduction of antagonistic organisms on natural microbial communities will be considered, as well as the host specificity of weed pathogens and the potential to spread to crops or other non target plants.

Another requirement, that is a "large use of biotechnologies", is fully met. These, developed for enhancement of efficacy, microbial production, fermentation, application, and risk assessment, could be further used in the future as guidelines to develop other biological control agents, attracting further interest of the scientific and industrial communities.

8. Potential impact

Concern over the evolution of fungicide-resistant strains of plant pathogens and of herbicide-resistant weeds, the loss of registration of some of the more effective pesticides or their phasing out, have generated an interest in the development of alternatives to synthetic agro-chemicals that are both effective and economically feasible. This has generated an increasing interest in biological control of plant diseases, pests, and weeds as an environmentally friendly practice to be used in conventional, low-input agriculture and organic farming.

The overcoming of some of the limits for the use of biocontrol agents can increase their use on horticultural, forest and field crops, in diverse habitats, helping in creating a "European" market.

The project will attempt to reinforce competitiveness of low-input agriculture and lower inputs. The end users to benefit from this project will be consumers asking for healthy food, organic farmers asking for alternative to agrochemicals, conventional farmers desiring to lower inputs, and European companies, especially small and medium enterprises developing or marketing biocontrol agents and their application technologies.

The broad use of molecular tools for precisely tracking the microbial strain released could be of great help in evaluating its real fate in the environment after the introduction, and mitigate the ephemeral worries of the public opinion about uncontrollable microbial dispersions.

Compared to previous project dealing with biological control funded by past EU frameworks, the project described differs because is not focused on just single targets, such us one noxious weed, or damping-off agents, it is not too narrow to protect a few crops, it is not only finalized to develop products such as commercial formulations or seed treatments, and covers important agriculture constraints interesting all European countries. It is hoped the results obtained could be easily adapted to different crops or exported and applied to different agents and against different targets.

9. Notes

The project "Enhancement and Exploitation of Soil Biocontrol Agents for Bio-Constraint Management in Crops" (Acronym 2E-BCAs; contract FOOD-CT-2003-001687; http://www.2E-BCAs.org) is co-funded by the European Commission.

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CHAPTER 14

POTENTIAL HEALTH PROBLEMS DUE TO EXPOSURE IN HANDLING AND USING BIOLOGICAL CONTROL AGENTS

Hermann Strasser and Martin Kirchmair

1. Introduction

Reviewing the European field of biocontrol, a wide range of biological control agents (BCAs) have been or are developed as commercial biopesticides, but little has been invested into the research and development of the products compared to the amount spent on the discovery of chemical pesticides (Butt *et al.,* 1999). This is in contradiction to the necessities for a successful registration because "green" Europe wants to meet high safety standards for BCAs. More than 270 active ingredients are listed in the second edition of The BioPesticide Manual (Copping 2001). The author reports that the number of products which are placed in different orders such as micro-organisms, macro-organisms, natural products, semiochemicals and genes increased to over 1000. Most of the commercialised BCAs in Europe are produced and distributed by small sized enterprises (SEs) which are companies which employ fewer than 50 employees and which have an annual turnover not exceeding € 10 million. These facts are important to point out because these enterprises must calculate with small profits, if any, and very often cannot afford the high costs for a successful registration of their BCAs, which are in most cases niche products.

Risk assessment procedures are necessary for the introduction and use of BCAs (Blum *et al.* 2003). While microbial control agents (bacteria, fungi, algae, protozoa, but also virus and viroids) have been practically regulated everywhere in Europe for a long time (e.g. Council Directive 91/414/EEC), macro-organisms (mites, insects, and entomopathogenic nematodes) have not in most countries. A reason for this policy has been that most of the macro-organisms are mainly used in glasshouses and plastic tunnels. This "indoor application" negotiates a type of security, even though more than sixty percent of the beneficial organisms used in central and northern Europe are defined as "exotics", imported from tropic and subtropics regions (i.e. in Germany more than 30 exotic species are commercialised; Zimmermann 2004). Experts in Germany concluded that there is no need for hazard and risk assessment neither for man nor the environment as it is for BCAs containing micro-organisms and viruses because of the specific climatic requirements. Nevertheless, there is an ongoing discussion in many OECD as well as EU member state countries considering the inclusion of the macrobials within a regulatory system to provide general basis data on the impact on human and animal health and the environment (Blum *et al.*, 2003).

The Council Directive 91/414/EEC identifies the requirements to be submitted by an applicant for the inclusion of an active substance in Annex I to that Directive and for the authorisation of this specific BCA. Until October 2004, only five micro-organisms have been

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evaluated in terms of hazard and risk assessment for man and the environment and are listed in Annex I (Table 1).

Table 1: Micro-organisms listed in Annex I of Directive 91/414/EEC (October 2004)

Micro-organisms	*Type*	*Commercial Name*	*Category*	*Rapporteur Member State*
Paecilomyces fumosoroseus	Fungus	Preferal	Insecticide	Belgium
Coniothyrium minitans	Fungus	Contans	Fungicide	Germany
Pseudomonas chlororaphis	Bacterium	Cedomon	Fungicide	Sweden
Gliocladium catenulatum	Fungus	PreStop	Fungicide	Finland
Ampelomyces quisqualis	Fungus	AQ10	Fungicide	France

This low number alarmed national delegates and they started to rethink how to balance the system for registration of biocontrol agents. Specific advice on the preparation of a complete dossier as provided in Directive 91/414/EEC, i.e. Annex VI B, however, is still missing. A draft version of Annex VI B concerning uniform principles for authorising micro-organisms as BCAs is still under discussion because it needs modification. This observation was reported by DG SANCO in their working document SANCO/108/2002 concerning the placing of plant protection products on the market in 2003. It was claimed that "specific guidance should be provided on which procedure should be used to assess operator exposure and risk". Regarding sensitisation, it is officially proclaimed that no methods for testing dermal sensitisation are available, which are suitable for testing micro-organisms (see amended Commission Directive 2001/36/EC, Annex II B, 5.2.2). What is the consequence? The Commission Directive reads, "As a consequence of the absence of proper test methods all micro-organisms will be labelled as potential sensitisers, unless the applicant wants to demonstrate the non-sensitising potential by submitting data. Therefore, this data requirement should be regarded as not obligatory but optional, on a provisional base."

The consequence of the comprehensive Directive mentioned above is that most of the appliers cannot fulfil the requirements neither today nor in the in near future. Data on specific safety aspects such as "operator exposure and risk" are simply not available to appliers. Only a few complete studies have been conducted in the last three decades, most of them, however, dealing with *Bacillus thuringiensis* products (Siegel 2001).

Even the implementation of a "complete" dossier based on OECD format, which was requested by 31st December 2004, would not solve the problem, however. The European member states demand sufficient information/data for operator/bystander exposure from the applicants (OECD 2003). Therefore, provision of additional or more detailed technical facts on the BCA and active substances, respectively, are in the interest of European rapporteur Member State (rMS) representatives (i.e. test concentration, exposure route and time of exposure). Applicants are currently advised by OECD to use the criteria and guidelines for evaluation and decision making from those countries to which the application is made (OECD 2004). But this policy contradicts the goals of harmonisation and equal treatment of appliers in MS, respectively.

The purpose of this chapter is to summarise the literature on the safety of biological control agents with specific reference to human infection, allergies, and intoxication. Secondly, it provides an overview of the European standards for testing the safety. Lastly, it will give an

updated review on the biological/ toxicological knowledge and will analyse if potential hazards will influence future biological control.

2. Risk related to exposure of biocontrol agents

Threshold limits for toxic or mutagenic substances to protect the workers' health are well defined, but no equivalents to "threshold limit value" or "biological value for occupational tolerability" have been established for BCAs. The Commission Directive 2000/54/EC provides a set of rules to protect workers from risks related to professional exposure to biological agents at work. In this Directive, biological agents include bacteria, fungi (yeasts and moulds), viruses, genetically modified micro-organisms, cell cultures and human endoparasites which may cause infections, allergies, or toxicity. Not included within this Directive, however, are macro-organisms like mites, nematodes or insects (OECD defines this category as Invertebrate BCAs or macrobials).

Nevertheless, macrobials are included and will be treated like BCAs based on micro-organisms in this chapter. BCAs can cause three types of disease: infections, allergies, and poisoning/toxic effects (Cook *et al.* 1996).

2.1. Infections

Pathogenic micro-organisms can enter the human body by penetrating damaged skin, through needle stick injuries and bites, or by their settling on mucous membranes. They can also be inhaled or swallowed, leading to infections of the upper respiratory tract or the digestive system. Whether or not an infection occurs depends on several factors: (i) the infectious dose, (ii) the characteristics of the biological agents and (iii) the susceptibility of the host to the pathogen.

Depending on the risk level of infection, biological agents are classified in four risk groups (Commission Directive 2000/54/EC).

- Group 1: biological agents which are unlikely to cause human disease.
- Group 2: biological agents which can cause human disease and may be hazardous to workers. They are unlikely to spread in the community and there is usually an effective prophylaxis or treatment available.
- Group 3: biological agents which can cause severe human disease and present a serious hazard to workers. There is a risk of spreading in the community, but there is usually an effective prophylaxis or treatment available. Some of them are unlikely dispersed into the air.
- Group 4: biological agents which cause severe human disease and are a serious hazard to workers. They may exhibit a high risk of spreading in the community and there is usually no effective prophylaxis or treatment available.

With the exception of *Pantoea agglomerans* (risk group 2), none of the organisms used as BCAs are listed in the risk groups 2 to 4.

2.2. Allergies

Fungi and some bacteria are important allergens, especially if people are exposed to very high concentrations of these biological agents for long-term periods. However, the allergenic potential of most fungal or bacterial species is not known. It is supposed that in the long run intensive contact with cells or cell components (such as enzymes) may lead to sensitation and allergisation.

Allergies are immunologically classified in distinct subtypes. The following allergies are specified in the context of exposure to biological agents.

- Type I allergy symptoms appear within a few minutes after a person having contact with the allergen (quick-type allergy). An example of this type of allergy is "hay fever".
- The exogen allergic alveolitis (EAA), a classic type III allergy, is triggered by repeated exposure to very high concentrations of bioaerosols. Symptoms are spontaneous fever, shivering fits, headaches, muscle and joint pains, breathing problems, and chronic cough. In addition, permanent damage of the lung tissue clinically associated with impairment of the lung function has been observed (e.g. farmer's lung, humidifier lung).
- Type IV allergies include dermal allergies of the delayed type. For example, contact dermatitis is caused by microbial exposure.

2.3. Toxic effects / poisonings

Some non-allergic conditions, for example asthma-like syndrome and organic toxic dust syndrome (ODTS), are not yet fully understood, but appear to be common among farm workers. The ODTS is a flu-like illness which is triggered by respiratory exposure to organic dusts. In contrast to EAA the underlying pathogenic mechanism is not immunogenic. The exact mechanisms of toxicity are unknown but endotoxins, fungal spores or mycotoxins are believed to play a crucial role.

Sick building syndrome (SBS) is a term used to describe symptoms in humans which result from problems with indoor air quality. Common complaints include dyspnea, flu-like symptoms, watery eyes, and allergic rhinitis. Although there most likely is no single cause for SBS, fungal contamination in buildings has increasingly been linked to the listed spectrum of symptoms. Microbial volatile organic compounds (MVOC) have been suggested to affect human health but the relevance of fungal metabolites in working environments remains investigated insufficiently.

3. Reports on health problems due to BCAs

In the OECD handout for "Biological Pesticides Registration" all BCAs used to control insects and micro-organisms are described as "generally to pose little or no risk to man and the environment" (Anonymous, 2005). To verify this claim, a literature research has been conducted and is summarised in this section. The databases SciFinder Scholar, Science Citation Index and PubMed were searched for literature regarding health risks caused by BCAs, for the BCAs which are listed in the second edition of The Biopesticide Manual (Copping, 2001).

3.1. Viral BCAs

Viral BCAs are very host specific and no impacts on animal or human health due to the BCAs themselves are assumed (Saik *et al.*, 1990). Toxicity tests on baculovirus have shown that the viruses pose no risk to humans and the environment. Problems may occur regarding the formulation type and one can predict allergic reactions, especially to contaminations with insect proteins remaining from the production process if individuals are exposed to viral BCAs over a long-term period. Therefore, workers are advised to wear protective clothing to prevent possible irritation from handling and applying these viral BCAs.

3.2. Bacterial BCAs

3.2.1. Infections

Since the discovery of the insecticidal activity of *Bacillus thuringiensis* (Bt) at the beginning of the twentieth century, the bacterium has been used increasingly against various insect pests. In spite of the extensive use of Bt products, only sporadic clinical case reports have been published (e. g. Damgaard *et al.,*1997, Samples & Buettner 1983). The same observation holds true for other bacteria used as BCAs: Bacteremia caused by *Agrobacterium radiobacter* (Amaya & Edwards 2003)*, Bacillus sphaericus (*Castagnola *et al.* 2001)*, Burkholderia cepacia* (Teng *et al.*, 2001) and *Burkholderia gladioli* (Shin *et al*., 1997), has been described mainly in catheterised patients. In cystic fibroses patients infections with *B. cepacia* were published (Rogers *et al.*, 2003, Tanser *et al.*, 2000). However, in all of these cases the infections were due to impaired general conditions of the patients.

More serious consequences are observed with infections following a traumatic inoculation like plant thorn or wood sliver injury. Septic arthritis caused by *Pantoea agglomerans* after such injuries were reviewed by Kratz *et al.* (2003).

3.2.2. Allergies

Inhalation of Gram-negative bacteria has a dual immunological significance. In infants exposure to high doses of these allergens might have a protective function against atopy. This is consistent with what has been reported for endotoxins (“hygiene hypothesis”). Whereas in established allergic inflammation the innate immune response evoked by allergens may contribute to the pathogenesis (Renz & Herz, 2002).

Little is known about allergic reactions against bacteria used in biocontrol. Exposure to Bt spray products may lead to either allergic skin sensitisation and induction of IgE and IgG antibodies, or both (Bernstein *et al.*, 1999). Doekes *et al.* (2004) conclude in a respiratory health study among Danish greenhouse workers that exposure to Bt microbial biopesticides may comprise a risk of IgE-mediated sensitisation. Once again the underlying message is that respiratory diseases are preventable by controlling harmful exposures to organic dust, toxic gases and chemicals. For this reason, all personnel have to use recommended protective equipment.

3.3. Fungal BCAs

3.3.1. Infections

Only few fungal species cause deep mycoses in immunocompetent people when inhaled. In general, the risk to acquire such an infection by opportunistic pathogenic fungi is very low. But there are several reported cases of such infections evolving after traumatic inoculation in literature. For example, cases of keratitis were caused by fungi such as *Beauveria bassiana* (Kisla *et al.*, 2000), *Colletotrichum gloesporoides* (Yamamoto *et al.*, 2001), *Metarhizium anisopliae* (Cepero de Garcia *et al.*, 1997) or *Paecilomyces lilacinus* (Anderson *et al.*, 2004) used as BCAs. Hall *et al.* (2004) have documented a case of cutaneous hyalohyphomycoses caused by *P. lilacinus*.

Nevertheless, fungal BCAs have not gained recognition as common health issues in literature. Therefore, an early effective exposure intervention is not stipulated. Section 5 will examine possible exposure routes on several occupational activities and assess whether fungal BCAs pose low risks, if any, to human and animal health.

3.3.2. Allergies

Along with pollens from trees, grasses, and weeds, fungal spores are an important cause of seasonal allergic rhinoconjunctivitis, asthma bronchiale and exogen allergic alveolitis (EAA). Allergic reactions are known from almost all fungal species used as BCAs. However, in the context of use of fungal BCAs allergenicity has been assessed herein for the first time in a systematic manner. Ward *et al.* (1998, 2000a, 2000b) studied the release of *M. anisopliae* into the environment as a prototype for other organisms used as pesticides or other beneficial applications. Using a mouse model, allergic immune and inflammatory responses due to this agent could be demonstrated.

3.4. Macro-organisms

Allergies caused by macrobials are well known for many years. Inhalant allergens are released by insects such as flies, beetles, moths, cockroaches and mites. Nevertheless, macro-organisms as BCAs have been used extensively for many decades without regulations and without obvious or documented hazards or harm to anyone (Blum *et al.*, 2003). Recently, the Asian ladybeetle *Harmonia axyridis* made headlines because its relationship with the incidence of allergic respiratory symptoms has been clearly demonstrated in several case reports (Ray & Pence, 2004).

4. Methods to measure exposure

Airborne microbial contaminants are increasingly gaining importance in view of health hazard to workers and consumers due to the emission of microbial propagules and metabolites in the production facilities and outdoors (Fischer & Dott 2003). Even microbial volatile organic compounds (MVOCs) have been suggested to affect human health, but their relevance in the working environment (indoor air) remains insufficiently studied. Exposure data is requested by the Commissions Directive 2001/36/EC, Annex II (part B, Section 5) and Annex III (part B, Section 7); however, standard methods for sampling and quantifying airborne contaminants and

MVOCs are still missing. Appliers are directed to use specific methods for the air analysis of the active substance and/or relevant metabolites formed during or shortly after application. However, at this moment appropriate validated methods and standard protocols are not available.

4.1. Micro-organisms

Air sampling provides information about the bio-aerosol composition of the surrounding air. Standard methods to collect volumetric samples include impaction and filtration. On the basis of these collection methods many instruments have been developed. The most widely used devices are slit- and sieve impactors.

Slit impaction samplers such as the commonly used Burkard spore traps (Burkard Manufacturing, Ltd, Rickmansworth, UK) with one-day and 7-day sampling heads allow time-discriminate sampling of bio-aerosols. Nevertheless, a differentiation on species level is usually not possible when total spores are collected on a tape or a coated microscope slide.

Sieve impactors with multiple holes deposit the samples through their multiple holes into a Petri dish filled with culture medium. Furthermore, a viable count can be conducted using filtration samplers where gelatine membrane filters are utilized to monitor micro-organisms. After taking a sample, the gelatine membrane filter is placed directly onto an agar plate. The gelatine dissolves on the moist surface so that the micro-organisms can come into direct contact with the nutrients.

These samplers can be used for the measurement of airborne fungal and bacterial propagules in both outdoor and indoor environments. Following sampling, the petri-dishes are incubated, and the resulting colonies are then counted and identified. Concentrations are expressed as colony forming units (CFU) m^{-3} of air.

If specific microbial BCAs (bacteria, fungi) should be monitored, selective culture media must be used. Otherwise, the overgrowth of naturally occurring airborne micro-organisms on full media would result in an understatement of BCA concentration.

Despite of the fact that analysis of samples by using microscopy and their culture are the most important approaches, molecular methods such as polymerase chain reaction (PCR) are becoming more common methods to analyse samples.

4.2. Detection of microbial volatile organic compounds (MVOCs)

In addition to cellular propagules, “biological risk” can emanate from volatile secondary metabolites produced by the microbial BCAs. It can be assumed that such substances will be diluted below any potential hazard level in the open air, but they may accumulate to relevant concentrations in indoor environments. As the secondary metabolite pattern changes when micro-organisms are grown under different conditions, it should first be demonstrated if the BCA is producing a potentially harmful volatile compound under certain production conditions. If so, monitoring might be necessary.

In general, sampling volatiles can be carried out in two different ways:

- Active sampling: a pump sucks a defined volume of surrounding air through an adsorbent tube (e.g. charcoal or tenax®)
- Passive sampling: sampling media are exposed to indoor air for a defined timeframe.

The MVOCs will be eluated from the adsorbent and analysed by using gas chromatography coupled to mass spectrometry.

5. Exposure study of fungal BCAs

Investigations of the environmental enrichment and the significance of secondary metabolites released by fungal BCAs have been conducted by the EU funded project RAFBCA (QLK1-CT2001-01391). These include mycoinsecticides (*Verticillium lecanii, M. anisopliae, B. brongniartii*), mycoparasites (*Trichoderma harzianum, Gliocladium* spp) and mycoherbicide (*Stagonospora convolvuli*). The major goal of the project was to detect and quantify the active substance and the relevant fungal metabolites in the crop or produce, to identify possible exposure routes, and to assess the risk metabolites pose to human and animal health. In this section *B. brongniartii* is used as the model organism in representation of the real exposure risk of those fungal BCAs commercialised in Europe.

5.1. State of the art

Fungi are considered as potentially harmful when humans are exposed to the spores in various environments, including hospitals (Rainer *et al.*, 2000). Fungal BCAs can be allergenic and produce substances which, in high dosages, have to be regarded as harmful (Strasser *et al.* 2000a). Methods to measure exposure as well as recommendations for precautions are therefore needed. As already mentioned, no national or international standard methods for sampling and quantifying airborne fungi exist. *Beauveria* spp*., M. anisopliae* and *V. lecanii* have been used as BCAs for many years with no use of protective clothing, and with very high degrees of exposure to conidia both in the production as well as in the application process. Thus there is a long history of exposure to these fungi. In most cases, data is still lacking from exposure monitoring of operators, bystanders, and workers during production, although it is a requirement listed in the Commissions Directive 2001/36/EC for a successful registration.

Until now companies have based their arguments for not monitoring exposure of group 1 organisms on the fact that no special containment measures are necessary for this category (Council Directive 98/24/EC). Nevertheless, often the following measures are taken by producers of fungal biomass to minimize the exposure of operators, bystanders, and workers to potentially allergenic fungal conidia: (i) It is stressed that once inoculated, fungal growth chambers are kept sealed which not only reduces the risk of accidental contamination, but also avoids the possibility of worker exposure to conidia. (ii) Factory workers are encouraged to wear gloves and face masks in the production area. (iii) Active ingredients are packaged in polyethylene or similar bags. The risk of such bags breaking is very low. Operators, farmers, and the public should only be exposed to a small amount of fungal colonised products or dry conidia by following these guidelines.

5.2. Evaluation of the exposure in the production of fungal BCAs

Reports of health problems among workers in biotechnology (i.e. BCA production facilities) are rare in scientific literature. The reason is that BCA production requires a containment which does not only ensure product purity, but also guarantees environmental safety. The containment allows the protection of the workers when handling the process organisms. Nevertheless, in BCA production facilities workers are exposed to the process micro-organisms and/or their

components. Particularly in the down stream processing stages (i.e. centrifugation, product concentration, waste handling) a high exposure risk exist and therefore, it is recommended to monitor the exposure risks in the production facilities (Figure 1, bordered zone).

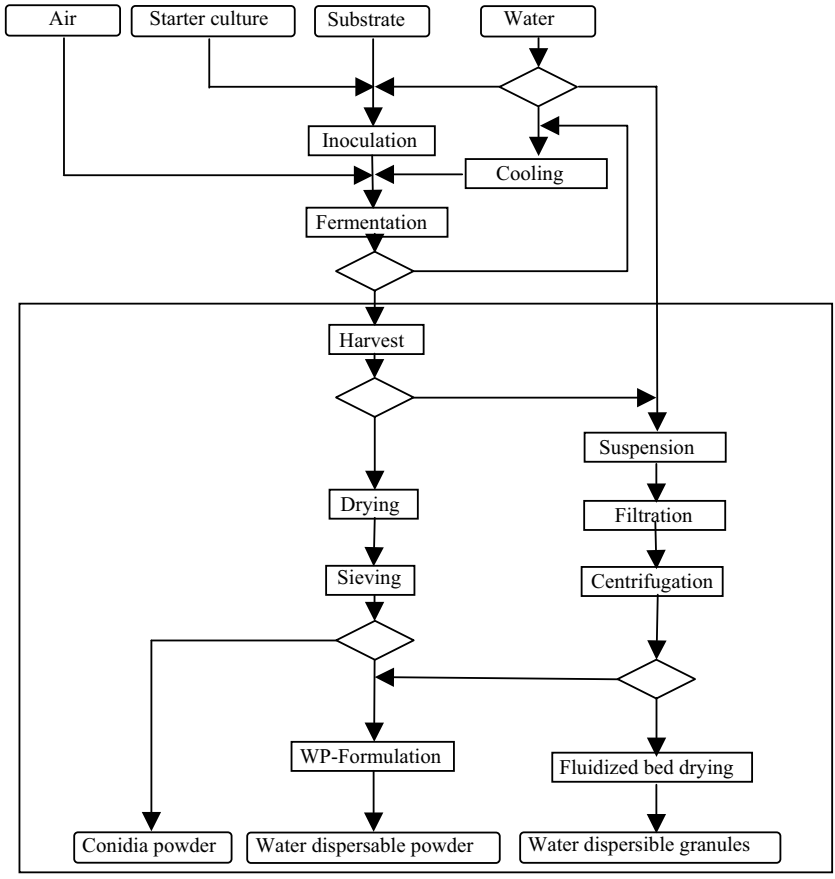

Figure 1: Flow diagram for the production of fungal BCA. Particularly in the down stream processing stages workers are exposed to the organisms and their components (bordered zone)

Rainer *et al.* (2003) studied the exposure risk of Melocont®-Pilzgerste, a fungal formulation based on *B. brongniartii* colonised barley, to workers producing the product in a diphasic fermentation process. The authors assessed the level of airborne propagules in the production facility and compared their findings to data obtained from a hospital environment (Rainer *et al.* 2000). A very low number of airborne fungal viables (5 and 7 CFU/m³) were found during the incubation of Melocont®-Pilzgerste. The CFU-numbers from the production facility were lower than the ones from the protected and unprotected hospital environment, where an average number of more than 320 CFU/m³ was found. According to Kurata (1994) the indoor air quality in the incubation unit can be classified as "bio-clean" (<60 CFU/m³). During the incubation period only one CFU of *B. brongniartii* was isolated on S2G nutrient agar.

In conclusion, regarding health hazards for workers and applicators due to the emission of fungal metabolites no health problems have been documented in literature [i.e. (i) respiratory infections, (ii) allergic respiratory diseases, (iii) intoxication by microbial cell components, metabolites and volatiles]. Although toxic secondary metabolites are expected to be present in airborne spores, and may be found in airborne dust and bio-aerosols, no health problems caused by *Beauveria* formulations have been reported either. Most importantly, though, there is no evidence that the presence of *B. brongniartii* can be attributed to the sick building syndrome (SBS). The serious illness effect SBSs is linked to the existence of MVOCs which may act as morbid agents.

5.3. Assessment of humans exposed to *Beauveria. spp. after field application*

When the focus is shifted from the production facilities to the field, only few studies have been conducted that specifically address the possibility of increased incidences of infections and allergies associated with the large – scale application of *Beauveria* products.

For many years *Beauveria* BCA's have been applied with no protective clothing and with very high degrees of exposure to conidia both in the production and application process. While allergies are reported for *B. bassiana,* not many other adverse medical effects have been recorded. Hussey & Tinsley (1981) mention that Chinese workers suffer form nose irritation during the production of *B. bassiana*, whereas Melnikova & Murza (1980) state that there are health risks for people who have permanent contact either by touch or inhalation with the fungus. The allergic reactions to *Beauveria* spores were caused by the fact that the product was handled in high concentration without any precautions for many years. However, Hussey & Tinsley (1981) point out that there was only little discomfort reported by the workers, and that more than 1,000 production units and about 20,000 people have been trained in the production and use since it was first developed in 1971. Since the late 80ies 1.3 million hectare of land per year have been successfully applied with *B. bassiana* in China, which amounts to an annual production output of more than 100,000 kg *Beauveria* spore powder product per year (Feng *et al.* 1994). China is the most encouraging country in the world for practical application of *B. bassiana* products in the last three decades. Its safety standards indicate that no prophylactic measures such as wearing masks and gloves are necessary while working in the crop (that is working in the green house and in the field, respectively).

What China stands for in the application of *B. bassiana*, Austria stands for *B. brongniartii*. In a ten year field study (1994 to 2004) conducted in Tyrol, Austria, Strasser & Pernfuss (2005) applied more than 50 tons Melocont®-Pilzgerste in the very densely settled Inntal valley. For

one year more than 1,600 ha of grass and agricultural land was applied with 30 to 50 kg per ha Melocont®-Pilzgerste. Over the entire testing period no complaints from the health departments in the region were received, and up to now there is no evidence that *B. brongniartii* is associated with any illness or infection. In Trentino, Italy, apple orchards were treated with first spray applications with second generation formulations of *B. brongniartii* (i.e. WP and WG formulations, Seger *et al.*, 2005a). Results showed that despite a small conidia driftage during spraying, no negative symptoms were reported by workers and bystanders despite the fact that exposure could not be ruled out. Even individuals who have a prior history of allergies (hay fever) did not complain during and after the spray applications.

5.4. Assessment of humans exposed to fungal BCA and their metabolites after field application

Do "model" fungal BCAs produce toxins after application when the product is present in the crop? This question seems to be utmost importance especially for the regulating authorities in Europe. Members of the RAFBCA consortium were confronted with this question because little is published: (i) about the range of metabolites produced by fungal BCAs; ii) about whether relevant metabolites enter the food chain, therefore posing a risk to human and animal health as well as the environment; (iii) about relevant examinations of workers who were tested for exposure risk to toxins with focus on exposure to fungal products and to toxicologically relevant compounds in the product, if any, under the proposed conditions of use.

As a result of the RAFBCA project none of the metabolites released by the "model" fungal BCAs must be defined as a "relevant" metabolite (i.e. metabolite of toxicological and/or ecotoxicological or environmental concern; see also amended EU Directive 91/414/EEC, Annex II, Section 4. Analytical Methods, p 43). Although secondary metabolites of fungal BCAs are often referred to as toxins (Vey *et al.*, 2001), no reports or publications in peer reviewed journals exist about this subject matter. Also, no information can be found in either MEDLINE or DIMDI (i.e. medical data banks) that indicate "model" fungal BCAs and their metabolites show unacceptable effects on human health and/or the environment during or after application.

Looking at our model organism *B. brongniartii* the fungal BCA can be characterised as follows:

(i) *B. brongniartii* is not a plant pathogen.

(ii) *Beauveria* production strains do not grow on plant material.

(iii) Data on metabolite production by commercial isolates of the genus *Beauveria* (e.g. Melocont®-Pilzgerste, Beauveria-Schweizer, Engerlingspilz-Andermatt, Boverol®, Melocont®-WG) is hard to come by. Only oosporein was characterised as a major secondary metabolite in submerged culture, in the final product and in mycosed pest organisms (Strasser *et al.*, 2000b, Seger *et al.*, 2005a).

(iv) There is no evidence of metabolites transferred to plants (RAFBCA studies, unpublished observations).

(v) As can be derived from the chemical and physical characterisation of oosporein (Seger *et al.*, 2005b), the metabolite degrades quickly under moderate alkaline conditions. Oosporein is not volatile and, therefore, cannot be inhaled/taken up by workers as MVOCs. An adsorption into soil and charged biological matrices is

nearly irreversible; however, oosporein can be washed off from the cuticula of crops and fruiting vegetables with tap water.

(vi) Exposure risks of toxins for workers and users are not relevant because formulated products are free of toxicologically "relevant" *Beauveria* metabolites. *Beauveria* metabolites have no relevant antibiotic activity, no cytotoxic or apoptotic effects (Abendstein & Strasser 2000 and unpublished results).

(vii) Hypothetically speaking, even if the fungus showed saprophytic growth on plant materials, the production of metabolites still is not relevant. Referring to the EU Directive 91/414/EEC, Annex IIB, item 2.8; no metabolites which are produced by *B. brongniartii* show unacceptable effects on human health and/or the environment during or after application.

In conclusion, there is sufficient information available from literature which demonstrates that *B. brongniartii* does not produce relevant metabolites (toxins) during or after application (Strasser *et al.*, 2000b, Seger *et al*., 2005a). No risks to humans are expected. There is no indication of environmental risk, nor do relevant metabolites enter the food chain. *B. brongniartii* is therefore an effective biological control agent which should be registered in Europe without any restrictions.

6. Discussion

Weighing the risks and benefits of the release of a BCA versus other control measurements (chemicals), one would expect that biological control could phase out many products which harm humans as well as the environment. The majority of commercialised BCAs in Europe and especially those active substances, for which a notification in accordance with Article 4 of Commission Regulation (EC) No 1112/2002 has been required, do not pose potential health problems, especially when looking at the exposure during handling and while using the products.

This recommendation should be in accordance with the official opinion of the EU and OECD countries, which have published the statement that biocontrol agents pose little or no risk to humans and the environment (Anonymous, 2005). This is why experts are astonished that despite considerable research efforts on biological control agents conducted during the last three decades, the number of such products on the market in Europe is still extremely low compared to the number of products used in the USA and Canada. It is public knowledge that many European researchers and experts are of the opinion that the major hurdle for prevention of the use of these products is the current legislation following the Councils Directive 91/414/EEC, which was originally developed to register synthetic chemical compounds. The following example should highlight the unsatisfying situation for BCA registration: The Directive reads that there is a need for a high quality assessment of BCAs regarding the environment, health and safety risks. Appliers have to come up with the data not only for the active substance (organism), but also for all the relevant metabolites, toxins and adjuvants. Assessment of the origin of the strain, the reproduction and the dispersal, providing information on the genetic stability of the micro-organism under the environmental conditions of proposed use, small and medium sized enterprises (SMEs) are discouraged from attempting to register biological control agents. In accordance with the information policy pertaining to a chemical substance (i.e. content of pure active substance, inactive isomers, impurities and additives),

appliers of BCAs have to identify whether a "relevant metabolite" (i.e. metabolite of toxicological, ecotoxicological, and/or environmental concerns) is produced, or may be produced, by the active substances (BCA) themselves or by species from the same genus. If the applicant has to answer with "yes," the following information has to be made available "on request" to the evaluators: (i) analytical standards of the pure active substance. (ii) samples of the active substance as it is manufactured. (iii) analytical standards of relevant metabolites and all other components included in the residue definition. (iv) if available, inclusion of samples of reference substances for the relevant impurities (see 4. Analytical Methods, Directive 91/414 Part A). From literature we know that fungi secrete a wide range of metabolites, and, therefore, appliers have to provide data to the regulating authorities on this subject.

Is this in accordance with the European agriculture policy to keep registration costs affordable for SMEs, which are the companies producing most of the successful biological alternatives? The costs for providing information on two major fungal metabolites produced by *Beauveria* and *Metarhizium*, oosporein and destruxin, were 12 Mio. €. The findings were realised in two different EU funded projects (i.e. BIPESCO- FAIR6-CT98-4105- and RAFBCA) and kept two teams busy for five years. The outcome of this project is that the BIPESCO and RAFBCA team could confirm that *Beauveria* and *Metarhizium* isolate, respectively, and their secreted major metabolites oosporein and destruxin do not harm humans and the environment. This information, however, has been available to experts for more than twenty years because both BCAs have been used in large amounts to control soil dwelling pests in Europe for many years. Nevertheless, more studies are necessary regarding monitoring whether both major metabolites enter the food chain. A rough calculation for oosporein monitoring in crops resulted in the need of six person months per crop or biological matrix to adapt the already validated sample preparation technique and analytics (Seger *et al.*, 2005a). In conclusion, it has to be obvious that the data requirements under the present Directive cannot be met by the European industry (i.e. SMEs) and on a larger scale will result in the prolongation of the time requirement to phase out unsustainable chemical pesticides.

7. Outlook

European industry and researchers are highly interested (i) in putting their BCAs on the market, (ii) making the products more attractive to the users by reducing the costs, and (iii) at the same time maintain the level of safety for producer, user and consumer. Therefore, it is indisputable that safety issues have to be taken very seriously. Among environmental concerns relating to biological control, there is their potential dispersal into and establishment in the environment, the accumulation of the active substance and/or metabolites in food, as well as non-targeted effects which can cause damage to the environment (van Lenteren *et al.*, 2003). Not to mention the human exposure evaluation to BCAs and their metabolites, which is necessary, but conclusive studies are not available as of yet. There is a need to integrate research on occupational health risks in relation to biological control at the European level. The concept of the "hazard analysis and critical control point" analysis (HACCP) could be a helpful instrument which has been defined in The Council Directive 93/43/EEC - Food Safety Regulation (Figure 2).

The HACCP system has been developed for the food production industry. Food business operators were asked to identify steps in their activities which are critical not only to ensure

food safety, but also to ensure that adequate safety procedures are identified, implemented, maintained, and reviewed on the basis of the following principles:

- analysing the potential food hazards in a food business operation,
- identifying the areas in those operations where food hazards may occur,
- deciding which of the areas identified are critical to food safety - the 'critical points',
- identifying and implementing effective control and monitoring procedures at those critical points, and
- reviewing the analysis of food hazards, the critical control points and the control and monitoring procedures periodically and whenever the food business operation changes.

These procedures can be easily implemented by BCA producers to identify any level in their production and application activities which is critical to ensure human safety and ensure that safety procedures are identified, implemented, maintained and reviewed on those principles.

EU-funded research shows impacts on Directive 91/414/EEC and Directive 2001/36/EEC and that the evaluation of biocontrol agents and their metabolites during registration of BCAs could be simplified (Blum *et al.*, 2003; Strasser & Pernfuss, 2005b). Expert consortia have generated new data that can be used to develop a new risk assessment strategy which could help accelerate risk assessment of BCAs and their metabolites as well as reduce registration costs. These experts have devised strategies that could lead to a more balanced system for risk assessment and registration, and enable the EU to compete with the USA and other countries.

The EU funded ERBIC (FAIR5-CT97-3489), BIPESCO and RAFBCA research produced data that could help the end users (policy makers, registration authorities, industry) as well as the public in making more informed decisions about biological control. Needless to say, new projects must be the next step to seriously promote the development and use of biological control for pest management. Currently, biological control researchers prepare themselves to follow up with a policy oriented research project funded by the 6th Framework Programme of the European Union (Call identifier: FP6-2004 - SSP-4). The goal of this proposal is to review

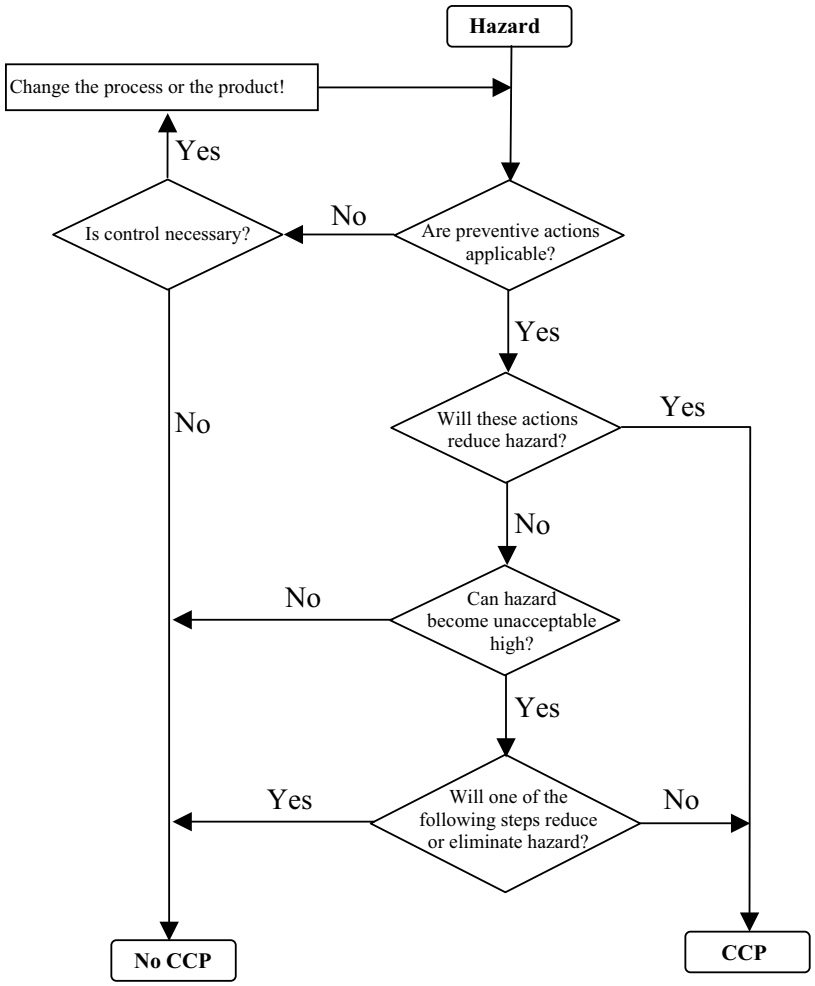

Figure 2: "Decision Tree" to determine a step or procedure at which control can be applied and a hazard can be prevented, eliminated or reduced to acceptable levels (Critical control point, CCP)

current legislation, guidelines and guidance documents at member sate and EU level, and to compare those to similar legislation in other countries where the introduction of new biopesticides has proven to be more successful. Scheduled future research activities will focus more on improving sustainable and quality-based crop systems (including non-food products and uses) and on developing techno-economic references to support the EU legislation. However, the research should be partly publicly funded (possibly with matching funds from the industry) and should result in a generic safety registration of each particular agent.

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CHAPTER 15

HARMONIA AXYRIDIS: A SUCCESSFUL BIOCONTROL AGENT OR AN INVASIVE THREAT?

Helen Roy, Peter Brown & Michael Majerus

1. Introduction

The harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is an aphidophagous coccinellid, native to central and eastern Asian (Kuznetsov, 1997). This predatory ladybird has been available in many countries for use as a biological control agent of pest insects including aphids and scale insects. Since 1916 *H. axyridis* has been repeatedly released as a classical biological control agent in the USA (Gordon, 1985). It established in the 1980s and has spread and increased in number dramatically so that it is now the dominant species of ladybird in many parts of North America (Hesler *et al.,* 2001; Smith *et al.,* 1996; Tedders and Schaefer, 1994). In mainland Europe it has been commercially available for both classical and inundative biological control strategies since 1982 (Iperti and Bertand, 2001) and has become established in France, Belgium, Holland, Germany, Luxemburg and Italy. It has also been released in South America and the Middle East. *Harmonia axyridis* has not been intentionally released in the UK, however given the proximity of the SE of Britain to the French, Belgium and Dutch coasts it was inevitable that it would arrive. In mid September 2004 a male was found in Essex (SE England).

The arrival of *H. axyridis* in the UK has been met with considerable concern both from ecological and anthropogenic perspectives. In this chapter we address both the perceived potential problems and the possible benefits associated with the arrival of this invasive species. In addition we report on methods of monitoring *H. axyridis* in the UK and the initial public response to a national survey involving this alien species and the native coccinellids with which it may interact.

2. Biology of *Harmonia axyridis*

2.1. Dietary range

Harmonia axyridis is a polyphagous species. It has a wider dietary range of essential prey than most other predatory coccinellids. It most often feeds on aphids, but will also consume coccids, psyllids and adelgids as essential prey. It can breed and develop on the immature stages of other coccinellids and, in captivity, the eggs of the flour moth *Ephestia kuhniella* are used as essential food. Alternative foods (*sensu* Hodek, 1996) include many other invertebrate prey, nectar, pollen, honeydew, plant sap and the juice of ripe fruit.

J. Eilenberg and H.M.T. Hokkanen (eds.),An Ecological and Societal Approach to Biological Control, 295–309.

2.2. Colour variation

The colour pattern of the pronota and elytra of *H. axyridis* are highly variable. The ground colour may be orange, red or black. The orange and red forms may be patterned with zero to 21 black spots (f. *succinea* complex) or may have a grid-like black pattern (f. *axyridis*). The black (melanic) forms commonly have two (f. *conspicua*) or four (f. *spectabilis*) orange or red spots. Many other forms with bars, stripes or splodges have been reported from its native range (e.g. Dobzhansky, 1933). The differences between forms are largely controlled by alleles of major genes (Hosina, 1933, 1936; Tan and Li, 1934; Komai, 1956), but environmental factors, such as temperature and diet also have some effect (Majerus and Roy, in press a). The phenotypic variability of *H. axyridis* facilitates the adaptability of this species in terms of life-history traits, competitive ability and habitat diversity (Grill *et al*., 1997).

2.3. Habitat range

Harmonia axyridis is widely reported as a semiarboreal species (Hodek, 1973; LaMana and Miller, 1996); however it also thrives and breeds in agricultural habitats (LaMana and Miller, 1996). The eurytopic nature of *H. axyridis* is further supported by the broad range of habitats, including coniferous woodland and reed-beds, in which it survives and reproduces in its extensive native Asian range. Native UK ladybirds tend to be more habitat and niche-specific than *H. axyridis* (Majerus, 1994).

2.4. Climatic range

The wide latitudinal and longitudinal range of *Harmonia axyridis* demonstrates the adaptability of this species to diverse climatic regimes.*H. axyridis* can survive winter temperatures below freezing and summer temperatures of 30°C (LaMana and Miller, 1998). These temperatures are within the range which is currently experienced across the UK. Furthermore, the effects of climate change are already evident across the UK and predictions suggest the trend of global warming will continue. The climatic adaptability of *H. axyridis* will provide it with a competitive advantage over less adaptable UK native coccinellids. Whereas some native species of ladybird (such as *Coccinella septempunctata*) require a period of dormancy prior to commencing reproduction, *H. axyridis* can breed continuously. Therefore, global warming would allow this species the possibility of reproducing over a longer time period. The polyphagous nature of *H. axyridis* would facilitate the survival of larvae regardless of the food type available at different times of the year. Indeed, in early November 2004, after native UK ladybirds had dispersed to overwintering sites, *H. axyridis* larvae and pupae were still active. Moreover, sightings of active *H. axyridis* in England in March and April 2005 demonstrate that it is capable of surviving the British winter climate.

2.5. Dispersal ability

In both Asia and America *H. axyridis* undertakes long migratory flights to and from overwintering sites (LaMana and Miller, 1996). Furthermore, this species is also highly dispersive during the breeding period as it searches for host plants with high densities of aphids. This high dispersal ability has certainly contributed to the rapid colonization of North America by *H. axyridis* and we predict that by 2008 *H. axyridis* will have spread across mainland Britain. Indeed the arrival of *H. axyridis* is in part attributed to its ability to disperse from

mainland Europe across the English Channel to the UK; although it is also known that some individuals have arrived on fruit and cut flowers (Majerus *et al*., in press).

3. *Harmonia axyridis*: A successful biological control agent?

Predatory ladybirds are widely considered as important biological control agents and there are examples of their use in all four biological control strategies: classical biological control, inoculation biological control, inundation biological control and conservation biological control. Indeed the Australian vedalia ladybird, *Rodolia cardinalis*, marked the advent of modern biological control. In 1888 the vedalia ladybird was released to control cottony cushion scale insects, *Icerya purchasi*, which were devastating the citrus industry of California. The introduction and subsequent establishment of this ladybird resulted in a dramatic decrease in scale insects (Majerus, 1994).

There have been many other examples of successful control of scale insects by various ladybird species (De Bach, 1964; Dixon, 2000). However, the control of aphids by ladybirds has largely been unsuccessful (Dixon, 2000). This has been attributed to the asynchrony of ladybirds with early season outbreaks of aphids and also the slow reproductive rate of ladybirds in comparison to aphids (Coderre, 1988; Majerus, 1994; Dixon, 2000). Furthermore, the release of generalist predators for biological control programmes is contentious for a number of reasons:

1. Inconsistent and long delays in post-release recovery (LaMana and Miller, 1996)
2. Potential to outcompete and displace other guild members (Rosenheim et al, 1994)
3. Impacts on non-target species (Howarth, 1991)

Harmonia axyridis has been used as a biological control agent around the world. Since 1982, *H. axyridis* has been commercially available in Europe and has had a much longer history in North America. It was first released in California in 1916 as a classical biological control agent but failed to establish. Numerous subsequent releases in various parts of North America also failed. However, in 1988 populations were found in Louisiana (Chapin and Brou, 1991). Whether these populations resulted from intentionally released beetles, or accidental migrants is still a matter of debate (Day *et al*., 1994; Tedders and Schaefer, 1994). Since the late 1980's *H. axyridis* has colonised much of the USA and latterly Canada. It is rapidly becoming the dominant species of ladybird in North America.

Harmonia axyridis has many attributes that contribute to its biological control potential but perhaps the most important is that it preys on a wide variety of homopteran insects such as aphids, psyllids, coccids and adelgids (Hodek, 1996; Koch, 2003). Both as an introduced biocontrol agent in North America and in its native Asia, *H. axyridis* has been reported to contribute to control of aphids on sweet corn (Musser and Shelton, 2003), alfalfa (Buntin and Bouton, 1997; Colunga-Garcia and Gage, 1998), cotton (Wells *et al*., 2001), tobacco (Wells and McPherson, 1999), winter wheat (Colunga-Garcia and Gage, 1998), soybean (Koch, 2003), pecans (Tedders and Schaefers, 1994; LaRock and Ellington, 1996) and apples (Brown and Miller, 1998). In China *H. axyridis* has also been used as an augmentative biocontrol agent for the control of coccids in pine forests (Wang, 1986). Therefore, the increase of *H. axyridis* throughout the UK may prove to be beneficial in reducing aphid numbers below economically damaging levels within many crop systems and so reducing the use of chemical pesticides.

Due to its wide dietary range, *H. axyridis* may also provide control of some other pest species, such as adelgids in conifer plantations and coccids generally. Furthermore, its impact may not be restricted to essential prey species. As *H. axyridis* feeds on many insects as alternative prey, when essential prey are not available, it may reduce populations of other herbivorous pests, as Yakhontov (1960) showed when *Brumus 8-signatus* and *Semiadalia 11-notata* were used to control the weevil *Phytonomus posticus* on lucerne.

Harmonia axyridis is compatible with many integrated pest management strategies. Koch (2003) reviewed the impact of pesticides on *H. axyridis* and although broad spectrum insecticides were found to be lethal to *H. axyridis*, synthetic pyrethroids and new pesticides, such as spinosad, indoxacarb and pyriproxyfen, were considerably less toxic to *H. axyridis* than to aphids. Fungicides also have little toxic effect on *H. axyridis* (Michaud and Grant 2003; Wells et al, 2001). Studies assessing the impact of insect resistant transgenic crops on *H. axyridis* have shown negative effects to be negligible (Musser and Shelton 2003; Wold et al, 2001; Ferry et al, 2003).

It can, therefore, be concluded that *H. axyridis* can be used as a compatible and effective component of integrated pest management schemes (Koch, 2003). However, the very traits that contribute to the success of *H. axyridis* as a biological control agent (size, diverse dietary range, predatory efficiency and wide niche colonisation ability) are of concern when the wider ecological impacts of this species are considered.

4. Problems associated with *Harmonia axyridis*

4.1. Ecological implications

The generalist diet of *H. axyridis* means that negative impacts on non-target prey species would appear to be inevitable and the native guild of predators, parasitoids and parasites that surround these prey will also be adversely affected. However, studies on this are sparse. Koch *et al.* (2003) have already identified *H. axyridis* as a potential predator of immature monarch butterflies, *Danaus plexippus*, even though these butterflies contain defensive chemicals. It is likely that many other species will be directly or indirectly affected by the arrival of *H. axyridis*.

There is evidence to suggest that *H. axyridis* is adversely affecting other aphidophages. Sato *et al.* (2005) reported that, in a laboratory study assessing the interactions between different species of ladybird larvae, 95% of *Adalia bipunctata* and 55% of *C. septempunctata* were consumed by *H. axyridis*. This supports field studies that identified declines in populations of *Brachiacantha ursine*, *Cycloneda munda* and *Chilocorus stigma* in Michigan and abundance of native coccinellids in apple orchards in West Virginia following the establishment of both *C. septempunctata* and *H. axyridis* (Brown and Miller, 1998; Colunga-Garcia and Gage, 1998). There are three ways in which *H. axyridis* is likely to negatively affect other aphidophages: resource competition, intraguild predation and inter-specific competition.

Harmonia axyridis is highly voracious (consuming up to 65 aphids per day), fertile and fecund and so has the potential to directly out-compete other aphidophages (Michaud, 2000). Furthermore, *H. axyridis* has a wider dietary range than many other aphidophagous coccinellids. This, coupled with its ability to disperse rapidly, forage widely and breed continuously, gives *H. axyridis* a considerable advantage over British aphidophages in competition for prey species.

It is clear that *H. axyridis* is one of the top predators within aphidophagous and coccidophagous guilds and can thrive on a varied diet, including other species of ladybird (Yasuda and Ohnuma, 1999; Sato *et al*., 2005) and parasitized aphids (Nakata, 1995). Furthermore, in Japan *H. axyridis* arrives in alfalfa fields just after a number of other ladybirds allowing *H. axyridis* to feed on the prepupae and pupae of other coccinellids (Takahashi, 1989). It has been shown that *H. axyridis* will prey on immature stages of three of the most common aphidophagous coccinellids in Britain, *C. septempunctata* (Yasuda *et al*., 2001), *A. bipunctata* (Burgio *et al*., 2002) and *Propylea quatuordecimpunctata* (Lynch *et al*., 2001). In contrast, evidence suggests that the immature stages of *H. axyridis* are rarely eaten by other aphidophagous insects. It appears that the defensive chemistry of *H. axyridis* ensures that larval stages of other coccinellid species find it unpalatable (Agarwala and Dixon, 1992). Impact studies into the effect of *H. axyridis* on other aphidophages, such as Neuroptera, the larvae of some syrphids and parasitoids of aphids are lacking and urgently needed. However, it is likely that all of these groups will suffer through inter-specific competition, and some may also be negatively affected by intraguild predation.

A recent methodology for risk assessment (developed within the EU-financed project "Evaluating Environmental Risks of Biological Control into Europe (ERBIC)") has been developed for the regulation and release of exotic natural enemies (Van Lenteren *et al*., 2003). This general framework integrates information on the potential of a prospective biological control agent to establish, its dispersal ability, host range and direct and indirect impacts on non-targets. It is notable that *H. axyridis* was allocated a high-risk index when this proposed methodology was applied to it as a biological control agent. Host range is regarded as a critical element in the risk evaluation process and it is stated that "*lack of host specificity might lead to unacceptable risk if the agent establishes and disperses widely whereas, in contrast, a monophagous biological control agent is not expected to create serious risk even when it establishes and disperses well*" (Van Lenteren *et al*., 2003).

Adequate risk assessment and regulation of potential biological control agents are essential if the continued good reputation of biological control is to be maintained. The use of high risk, exotic species should be implemented with extreme caution. It has been recognised that for some species there may be a high probability of adverse ecological effects but these may not be realised under the specific conditions of release, for example in greenhouses (Van Lenteren *et al.,*2003). Biological control theory dictates that host specific agents are the most acceptable, both from efficacy and safety perspectives, however economics ensures that voracious species with wide host ranges are attractive to commercial producers. However, in the case of highly polyphagous, dispersive and adaptable species, such as *H. axyridis*, potential risks of use, even in restricted circumstances, may be greater than potential benefits.

In summary, *H. axyridis* is undoubtedly a dominant unidirectional intraguild predator and, although levels of intraguild predation are inversely correlated to aphid or coccid density (Hironori and Katsuhiro, 1997; Burgio *et al*.,2002), it has the potential to dramatically disrupt native aphidophagous and coccidophagous guilds in Britain.

4.2. Anthropogenic implications

In North America *H. axyridis* has become a nuisance to humans and is now, ironically, widely considered to be a pest. This is mainly a consequence of its behaviour when conditions become unfavourable both in late summer, when aphid populations decline, and in autumn, as adverse climatic conditions stimulate *H. axyridis* to undertake long-range migrations from their feeding

habitats to overwintering sites (Huelsman *et al*.,2002). Both scenarios lead adult *H. axyridis* to aggregate in large numbers.

In late summer it is essential that *H. axyridis* adults build up their energy reserves to ensure overwintering survival. At this time aphids are often scarce and so *H. axyridis* switch to feeding on the sweet sap of ripe fruits, such as apples and pears, blemishing the fruits and reducing the value of the crop. This has gained *H. axyridis* the status of a potential fruit production and processing pest in North America. This ladybird is also problematic in vineyards because *H. axyridis* feeds on the ripe grapes. The beetles are difficult to separate from the grapes at harvest and so they are crushed with the crop and their bitter-tasting, alkaloid defensive chemicals can seriously taint the vintage (Ratcliffe, 2002).

As adverse winter conditions approach, *H. axyridis* adults aggregate in large numbers in suitable overwintering sites such as houses, sheds and garages (Kidd *et al*.,1995). As the temperature increases, either through central heating or the onset of spring, the ladybirds increase in activity and begin to crawl and fly inside homes (Huelsman *et al*., 2002). Reflex blood is exuded from the femoro-tibial joints if the ladybirds are aggravated and this has a foul odour and stains soft furnishings. Once *H. axyridis* becomes active, it seeks food, and will then bite people to assess whether they are palatable. The resultant bites feel like a sting as a result of the predigestive enzymes injected into the incision, and a small bump usually develops. A few people have shown a hyperallergic reaction to *H. axyridis*, in the form of allergic rhinoconjunctivitis (Huelsman *et al*.,2002; Yarbrough *et al*., 1999).

5. The Harlequin Ladybird survey

The arrival of *H. axyridis* in the UK stimulated researchers from Cambridge University, the Centre for Ecology and Hydrology and Anglia Polytechnic University to initiate the Harlequin Ladybird Survey. We recognized that *H. axyridis* should be monitored for a number of reasons. First, it is critical to assess whether the potential ecological and anthropogenic impacts will be realized in the UK, using North America as a case study. Second, the discovery of *H. axyridis* in Britain provides a unique opportunity to study an invasive species from the time of arrival and so provides a model system for monitoring invasive species. Furthermore, the arrival presents the rare opportunity to address a range of evolutionary questions as a Founder population adapts phenotypically and genotypically to equilibrium states under selection. In *H. axyridis*, studies on changes in frequencies of colour-pattern morphs and changes in the prevalence of male-killing bacteria following arrival provide unique opportunities to investigate the evolution and maintenance of melanic polymorphism and sex ratio distorters respectively (Majerus and Roy, in press b).

5.1. What is the Harlequin Ladybird Survey?

The Harlequin Ladybird Survey and the UK Ladybird Survey are collaborative projects designed to monitor both the spread of *H. axyridis* across the UK and to assess the status of native ladybird populations. These projects are funded by the Department of Food and Rural Affairs (Defra) through the National Biodiversity Network Trust (NBNT). The further support that this research has received from many organizations including: Centre for Environmental Data and Recording (CEDaR), Joint Nature Conservancy Council (JNCC), Field Studies

Council (FSC), the Royal Society for the Protection of Birds (RSPB), Natural History Museum (London), British Wildlife Trusts and the National Trust is testament to the perceived importance of this study.

The surveys were launched on the 15th March 2005 with the support of two websites: the first for specific monitoring of the *H. axyridis* (www.harlequin-survey.org) and the second for recording native ladybird species (www.ladybird-survey.org). Both websites have been designed to engage the public to contribute to the survey and so provide general information on ladybirds and enable recording either on-line or using conventional post (Figure 1).

Ladybird records are highlighted as verified or unverified within the database. A verified record is one in which the specimen or a photo of the specimen has been seen and identified by one of the contributing scientists. Recorders are strongly encouraged to seek verification of their record and many have done so. This data enables distribution maps to be plotted and these are updated frequently on the website.

In addition to these general surveys other approaches have been developed to target specific groups including: young people (through the RSPB Wildsquare initiative), wildlife enthusiasts (through talks at wildlife societies) and experienced entomologists (through journals such as *Antenna*, *Entomologist's Monthly Magazine* and *The Bulletin of the Amateur Entomologists' Society*). The latter two groups are encouraged to establish transects (approximately 1-2 Km) to be monitored repeatedly through the active season for ladybirds and to replicate in following years, so that the impact of the arrival of *H. axyridis* on native coccinellids can be assessed.

5.2. Media attention

As outlined, engaging the public to help record *H. axyridis* and other ladybirds is a significant part of the project and the public response has been enormous. This has been aided by the publicity that the project has received through the national press. Just after the first report of *H. axyridis* was confirmed, on 4th October 2004 a press release was issued from Cambridge University, it began:

"The Ladybird Has Landed

A new ladybird has arrived in Britain. But not just any ladybird: this is *Harmonia axyridis*, the most invasive ladybird on Earth."

From this the press coverage that *H. axyridis* received was vast. Most of the national papers and many of the national and regional radio and television stations produced pieces highlighting the arrival of *H. axyridis* and the threat it posed both ecologically and anthropogenically. Very few highlighted the benefits of this species as a successful biological control agent. Following this initial publicity there were 110 confirmed reports of *H. axyridis* mostly in SE England (Figure 2).

On the 15th March 2005 another press release was issued to announce the launch of the websites for the surveys. This one began:

"Hunt for the harlequin – A UK survey for the world's most invasive ladybird

Britain's best loved beetle, the ladybird, is under threat from the world's most invasive species – the harlequin ladybird (*Harmonia axyridis*)."

The Harlequin Ladybird Survey

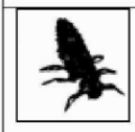
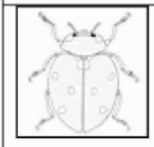
Harlequin Ladybird Recording Form

Name: **Date**:

Address:

Postcode:

Email address: (*Please include this if you have one!*)

What you found (circle)		**How many?** (circle)
	Larvae	1 / 1-5 / 6-10 / more than 10
	Adults	1 / 1-5 / 6-10 / more than 10

Where you found this:
(*Please include a grid reference, a postcode and/or name of the site*)

What was it doing?
(*E.g. flying, walking on the ground, sitting on a leaf, eating a greenfly…)*

Please send us photos or live specimens if you are able

http://www.harlequin-survey.org/

Figure 1: Harlequin ladybird recording form used for either on-line recording or postal submissions

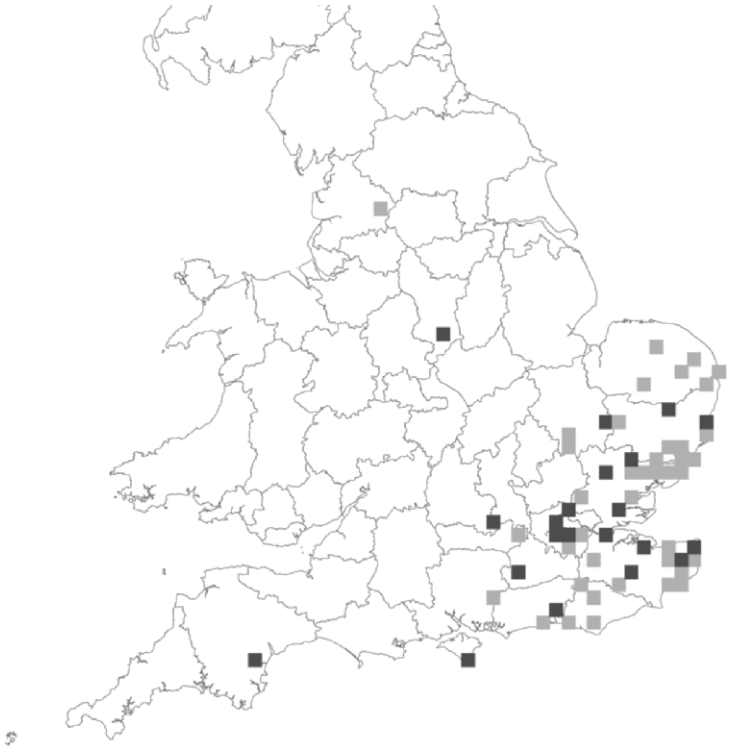


Figure 2: Distribution of Harmonia axyridis *in the UK (records up to 19th April 2005). Orange squares = 2004; Red squares = 2005*

The press coverage of this launch was even greater than the initial response in October 2004 and made front page news in *The Times*. Again the emphasis was on the negative impacts of *H. axyridis*, and in particular its threat to native ladybirds, which was emphasized as having implications to both the biodiversity and biological control potential of native species. The publicity gained from this launch was highly beneficial in advertising the websites. Within a few hours the Harlequin Survey website had received over 4000 visits. Over the first five weeks immediately following the launch of the surveys 592 online records were submitted, of which 24 were confirmed as *H. axyridis* (Figure 2 and 3), 174 were verified as native coccinellids and the remainder are currently unverified (Figure 3). It appears that many members of the public have been stimulated to look out for ladybirds, have noticed less familiar native species for the first time and suspected them to be *H. axyridis.* The number of verified *H. axyridis* reports is expected to rise as the spring dispersal continues and the breeding period begins.

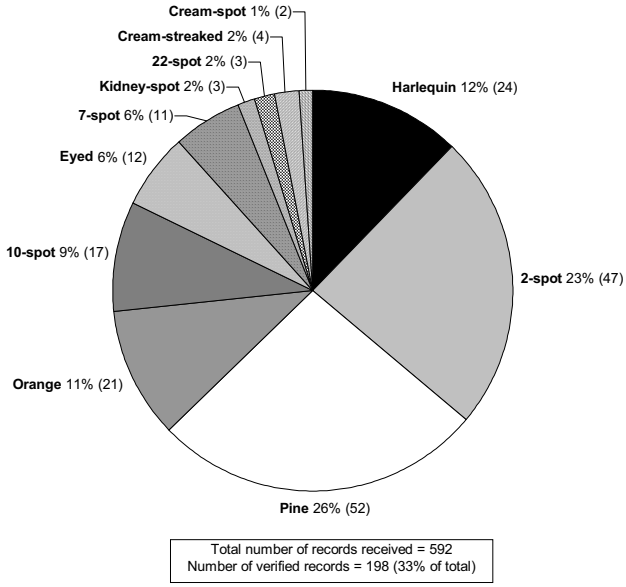


Figure 3: Percentage and actual number (in brackets) of verified ladybird records by species received from the UK public by the Harlequin Ladybird Survey from 15th March 2005 to 19th April 2005

(Harlequin = *Harmonia axyridis*; 2-spot = *Adalia 2-punctata*; Pine = *Exochomus 4-pustulatus*; Orange = *Halyzia 16-guttata*; 10-spot = *Adalia 10-punctata*; Eyed = *Anatis ocellata*; 7-spot = *Coccinella 7-punctata*; Kidney spot = *Chilocorus renipustulatus*; 22-spot = *Psyllobora 22-punctata*; Cream-streaked = *Harmonia 4-punctata*; Cream-spot = *Calvia 14-guttata*)

5.3. Anecdotal evidence of the UK public opinion on the arrival of H. axyridis

Ladybirds are extremely popular insects and so it is perhaps not surprising that the public response to these surveys has been great. The correspondence that has resulted from the publicity associated with the launch of the Harlequin Ladybird Survey in the UK has provided anecdotal evidence of the public's opinion on the arrival of *H. axyridis*. Undoubtedly the overwhelming response is of concern for the native fauna from a biodiversity perspective, with little consideration of the origins of *H. axyridis* as a biological control agent. However, the general concern for native species has highlighted the perceived importance of natural enemies, such as ladybirds, of insect pests as biological control agents. So, while to most people *H. axyridis* is an unwelcome arrival, this has not altered their affection for ladybirds. However, although there still appears to be wide support for biological control as a pest control strategy, the arrival of *H. axyridis* may have emphasized the different methods that can be employed and classical biological control may now be viewed more cautiously. Indeed some people have

expressed frustration that risk assessment and cost-benefit analysis have not prevented the use of *H. axyridis* in biological control programmes.

There have been a minority of people who have asked why the impact to our native fauna is a concern and whether in fact this is just a natural process of colonization by one species coupled with potential extinction of others. A similarly low number of people have recognized that *H. axyridis* may benefit agricultural systems and thus feel that it's arrival is advantageous. However, the overwhelming balance of public opinion is that *H. axyridis* represents a real threat to UK ecosystems.

5.4. Future plans

The Harlequin Ladybird Survey and the UK Ladybird Survey will continue to monitor ladybirds within the UK for at least the next five years. The data accumulated, along with historic data, will enable detailed evaluation of the direct and indirect effects of *H. axyridis* on native coccinellids and other insects. From this quantitative approach we will gain a greater understanding of the complex interactions between multiple species within the aphidophagous predatory guild with particular reference to an invasive species. Many studies have assessed the use of multiple natural enemies for biological control but generally on a small spatial scale (Roy and Pell, 2000). The UK Ladybird Survey, and projects generated from it, will enable multiple species interactions to be considered on much larger spatial and temporal scales. This will not be without its challenges but continued research in this area is critical to create a strategy for controlling this invasive species, protecting the native fauna and implementing sustainable strategies for pest management.

6. *Harmonia axyridis*: a global problem

Coccinellids have been used widely as classical biological control agents. In some cases their use has been highly successful as typified by the vedalia ladybird introduced to control cottony cushion scale insects in the Californian citrus industry in 1888 (Majerus, 1994). In other cases introduced coccinellids have failed to adequately control the target pest below economically damaging thresholds or, indeed, to thrive at all. Up to 1985, of 179 species of coccinellid introduced to North America only 18 became established (Gordon, 1985). However, perhaps the worst case scenario of biological control is an introduced species adversely affecting non-target species, as, for example, in the infamous case of cane toads in Australia. *Harmonia axyridis* is not the first introduced coccinellid to present a threat to the wider ecosystem. The 7-spot ladybird, *C. septempunctata* was repeatedly released in North America and the decline of the convergent ladybird, *Hippodamia convergens*, and two endangered lycaenid butterflies are now attributed to its success in establishing (Horn, 1991).

Invasive species, whether they are intentionally or unintentionally introduced, represent a global problem. However, even within Europe there is considerable variation in the regulation of introduced invertebrate biological control agents, from none at all to stringent. Cooperation and collaboration between countries both within Europe and beyond will undoubtedly accelerate progress and understanding of *H. axyridis* and other invasive species. Hopefully, lessons will be learnt before homogenization of species occurs on a global scale. The threat that voracious predators, such as *H. axyridis*, pose to indigenous species are an unacceptable consequence of biological control. The prevalence of *H. axyridis* around the world is testament

to its adaptability and competitive ability, both traits that ensure *H. axyridis* is successful in controlling pest insects. Unfortunately it is these same traits that also make it a threat to other species on a global scale. The high profile of *H. axyridis* as both an invasive species and a biological control agent may hinder the promotion of modern biological control but safety and sustainability must be the prime consideration. Biological control is and will remain an essential component of sustainable agriculture, but the distinction between a successful biological control agent and an invasive species can be narrow.

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