DIAS report

Implementation of Biocontrol in Practice in Temperate Regions - Present and Near Future

Proceedings of the International Workshop at Research Centre Flakkebjerg, Denmark on November 1 to 3, 2005

Lise Stengård Hansen, Annie Enkegaard, Tove Steenberg, Sabine Ravnskov and John Larsen

DIAS report No. 119 • February 2006

Ministry of Food, Agriculture and Fisheries
Danish Institute of Agricultural Sciences

DIAS report Plant Production No. 119 • February 2006

Implementation of Biocontrol in Practice in Temperate Regions - Present and Near Future

Proceedings of the International Workshop at Research Centre Flakkebjerg, Denmark on November 1 to 3, 2005

Lise Stengård Hansen and Tove Steenberg
Department of Integrated Pest Management
Research Centre Sorgenfri
Skovbrynet 14
DK-2800 Kgs. Lyngby

Annie Enkegaard, Sabine Ravnskov and John Larsen
Department of Integrated Pest Management
Research Centre Flakkebjerg
Forsøgsvej 1
DK-4200 Slagelse

DIAS reports primarily contain research results and trial statements aimed at Danish conditions. Also, the reports describe larger completed research projects or acts as an appendix at meetings and conferences. DIAS reports are published in the series:
Plant production, Animal Husbandry and Horticulture.

Subscribers obtain 25% discount. Subscription can be taken out by contacting:
Danish Institute of Agricultural Sciences
P.O. Box 50, DK-8830 Tjele
Tlf. +45 8999 1028

All DIAS publications can be ordered on the internet:
www.agrsci.dk

Print: www.digisource.dk
ISBN 87-88976-88-2
ISSN 1397-9884

Contents

Development, registration, industrial production and commercialisation of BCAs

Biocontrol of pests and diseases in stored products, food processing, post harvest

Inoculative biocontrol of insects, weeds and diseases in outdoor crops, forestry, glasshouses

Inundative biocontrol of pests, diseases and weeds in outdoor crops, forestry, glasshouses

Summary and conclusions

Posters

Foreword

The international workshop on "Implementation of Biocontrol in Temperate Regions in Practice – Present and Near Future" took place at Research Centre Flakkebjerg, Denmark on November 1 to 3, 2005. The workshop was initiated by the Danish Centre for Biological Control, and arranged by the Department of Integrated Pest Management, Danish Institute of Agricultural Sciences. The workshop attracted 80 participants from 10 countries, mostly from Northern Europe. A number of specialists participated as invited speakers to give a state-of-the-art in a specific sector. The programme contained 40 oral presentations and 19 posters addressing practical application of biological pest control in a wide range of areas: field crops, greenhouses, forestry, animal husbandry and stored products. The participants thus had a unique opportunity to draw on the experience obtained from biocontrol application in other sectors.

The overall impression from the workshop is that biological control has great potential in temperate regions and that a substantial amount of research has led to successful application in several sectors. It is my hope that the workshop will promote further application of biological control in temperate regions.

I would like to thank the Ministry of Science, Technology and Innovation for providing funds for the Danish Centre for Biological Control via the research programme FØTEK III. The contributions from the sponsors of this specific workshop: Borregaard BioPlant, EWH Bio-Production, Garta and Mortalin A/S, are gratefully acknowledged. I would like to thank Kirsten Jensen and Sonja Graugaard for editing the language and compiling the proceedings, respectively. Finally the members of the organizing committee are thanked for delightful collaboration.

Lise Stengård Hansen
Head of organizing committee

Organizing committee
Lise Stengård Hansen
Annie Enkegaard
Sabine Ravnskov
Tove Steenberg
Sonja Graugaard

Introduction to different strategies of biocontrol

Jørgen Eilenberg
Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: Biological control (or biocontrol) is defined as the use of living organisms to control pest organisms. I use the term 'pest' for insects, mites and vertebrate pests, plant diseases, and weeds. Four complementary strategies of biocontrol exist and these are defined and briefly outlined.

Key words: Biocontrol, biological control, classical biological control, inoculation biological control, inundation biological control, conservation biological control

Introduction

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'*

Biological control in plant crops is placed among other possibilities in integrated pest management. The main point is that in biological control the agent must be a living entity (vira included).

Biological control has traditionally often been seen as a vision. DeBach (1964) gave this wording of 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'*

Biological control can be divided into four complementary strategies: classical, inoculation, inundation and conservation. The strategies are defined (based on Eilenberg *et al.* (2001) and Eilenberg (2005)) and briefly described below. It should be mentioned that some authors have preferred to write the prefixes as adjectives for the latter three strategies, thus naming the

strategies: classical, inoculative, inundative and conservative (Hajek, 2004). I prefer to use the noun form of all prefixes, but these differences in grammar are not significant.

Classical biological control

> *'The intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest 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's. The early successes were the reason for the term 'classical', which cannot be understood without this historical dimension.

The strategy was (and still is) among the most successful 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.

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'*

The main principle of inoculation biological control is that 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. The goal is that the natural enemy increases in population size and thus controls the pest over a period of time. The inoculated biocontrol organisms will, however, not establish permanently at a sufficiently high population density to prevent damage. New inoculations must be carried out after a period of time.

It can be postulated that inoculation biological control represents a reestablishment of a natural balance, temporarily distorted by man. Soil for cropping is 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.

Inundation biological control

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

The principle is as follows: a pest population increases in size, but at a certain time 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 a biocontrol agent is needed. The term 'biopesticide' is often associated with inundation biological control, linking the concept rather closely to the use of chemical pesticides. Inundation can be hard to separate from inoculation and these two strategies are often together called 'augmentation'.

Inundation is probably the strategy for biological control, which most easily can be explained to everybody: the population density of a nasty pest is too high, and a biocontrol agent is applied to control the pest.

Conservation biological control

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

A pest species 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'. To perform conservation biocontrol, 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.

Among the four biological control strategies, conservation biological control can be seen as the one most tightly connected to the main principles of organic farming, which has protection of the existing natural enemies as one of its main principles.

References

DeBach, P. (ed.) 1964: Biological control of insect pests and weeds. Chapman and Hall, London, 844 pp.

Eilenberg, J. 2005: The concepts and vision for biological control. In: An Ecological and Societal Approach to Biological Control, eds. Eilenberg, J. and Hokkanen, H.T.M. Springer (in press).

Eilenberg, J., Hajek, A. & Lomer, C. 2001: Suggestions for unifying the terminology in biological control. BioControl 46: 387-400.

Hajek, A. 2004: Natural Enemies. An Introduction to Biological Control. Cambridge University Press, Cambridge, 378 pp.

Biological control through conservation of natural enemies of agricultural and forest arthropod pests – recent practices and possibilities

Barbara Ekbom
Department of Entomology, Swedish University of Agricultural Sciences, Box 7044, S-750 07 Uppsala, Sweden

Abstract: Many insect pests are subject to the action of natural enemies that commonly occur in their ecosystems. These naturally occurring predators and parasitoids often play an important role in suppressing pest populations. A better understanding of the factors that influence natural enemy efficacy under field conditions in agriculture and forestry will indicate how best to conserve and enhance endemic species that contribute to mortality in pest populations. Heterogeneity is an important factor influencing populations of insect pests and their natural enemies in agricultural landscapes. We need to consider the landscape in terms of how insects move between habitats. Insects require a variety of resource types and these resources should be within their movement range. One example presented is from a forest ecosystem where a potential pest, the pine sawfly is most often held in check by natural enemies, but a slight change in environmental factors may allow the pest to escape natural enemy control. Another well-studied example presented is the interaction between polyphagous predators and the bird cherry-oat aphid in spring cereal agroecosystems. Evidence is presented to show that ground-living generalist predators have an important impact on the size of aphid populations. This impact prevents yield loss. The results of empirical and theoretical studies have indicated that predation during the time when aphids are colonizing the fields is very important for biological control. Knowledge of the factors that may upset the biological control balance between enemies and pests may help predict outbreaks where additional control measures must be used.

Key words: Insect pests, predators, parasitoids, cereal aphids, pine sawflies, outbreak, ephemeral habitat

Introduction

Many natural occurring predators and parasitoids often play an important role in suppressing pest populations. Sometimes, however, this "natural" control does not limit pest numbers sufficiently to avoid damage of economic importance. To enhance "natural" control we need better knowledge of the factors that influence natural enemy efficacy.

Pest dynamics in forests, which are perennial habitats, are often characterized by episoic outbreaks (Myers, 1988). The reason for these outbreaks is often unclear (Liebhold & Kamata, 2000) and two competing theories are invoked to explain insect outbreaks. One puts climatic factors at the forefront while the other suggests that biotic interactions such as natural enemy attack are most important. Recent work (Dwyer *et al.*, 2004) proposes that at low pest

densities generalist enemies may hold pest populations at a low level and at high pest densities cycles may be driven by specialist parasitoids and pathogens. Complex dynamics are generated when effects of weather cause unpredictable shifts between low and high pest densities (Stone, 2004).

Agricultural systems are often dominated by ephemeral habitats. Pests in agricultural fields are usually not permanent residents and will recolonize fields each season (Wissinger, 1997) making it difficult for pests and enemies to have a close relationship. Traits of natural enemies in disturbed habitats are not the same as those associated with natural enemies in perennial habitats (Wiedenmann & Smith, Jr., 1997). Annual crop systems are frequently disturbed, which can make it difficult for natural enemies to perform successful pest suppression. Therefore, for predators to occur in fields after disturbances, farmers are dependent on the predators' ability to recolonize. It is important that the predators maintain a high population in proximity to the field and have a high dispersal ability to reduce the time lag between recolonization of natural enemies and establishment of pest. If this is the case, a synchrony will be created between predator and prey, which will lead to more successful pest suppression (Wiedenmann & Smith, Jr., 1997).

Forest Pest: Pine Sawfly (*Neodiprion sertifer*)

This insect is well studied (Larsson *et al.*, 2000), and a great deal of knowledge has accumulated on demographic parameters and population dynamics. Research has shown that ground-living predators, such as small mammals (Hanski, 1987), and cocoon parasitoids can have an important impact on the population dynamics of the pine sawfly. These enemies are assumed to have a typical type III functional response, which is often found for vertebrates where learning is involved and where prey is aggregated.

A model was built to analyse population dynamics of the pine sawfly (Larsson *et al.*, 2000). Using demographic parameters from both the laboratory and the field, the action of predators, both on larvae and cocoons, as well as plant quality were considered. We found a system that had multiequilibria (Figure 1) (Belovsky & Joern, 1995). At low population levels the cocoon predators held the population in check, but it was possible for the population to escape control when plant quality changed.

When trees grow on good soils the generalist cocoon predators may often be able to hold pine sawfly levels low. Work by Herz & Heitland (2003) showed that predation on another sawfly, *Diprion pini*, was highest in stands where the soils were most fertile and where there was dense understory vegetation.

Potential applications of these results are 1) monitoring programs that target poor soils and pure pine stands where the risk of escape from cocoon predation is highest, 2) identification of abiotic factors that will influence plant quality. In order to be able to suggest methods of enhancement for better pest control in forests, it is important that dominant species in the natural enemy communities of forest arthropod pests are identified. It is also

essential to consider tri-trophic interactions as well as the possibility that there may be interference between different groups of natural enemies.

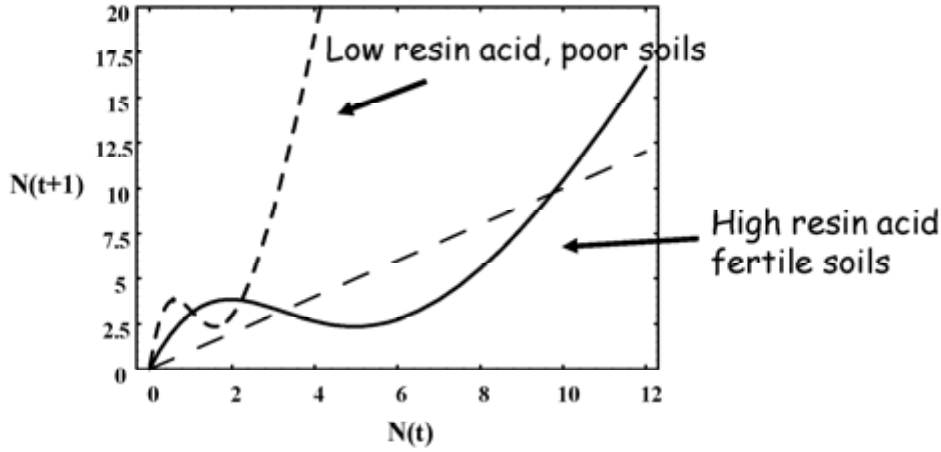

Figure 1. Recruitment curves for the pine sawfly. Modified from Larsson *et al.* (2000). The straight dashed line is the equilbrium value.

Agricultural pest: Bird cherry-oat aphid (*Rhopalosiphum padi*)

The bird cherry-oat aphid is a migratory pest. It spends winters on the bird cherry tree (*Prunus padus*) as an egg. The aphid migrates to grass hosts after 2 to 3 generations on the winter host. Many aphids will land in annual crop fields where cereals are grown. In at least 4 of every 10 years the migrating population is so large that reproduction in cereal fields leads to aphid numbers that will cause economic losses. There are many natural enemies in the agricultural landscape; both specialists such as ladybugs and parasitoids as well as generalists like carabids and spiders. The generalist predators most often belong to local populations. Field inventories have shown that high numbers of generalist predators are often correlated with low numbers of aphids in cereals, while there is no such correlation with specialist natural enemies (Ekbom & Wiktelius, 1985).

Simulation studies (Ekbom *et al.*, 1992) have shown that predation during the establishment phase of aphid migration will have a great impact on the population development of the bird cherry-oat aphid in cereal fields (Figure 2). We also had reason to believe that landscape surrounding the fields could be important as many natural enemies utilize field margins and other perennial habitats for overwintering (Bommarco & Ekbom, 2000).

Figure 2. Development of the bird cherry-oat aphid population in a cereal field. Model example. The broken line is population development without predation during establishment. The solid line is population growth with predation.

Landscape, farm management and biological control

Studies were carried out on 10 farms in the Uppsala area of Sweden. Five organic farms and 5 conventional farms that were paired according to landscape features were inventoried. We looked at number of aphids arriving at the field, the predation rated during establishment and aphid growth rate with and without natural enemies (Östman *et al.*, 2001a).

We found that both landscape features and farm management influence biological control. Low numbers of establishing aphids and high predation rates were associated with high perimeter to area ratios (generally smaller fields) and a high proportion of perennial crops in the surroundings. Number of establishing aphids was lower and predation rates were higher on organic farms in comparison with conventional farms with similar landscapes. We also estimated the value of biological control as an ecosystem service in cereal fields (Östman *et al.*, 2003).

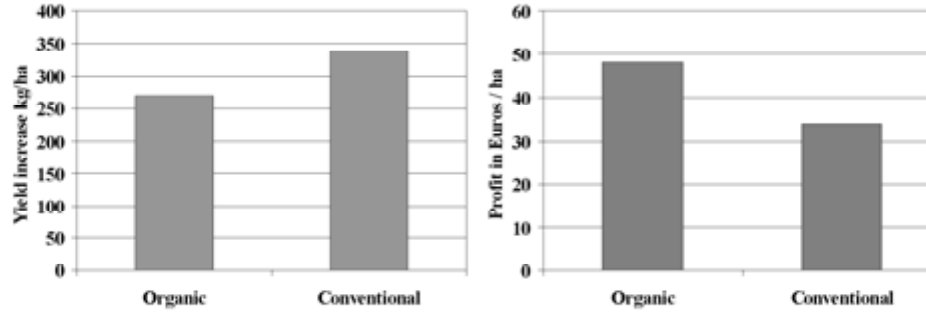

Figure 3. Average yield increase and monetary profit attributable to natural enemies in Swedish cereal fields. From information in Östman *et al.* 2003.

The results of our studies showed that predation on aphids arriving to the fields is most important for successful biological control. Landscapes with high perimeter to area ratios (small fields and many non-crop habitats) and high proportions of perennial crops such as meadows and leys facilitate biological control. Predators on organic farms and in complex landscapes are in good condition (Östman *et al*., 2001b). Availability of alternative prey may be important and thereby result in enhanced predator abundance on organic farms and in complex landscapes.

Conclusions

Natural enemies are doing an important job for us, in both perennial habitats like forests and ephemeral habitats found in annual crops in agriculture. We must find ways to enhance their biological control potential so that they can hold pest populations at levels where no economic damage occurs. To do this we should seek ways to increase natural enemy numbers and enhance their predation rates. Some factors that have been shown to be important and warrant further investigation are the influence of habitat quality, landscape structure and management practices. We also need to know more about the composition of communities of natural enemies found in differently managed ecosystems and the interactions occurring between different species of enemies.

Acknowledgements

Jan Bengtsson, Christer Björkman, Riccardo Bommarco, Philip Chiverton, Solveig Eriksson, Sascha Firle, Carol Högfeldt, Stig Larsson, Mario Natiello, Örjan Östman, Mette Petersen, Henrik Wallin, Anki Weibull, and Staffan Wiktelius are all thanked for high-quality collaboration over the years. Financial support came from SLU, SJFR and FORMAS.

References

Belovsky, G.E. & Joern, A. 1995: The dominance of different regulating factors for rangeland grasshoppers. In: Population dynamics: new approaches and synthesis. Eds. Cappuccino and Price: 359-386.

Bommarco, R. & Ekbom, B. 2000: Landscape management and resident generalist predators in annual crop systems. In: Ekbom, B., Irwin, M.E. & Robert, Y. (eds.) Interchanges of insects between agricultural and surrounding landscapes. Kluwer, pp. 169-182.

Dwyer, G., Dushoff, J. & Yee, S.H. 2004: The combined effects of pathogens and predators on insect outbreaks. Nature 430: 341-345.

Ekbom, B.S. & Wiktelius, S. 1985: Polyphagous arthropod predators in cereal crops in central Sweden, 1979-1982. Z. Ang. Ent. 99: 433-442.

Ekbom, B., Wiktelius, S. & Chiverton, P.A. 1992: Can polyphagous predators control the bird cherry-oat aphid in spring cereals?: A simulation study. Ent. Exp Appl. 65: 215-223.

Hanski, I. 1987: Pine sawfly population dynamics: patterns, processes, problems. Oikos 50: 327-335.

Herz, A. & Heitland, W. 2003: Impact of cocoon predation and parasitism on endemic populations of the common pine sawfly, *Diprion pini* (L.) in different forest types. Agric. Forest. Entomol. 5: 35-41.

Larsson, S., Ekbom, B. & Björkman, C. 2000: Influence of plant quality on pine sawfly population dynamics. Oikos 89: 440-450.

Liebhold, A. & Kamata, N. 2000: Are population cycles and apatial cynchrony a universal characteristic of forest insect populations? Popul. Ecol. 42: 205-209.

Myers, J.H. 1988: Can a general hypothesis explain population cycles of forest Lepidoptera? Advances in Ecological Research 18: 179-242.

Östman, Ö., Ekbom, B. & Bengtsson, J. 2001a: Landscape heterogeneity and farming practice influence biological control. Basic and Applied Ecology 2(4): 365-371.

Östman, Ö., Ekbom, B., Bengtsson, J. & Weibull, A-C. 2001b: Landscape Complexity and Farming Practice influence the Condition of Polyphagous Carabid Beetles. Ecological Applications 11(2): 480-488.

Östman, Ö., Ekbom, B., & Bengtsson, J. 2003: The yield increase due to aphid predation by groundliving polyphagous predators in spring barley. Ecological Economics 45: 149-158.

Stone, L. 2004: A three-player solution. Nature 430: 299-300.

Wiedenmann, R.N. & Smith, Jr., J.W. 1997: Attributes of natural enemies in ephemeral crop habitats. Biological Control 10: 16-22.

Wissinger, S.A. 1997: Cyclic colonization in predictably ephemeral habitats: a template for biological control in annual crop systems. Biological Control 10: 4-15.

Organic amendment and disease control. Perspectives for biological control of soil borne diseases

Lars Bødker[1], Mariann Wikström[2], Lars Persson[3], Sabine Ravnskov[1], John Larsen[1], Åsa Olsson[3]

[1]Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark; [2]Findus R &D AB, P.O. Box 530, S-267 25 Bjuv, Sweden; [3]Sockernäringens BetodlingsUtveckling AB (SBU), Borgeby Slottsväg 11, 237 91 Bjärred, Sweden

Abstract: Several field trials in Sweden and Denmark have shown promising effects using organic amendment for prevention and control of soil borne diseases. However, these results have often been difficult to reproduce in field studies, where the variation in the natural level of infestation varies and the interaction with many different growth factors blurs the result. Field studies are often carried out in fields with high infestation levels where a biological control using green manure has insufficient effect as soil disinfection method. Several mechanisms are included in biological control of soil borne diseases using organic amendments. The paper will raise a discussion on the perspectives, dogmas and facts for biological control of soil borne diseases by showing examples of field studies in different horticultural crops and forest nurseries.

Biocontrol of the pollen beetle in rapeseed: which strategy?

Heikki M.T. Hokkanen
Department of Applied Biology, Helsinki University, Box 27, FIN-00014 Helsinki, Finland

Abstract: Parasitoids, predators, insect pathogenic fungi, nematodes and microsporidia have all shown promise in the biological suppression of the pollen beetle *Meligethes aeneus* in rapeseed. Under favourable conditions, in Finland pollen beetles are locally and temporarily already under efficient natural control by one or several of these agents, but overall the pest remains as the number one pest of rapeseed in Finland, as well as elsewhere in Europe. Conservation biological control to enhance natural control, particularly by the parasitoid *Phradis morionellus*, appears as the most feasible approach to solve the problem. Field and simulation data are used to demonstrate how a simple change in the tillage practice may be enough to obtain efficient control. Several other options are, or will be, available subject to successful registration of microbial products based on the fungi *Metarhizium anisopliae* and *Beauveria bassiana* and the nematode *Steinernema feltiae*. Strategies and methods of applying these agents have been developed to facilitate either inundative treatments or ecosystem restoration to (re-) establish the presence of these agents in agricultural fields. Several critical components of agricultural practice have been identified for maximising the control by biological agents. These include direct drilling of the crop after rapeseed, undersowing the rapeseed crop with white clover and either complete avoidance or strict targeting of chemical insecticide applications in time and space. Crop rotation should be designed also to consider the mobility of the key insects (rapeseed fields close to previous year's rapeseed fields) as well as the need for soil-borne antagonists to recycle in alternative host species when rapeseed is not grown (e.g. clover to harbour *Sitona* root-weevils). The microsporidium *Nosema meligethi* could be (re?)introduced to rapeseed growing areas using the classical biocontrol strategy to dampen natural fluctuations in pollen beetle densities and to further suppress the mean population densities of the pest. The increasing popularity of direct drilling gives good reason to believe that with further help from the IPM components described here, permanent biological control of the pollen beetle can be achieved in Northern Europe.

Key words: Parasitoids, predators, entomopathogenic fungi, entomopathogenic nematodes, microsporida, biological control, integrated control, pest management, turnip rapeseed

Introduction

In Finland, the natural control factors and management of the pollen beetle *Meligethes aeneus* F. have been studied by my group since 1983 (see e.g. Hokkanen *et al.*, 1986, 1988; Hokkanen, 1993, 2000; Hokkanen & Lipa, 1995). The aim of this paper is to review the role of different antagonists in limiting the numbers of the pollen beetle and to estimate their potential for use in biological control of the pest in northern Europe.

Parasitoids

Two species of parasitoids occur commonly on *M. aeneus* in Finnish oilseed rape (OSR) fields: *Phradis morionellus* (Ichneumonidae) and *Diospilus capito* (Braconidae) (Hokkanen *et al.*, 1988). The former is the dominant species, *D. capito* being virtually absent in most locations in most years but occasionally reaching up to 20% level of parasitism locally. The per cent parasitism of pollen beetle larvae by *P. morionellus* was studied in Finland for 11 years (1985-1995) at 35-70 different locations (13 regions), covering the total area of rapeseed cropping in Finland (Hokkanen, 2006). The total proportion of pollen beetles removed (*sensu* van Driesche *et al.*, 1991) from the new generation adult population (% parasitism at each region weighted by the area of rapeseed grown in that area) varied between the highest level in 1987 at 49.5% to the lowest in the following year (1988) at 7.5%; usually the proportion was around 30% (Figure 1). Pollen beetle attack decreased from severe in the early 1980s to moderate in 1985-1988, with rising levels of parasitism by *P. morionellus*. After the 1988 crash in *Phradis* populations, pollen beetle attack jumped again to severe until the early 1990s, after which some balance appears to have been reached (Figure 1). Insecticide sprays to control the all-time high populations of the cereal aphid *Rhopalosiphum padi* in 1988 are a likely explanation for the parasitoid crash in 1988.

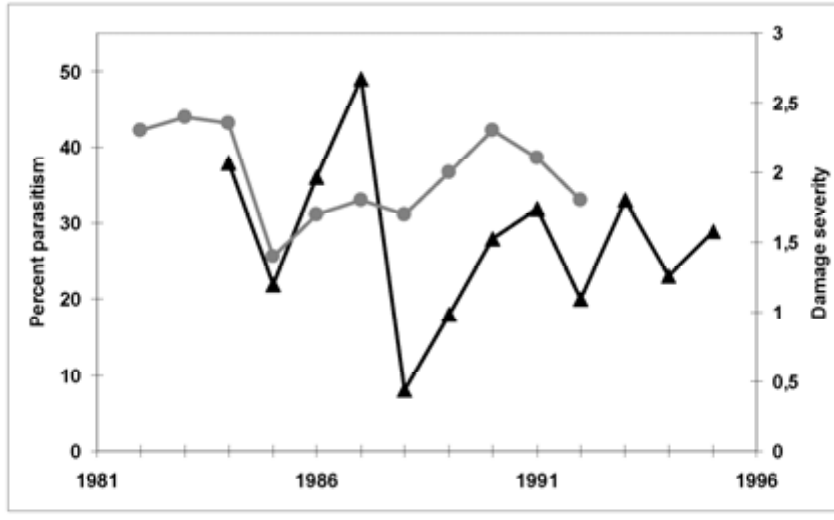

Figure 1. Pollen beetle overall damage severity 1982-1992 (grey line, dots) and per cent parasitism 1984-1995 (black line, triangles) in Finland. Damage severity data: Agricultural Research Centre of Finland survey data. (From Hokkanen, 2006).

Parasitoids appeared to significantly lower the pollen beetle populations as soon as 30-40% parasitism was reached (Figure 1). In 1985-88 the drop in pollen beetle damage severity may

have been caused by the high levels of parasitism. The parasitoid crash in 1988 appears to have released the pollen beetles from this natural control, which afterwards seemed to catch up only slowly (Figure 1). To further study this situation, a dynamic simulation model was constructed (Kaukoranta & Hokkanen, unpublished) to describe the rapeseed plant, pollen beetle and parasitoid interaction. This suggested that pollen beetle populations can indeed be held at a very low level by *Phradis* if the interaction is not disrupted by pesticide applications, or if the parasitoid population is enhanced by some other simple means, such as using direct drilling in establishing the crop following rapeseed. This avoids the mechanical destruction of the parasitoid pupae, which overwinter in the field soil, and can result in a four-fold increase in parasitoid numbers in the following year (Hokkanen *et al.*, 1988). This prediction of the model still awaits validation in practice, but all available data suggest that in Finland biocontrol of the pollen beetle is possible using simple conservation biological control methods.

Predators

Polyphagous predators have been shown to be important natural enemies of pests of oilseed rape under certain conditions. In Germany, Thiele (1977) concluded that about 50% of pollen beetle larvae/pupae may be eaten by predatory ground beetles during larval drop and pupation in soil. In Finnish rapeseed fields, carabid and staphylinid beetles along with spiders are the most common predators (Hokkanen, 2004). Over 40 species of ground beetles were determined over several years of studies, but there was a large variation between years and separate fields in the species composition, with only a few species occurring predictably (euconstant species). These include *Pterostichus melanarius, Amara eurynota, Harpalus rufipes, Calathus melanocephalus* and *Clivina fossor* (Hokkanen, 2004).

Besides ground beetles, other potentially important predatory groups in the rapeseed agroecosystem in Finland have been encountered as follows (data from Hokkanen, 2004): *Staphylinid beetles* and *spiders* are very common in pitfall traps, yellow water traps and in photoeclector catches. Abundance of both groups is roughly one half of the number of carabid beetles. *Lacewings* are encountered only occasionally, but regularly, as are *predatory bugs*; *Nabis ferus* and *Anthocoris nemorum* have been identified from rapeseed fields. *Predatory ladybeetles* occur usually in very low numbers on rapeseed. However, in the exceptional year of 1988, following a 'ladybeetle explosion', they were significant predators of pollen beetle larvae on all rapeseed fields in southern Finland: typical densities were 40-50 ladybeetles/m^2 over all of the rapeseed fields (*Coccinella septempunctata, Adalia bipunctata*).

Impact of insecticide treatment on predator activity has been studied on two occasions: in 1983 fenitrothion was sprayed on one part of a field, and the other half was untreated. Spraying had a drastic effect on carabid activity: three times as many were captured on the untreated part. Species that appeared particularly affected were *Lasiotrechus discus, Patrobus atrorufus* and *Synuchus nivalis*. However, studies in 1999-2000 with lambda-cyhalotrhin revealed no differences in carabid activity densities between untreated, insecticide treated or *Metarhizium*-treated plots (Hokkanen, 2004).

Impact of predators on pollen beetle numbers has been studied on two occasions in Finland, indicating that with proper management they also can be highly effective antagonists of the pest (Hokkanen, 2004):

(1) Exclusion experiments in 1985 showed that there was no significant impact on the number of emerging pollen beetles from predator exclusion (total of 11,105 beetles were collected from the emergence traps). A likely explanation is the seasonal dynamics of carabids in annual crops in Finland: the activity is at its lowest during pollen beetle pupation, 2nd and 3rd week of July. Activity density dynamics differ, however, depending on the crop: data from rapeseed, sugarbeet, cabbage, and timothy indicate that in more permanent crops (e.g. timothy) some key carabid species maintain activity throughout the summer (Varis *et al.*, 1984).

(2) An intercropping experiment in 1993 revealed no differences in the number of pollen beetles/plant, % parasitism or overall predator activity densities between the monocrop and intercrop. The number of emerging new generation pollen beetles, however, was drastically reduced in the intercrop as compared with the monocrop, which produced about 5 times as many F1 pollen beetles as the intercrop per surface area, or about 2.5 times as many per rapeseed plant (Figure 2). Higher predator pressure (lower total number of prey, but equal number of predators) in the intercrop is a likely explanation, worthy of further investigations.

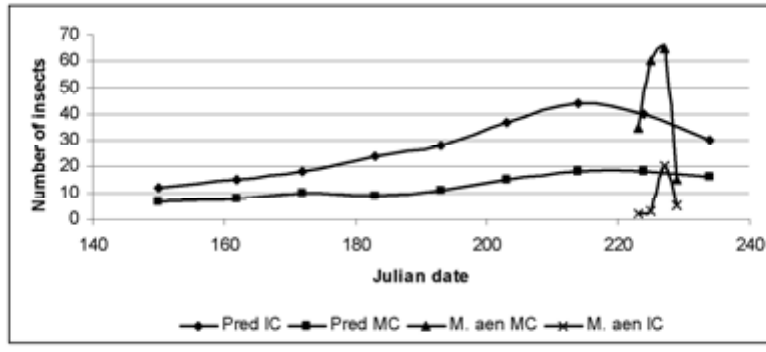

Figure 2. Number of predators per pitfall trap, relative to rapeseed plant density, in the monocrop (MC) and intercrop (IC), and number of *M. aeneus* F1/m^2 from both systems. Pupation occurs around day 200 in Finland. (from Hokkanen, 2004).

Insect pathogenic fungi

The entomopathogenic fungi (EPF) *Metarhizium anisopliae* and *Beauveria bassiana* have repeatedly been shown to kill effectively the adults and larvae of the pollen beetle (e.g. Butt *et al.*, 1994; Husberg & Hokkanen, 2001; Hokkanen *et al.*, unpublished). For example, spray treatment in the field with *M. anisopliae* caused 75% mortality in pollen beetle larvae (Hokkanen *et al.*, unpublished). Field treatments, however, have consistently not resulted in any

significant reduction in the new generation pollen beetle numbers, either via soil treatment or via fungal sprays (Hokkanen, 1993; Hokkanen *et al.*, unpublished).

Soil treatment with EPF has, however, been shown to cause dramatic indirect effects via increased overwintering mortality in the pollen beetle. Hokkanen (1993) demonstrated that although soil treatment with *B. bassiana* did not reduce F1 numbers in the autumn, the overwintering survival was halved compared with that in untreated controls (from 14% to 7%). Fungal treatment affected significantly the beetle weights: even under optimum food resources the beetles emerging from treated soils were 16% lighter than beetles emerging from control soils. Reference beetles collected from the wild were before overwintering still much lighter than either of the experimental groups (46% lighter than the experimental reference), resulting in only 3% survival over the winter (Hokkanen, 1993).

As agricultural soils throughout Europe have been shown to be almost deserts with respect to the occurrence of EPF (Zec-Vojinovic *et al.*, 2006), they could easily be (re)colonised with these fungi in order to add a significant new natural mortality factor of not only the pollen beetle but also of many other key pests of OSR. The technology to do this exists and is used for example in Switzerland to colonise meadows with *Beauveria brongniartii* to control *Melolontha melolontha* (Enkerli *et al.*, 2004).

Another approach for using EPF is to knock down pollen beetle peaks early in the season using trap crops and honeybee-mediated, targeted application of EPF. We are experimenting with *M. anisopliae*, spread to a turnip rape trap crop by honeybees before the main crop is attractive to the beetles, in order to infect the beetles before they damage the main crop (Cook & Hokkanen, in prep.).

Insect pathogenic nematodes

Biological control using entomopathogenic nematodes (EPN) has with a few exceptions been restricted to intensive cultivation systems in greenhouses and nurseries. So far, they have not found their place in the management of pests in any of the large-scale agricultural crops, and therefore, the markets for nematodes have remained small.

My group has studied the potential application of EPN in controlling OSR pests, in recent years in collaboration within the MASTER consortium (see Williams *et al.*, 2002). Series of field tests in Finland have consistently shown excellent pollen beetle control, using optimum timing (beginning of pupation of *M. aeneus*, early July) and a 'sufficient' dose (1 million IJ/m^2) of the EPN *Steinernema feltiae*. For example in 2002 the treatment provided 93.8% control (Menzler-Hokkanen & Hokkanen, 2005). Flea beetles were reduced by 50.1%, and no non-target effects were detected, except that the pollen beetle parasitoid *Phradis morionellus* was reduced to the same extent as its host, by 94.4% (via host depletion). Another study in 2004 showed that even with a much lower dose, excellent pollen beetle control results can be achieved (Figure 3); similar results have been obtained in other countries against the pollen beetle and other OSR beetle pests within the collaborative field trials in the MASTER project (Hokkanen *et al.*, 2006).

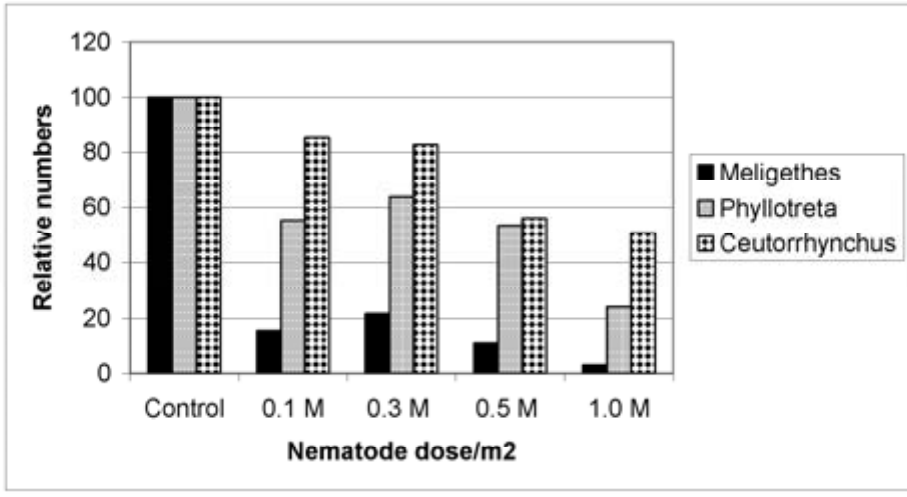

Figure 3. Relative effect of *Steinernema feltiae* treatments at different doses on some rapeseed target pests in Finland in 2004. Watertreated control = 100.

Problems in translating these excellent results into practice include: (1) it is usually not possible for a farmer to carry out treatments at the optimum time (end of flowering) and (2) the high dose often required for good control is prohibitively expensive. To overcome these, my group is investigating a low dose, controlled-release nematode delivery system ('NemaBag') that can be applied at a suitable time (e.g. at sowing). Another approach that we study is conservation biological control following inoculative release of EPN; this work focuses on designing optimum crop rotations and the use of white clover as undercrop in rapeseed to support alternative hosts such as *Sitona* weevils for the EPN. It can be concluded that EPN show good activity against key oilseed rape pests (Hokkanen *et al*., 2006) and that the controlled-release, lowrate delivery system merits intensified study as a possible way of making progress towards practical use of EPN in controlling pests on OSR and other major outdoor crops.

Insect pathogenic microsporidia

The intracellular obligate parasite *Nosema (Amncaliia) meligethi* occurs at endemic levels in Finland outside the rapeseed growing areas but is virtually absent from areas where rapeseed is grown (Hokkanen & Lipa, 1995). The parasite is specific to the genus *Meligethes*, occurs typically as a chronic disease with lowered fecundity and lifespan of the host and causes high overwintering mortality. It is transmitted both horizontally and vertically and is an 'ideal' insect pathogen from the population dynamics point of view (*sensu* Anderson & May, 1982). Our studies in Finland indicate that *N. meligethi* probably is incompatible with current OSR cropping practices, because it hardly ever occurs in areas where rapeseed is grown. Such an

incompatibility is probably a result of frequent insecticide treatments, which probably kill diseased (weakened) individuals more effectively than healthy ones, thus practically curing the population of the *Nosema* disease. Our studies further show that *Nosema* infections lower beetle weights on the average by 13%, which is enough to explain the observed increases in the overwintering mortality of the beetles (Hokkanen & Lipa, 1995). It is possible to artificially spread the disease, but probably the only feasible way to use it in biological control might be as classical (re?)introductions into pollen beetle populations. These would have to be associated with some changes in cropping practices in order to create a conducive environment for the disease to act. Its role could then be to stabilize natural fluctuations in pollen beetle population levels, after these otherwise are kept at low levels by parasitoids, predators and other pathogens (EPF, EPN).

Conclusions

There appear to be several feasible ways to achieve biological control of the pollen beetle in OSR cultivation. Some techniques can be implemented at any time (e.g. parasitoid and predator management; use of direct drilling and undercrop; pesticide use patterns), while others may need further research and development - and in some cases, registration (e.g. use of EPF, EPN and *Nosema*).

In brief, in order to maximise bio-control by pollen beetle natural enemies, we should:

- not plough the field after OSR harvest (parasitoid management)
- not spray insecticides on OSR, nor on the following crop; or use very tight spray windows if treatments are absolutely necessary (parasitoid management; *Nosema* enhancement)
- undersow or intercrop with clover (predator enhancement; alternative hosts for nematode replication such as *Sitona* root weevils)
- design crop rotation to consider the mobility of the key insects (rapeseed fields close to previous year's rapeseed fields to enhance parasitoid migration)
- re-introduce insect pathogenic fungi and nematodes to the OSR cropping systems (inundative, inoculative applications)
- knockdown pollen beetle peaks using trap crops and honeybee mediated, targeted application of EPF
- re-introduce *Nosema meligehti* (inoculative or classical introductions)

Acknowledgements

Support from the EU project MASTER: MAnagement STrategies for European Rape pests (QLK5-CT-2001-01447) is gratefully acknowledged.

References

Anderson, R.M. & May, R.M. 1982: Coevolution of hosts and parasites. Parasitology 85: 411-426.

Butt, T.M., Ibrahim, L., Ball, B.V. & Clark, S.J. 1994: Pathogenicity of the entomogenous fungi *Metarhizium anisopliae* and *Beauveria bassiana* against crucifer pests and the honey bee. Biocontrol Science & Technology 4: 207-14.

Enkerli, J., Widmer, F. & Keller, S. 2004: Long-term field persistence of *Beauveria brongniartii* strains applied as biocontrol agents against European cockchafer larvae in Switzerland. Biological Control 29: 115-123.

Hokkanen, H.M.T. 1993: Overwintering survival and spring emergence in *Meligethes aeneus*: effects of body weight, crowding, and soil treatment with *Beauveria bassiana*. Entomologia Experimentalis et Applicata 67: 241-246.

Hokkanen, H.M.T. 2000: The making of a pest: recruitment of *Meligethes aeneus* onto oilseed brassicas. Entomologia Experimentalis et Applicata 95: 141-149.

Hokkanen, H.M.T. 2005: Impact of predators on pollen beetle *Meligethes aeneus* on rapeseed in Finland. IOBC/WPRS Bulletin 27: 295-298.

Hokkanen, H.M.T. 2006: *Phradis morionellus* on *Meligethes aeneus*: long-term patterns of parasitism and impact on pollen beetle populations in Finland. IOBC/WPRS Bulletin (in press).

Hokkanen, H., Granlund, H., Husberg, G.-B. & Markkula, M. 1986: Trap crops used successfully to control *Meligethes aeneus* (Col., Nitidulidae), the rape blossom beetle. Annales Entomologici Fennici 52: 115-120.

Hokkanen, H., Husberg, G.-B. & Söderblom, M. 1988: Natural enemy conservation for the integrated control of the rape blossom beetle *Meligethes aeneus* F. Annales Agriculturae Fenniae 27: 281-294.

Hokkanen, H.M.T. & Lipa, J.J. 1995: Occurrence and dynamics of *Nosema meligethi* (Microsporida) in populations of *Meligethes aeneus* (Coleopera, Nitidulidae) in Finland. Entomologica Fennica 6: 11-18.

Hokkanen, H.M.T., Zec-Vojinovic, M., Büchs, W., Husberg, G.-B., Klukowski, Z., Luik, A., Menzler-Hokkanen, I., Nilsson, C., Ulber, B. & Williams, I. 2006: Effectiveness of entomopathogenic nematodes in the control of OSR pests. Proceedings of the MASTER Final Symposium, Göttingen (in prep.).

Husberg, G.-B. & Hokkanen, H.M.T. 2001: Efects of *Metarhizium anisopliae* on the pollen beetle *Meligethes aeneus* and its parasitoids *Phradis morionellus* and *Diospilus capito*. BioControl 46: 261-273.

Lipa, J.J. & Hokkanen, H.M.T. 1992: *Nosema meligethi* I. & R. (Microsporida) in populations of *Meligethes* spp. in Europe. Biocontrol Science and Technology 2: 119-125.

Menzler-Hokkanen, I. & Hokkanen, H.M.T. 2005: Developing entomopathogenic nematode delivery systems for biological control of oilseed rape pests. IOBC/wprs Bulletin 28(3): 19-22.

Thiele, H.-U. 1977: Carabid beetles in their environments. Zoophysiol. Ecol. 10: 1-369.

van Driesche, R.G., Bellows, T.S., Elkinton, J.S., Gould, J.R. & Ferro, D.N. 1991: The meaning of percentage parasitism revisited: solutions to the problem of accurately estimating total losses from parasitism. Environ. Entomol. 20: 1-7.

Varis, A.-L., Holopainen, J.K. & Koponen, M. 1984: Abundance and seasonal occurrence of adult Carabidae (Coleoptera) in cabage, sugar beet and timothy fields in southern Finland. Z. ang. Ent. 98: 62-73.

Vänninen, I., Tyni-Juslin, J. & Hokkanen, H. 2000: Persistence of augmented *Metarhizium anisopliae* and *Beauveria bassiana* in Finnish agricultural soils. BioControl 45: 201-222.

Williams, I.H., Büchs, W., Hokkanen, H., Menzler-Hokkanen, I., Johnen, A., Klukowski, Z., Luik, A., Nilsson, C. & Ulber, B. 2002: MASTER: Management Strategies for European Rape Pests - a new EU Project. The BCPC Conference, Pests & Diseases, Brighton, 18-21 November 2002, p. 641-646.

Zec-Vojinovic, M., Hokkanen, H.M.T., Büchs, W., Klukowski, Z., Luik, A., Nilsson, C., Ulber, B. & Williams, I. 2006: Natural occurrence of pathogens of oilseed rape pests in agricultural fields in Europe. Proceedings of the MASTER Final Symposium, Göttingen (in prep.).

Predator preferences – implications for conservation biological control

Lene Sigsgaard
Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: Preference for prey is an important criterion when assessing the control potential of a given predator. It was found that *Anthocoris nemorum* L. prefers *Aphis pomi* De Geer to *Dysaphis plantaginea* (Passerini). Earlier choice experiments with *A. nemorum* and *A. nemoralis* (Fabr.) for apple and pear pests showed preferences that differed between the two anthocorid species. For example in a two-choice experiment *A. nemoralis* preferred *Cacopsylla pyri* (L.) while *A. nemorum* preferred *A. pomi*. Oviposition preference experiments showed that *A. nemorum* prefers to oviposit on apple leaves and *A. nemoralis* on pear leaves. Since the oviposition site selected by overwintering adult anthocorids in spring will determine the distribution of the far more numerous – and less mobile – offspring, oviposition preference will have a major impact on resulting biological control. Field samplings show that *A. nemoralis* is more common in pear and *A. nemorum* in apple, thus confirming results from preference experiments. It was observed that *A. nemorum* was common in stinging nettle. Knowledge of prey and plant preferences of individual predator species can be used for predicting their contribution to biological control in a given habitat. Such information can also be used to optimise functional biodiversity for conservation biological control.

Key words: *Aphis pomi, Dysaphis plantaginea, Anthocoris nemorum*, preference

Introduction

Anthocoris nemorum and *A. nemoralis* are important predators in apple and pear orchards. They are polyphagous, preying on aphids, mites, psyllids and lepidopteran eggs and young larvae. *A. nemoralis* is particularly important in controlling pear psyllids (Solomon *et al.*, 2000). *A. nemorum* has been considered to have little preference for any prey, but a recent study documented differences in its preferences for some aphids of importance in greenhouses (Meyling *et al.*, 2003). Several studies have documented the distribution of anthocorids among various habitats and annual changes in distribution among habitats (Fauvel, 1999). Still, little is known about what guides the distribution. One mechanism documented is attraction of anthocorids to psyllid induced volatiles (Scutareanu *et al.*, 1997). This paper presents results from a prey preference experiment and relate this to results from previously reported prey preference studies and to two studies of anthocorid oviposition preferences.

Materials and methods

Anthocoris nemorum females were field collected five days prior to the experiment and were kept in thermo cabinets (L16:D8 photoperiod, 20 ± 1°C) and fed surplus grain moth (*Sitotroga cerealella* (Oliver)) eggs until use in assays. For details about Anthocorid rearing, see Sigsgaard (2005a). Fourth instar of *A. pomi*, green apple aphid and *D. plantaginea*, rosy apple aphid were field-collected on unsprayed apple trees immediately before the experiment. Prey preference was assessed in small units (30-ml plastic cups). Aphids were provided fresh pieces of apple leaves, and ten individuals of each prey were used. Aphids were allowed to settle for at least 15 min. before a predator was introduced. Anthocorids were starved for 24 h prior to experiments. Aphid nymphs were selected to obtain comparable sizes. Preference was assessed after 1.5 h. Control was used to assess mortality in the absence of predators. Low control mortality allowed analysis without corrections. A paired t-test was used to analyse difference in numbers of the two species consumed (SAS Institute, 1999) Preference (α) was analysed following the method of Chesson (1983) that allows for analysis of preference in experiments with food depletion (i.e. where the number of prey available is not assumed constant during the experiment).

Results and discussion

Anthocoris nemorum killed (dead and eaten) significantly more green apple aphids than rosy apple aphids (Figure 1). A paired t-test of data revealed significant preference for the green apple aphid. The difference between numbers of the two species eaten was highly significant ($t = 5.4$, $df = 16$, $P < 0.0001$) and the calculated prey preference for green apple aphid was 0.67 (c.i.: 0.60-0.75).

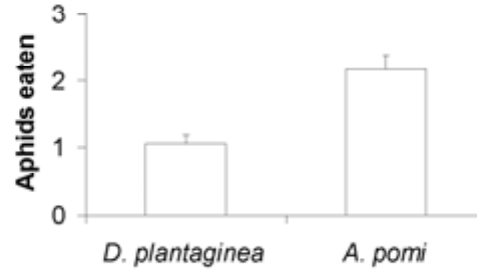

Figure 1. Numbers of *D. plantaginea* and *A. pomi* eaten by *A. nemorum* in 1.5 h.

Anthocoris nemorum also killed more green apple aphids than pear psyllids (*C. pyri*), with significant difference between the proportions killed of the two species. This was in contrast to *A. nemoralis* which preferred pear psyllids (Sigsgaard, 2005b). In the same study it was found that both species strongly preferred pear psyllids to female spider mites (*Panonychus ulmi*). Prey preference studies suggest that *A. nemorum* is a better aphid predator than *A. nemoralis* and thus a better candidate for biological control in apple. In a situation where apple trees have a mixed infestation of rosy and green apple aphid, results suggest that a higher proportion of green apple aphid will be removed. Adult spider mites were the least preferred, and a major role in controlling these by anthocorids should not be expected if alternative prey is available. However, immature spider mites may be a more preferred prey.

While *A. nemorum* preferred to oviposit on apple leaves (75% eggs laid on apple), *A. nemoralis* preferred pear leaves (71% eggs laid on pear leaves) (Sigsgaard, 2004). Oviposition was also guided by the presence of prey, honeydew and leaf damage (Sigsgaard, 2005a). Since the oviposition site selected by overwintering adult anthocorids in spring will determine the distribution of the far more numerous – and less mobile – offspring, oviposition preference will have a major impact on resulting biological control. Orchard samplings show that *A. nemoralis* is more common in pear and *A. nemorum* in apple (Sigsgaard, 2005c), thus confirming results from prey preference and oviposition preference experiments. The densities of the two anthocorid species on non-crop plants also vary. It was observed that *A. nemorum* was abundant on stinging nettle. Certain flowers have also been shown to attract *A. nemorum* and *A. nemoralis*. For example cornflower and corn camomile are attractive to *A. nemorum* (Fitz Gerald & Solomon, 2004). Knowledge of prey and plant preferences of individual predator species can be used for predicting their contribution to biological control in a given habitat. Such information can also be used to optimise functional biodiversity for conservation biological control.

References

Chesson, J. 1983: The estimation and analysis of preference and its relationship to foraging models. Ecology 64: 1297-1304.

Fauvel, G. 1999: Diversity of Heteroptera in agroecosystems: role of sustainability and bioindication. Agric. Ecosyst. & Env. 74: 275-303.

Fitzgerald, J.D. & Solomon, M.G. 2004: Can flowering plants enhance numbers of beneficial arthropods in UK apple and pear orchards? Biocontr. Sci. Techn. 14: 291-300.

Meyling, N.V., Enkegaard, A. & Brødsgaard, H.F. 2003: Two Anthocoris bugs as predators of glasshouse aphids - voracity and prey preference. Ent. Exp. Appl. 108: 59-70.

SAS Institute 1999: SAS/STAT User's Guide Version 8. SAS/STAT Inc., Cary N. C.

Scutareanu, P., Drukker, B., Bruin, J., Posthumus, M.A. & Sabelis, M.W. 1997: Volatiles from Psylla-infested pear trees and their possible involvement in attraction of anthocorid predators. J. Chem. Ecol. 23: 2241-2260.

Sigsgaard, L. 2004: Oviposition preference of *Anthocoris nemorum* and *A. nemoralis* for apple and pear. Ent. Exp. Appl. 111: 215-223.

Sigsgaard, L. 2005a: Oviposition preference of *Anthocoris nemoralis* and *A. nemorum* (Heteroptera: Anthocoridae) on pear leaves affected by leaf damage, honeydew and prey. Biocontr. Sci. Techn. 15: 139-151.

Sigsgaard, L. 2005b: Prey preferences of *Anthocoris nemoralis* and *A. nemorum* (Heteroptera: Anthocoridae) and their predation behaviour towards Pear psyllid, *Cacopsylla pyri*. IOBC-WPRS Bull. 28(*in press*): 98-201.

Sigsgaard, L. 2005c: Occurrence of the anthocorids *Anthocoris nemorum* and *A. nemoralis* in apple and pear in Denmark. IOBC-WPRS Bull. 28(*in press*): 122-125.

Solomon, M.G., Cross, J.V., Fitzgerald, J.D., Campbell, C.A.M., Jolly, R.L., Olszak, R.W., Niemczyk, E. & Vogt, H. 2000: Biocontrol of pests of apples and pears in northern and central Europe - 3. Predators. Biocontr. Sci. Techn. 10: 91-128.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biological control as an ecosystem service and its relevance to GMO biosafety testing

Gábor L. Lövei

Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark

Abstract: Biodiversity is essential for the proper functioning of ecosystems. Several of such functions (ecosystem services) have direct or indirect benefits for humans. One of these is biological control of pests and weeds. Biological control is important for agriculture, yet agriculture has a profound influence on this function. A novel agricultural technology, which can have a role in increasing food security is the field growing of genetically modified (GM) crop plants. The GM plants, when released, will influence the habitats they are grown in, and conservation biological control as an important service should be considered. This poses several challenges to biocontrol specialists. The important steps in testing whether GM plants have an impact on biological control agents are yet imperfect (limited species range, inconsistency in selecting species, methodological imperfections).

Key words: Biological control, GM plants, risk assessment, environmental impact

Introduction

Biodiversity is essential for the proper functioning of ecosystems (Vitousek & Hooper, 1993). Several such functions, termed "ecosystem services" (Daily *et al.*, 1997) have direct or indirect benefits for humans. Ecosystem services have traditionally been considered inexhaustible, due to their scale and intensity. With a human population currently at >6 billion, our impact on global scale reached a point where we can, and do, modify ecosystem services (Vitousek *et al.*, 1997). Ecosystem services cannot be replaced by technology, and their functioning is arguably crucial in both developed and developing regions of the world.

Food security and resource use are important concerns for societies. Science and technology can have important roles to help achieve these societal goals, but any new technology should consider the impact on the environment (Hails, 2002). A novel agricultural technology, which can have a role in increasing food security, is the field growing of genetically modified (GM) crop plants. Currently, GM plants are grown on an estimated 80 million ha worldwide, mostly in developed countries (James, 2004). The theory and practice of GM plant growing has a wide range of relevant questions that are currently unsettled.

In this paper I argue that the environmental impact assessment of GM crops should be done using the theoretical framework of ecosystem services, and that one of the major ecosystem functions to consider is natural biological control of pests and weeds. Subse-

quently, aspects related to biological control, especially species selection and laboratory test conditions, are discussed.

The important steps in testing whether GM plants have an impact on biological control agents are not consistently developed and there are several shortcomings (limited species range, inconsistency in selecting species). The GM plants, when released, will influence the habitats they are grown in, and conservation biological control as an important service should be considered. This poses several challenges to biocontrol specialists.

Review and discussion

Ecosystem services

Ecosystem services (ESs) operate on vast scales and have been recognised as irreplaceable (Daily, 1997). ES have been classified into several groups. The *production of goods* includes products that are harvested or used directly, such as food, medicine, fibre and timber, energy, industrial products and genetic resources. *Regeneration processes* include detoxification, decomposition, soil fertility maintenance, air and water purification, seed dispersal and pollination. *Stabilising processes* encompass coastal and river channel stability, moderation of weather extremes, floods and drought and the compensation of one species for another. Biological control also falls into this category of ESs. ESs also include *life-fulfilling functions* of aesthetic beauty, cultural and spiritual inspiration and inspiration for scientific discovery.

Human domination of the Earth

ESs have traditionally been considered inexhaustible (Daily *et al.*, 1997) but the continuing increase in the population size of humankind brought impacts of previously unimaginable magnitude on the Earth's ecosystems (Vitousek *et al.*, 1997). Examples range from the 40-50% of ice-free land surface heavily transformed by humans through the doubling of fixed nitrogen available to plants via the production of fertilisers (Vitousek *et al.*, 1997) to the current, human-driven extinction wave (Lövei, 2001a).

Agriculture and environmental impacts

The basis of human population growth is agriculture, which uses only a small fraction of plant and animal species that exist but produces most of the calories the human population needs (Tilman *et al.*, 2002). During historic times, the intensity of agriculture has been increasing. Important elements of this process include a) the continuing decrease of the part of the population involved in agriculture, b) replaced by more energy-intensive methods of production and c) with subsequent increasing impacts on environment. Even in developed countries, where agriculture involves only a small segment of the population and the share of such production may be a small % of national income, agriculture remains an important environmental quality driver (Tilman *et al.*, 2002; Hails, 2002). New technologies continue to emerge, and their spread is increasingly faster. The latest such development is the growing of genetically modified crops (Conway, 1997).

GM crops in the world

The first GM crops were commercially grown in 1996. At the latest estimate (James, 2004)

there were about 80 million ha of genetically modified crops grown in 17 countries of the world. Since the beginning, the same 4 crops (cotton, maize, soybean, canola) make up >95% of the area under GM crops, and most of the growing area is found in 5 countries: USA, Canada, China, Argentina and Brazil. Two traits: herbicide resistance and insect resistance dominate, with a smaller share of cultivars containing both traits. Currently only one crop has more GM than non-GM area, that of soybean (James, 2004).

GM crops and biological control

GM crops have significant potential environmental impact and risks (NRC, 2002). Therefore, pre-release risk assessment is required before such crops can be grown in the field. This often includes the demonstration of "no environmental harm", although the kinds of proof necessary for such an such assertion are not specified (NRC, 2002). One reason that we should be concerned about "environmental harm" is indeed the potential impact on ESs (Lövei, 2001b). Beneficial organisms are often included in pre-release risk assessment, although the expressed link between them and the ES they provide is not made clear (Birch *et al*., 2004). Biological control by natural enemies is one such ES, and this is the rationale to include species that are important in biological control (Lövei, 2001b).

Pre-release testing of natural enemy impacts

The number of natural enemies tested so far is not large. A recent review (Lövei & Arpaia, 2005) could only list 32 species of natural enemies that have ever been examined for the effect of GM plants, directly or indirectly. These included 35 studies on 18 species of predators in 3 orders. Seventeen studies were on Heteroptera (involving 11 species), 7 on Neuroptera (all on the green lacewing, *Chrysoperla carnea*) and 11 on Coleoptera (including species of two families, Coccinellidae and Carabidae). More than a single study was done on a handful of species: the coccinellids *Adalia bipunctata* (3 studies) and *Coleomegilla maculata* (4) and the heteropterans *Perillus bioculatus* (3), *Geocoris puncticeps*, *Orius tristicolor* and *O. insidiosus* (2 each). For parasitoids, a similarly small and fragmented database exists. No test has been done on other types of natural enemies (e.g. pathogens). Summarising the available quantitative data, while most of the parameters indicated neutral impact, nearly 30% of the quantified characters showed a significantly negative impact, underlining the necessity for pre-release testing.

Selection of species for pre-release testing

As pre-release testing is necessarily limited, preferably species that are "important" in providing a biocontrol service should be selected for pre-release testing. However, ecological importance is not an obviously clear concept (Hurlbert, 1997). Criteria for selecting natural enemy species for testing seems to be more or less ad hoc (Lövei & Arpaia, 2005), at maximum using limited surveys (Cowgill & Atkinson, 2003) or existing information (Romeis *et al.*, 2004). To put this on a more solid basis, Birch *et al.* (2004) suggested a method to organise available information and defined the criteria to arrive at a defendable selection of natural enemies for such testing. The criteria to be scored include feeding habit, occurrence/distribution in the habitat, abundance, presence over season, linkage to habitat and significance of the function provided by that species. All these criteria are scored on scale 1 (high) to 3 (small). All crite-

ria have equal weight, and species with the lowest combined scores are selected for further assessment, then testing.

Future challenges related to biological control

While the above selection method is an improvement over the current practice, there remain significant challenges to biological control practitioners to be able to correctly assess the impact of GM crops on natural enemies and to harmonise GM crops with biological control. These are, not surprisingly, location- and crop-specific. Here only salient points for (North, but not only) European conditions are mentioned.

The first challenge is to perfect the process by which species significant in biological control can be selected for pre-release tests. First, the empirical base has to be expanded, as arguably significant natural enemy groups, both arthropods (e.g. spiders, predatory flies) and representatives of other groups (virtually all) have never been tested. Test conditions have to be more realistic (Lövei & Arpaia, 2005).

Another challenge is to improve the understanding of the significance of biological control at field vs. landscape level to better understand the movement patterns by natural enemies and the conditions influencing them (Clough *et al.*, 2005).

Several natural enemies are omnivores, and the complexity of exposure routes and impacts from host plants and prey need clarification.

The link between biodiversity and biological control effectiveness is not yet sufficiently clear, and this is also linked to the urgent need to design monitoring regimes for the field impacts of crops authorised for commercial growing.

Conclusion

There is a good understanding of the importance of biological control in agriculture, and numerous papers on risk assessment of GM crops mention tests on natural enemies. As argued by Lövei (2001b), the inclusion of the ESs concept is more than a mere semantic change. Agriculture has a profound impact on environmental quality in Europe, and due to a combination of factors mentioned above, all new technology will have to be closely scrutinised for its environmental impact (Hails, 2002). The underlying reason is that humankind cannot afford to further degrade ESs. Biological control is an important ES, and therefore such impact assessment studies need to be extended to specifically consider the impact on biological control. This should not only extend to a careful selection of important species for laboratory, pre-release studies, but to more careful test conditions, and specifically to test the impact of such plants on biological control function on semi-field and field scales.

Acknowledgements

Work related to this paper was funded by the Danish Research Council, the European Union, DIAS, and DANIDA. I thank J. Hughes Matiny, E. Vincze, S. Bowra, K.H. Madsen, D.A.

Andow for discussions, H.-B. Christiansen, K. Frank, J.-Y. Guo, J. Lilholt, I.W. Nielsen, U. Sandberg, B.P. Pedersen and L. Pedersen for technical assistance.

References

Birch, A.N.E., Wheatley, R. & Anyango, B. *et al.* 2004: Biodiversity and Non-Target Impacts: a Case Study of Bt Maize in Kenya. In: Environmental Risk Assessment of Transgenic Organisms: A Case Study of Bt Maize in Kenya. eds. Hilbeck, A. & Andow, D.A., CABI International, Wallingsford. U.K.: 117-185.

Clough, Y., Kruess, A., Kleijn, D. & Tscharntke, T. 2005: Spider diversity in cereal fields: comparing factors at local, landscape and regional scales. J. Biogeog. 32. 2007-2014.

Conway, G.D. 1997: The doubly green revolution. Penguin, London.

Cowgill, S.E. & Atkinson, H.J. 2003: A sequential approach to risk assessment of transgenic plants expressing protease inhibitors: effects of nontarget herbivorous insects. Transgenic Research 12: 439-449.

Daily, G.C. (ed.) 1997: Nature's services. Island Press, Washington, D.C.

Daily, G.C., Alexander, S.E., Ehrlich, P.R., Goulder, L., Lubchenco, J., Matson, P.A., Mooney, H.A., Postel, S., Schneider, S.H., Tilman, D. & Woodwell, G.M. 1997: Ecosystem services: Benefits supplied to human societies by natural ecosystems. Issues in Ecol. 2: 1-18.

Hails, R.S. 2002: Assessing the risks associated with new agricultural practices. Nature 418: 685-688.

Hurlbert, S.H. 1997: Functional importance vs. keystoneness: reformulating some questions in theoretical biocenology. Aus. J. Ecol. 22: 369-382.

James, C. 2004: Global status of commercialized biotech/GM crops: 2004. ISAAA Brief No. 32.

Lövei, G.L. 2001a: Extinctions, modern examples of. In: Encyclopaedia of biodiversity, vol. 2., ed. Levin, S.A., Academic Press, New York: 731-743.

Lövei, G.L. 2001b: Ecological risks and benefits of transgenic plants. N. Z. Plant Prot. 54: 93-100.

Lövei, G.L., & Arpaia, S. 2005: The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomol. Exp. Appl. 114: 1-14.

National Research Council (NRC). 2002: Environmental effects of transgenic plants. National Academy Press, Washington, DC.

Romeis, J., Sharma, H.C., Sharma, K.K., Das, S. & Sarmah, B.K. 2004: The potential of transgenic chickpeas for pest control and possible effects on non-target arthropods. Crop Prot. 23: 923-938.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. & Polasky, S. 2002: Agricultural sustainability and intensive production practices. Nature 418: 671-677.

Vitousek, P.M. & Hooper, D.U. 1993: Biological diversity and terrestrial ecosystem biogeochemistry. In: Biodiversity and ecosystem function, eds. Schulze & Mooney, Springer, Berlin: 3-14.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. 1997: Human domination of earth's ecosystems. Science 277: 494-499.

Biological control of small mammal pests by improvement of perch and nesting sites for avian predators

Solveig Vibe-Petersen

Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Danish Institute of Agricultural Sciences, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark

Abstract: The main prey of many avian predators is rodents. Avian predators may consume enormous amounts of prey and are therefore frequently considered potential biocontrol agents of rodent pests. The use of perch poles and nest boxes for attracting avian predators and increasing their activity locally has proven successful by several studies worldwide. Perhaps for this reason such methods are commonly used in forestry in Denmark. Scientific documentation in general on the effects of perch poles and nest boxes on the prey population, however, is sparse, and even less when it comes to the effect on the damage caused by the pest rodent population. In some places, the methods have lead to successful control of rodent pests, but other studies have failed to prove any effect on the rodent population. The results of a predator attraction vs. predator exclusion study carried out in Denmark and a similar study carried out in Tanzania will be summarised. Whereas the Danish study showed divergent results, the Tanzanian study showed positive effect of perch pole and nest box use on the crop yield. Despite of that there was no further effect from control on the rodent population. This indicates that predators affect the foraging behaviour of the rodents.

Biological control of pests in livestock production

Christopher J. Geden
USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, PO Box 14565, Gainesville, Florida, 32607, USA

Abstract: Filth flies have a wide array of natural enemies that can be exploited for augmentative biological control. There are several important predators of fly eggs and larvae, including the mite *Macrocheles muscaedomesticae*, the histerid beetle *Carcinops pumilio*, and the black dump fly *Hydrotaea aenescens*. Mass rearing methods have been developed for these predators, and *C. pumilio* and *H. aenescens* are now available as products from commercial insectaries. The most widely used natural enemies are pteromalid pupal parasitoids in the genera *Muscidifurax* and *Spalangia*. Rearing methods for these parasitoids are straightforward but require close attention to detail and the biology of the species being produced. The first requirement is an efficient method for mass-rearing hosts; house flies can be grown for $0.50 per 10,000 fly pupae. Pupae are exposed to hosts for about 3 days, using host:parasitoid ratios that ensure high rates of parasitism without causing excessive mortality because of superparasitism. Rearing protocols also need to take into account whether the species is solitary or gregarious and the aggressiveness of parasitoid larvae in superparasitized hosts. Parasitoid releases have been demonstrated to be an effective fly management tool in several studies, including tests in Denmark, New York, Maryland, Nebraska, and Florida. Most filth fly parasitoids are vulnerable to *Nosema* disease that can cause a 90% reduction in wasp fecundity, but infection can be prevented by careful screening at the time of colony founding and can be controlled by drug and heat therapy. Adult flies are subject to infection with parasitic nematodes and the fungal pathogens *Entomophthora muscae* and *Beauveria bassiana*. Of these, *B. bassiana* has the greatest potential for operational fly control because it is economical to produce, has a long shelf life and can be deployed in a variety of ways. A baculovirus (SGHV) was discovered recently that may hold promise as well; the virus causes hyperplasia of the salivary glands and shuts down ovarian production.

Key words: House fly, stable fly, biological control

Introduction

At present, the most promising targets for biological control of livestock pests are muscoid flies. The house fly (*Musca domestica* L.) and stable fly (*Stomoxys calcitrans*) have received the greatest attention, and this review concentrates on the status of biological control programs to control these pests. Pasture pests such as horn fly and face fly are vulnerable to many of the same natural enemies as pests of confined animals, but the operational challenges of biocontrol in isolated manure pats on pasture have not been addressed.

Filth flies are vulnerable to attack, parasitism or infection at every life stage. Table 1 outlines the various natural enemies of flies and the fly life stage that is attacked. Eggs and young larvae are preyed on by predacious mites and beetles, and older larvae are parasitized by nematodes and attacked by larvae of the black dump fly *Hydrotaea aenescens*. Adult flies are subject to infection by fungal and viral pathogens. The biocontrol potential and status of these natural enemies will be addressed in the sections that follow in an attempt to summarize and update more comprehensive reviews that have been done on this topic in the past (Patterson *et al.*, 1981; Axtell, 1986; Patterson and Rutz, 1986; Rutz and Patterson, 1990; Axtell and Arends, 1990; Legner, 1995; Geden and Hogsette, 2001).

Table 1. Natural enemies with potential use for biological control of filth flies.

Natural enemy	**Fly stage attacked**
Predators	
Macrochelid, uropodid and parasitid mites	Eggs
Carcinops pumilo (Histeridae)	Eggs & young larvae
Hydrotoaea aenescens(Anthomyiidae)	Larvae
Nematodes	
Steinernematids, heterorhabditids	Larvae
Paraiotonchium muscaedomesticae	Larvae
Parasitoids	
Encyrtids (*Tachinaephagus zealandicus*)	Larvae
Diapriids (*Trichopria* spp.)	Young pupae
Pteromalids & chalcids (*Muscidifurax*, *Spalangia*)	Older pupae
Pathogens	
Entomophthora muscae complex	Adults
Beauveria, Metarhizium	Adults
SGHV virus	Adults

Predators

Mites

A large and varied guild of predator mites attacks eggs and newly hatched larvae of muscoid flies in livestock and poultry facilities. Although urpodid and parasitid mites are commonly found, the most abundant and important species is the macrochelid *Macrocheles muscaedomesticae*. This mite has a cosmopolitan distribution and is found in accumulations of cattle, poultry, pig and sheep manure. Adult females feed on fly eggs and newly hatched

larvae as well as alternate prey including other flies, acarid mites and nematodes (O'Donell and Axtell, 1965; Geden and Axtell, 1988). Attack rates on house fly prey generally are in the range of 10-20 fly immatures killed per female per day under normal conditions but are suppressed when mites are crowded or presented with an abundance of alternate prey (reviewed in Geden, 1990). Development from egg to adult is completed in only about 2 days at 27-30°C (Wade and Rodriguez, 1961). Female mites live for about 24 days and lay most of their eggs in the first two weeks after emergence (Filipponi and Petrelli, 1967). Dispersal to new habitats is via phoretic transport of adult mites on flies (Farish and Axtell, 1971; Borden, 1989).

M. muscaedometicae is an important natural regulator of fly populations in the field (Legner, 1971; Legner *et al.*, 1973; Axtell, 1963). The short development time of the mite allows populations to fluctuate widely in response to changes in the abundance of flies and other prey. This species has considerable potential as an augmentative biological control agent that could be particularly useful in situations such as calf hutches or newly cleaned poultry houses where resident natural enemy activity is low. The mites can be easily reared using nematodes as prey (Singh *et al.*, 1966), and Ho *et al.* (1990) decribed a mass-rearing protocol in which mite populations could be increased 70-fold per generation. At this time there are no known commercial producers of *M. muscaedomesticae*.

Beetles

Animal manure is home to a variety of predacious beetles, especially in the families Histeridae and Staphylinidae (Legner and Olton, 1970; Peck and Anderson, 1969; Pfeiffer and Axtell, 1980; Aak and Ottesen, 2002). The most common and important beetle predator of filth flies is the histerid beetle *Carcinops pumilio*. Although *C. pumilio* is most abundant in poultry manure, this species occurs over a range of habitats throughout much of the world and is often found in calf bedding. There are two larval instars and all motile stages attack fly eggs and other prey items such as acarid mites. Development from egg to adult takes about three weeks under normal conditions (Fletcher *et al.*, 1991), and adults can live for up to two years. Adult fecundity varies depending on the quality of food sources and competition but can be up to 10 eggs per female per day (Geden, 1984; Achiano and Giliomee, 2004; Kaufman *et al.*, 2001a). Adults disperse by flight in response to declining availability of prey (Geden *et al.*, 1988; Kaufman *et al.*, 2000, 2002).

Attack rates of *C. pumilio* adults range from 10-100 fly immatures killed per adult per day depending on temperature, competition and the presence of alternate prey (Geden and Axtell, 1988; Geden *et al.*, 1988). Populations in the field tend to be relatively stable and beetles form aggregations in preferred subhabitats (Tobin *et al.*, 1999; Tobin and Bjoernstad, 2003; Geden and Stoffolano, 1988). Beetle populations take a heavy toll on fly immatures, and their populations can be conserved and promoted by manure management practices (Peck and Anderson, 1970; Hinton and Moon, 2003; Kaufman *et al.*, 2002; Mullens *et al.*, 1996; Geden and Stoffolano, 1988). In addition, beetles can be collected using traps baited with prey or UV light and moved to other facilities (Kaufman *et al.*, 2002). *C. pumilio* can be reared in the laboratory using house flies or other prey, but they are relatively costly to

produce because of cannibalism and their long development time. The beetles are currently being produced by a commercial insectary in the US (IPM Labs, Locke, NY), who recommends making small inoculative releases after manure cleanout.

Hydrotaea aenescens.

Larvae of the black dump fly *H. aenescens,* also commonly known by the former generic name *Ophyra,* are facultative predators that can kill about 15 house fly larvae each per day (Geden *et al.*, 1988). Adults of this species tend to prefer dark places, are not prone to emigration from animal production facilities and are generally less pestiferous than are those of the house fly (Nolan and Kissam, 1987). They are commonly found in poultry houses, where they can achieve dominance over house flies when their populations are augmented by rearing and release programs (Turner and Carter, 1990; Turner *et al.*, 1992). The flies also develop in manure of cattle and pigs (Hogsette *et al.*, 2002; Farkas *et al.*, 1998). Mass-rearing methods have developed for this species (Hogsette and Washington, 1995). Although they are available from several commercial insectaries in the US and Europe, there is little documented information on the efficacy of black dump fly releases in production systems other than poultry.

Nematodes

Steinernematids and Heterorhabditids

Steinernematid and heterorhabditid nematodes have been studied extensively for control of filth flies, with mixed results. In laboratory studies using substrates that are favorable for nematode survival, fly larvae are highly susceptible to most of the entomogenous nematodes that have been tested (Renn *et al.*, 1985; Geden *et al.*, 1986; Mullens *et al.*, 1987a; Taylor *et al.*, 1998). However, results on more natural substrates have generally been disappointing. An early report suggested that nematodes were effective for controlling fly populations in British Columbia poultry houses (Belton *et al.*, 1987), but several other studies have demonstrated that the nematodes perform poorly in poultry and pig manure (Geden *et al.*, 1986; Georgis *et al.*, 1987; Mullens *et al.*, 1987; Renn, 1995, 1998). Cow manure, especially when mixed with soil or bedding, may be a more suitable habitat for nematode use (Taylor *et al.*, 1998). Adult flies are less susceptible to parasitism than larvae on treated filter paper but can be infected by visiting bait stations with parasites (Renn *et al.*, 1985; Renn, 1998). In spite of these mixed results, nematodes are widely available from commercial sources that promote their effectiveness for control of fly larvae.

Paraiotonchium muscadomesticae

The life cycle of *P. muscadomesticae* is similar to that of *P. (Heterotylenchus) autumnalis* in the face fly, *Musca autumnalis* (Coler and Nguyen, 1994; Geden, 1997a). Young adult nematodes are deposited from the ovaries of infected femal flies into fly breeding habitats, where they mate and females seek mature fly larvae. Mated female nematodes penetrate the larval cuticle and enter the haemacoel. As the fly develops into the adult stage, the nematodes go through first a parthenogenetic and then a gametogenetic generation resulting in the production of about 30,000 nematodes per fly. The nematodes then move into the ovaries of

the fly where they are deposited during "mock oviposition". Infected flies live about half as long as uninfected flies and do not produce any eggs. The parasite, which has only been found in Brazil, appears to be fairly specific for house flies. So far, two attempts to get *P. muscaedomesticae* established in the US have been unsuccessful, but it may have potential as an inundative biological control agent.

Parasitoids

Pteromalids and chalcids

About a dozen common pteromalid and chalcid parasitoids are found attacking fly pupae throughout the world. Most species are cosmopolitan in their distribution and nearly all are solitary parasitoids; that is, produce a single parasitoid progeny per host. There are a few gregarious parasitoids, most notably *Muscidifurax raptorellus*, *Nasonia vitripennis*, *Trichomolopsis dubius* and *T. sarcophagae*. The biology of these species is quite similar; females sting host pupae, host-feed, then deposit their eggs in the space between the puparium and the developing fly immature within. Parasitoid larvae feed externally on the host and pupate within the puparium, after which the adult chews an exit hole and escapes. Development of most species is complete in 2-4 weeks. The literature on fly parasitoids is voluminous and beyond the scope of this article to review; reviews can be found in Patterson *et al.* (1981), Axtell (1986), Patterson and Rutz (1986), Rutz and Patterson (1990), Axtell and Arends (1990), Legner (1995) and Geden and Hogsette (2001).

The two most common and important genera of pteromalids are *Spalangia* and *Muscidifurax*, and of those the two most frequently encountered species are *S. cameroni* and *M. raptor* (e.g. Birkemoe *et al.*, 2004; Gibson and Floate, 2004; Hernandez *et al.*, 2004; Hogsette *et al.*, 2001; Skovgard and Jespersen, 1999; Floate *et al.*, 1999). These two species differ in several key regards, with characteristics that compliment each other. *M. raptor* has a short development time (about 14 days at 27°C), a short lifespan (about 2 weeks), a high attack rate (up to 20 hosts killed/female/day), is very sensitive to pesticides, and tends to forage for pupae near the surface of the flybreeding substrate. In contrast, *S. cameroni* has a longer development time (about 26 days), a long lifespan (about 3 weeks) and a lower attack rate (up to 10 hosts/female/day) but is less sensitive to pesticides and is highly effective at locating pupae buried deep in the breeding substrate (Geden 1996, 1997b, 1999, 2002; Geden *et al.*, 2005). Both of these species are found in a diverse range of habitats worldwide. There are some situations in which other species can predominate. For example, *Splangia endius* (found in warmer climates) and *S. nigroaenea* (cosmopolitan) are most commonly found in outdoor habitats such as feedlots, spilled silage, stockpiled manure and the open-sided poultry houses that were often seen in California and the southeastern US.

Releases of parasitoids, especially when combined with other IPM components, can increase rates of parasitism to levels that provide an adequate degree of fly control (Morgan and Patterson, 1990; Geden *et al.*, 1992; Petersen and Cawthra, 1995; Crespo *et al.*, 1998; Skovgard and Nachman, 2004). In other instances, parasitoid releases have had little impact

on fly populations or parasitism levels (Meyer *et al.*, 1990; Andress and Campbell, 1994; Weinzierl and Jones, 1998; McKay and Galloway, 1999; Kaufman *et al.*, 2001b). The reasons for success and failure of parasitoid releases for fly control are not obvious and in many cases may have been site-specific. In general, releases have been most effective when they were preceded by local surveys to determine the most appropriate species for release and when releases were coupled with improved sanitation and curtailed use of hard pesticides as premise treatments. Releases of combinations of species with complimentary niche characteristics may be more effective than single-species releases, but little work has been done on this subject (Kaufman *et al.*, 2001b; Geden and Hogsette, 2005).

Fly parasitoids can be reared for as little as $0.50 per 10,000 parasitized pupae in material costs using inexpensive flyrearing media (Hogsette, 1992). Several species are available to farmers from many commercial insectaries in the Americas and Europe. They are shipped as parasitized pupae, which can then be either scattered or deployed in release stations. Release stations have the virtue of providing a degree of protection from the environment and predation by rodents and arthropods, but they must be deployed in areas near fly breeding sites because parasitoid dispersal can be limited to a fairly small range (Tobin and Pitts, 1999; Floate *et al.*, 2000; Skovgard, 2002). The quality of commercial parasitoids is variable. Parasitism can vary considerably between shipments (Geden and Hogsette, 2005), and colonies of solitary parasitoids can easily become contaminated with fast-developing gregarious species such as *N. vitripennis* and *M. raptorellus*. In addition, many colonies of parasitoids are infected with a debilitating *Nosema* disease that can reduce the wasps' fitness by as much as 90% (Zchori-Fein *et al.*, 1992; Becnel and Geden, 1994; Geden *et al.*, 1995a). Fortunately, the disease can be prevented by careful colonyfounding practices and managed using heat and drug remediation methods (Boohene *et al.*, 2003a,b).

Other parasitoids

The pteromalids are ectoparasitic, mostly solitary parasitoids of fly pupae. In most regions there are no parasitoids that attack the fly's larval stage. One species that holds promise for filling this empty niche is the encyrtid *Tachinaephagus zealandicus*. *T. zealandicus* is a gregarious endoparasitoid that attacks mature larvae of several muscoid flies in the southern hemisphere. The adults are proovigenic, do not need to host-feed, and can kill up to 25 fly larvae/day (Oton and Legner, 1974; Ferreira de Almeida *et al.*, 2002a,b). Massrearing this species is challenging because the parasitoids are more easily reared on calliphorid and sarcophagid hosts than on house flies. *T. zealandicus* is also susceptible to *Nosema* disease, but the disease can be managed by a combination of drug therapy followed by isolation of individual female lines (Ferriera de Almeida *et al.*, 2002c; Geden *et al.*, 2003). The diapriid parasitoid *Trichopria nigra* also has potential as a classical biocontrol agent for filth flies. *T. nigra* is a proovigenic endoparasitoid that attacks pupae of all ages of a variety of muscoid fly pests and is highly gregarious, producing 5-50 parasitoids/host depending on host size and species. Both *T. zealandicus* and *T. nigra* need to be evaluated for their host range and compatibility with native species to determine whether these exotic species are appropriate for release. In

the current regulatory climate it may be difficult to obtain permission to import and release these "empty niche" biocontrol candidates.

Pathogens

E. muscae complex

Adult house flies are susceptible to infection with the fungal pathogens *Entomophtora muscae* and *E. schizophorae*, which typically kill the flies 4-6 days after exposure to conidia. Flies become infected when exposed to conidia discharged from cadavers of infected flies. The intensity and duration of conidial discharge and the survival of conidia depend on temperature and relative humidy conditions (Krasnoff *et al.*, 1995; Kalsbeek *et al.*, 2001a; Madeira, 1998; Six and Mullens, 1996, Mullens and Rodriguez, 1985). Natural epizootics are common in the fall months in temperate regions, with infection rates commonly exceeding 50% (Mullens *et al.*, 1987b; Six and Mullens, 1996; Watson and Petersen, 1993; Steinkraus *et al.*, 1993). Although *E. muscae* may be an important natural regulator of fly populations it remains unclear whether this pathogen can be manipulated for augmentative biological control. Mass-rearing methods have been developed to produce large numbers of infected flies (Mullens, 1986), and field releases of *E. muscae* and *E. schizophorae* have resulted in increased disease prevalence (Kramer and Steinkraus, 1986; Steinkraus *et al.*, 1993; Geden *et al.*, 1993; Six and Mullens, 1996). The impact of releases on fly control may be dampened by the need for high fly populations to sustain epizootics (Geden *et al.*, 1993) and by the ability of the flies to mitigate the effects of infection by resting in warm areas to raise their body temperature (behavioral fever) (Watson *et al.*, 1993; Kalsbeek *et al.*, 2001b).

Beauveria and Metarhizium

Field populations of house flies and stable flies usually have low rates of infection with *B. bassiana and M. anisopliae* (Steinkraus *et al.*, 1990; Skovgard and Steenberg, 2002). In laboratory bioassays, larval and adult flies are highly susceptible to these entomopathogens. Virulence varies widely depending on strain and formulation, and adult house flies are particularly susceptible to sugar baits with *B. bassiana* conidia (Kuramoto and Shimazu, 1992; Geden *et al.*, 1995b; Watson *et al.*, 1995; Darwish and Zayed, 2002; Lecuona *et al.*, 2005). Laboratory and field data indicate that use of entomopathogenic fungi is compatible with other natural enemies including *C. pumilio*, *S. cameroni* and *M. raptor* (Geden *et al.*, 1995; Kaufman *et al.*, 2005; Nielsen *et al.*, 2005). Field data on efficacy at present are limited to two studies. Watson *et al.* (1996) applied *B. bassiana* to the inside walls of calf hutches and observed up to 47% infection among house flies in the treated hutches. Kaufman *et al.* (2005) found that space sprays with *B. bassiana* in poultry houses provided fly control comparable to that observed in houses treated with pyrethrin. *B. bassiana* is currently being commercially produced for fly control by JABB, Inc., Pine Level, NC, USA.

SGHV virus

In 1993, Coler *et al.* (1993) reported the discovery of a double-stranded DNA non-occluded baculovirus infecting house flies on dairy farms in Florida. Infected flies develop hyperplasic

salivary glands and infected females fail to develop their ovaries if they are infected soon after after emergence. The primary route of infection appears to be *per os*, when uninfected flies visit food onto which infected flies have regurgitated virions. In a survey conducted in the summer of 2005, we found infection rates of up to 30% of field-collected flies; nearly all of the infected flies had undeveloped ovaries. High levels of infection are correlated with high populations of flies, presumably due to the increased opportunities for co-feeding of infected and uninfected flies. SGHV of house flies, which appears to be related to a baculovirus of tsetse flies (Jaensen, 1978; Sang *et al*., 1996), may have potential for microbial control of adult flies if it can be incorporated into baits with no loss of potency.

References

Aak, A. & Ottesen, P.S. 2002: Factors affecting diversity of poultry house insects, with emphasis on beetles (Coleoptera). Norwegian J. Entomol. 49: 1-17. 2002.

Achiano, K.A. & Giliomee, J.H. 2004: Effect of crowding on fecundity, body size, developmental time, survival and oviposition of *Carcinops pumilio* (Erichson) (Coleoptera: Histeridae) under laboratory conditions. African Entomol. 12: 209-215.

Andress, E.R. & Campbell, J.B. 1994: Inundative releases of pteromalid parasitoids (Hymenoptera: Pteromalidae) for the control of stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) at confined cattle installations in west central Nebraska. J. Econ. Entomol. 87: 714-722.

Axtell, R.C. 1963: Effect of Macrochelidae (Acarina: Mesostigmata) on house fly production from dairy cattle manure. J. Econ. Entomol. 56: 317-321.

Axtell, R.C. 1986: Fly management in poultry production: cultural, biological, and chemical. Poultry Sci. 65: 657-667.

Axtell, R.C. & Arends, J.J. 1990: Ecology and management of arthropod pests of poultry. Ann. Rev. Entomol. 35: 101-126.

Becnel, J.J. & Geden, C.J. 1994: Description of a new species of microsporidia from *Muscidifurax raptor* (Hymenoptera: Pteromalidae), a pupal parasitoid of muscoid flies. J. Eukaryotic Microbiol. 41: 236-243.

Belton, P., Rutherford, T.A., Trotter, D.B. & Webster, J.M. 1987: *Heterorhabditis heliothidis*: A potential biological control agent of house flies in caged-layer poultry barns. J. Nematol. 19: 263-266.

Birkemoe, T., Soleng, A. & Riddervold, K.W. 2004: Abundance of parasitoid Hymenoptera on pupae of *Musca domestica* and *Stomoxys calcitrans* (Diptera, Muscidae) on pig farms in Vestfold, Norway. Norwegian J. Entomol. 51: 159-164).

Borden, E.E.R. 1989: The phoretic behavior and olfactory preference of *Macrocheles muscaedomesticae* (Scopoli) (Acarina: Macrochelidae) in its relationship with *Fannia canicularis* (L.) (Diptera:Muscidae). Pan-Pacific Entomol. 65: 89-96.

Coler, R.R. & Nguyen, K.B. 1994: *Paraiotonchium muscadomesticae* n. sp. (Tylenchida: Iotonchidae) a parasite of house fly (*Musca domestica*) in Brazil and key to species of the genus *Paraiotonchium*. Journal of Nematology 26: 392- 401.

Coler, R.R., Boucias, D.G., Frank, J.H., Maruniak, J.E., Garcia-Canedo, A. & Pendland, J.C. 1993: Characterization and description of a virus causing salivary gland hyperplasia in the housefly, *Musca domestica*. Med. Vet. Entomol. 7: 275-282.

Crespo, D.C., Lecuona, R.E. & Hogsette, J.A. 1998: Biological control: an important component in integrated management of *Musca domestica* (Diptera: Muscidae) in caged-layer poultry houses in Buenos Aires, Argentina. Biol. Control 13: 16-24.

Darwish, E. & Zayed, A. 2002: Pathogenicity of two entomopathogenic hyphomycetes, *Beauveria bassiana* and *Metarhizium anisopliae*, to the housefly *Musca domestica* L.J. Egyptian Soc. Parasitol. 32(3): 785-96.

Farish, D.J. & Axtell, R.C. 1971: Phoresy redefined and examined in *Macrocheles muscaedomesticae* (Acarina: Macrochelidae). Acarologia 13: 16-29.

Farkas, R., Hogsette, J.A. & Boerzsoenyi, L. 1998: Development of *Hydrotaea aenescens* and *Musca domestica* (Diptera: Muscidae) in poultry and pig manures of different moisture content. Environ. Entomol. 27: 695-699.

Ferreira de Almeida, M.A., Pires do Prado, A. & Geden, C.J. 2002a: Influence of temperature on development time and longevity of *Tachinaephagus zealandicus* (Hymenoptera: Encyrtidae), and effects of nutrition and emergence order on longevity. Environ. Entomol. 31: 375-380.

Ferreira de Almeida, M.A., Geden, C.J. & Prese do Prado, A. 2002b: Influence of feeding treatment, host density, temperature and cool storage on attack rates *of Tachinaephagus zealandicus*. Environ. Entomol. 31: 732-738.

Ferriera de Almeida, M.A., Geden, C.J., Boohene, C.K., Becnel, J.J. & Pires do Prado, A. 2002c: Microsporidiosis of *Tachinaephagus zealandicus* (Hymenoptera: Encyrtidae). Mem. Inst. Oswaldo Cruz 97: 527-530.

Filipponi, A. & Petrelli, M.G. 1967: Autecologia capacita' moltiplicativa di *Macrocheles muscaedomesticae* (Scopoli) (Acari: Mesostigmata). Riv. Parassitol. 28: 129-156.

Fletcher, M.G., Axtell, R.C., Stinner, R.E. & Wilhoit, L.R. 1991: Temperature-dependent development of immature *Carcinops pumilio* (Coleoptera: Histeridae), a predator of *Musca domestica* (Diptera: Muscidae). J. Entomol. Sci. 26: 99-108.

Floate, K., Khan, B. & Gibson, G. 1999: Hymenopterous parasitoids of filth fly (Diptera: Muscidae) pupae in cattle feedlots. Can. Entomol. 131: 347-362.

Floate, K., Coghlin, P. & Gibson, G.A.P. 2000: Dispersal of the filth fly parasitoid *Muscidifurax raptorellus* (Hymenoptera: Pteromalidae) following mass releases in cattle confinements. Biological Control: theory and applications in pest management. 18: 172-178.

Geden, C.J. 1984: Population dynamics, spatial distribution, dispersal behavior and life history of the predaceous histerid, *Carcinops pumilio* (Erichson), with observations of other members of the poultry manure arthropod community. Ph.D. dissertation, Univerity of Massachusetts, Amherst.

Geden, C.J. 1990: The role of coleopteran and acarine predators in house fly population regulation in poultry production facilities. Pp. 177-200 in: Biocontrol of Arthropods Affecting Livestock and Poultry, D. A. Rutz and R. A. Patterson (eds). Westview Press, Boulder.

Geden, C.J. 1996: Modeling host attacks and progeny production of *Spalangia gemina, S. cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) at constant and variable temperatures. Biological Control 7: 172-178.

Geden, C.J. 1997a: Evaluation of *Paraiotonchium muscadomesticae*, a potential biological control agent of the house fly. Biological Control 10: 42-47.

Geden, C.J. 1997b: Development models of the filth fly parasitoids *Spalangia gemina*, *S. cameroni*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) under constant and variable temperatures. Biological Control 9: 185-192.

Geden, C.J. 1999: Host location by house fly parasitoids in poultry manure at different moisture levels and host densities. Environmental Entomology 28: 755-760.

Geden, C.J. 2002: Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: Pteromalidae, Chalcididae) of house flies, *Musca domestica* L. (Diptera: Muscidae), in three types of substrates. Environ. Entomol. 31: 411-417.

Geden, C.J. & Axtell, R.C. 1988: Predation by *Carcinops pumilio* (Coleoptera: Histeridae) and *Macrocheles muscaedomesticae* (Acarina: Macrochelidae) on the house fly (Diptera: Muscidae): functional response, and effects of temperature and availability of alternative prey. Environ. Entomol. 17: 739-744.

Geden, C.J. & Hogsette J.A. (eds) 2001: Research and Extension Needs for Integrated Management Programs for Livestock and Poultry, (269 pp). http://cmave.usda.ufl.edu/lincoln.html.

Geden, C.J. & Hogsette, J.A. 2005: Suppression of house flies (Diptera: Muscidae) in Florida poultry houses by sustained releases of *Muscidifurax raptorellus* and *Spalangia cameroni* (Hymenoptera: Pteromalidae). Environ. Entomol. (in press).

Geden, C.J. & Stoffolano, J.G. 1988: Dispersion patterns of arthropods associated with poultry manure in enclosed houses in Massachusetts: spatial distribution and effects of manure moisture and accumulation time. J. Entomol. Sci. 23: 136-148.

Geden, C.J., Axtell, R.C. & Brooks, W.M. 1986: Susceptibility of the house fly, *Musca domestica* (Diptera: Muscidae) to the entomogenous nematodes *Steinernema feltiae*, *S. glaseri* (Steinernematidae) and *Heterorhabditis heliothidis* (Heterorhabditidae). J. Med. Entomol. 23: 326-332.

Geden, C.J., Stoffolano, J.G. Jr. & Elkinton, J.S. 1987: Prey-mediated dispersal behavior of the predaceous histerid, *Carcinops pumilio*. Environ. Entomol. 16: 415-419.

Geden, C.J., Stinner, R.E. & Axtell, R.C. 1988: Predation by predators of the house fly in poultry manure: effects of predator density, feeding history, interspecific interference, and field conditions. Environ. Entomol. 17: 320-329.

Geden, C.J., Steinkraus, D.C., Miller, R.W. & Rutz, D.A. 1992: Suppression of house flies on New York and Maryland dairies using *Muscidifurax raptor* in an integrated management program. Environ. Entomol. 21:1419-1426.

Geden, C.J., Steinkraus, D.C. & Rutz, D.A. 1993: Evaluation of two methods for release of *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) to infect house flies (Diptera: Muscidae) on dairy farms. Environ. Entomol. 20: 1201-1208.

Geden, C.J., Long, S.J., Rutz, D.A. & Becnel, J.J. 1995a: *Nosema* disease of the parasitoid *Muscidifurax raptor* (Hymenoptera: Pteromalidae): prevalence, patterns of transmission, management, and impact. Biological Control 5: 607-614.

Geden, C.J., Rutz, D.A. & Steinkraus, D.C. 1995b: Virulence of different isolates and formulations of *Beauveria bassiana* for house flies and the parasitoid *Muscidifurax raptor*. Biological Control 5: 615-621.

Geden, C.J., Ferreira de Almeida, M.A. & Pires do Prado, A. 2003: Effects of *Nosema* disease on fitness of the parasitoid *Tachinaephagus zealandicus* (Hymenoptera: Encyrtidae). Environ. Entomol. 32: 1139-1145.

Geden, C.J., Moon, R.D. & Butler, J.F. 2005: Host attacks and progeny production by six species of solitary fly parasitoids on pupae of house fly, horn fly, stable fly, black dump fly and a flesh fly. Environ. Entomol (in press).

Georgis, R., Mullens, B.A. & Meyer, J.A. 1987: Survival and movement of insect parasitic nematodes in poultry manure and their infectivity against *Musca domestica*. J. Nematol. 19: 292-295.

Gibson, G.A.P & Floate, K.D. 2004: Filth fly parasitoids on dairy farms in Ontario and Quebec, Canada. Canadian Entomologist 136: 407-417.

Hernandez-Hernandez, B., Cruz-Vazquez, C., Gonzalez-Hernandez, A., Perales-Segovia, C. & Martinez-Martinez, L. 2004: Pupae parasitoids (Hymenoptera: Pteromalidae) of flies (Diptera: Muscidae) associated with dairy cattle manure in Aguascalientes, Mexico. Folia Entomol. Mex. 43(1): 9-15.

Hinton, J.L. & Moon, R.D. 2003: Arthropod populations in high-rise, caged-layer houses after three manure cleanout treatments. J. Econ. Entomol. 96: 1352-1361.

Ho, C.C., Cromroy, H.L. & Patterson, R.S. 1990: Mass production of the predaceous mite, *Macrocheles muscaedomesticae* (Scopoli) (Acarina: Machrochelidae), a predator of the house fly. Pp 201 in: Biocontrol of Arthropods Affecting Livestock and Poultry, D. A. Rutz & R. A. Patterson (eds). Westview Press, Boulder.

Hogsette, F.A. 1992: New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. J. Econ. Entomol. 85: 2291-2294.

Hogsette, J.A. & Washington, F. 1995: Quantitative mass production of *Hydrotaea aenescens* (Diptera: Muscidae). J. Econ. Entomol. 88: 1238-1242.

Hogsette, J.A., Farkas, R. & Thuroczy, C. 2001: Hymenopteran pupal parasitoids recovered from house fly and stable fly (Diptera: Muscidae) pupae collected on livestock facilities in southern and eastern Hungary. Environ. Entomol. 30: 107-111.

Hogsette, J.A., Farkas, R. & Coler, R.R. 2002: Development of *Hydrotaea aenescens* (Diptera: Muscidae) in manure of unweaned dairy calves and lactating cows. J. Econ. Entomol. 95: 527-530.

Jaenson, T.G.T. 1978: Virus-like rods associated with salivary gland hyperplasia in tsetse *Glossina pallidpes*. Trans. Roy. Soc. Trop. Med. Hyg. 67:234-238.

Kalsbeek, V., Pell, J.K. & Steenberg, T. 2001a: Sporulation by *Entomophthora schizophorae* (Zygomycetes: Entomophthorales) from housefly cadavers and the persistence of primary conidia at constant temperatures and relative humidities. J. Invertebr. Pathol. 77: 149-157.

Kalsbeek, V., Mullens, B.A. & Jespersen, J.B. 2001b: Field studies of *Entomophthora* (Zygomycetes: Entomophthorales) - induced behavioral fever in *Musca domestica* (Diptera: Muscidae) in Denmark. Biol. Control 21: 264-273.

Kaufman, P.E., Long, S.J., Rutz, D.A. & Glenister, C.S. 2000: Prey- and density-mediated dispersal in *Carcinops pumilio* (Coleoptera: Histeridae), a predator of house fly (Diptera: Muscidae) eggs and larvae. J. Med. Entomol. 37:929-932.

Kaufman, P.E., Long, S.J., Rutz, D.A. & Glenister, C.S. 2001a: Larval production from field-collected *Carcinops pumilio* (Coleoptera: Histeridae) following three starvation periods. J. Med. Entomol. 38: 278-281.

Kaufman, P.E., Long, S.J. & Rutz, R.A. 2001b: Impact of exposure length and pupal source on *Muscidifurax raptorellus* and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) parasitism in a New York poultry facility. J. Econ. Entomol. 94: 998-1003.

Kaufman, P.E., Long, S.J., Rutz, D.A. & Waldron, J.K. 2001c: Parasitism rates of *Muscidifurax raptorellus* and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) after individual and paired releases in New York poultry facilities. J. Econ. Entomol. 94: 593-598.

Kaufman, P.E., Burgess, M., Rutz, D.A. & Glenister, C. 2002: Population dynamics of manure inhabiting arthropods under an integrated pest management (IPM) program in New York poultry facilities. 3. Case studies. J. Appl. Poultry Res. 11: 90-103.

Kaufman, P.E., Rutz, D.A. & Waldron, J.K. 2002: Seasonal variation in *Carcinops pumilio* (Coleoptera: Histeridae) dispersal and potential for suppression of dispersal behavior. J. Med. Entomol. 39: 106-111.

Kaufman, P.E., Reasor, C., Rutz, D.A., Ketzis, J.K. & Arends, J.J. 2005: Evaluation of *Beauveria bassiana* applications against adult house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York state. Biol. Control 33: 360-367.

Kramer, J.P. & Steinkraus, D.C. 1987: Experimental induction of the mycosis caused by *Entomophthora muscae* in a population of house flies (*Musca domestica*) in a poultry building. J. N. Y. Entomol. Soc. 95: 114-117.

Krasnoff, S.B., Watson, D.W., Gibson, D.M. & Kwan, E.C. 1995: Behavioral effects of the entomopathogenic fungus, *Entomophthora muscae* on its host *Musca domestica*: Postural changes in dying hosts and gated pattern of mortality. J. Insect Physiol. 41: 895-903.

Kuramoto, H. & Shimazu, M. 1992: Pathogenicity of some entomogenous fungi of the adult housefly, *Musca domestica* (Diptera: Muscidae). Jap. J. Entomol. Zool. 36: 202-203.

Lecuona, R.E., Turica, M., Tarocco, F. & Crespo, D.C. 2005: Microbial control of *Musca domestica* (Diptera: Muscidae) with selected strains of *Beauveria bassiana*. J. Med. Entomol. 42: 332-336.

Legner, E.F. 1971: Some effects of the ambient arthropod complex on the density and potential parasitization of muscoid Diptera in poultry wastes. J. Econ. Entomol. 64: 111-115.

Legner, E.F. 1995: Biological control of Diptera of medical and veterinary importance. J. Vector Ecol. 20: 59-120.

Legner, E.F. & Olton, G.S. 1968: Activity of parasites from Diptera: *Musca domestica*, *Stomoxys calcitrans* and species of *Fannia*, *Muscina*, and *Ophyra*. II. At sites in the Eastern Hemisphere and Pacific area. Annals, Entomol. Soc. Amer., 61, 1306-1314.

Legner, E.F. & Olton, G.S. 1970: Worldwide survey and comparison of adult predator and scavenger insect populations associated with domestic animal manure where livestock is artificially congregated. Hilgardia 40: 225-266.

Legner, E.F. & Olton, G.S. 1971: Distribution and relative abundance of dipterous pupae and their parasitoids in accumulations of domestic animal manure in the southwestern United States. Hilgardia 40: 505-535.

Legner, E.F., Bowen, W.R., McKeen, W.D., Rooney, W.F. & Hobza, R.F. 1973: Inverse relationship between mass of breeding habitat and synanthropic fly emergence and the measurement of population densities with sticky tapes in California inland valleys. Environ. Entomol. 2: 199-205.

Madeira, N.G. 1998: Persistence of conidia of *Entomophthora muscae* in relation to age, temperature, and humidity. BioControl 43: 87-95.

McKay, T. & Galloway, T.D. 1999: Survey and release of parasitoids (Hymenoptera) attacking house and stable flies (Diptera: Muscidae) in dairy operations. Can. Entomol. 131. no. 6, pp. 743-756.

Meyer, J.A., Mullens, B.A., Cyr, T.L. & Stokes, C. 1990: Commercial and naturally occurring fly parasitoids (Hymenoptera:Pteromalidae) as biological control agents of stable flies and house flies (Diptera: Muscidae) on California dairies. J. Econ. Entomol. 83: 799-806. Patterson, R.S. and D.A. Rutz (eds). 1986. Biological control of muscoid flies. Entomol. Soc. Amer. Misc. Publ. 62, Lanham, MD.

Morgan, P.B. & Patterson, R.S. 1990: Efficiency of target formulations of pesticides plus augmentative releases of *Spalangia endius* Walker (Hymenoptera: Pteromalidae) to suppress populations of *Musca domestica* L. (Diptera: Muscidae) at poultry ranches in the southeastern United States, pp. 69-78 In: D. A. Rutz & R. S. Patterson (eds). Biocontrol of arthropods affecting livestock and poultry, Westview, Boulder.

Mullens, B.A. & Rodriguez, J.L. 1985: Dynamics of *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) conidial discharge from *Musca domestica* (Diptera: Muscidae) cadavers. Environ. Entomol. 14:317-322.

Mullens, B.A., Meyer, J.A. & Georgis, R. 1987: Field tests of insect-parasitic nematodes against larvae of manure-breeding flies on caged-layer poultry facilities. J. Econ. Entomol. 80: 438-442.

Mullens, B.A., Rodriguez, J.L. & Meyer, J.A. 1987: An epizootiological study of *Entomophthora muscae* in muscoid fly populations on Southern California poultry facilities, with emphasis on *Musca domestica*. Hilgardia 55(3): 1-41.

Nielsen, C., Skovgård, H. & Steenberg, T. 2005: Effect of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on survival and reproduction of the filth fly parasitoid, *Spalangia cameroni* (Hymenoptera: Pteromalidae). Environ. Entomol. 34: 133-139.

Nolan, M.P. III & Kissam, J.B. 1987: Nuisance potential of a dump fly, *Ophyra aenescens* (Diptera: Muscidae) breeding at poultry farms. Environ. Entomol. 16: 828-831.

O'Donnell, A.E. & Axtell, R.C. 1965: Predation by *Fuscuropoda vegetans* (Acarina: Uropodidae) on the house fly (*Musca domestica*). Annals Entomol. Soc. Amer. 58: 403-404.

Olton, G.S. & Legner, E.F. 1974: Biology of *Tachinaephagus zealandicus* (Hymenoptera: Encyrtidae), parasitoid of synanthropic Diptera. Can. Entomol. 106 (8): 785-800.

Patterson, R.S & Rutz, D.A. (eds). 1986: Biological control of muscoid flies. Entomol. Soc. Amer. Misc. Publ. 62., Lanham, MD.

Patterson, R.S., Koehler, P.G., Morgan, P.B. & Harris, R.L. (eds). 1981: Status of Biological Control of Filth Flies. USDA, ARS, SEA A106.2:F64. New Orleans, La.

Peck, J.H. & Anderson, J.R. 1969: Arthropod predators of immature Diptera developing in poultry droppings in northern California. Part I. Determination of seasonal abundance and natural cohabitation with prey. J. Med. Entomol. 6: 163-167.

Peck, J.H. & Anderson, J.R. 1970: Influence of poultry manure-removal-schedules on various Diptera larvae and arthropod predators. J. Econ. Entomol. 63: 163-167.

Petersen, J.J. & Cawthra, J.K. 1995: Release of a gregarious *Muscidifurax* species (Hymenoptera: Pteromalidae) for the control of filth flies associated with confined beef cattle. Biol. Control 5: 279-284.

Pfeiffer, D.G. & Axtell, R.C. Coleoptera of poultry manure in caged layer houses in North Carolina. Environ. Entomol. 9: 21-28.

Renn, N. 1995: Mortality of immature houseflies (*Musca domestica* L.) in artificial diet and chicken manure after exposure to encapsulated entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Biocontrol Sci. Tech. 5: 349-359.

Renn, N. 1998: The efficacy of entomopathogenic nematodes for controlling housefly infestations of intensive pig units. Med. and Vet. Entomol. 12: 46-51.

Renn, N., Barson, G. & Richardson, P.N. 1985: Preliminary laboratory tests with two species of entomophilic nematodes for control of *Musca domestica* in intensive animal units. Annals Appl. Biol. 106: 229-233.

Rutz, D.A & Patterson, R.S. (eds). 1990: Biocontrol of arthropods affecting livestock and poultry. Westview, Boulder, CO.

Sang, R.C., Jura, W.G.Z.O., Otieno, L.H. & Ogaja, P.T. 1996: Ultrastructural changes in the milk gland of tsetse *Glossina morsitans centralis* (Diptera: Glossinidae) female infected by a DNA virus. J. Invertebr. Pathol. 68: 253-259.

Singh, P., King, W.E. & Rodriguez, J.G. 1966: Biological control of muscids as influenced by host preference of Macrocheles muscaedomesticae. J. Med. Entomol. 3: 78-81.

Six, D.L. & Mullens, B.A. 1996: Seasonal prevalence of *Entomophthora muscae* and introduction of *Entomophthora schizophorae* (Zygomycotina: Entomophthorales) in *Musca domestica* (Diptera: Muscidae) populations on California dairies. Biological Control 6: 315-323.

Skovgård, H. 2002: Dispersal of the filth fly parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) in a swine facility using fluorescent dust marking and sentinel pupal bags. Environ. Entomol. 31: 425-431.

Skovgård, H. 2004: Sustained releases of the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) for control of house flies, *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) on dairy farms in Denmark. Biol. Control 30: 288-297.

Skovgård, H. & Nachman, G. 2004: Biological control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) by means of inundative releases of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 94: 555-567.

Skovgård, H. & Jespersen, J.B. 1999: Activity and relative abundance of hymenopterous parasitoids that attack puparia of *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) on confined pig and cattle farms in Denmark. Bull. Entol. Res. 89: 263-269.

Skovgård, H. & Steenberg, T. 2002: Activity of pupal parasitoids of the stable fly *Stomoxys calcitrans* and prevalence of entomopathogenic fungi in the stable fly and the house fly, *Musca domestica*. BioControl 47: 45-60.

Steinkraus, D.C., Geden, C.J. & Rutz, D.A. 1990: First report of the natural occurrence of *Beauveria bassiana* (Moniliales: Moniliaceae) in *Musca domestica* (Diptera: Muscidae). J. Med. Entomol. 27: 309-312.

Steinkraus, D.C., Geden, C.J. & Rutz, D.A. 1993: Prevalence of *Entomophthora muscae* (Cohn) Fresenius (Zygomycetes: Entomophthoraceae) in house flies (Diptera: Muscidae) on dairy farms in New York and induction of epizootics. Biol. Control 3: 93-100.

Taylor, D.B., Szalanski, A.L., Adams, B.J. & Peterson, R.D. II. 1998: Susceptibility of house fly (Diptera: Muscidae) larvae to entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae). Environ. Entomol: 27:1514-1519.

Tobin, P.C. & Pitts, C.W. 1999: Dispersal of *Muscidifurax raptorellus* Kogan and Legner (Hymenoptera: Pteromalidae) in a high rise poultry facility. Biological Control 16: 68-72.

Tobin, P.C. & Bjoernstad, O.N. 2003: Spatial dynamics and cross-correlation in a transient predator-prey system. J. Anim. Ecol.: 72: 460-467.

Tobin, P.C., Fleischer, S.J. & Pitts, C.W. 1999: Spatio-temporal dynamics of resident and immigrating populations of *Carcinops pumilio* (Coleoptera: Histeridae) in high-rise poultry facilities. J. Med. Entomol. 36: 568-77.

Turner, E.C. & Carter, L. 1990: Mass rearing and introduction of *Ophyra aenescens* (Wiedemann) (Diptera: Muscidae) in high-rise caged layer houses to reduce house fly populations. J. Agric. Entomol. 7: 247-257.

Turner, E.C., Ruszler, P.L., Dillon, P., Carter, L. & Youngman, R. 1992: An integrated pest management program to control house flies in commercial high rise houses. J. Appl. Poultry. Res. 1: 242-250.

Wade, C.F. & Rodriguez, J.G. 1961: Life history of *Macrocheles muscaedomesticae* (Acarina: Macrochelidae), a predator of the house fly. Annals Entomol. Soc. Amer. 54: 776-781.

Watson, D.W. & Petersen, J.J. 1993: Seasonal activity of *Entomophthora muscae* (Zygomycetes: Entomophthorales) in *Musca domestica* L. (Diptera: Muscidae) with reference to temperature and relative humidity. Biol. Control 3: 182-190.

Watson, D.W., Mullens, B.A. & Petersen, J.J. 1993: Behavioral fever response of *Musca domestica* (Diptera: Muscidae) to infection by *Entomophthora muscae* (Zygomycetes: Entomophthorales). J. Invert. Pathol. 61: 10-16.

Watson, D.W., Geden, C.J., Long, S.J. & Rutz, D.A. 1995: Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). Biol. Control 5: 405-411.

Watson, D.W., Rutz, D.A. & Long, S.J. 1996: *Beauveria bassiana* and sawdust bedding for the management of the house fly, *Musca domestica* (Diptera: Muscidae) in calf hutches. Biol. Control 7: 221-227.

Weinzierl, R.A. & Jones, C.J. 1998: Releases of *Spalangia nigroaenea* and *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae) increase rates of parasitism and total mortality of stable fly and house fly (Diptera: Muscidae) pupae in Illinois cattle feedlots. J. Econ. Entomol. 91: 1114-1121.

Zchori-Fein, E., Geden, C.J. & Rutz, D.A. 1992: Microsporidioses of pteromalid parasitoids of muscoid flies. J. Invert. Pathol. 60: 292-298.

Biological control of parasitic nematodes in grazing livestock – experiences from Danish and Swedish experiments

Michael Larsen[1], Stig M. Thamsborg[1], Jørn R. Grønvold[2]

[1]Veterinary Pathobiology, Section for Parasitology, The Royal Veterinary and Agricultural University, Dyrlægevej 10, DK-1870 Frederiksberg C, Denmark; [2]Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: Parasitic nematodes compromise welfare and cause production losses in grazing ruminants. Biological control (BC) by means of nematode-destroying fungi has been investigated against the most important gastrointestinal parasitic nematodes of ruminants.

In Denmark the fungus *Duddingtonia flagrans* has been tested in in-door studies and in field trials with set-stocked, first season grazing (FSG) calves. Dose titration studies showed an increasing activity of *D. flagrans* with increasing number of parasite eggs in faeces and increasing dose. In several field trials daily feeding of fungal spores (0.25-1 million/kg body weight) for 2 to 3 months reduced the level of infective larvae on pasture and the subsequent overall production increased significantly for fungus treated calves compared to untreated controls. Swedish trials have confirmed the promising prospects of *D. flagrans*. In a plot trial where dung from FSG calves treated with either ivermectin bolus, fed fungal spores or non-treated controls was placed on the plots, none, or very few larvae were recovered around ivermectin pats, while the numbers of larvae were significantly reduced around fungus pats compared to samples from control plots. In a grazing trial 5 groups FSG calves were compared (first 4 groups set-stocked): controls, ivermectin bolus treated, fungus fed for 3 months, treated with a copper oxide wire particle bolus, or evasive grazing. Evasive grazing caused slightly higher or equal weight gains to that found for the ivermectin group while the fungus group showed as good as or slightly lower gain than the ivermectin group.

Two 3-year trials with lambs, applying 0.25 million spores/kg body weight daily for 6 weeks up to 3½ months, showed significantly increased production benefit (weight gain) both under set-stocked experimental (DK) and normal (S) farm management conditions. This effect was only found when ewes were parasite-free at time of turn-out, and it was found that not all measured parasitological parameters during the season managed to support the resulting production effect.

In conclusion it can be said that BC as principle have been shown to work, but to be implemented under practical farming conditions in Scandinavia, further technological development is needed and subsequent testing on-farm is required.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

The pupal parasitoid, *Spalangia cameroni*, as biocontrol agent of house flies and stable flies on confined Danish dairy cattle farms

Henrik Skovgård

Department of Integrated Pest Management, The Danish Pest Infestation Laboratory, Danish Institute of Agricultural Sciences, Skovbrynet 14, DK-2800 Kgs. Lyngby

Abstract: In the period 1996–2004 extensive research activities took place on hymenopteran pupal parasitoids (Pteromalidae) as possible biological control agents against house flies, *Musca domestica* (L) and stable flies, *Stomoxys calcitrans* (L.) on confined Danish dairy cattle and swine installations. Based on initial studies, which aimed to describe the relative abundance and parasitism of the parasitoids, it soon became clear that the solitary pupal parasitoid *Spalangia cameroni* Perkins was a candidate for field releases. Subsequently, an inundative approach was determined for the field releases of *S. cameroni* on two organic dairy cattle farms one of which served as control without parasitoids. Every second week from mid-April and up to October in the release year between 100 and 200 female *S. cameroni* were manually introduced per m^2 of the straw beddings. Season-long parasitism of sentinel house fly pupae averaged 35.8% in the release farm versus 8.4% in the control farm, respectively. Compared with the control, the mean density of house flies and stable flies per animal was distinctly suppressed due to *S. cameroni* releases in the period August-September of normal peak abundance of flies. From early summer and onwards the pteromalid, *Muscidifurax raptor* Girault and Sanders occurred frequently along with *S. cameroni* in the stable environments. Based on the results, the cost-effectiveness of using *S. cameroni* in fly control is discussed.

Key words: Biological control, Spalangia cameroni, house fly, stable fly, dairy cattle

Introduction

The house fly, *Musca domestica* (L) and the haematophagous stable fly, *Stomoxys calcitrans* (L.) are pests on livestock farms. They develop in decomposing organic manure or other decaying organic matter and can both be a nuisance to the animals, farm operators and neighbours of the farm. Especially the stable fly can affect animal welfare and cause reduction in weight gain of beef cattle and milk production of lactating cows if the animals remain unprotected (Berry *et al.*, 1983; Campbell *et al.*, 1987). Although house flies have not directly been proven to reduce the performance of the animals, suppression of this fly is necessary due to nuisance or public health problems (Chavasse *et al.*, 1999; Iwasa *et al.*, 1999).

Pupal parasitoids (Hymenoptera: Pteromalidae) have been extensively studied as biological agents for fly control (Legner & Olton, 1968; Rueda & Axtell, 1985a; Bellini & Maini, 1988; Hogsette *et al.*, 1994; Klunker, 1994; Lysyk, 1995; Floate *et al.*, 2000). In

Denmark the solitary ectoparasitoid, *Spalangia cameroni* Perkins is abundant on most dairy cattle farms with straw embedded pens or boxes for calves. On modern dairy cattle units with loose housing systems *S. cameroni* is common as well in the straw embedded areas for animal resting. *Spalangia cameroni* seems therefore to have the potential for control of house flies and stable flies at indoor sites (Skovgård & Jespersen, 1999; 2000). Furthermore, based on the literature, successful fly control is mainly linked to the inundative approach where large numbers of parasitoids are released into such more or less enclosed animal units as can be found in dairy cattle operations in Denmark.

The aim of the present paper is to present the results of studies conducted in 2001 and published in Skovgård, (2004) where *S. cameroni* was released into organic dairy cattle farms to assess the controlling effect on house fly and stable fly populations. Further, the cost-effectiveness of using *S. cameroni* compared with larvicides as used in the traditional farming systems will be briefly discussed.

Material and methods

Experimental sites

The study was performed on two organic dairy cattle farms near Copenhagen. One farm was designated as 'release' unit, i.e. received regular releases of *S. cameroni*, and one was untreated.

Each farm hosted from 70 to 100 milking cows, 30 to 60 heifers and 30 to 40 calves. Young calves were abundant during April from June and again in September to November. Fly-breeding sites on the farms were associated with the straw embedded areas and accumulated organic matter below water bowls and feed bunks. Lactating cows were on pasture from May to October and during early morning and late afternoon were only in the stables for milking.

No releases of *S. cameroni* were conducted outdoors as removed manure was deposited more than 1 km from the two farms and with little immature house fly and stable fly development.

Rearing of S. cameroni

The laboratory culture of *S. cameroni* used for massrearing was established from individuals collected on a single farm in 1997 and later supplemented each year with new specimens from other farms to minimize the risk of inbreeding. Massrearing of parasitoids took place by weekly exposure of *M. domestica* pupae (12-36 h) to adult *S. cameroni* for 2-3 days. The pupae were then incubated at 25°C, 60% r. h. and 12:12 (L:D) for the parasitoids to emerge, which occurred between day 21 and 25. To determine the number of parasitoids emerged and their sex ratio, 100 parasitized pupae were randomly sampled each week and incubated under similar conditions as described above. Adult parasitoids were occasionally stored at 15°C for 4-6 days prior to release.

Releases of S. cameroni

Every second week starting in mid-April 100 female *S. cameroni* per m^2 of the bedding areas were spread out by hand. From July and until October this release rate was increased to 200 females per m^2, mainly, to cope with the rapidly growing fly populations.

Activity of parasitoids

The activity of *S. cameroni* was determined by means of sentinel mesh bags (5 x 10 cm), each containing 30 laboratory-reared house fly pupae (12-36 h-old) (Rutz & Axtell, 1979). On each of the two farms 20 bags were distributed in sites of fly development and covered with organic material to reduce desiccation of the exposed fly pupae. *Spalangia cameroni* generally requires live fly pupae for complete development. Hence, to determine the percentage of sentinel fly pupae alive at the time of exposure, two bags were kept in the laboratory in a similar environment as described above. After seven days of exposure, the bags were returned to the laboratory and incubated at 25°C and 60% r. h. for another seven days. Intact fly puparia were then placed in 25 ml ventilated plastic vials, covered with 4-5 cm of sawdust and held for approximately 60 d for parasitoid emergence. Dead individuals were stored in 70% ethanol for later identification of species. Fly puparia without emergence holes were dissected to detect if parasitoids had died.

Fly abundance

The relative densities of flies were estimated by weekly observations using visual indices (Kristiansen & Skovmand, 1985) on approximately 8-10 animals per farm, where index 0 denotes 0-3 flies, 1: 4-6, 2: 7-12, 3: 13-25, 4: 26-50, 5: 51-100, 6: 101-200 and 7: 201-400 flies per adult animal, respectively. The indices were converted to a measure of fly density by use of the median of the various intervals. In order to provide a mean number of flies per animal and week, the flies were pooled and divided by the total number of scouted animals. At high fly densities it was difficult to distinguish reliably between adults of *M. domestica* and *S. calcitrans*.

Results and discussion

Fly abundance and parasitism

The numbers of house flies and stable flies on the control farm increased from June-July to a maximum of about 300 flies per animal in August-September, followed by a decline in late September due mainly to decreasing ambient temperatures. Compared with the untreated farm this study demonstrates that releases of *S. cameroni* every second week had a significant positive effect on the overall percentage parasitism of sentinel house fly pupae, with 8.4% ± 1.5% (SE) for the untreated farm and 35.8% ± 6.3% (SE) for the treated farm, respectively. This increase in parasitism clearly reduced the abundance of house flies and stable flies per animal to acceptable low levels (Figure 1).

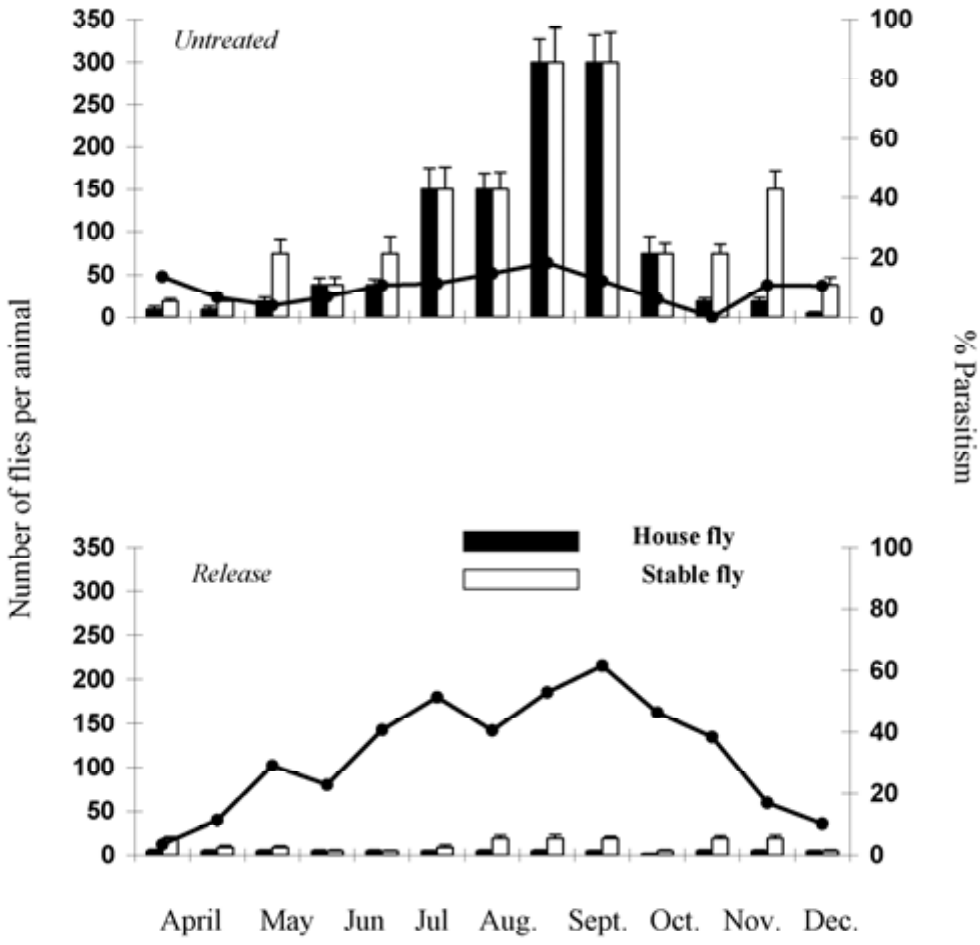

Figure 1. Density (±SE) of house flies (solid bar) and stable flies (open bar) per adult animal, and percentage parasitism (solid line) of untreated farm and *S. cameroni* release farm. Abundance of flies was recalculated from fly indices where median values are used (see text and Skovgård, 2004).

It was surprising that the stable fly was suppressed to a similar low level as the house fly although larvae and puparia of the stable fly are often located at deeper depths. Puparia of house fly aggregate more often in dryer materials and along the periphery of less trampled and cooler parts of the confined areas (McPheron & Broce, 1996; Thomsen, 1938; Tobin & Pitts, 2002). An explanation may be that since immature larvae and puparia of the house fly and stable fly were mainly found in the upper 5-10 cm due to extreme heat produced in the bed-

ding (>40°C) and anaerobic conditions below, *S. cameroni* was able to locate the stable fly puparia with relative ease. Furthermore, *S. cameroni* is known to search for fly pupae in the depth of the organic matter and does not completely avoid moist or moderately heated micro-environments (Smith & Rutz, 1991; Geden, 1999; Geden, 2002).

Relative abundance of parasitoids

Spalangia cameroni dominated in the sentinel mesh bags with 80.5% to 93.1% of the parasitoids recovered on the release and untreated farms. Other species of parasitoids recovered were *Spalangia nigripes* Curtis (0.8-8.5%), *Muscidifurax raptor* Girault & Sanders (4.0-6.0%), *Pachycrepoideus vindemiae* (Rondani) (<5%) and the ichneumonid *Phygadeuon fumator* Gravenholst (0.3-6.9%). The findings of *S. nigripes*, *M. raptor*, *P. vindemiae* and *P. fumator* in addition to *S. cameroni* were expected as they have been reported in previous investigations (Skovgård & Jespersen, 1999; 2000; Skovgård & Steenberg, 2002). *M. raptor* is of particular interest as this species has a shorter development time than *S. cameroni* (Geden, 1997; Mann *et al.*, 1990) and is known to prefer to search in the upper layers of the manure (Rueda & Axtell, 1985b). This behaviour complements that of *S. cameroni*, which mainly searches deeper in the organic matter. Hence, *M. raptor* may be a potential candidate if mixed with *S. cameroni* to cover a larger range of flybreeding microhabitats.

Costs of producing S. cameroni

Farmers need to be convinced of the economic feasibility of using *S. cameroni* as a method of fly control. Economic feasibility will reflect, in part, the number of parasitoid wasps needed for effective control. Release rates based on the abundance of flies exist (Morgan *et al.*, 1981). These may not work in practice for the farm operator, where more simple methods are needed, based either on the number of female parasitoids as a function of the area to be protected or on the number of animals. Although such information can be found in the literature, the numbers of female parasitoids to be released may differ considerably as suggested by suppliers or the numbers applied by researchers in their attempt to control the nuisance flies. Cranshaw *et al.* (1996) reported that suppliers recommended 56 females per m^2 for sufficient fly control, whereas in the study by Rutz & Axtell (1979) in poultry facilities 178-381 females per m^2 of *M. raptor* were released. In the present study 100-200 female parasitoids per m^2 were released every second week to achieve acceptable control of the house fly and the stable fly. Whether this level can be reduced further or has to be increased depends on several factors, e.g., time of the year, sanitation practice, and activity of naturally occurring parasitoids. For example, from April to and including May the activity of released *S. cameroni* was low as a result of low temperatures, whereas it increased markedly later in the fly season where 200 females per m^2 resulted in a significantly higher level of parasitism (Figure 1).

Commercial production costs for *S. cameroni* on the black dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae) that is used for house fly control in swine facilities is €0.41 per thousand parasitoids (Mortalin production Aps. Denmark-DK). Based on a release rate of 100 female *S. cameroni* per m^2 every second week from mid-April to July (six releases), subsequently increased to 200 females from July to October (six releases), the estimated annual

costs of fly control per farm and e.g., 100 m^2 of straw bedding will be €148 - based on a sex ratio of 1:1. In comparison, annual costs of fly control with larvicides (2% cyromazin, Mortalin production Aps. Denmark-DK) on a farm of a similar size (€0.68 per m^2 per treatment) will be €408, assuming six treatments are performed during the fly season. The present example shows that *S. cameroni* clearly is competitive on the market compared with a larvicidal product. The low price of parasitoids produced on *H. aenescens* is explained by the fact that the firms already have a massive production of *H. aenescens* pupae and additional demands for parasitoid production will add little to the total costs.

The production of *S. cameroni* on pupae of the black dump fly may lead to speculations whether field-released *S. cameroni* will have similar effect had they been cultured on house fly or stable fly pupae instead. Based on a laboratory study (no choice/choice experiments) by Torp (2005), which aims to examine whether the natal host influences on *S. cameronis*' later host choice showed that rearing *S. cameroni* on pupae of *H. aenescens* for many generations had insignificant influence on the females performance when offered either pupae of *M. domestica* or *Stomoxys calcitrans*.

Future perspectives

Steps that might lead to further progress in the biological control of house flies and stable flies could be to expand the interval between two releases of parasitoids early in the spring by more than two weeks. Later, in the fly season, it is likely that there will be a demand for a high number of released parasitoids, which could be a mixture of both newly hatched individuals and individuals that will hatch with time in the bedding. This could be a solution that will make the use of parasitoids highly cost-effective compared with other control methods. Such an approach needs, however, to be confirmed from field experiments before being recommended.

Acknowledgements

The author sincerely thanks Aa. Borges and C. Dahl for technical assistance. Many thanks also to L. Peschel for her assistance in rearing the house flies, and the two organic dairy producers for hosting the experimental part. The study was financed by the Danish Ministry of Food, Agriculture and Fisheries.

References

Bellini, R. & Maini, S. 1988: Presenza stagionale e attività di parassitoidi (Hymenoptera: Pteromalidae) di Ditteri sinantropici in allevamenti zootecnici della Romagna. Bollettino dell'Istituto di Entomologia. Guido Grandi dell'Università di Bologna (English summary) 43: 207-222.

Berry, I.L., Stage, D.A. & Campbell, J.B. 1983: Populations and economic impacts of stable flies on cattle. Am. Soc. Agric. Eng. 26: 873-877.

Campbell, J.B., Berry, I.L., Boxler, D.J., Davis, R.L., Clanton, D.C. & Deutscher, G.H. 1987: Effects of stable flies (Diptera: Muscidae) on weight gain and feed efficiency of feedlot cattle. J. Econ. Entomol. 80: 117-119.

Chavasse, D.C., Shier, R.P., Murphy, O.A., Huttly, S.R.A., Cousens, S.N. & Akhtar T. 1999: Impact of fly control on childhood diarrhoea in Pakistan: community-randomised trial. Lancet 353: 22-25.

Cranshaw, W., Sclar, D.C. & Cooper, D. 1996: A review of 1994 pricing and marketing by suppliers of organisms for biological control of arthropods in the United States. Biological Control 6: 291-296.

Floate, K., Coghlin, P. & Gibson, G. 2000: Dispersal of the filth fly parasitoid Muscidi*furax raptorellus* (Hymenoptera: Pteromalidae) following mass releases in cattle confinements. Biological Control. 18: 172-178.

Geden, C.J. 1997: Development models for the filth fly parasitoids *Spalangia gemina*, *S. cameroni*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) under constant and variable temperatures. Biological Control 9: 185-192.

Geden, C.J. 1999: Host location by house fly (Diptera:Muscidae) parasitoids in poultry manure at different moisture levels and host densities. Environ. Entomol. 28: 755-760.

Geden, C.J. 2002: Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: Pteromalidae, Chalcididae) of house flies (Diptera: Muscidae) in three types of substrates. Environ. Entomol. 31: 411-417.

Hogsette, J.A., Farkas, R. & Coler, R. 1994: Hymenopteran pupal parasites recovered from house fly and stable fly (Diptera: Muscidae) pupae collected on livestock and poultry facilities in northern and central Hungary. Environ. Entomol. 23: 778-781.

Iwasa, M., Makino, Sou-Ichi., Asakura, H., Kobori, H. & Morimoto, Y. 1999: Detection of *Escherichia coli* O157:H7 from *Musca domestica* (Diptera: Muscidae) at a cattle farm in Japan. J. Med. Entomol. 36: 108-112.

Klunker, R. 1994: Zum Auftreten von Puppariumparasitoiden als natürliche Feinde von Stallfliegen. Applied Parasitology (English summary) 35: 36-50.

Kristiansen, K. & Skovmand, O. 1985: A method for the study of population size and survival rate of house flies. Entomol. Exp. Appl. 38: 145-150.

Legner, E.F. & Olton, G.S. 1968: Activity of parasites from Diptera: *Musca domestica*, *Stomoxys calcitrans*, and species of Fannia, Muscina, and Ophyra. II. At sites in the eastern hemisphere and pacific area. Ann. Entomol. Soc. Am. 61: 1306-1314.

Lysyk, T.J. 1995: Parasitoids (Hymenoptera: Pteromalidae), Ichneumonidae) of filth fly (Diptera: Muscidae) pupae at dairies in Alberta. J. Econ. Entomol. 88: 659-665.

Mann, J.A., Axtell, R.C. & Stinner, R.E. 1990: Temperature-dependent development and parasitism rates of four species of Pteromalidae (Hymenoptera) parasitoids of house fly (*Musca domestica*) pupae. Med. Vet. Entomol. 4: 245-253.

McPheron, L.J. & Broce, A.B. 1996: Environmental components of pupariation-site selection by the stable fly (Diptera: Muscidae). Environ. Entomol. 25: 665-671.

Morgan, P.B., Weidhaas, D.E. & Patterson, R.S. 1981: Host-parasite relationship: Augmentative releases of *Spalangia endius* Walker used in conjunction with population modeling to suppress field populations of *Musca domestica* L. (Hymenoptera: Pteromalidae and Diptera: Muscidae). J. Kansas Entomol. Soc. 54: 496-504.

Rueda, L.M. & Axtell, R.C. 1985a: Comparison of hymenopterous parasites of house fly, *Musca domestica* (Diptera: Muscidae), pupae in different livestock and poultry production systems. Environ. Entomol. 14: 217-222.

Rueda, L.M. & Axtell, R.C. 1985b: Effect of depth of house fly pupae in poultry manure on parasitism by six species of pteromalidae (Hymenoptera). J. Entomol. Sci. 20: 444-449.

Rutz, D.A. & Axtell, R.C. 1979: Sustained releases of *Muscidifurax raptor* (Hymenoptera: Pteromalidae) for house fly (*Musca domestica*) control in two types of caged-layer poultry houses. Environ. Entomol. 8: 1105-1110.

Skovgård, H. & Jespersen, J.B. 1999: Activity and relative abundance of hymenopterous parasitoids that attack puparia of *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) on confined pig and cattle farms in Denmark. Bull. Entomol. Res. 89: 263-269.

Skovgård, H. & Jespersen, J.B. 2000: Seasonal and spatial activity of hymenopterous pupal parasitoids (Pteromalidae and Ichneumonidae) of the house fly (Diptera: Muscidae) on Danish pig and cattle farms. Environ. Entomol. 29: 630-637.

Skovgård, H. & Steenberg, T. 2002: Activity of pupal parasitoids in the stable fly *Stomoxys calcitrans* (Diptera: Muscidae) and prevalence of entomopathogenic fungi in the stable fly and the house fly *Musca domestica* (Diptera: Muscidae) in Denmark. BioControl 47: 45-60.

Skovgård, H. 2004: Sustained releases of the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) for control of house flies, *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) on dairy farms in Denmark. Biological Control 30: 288-297.

Smith, L. & Rutz, D.A. 1991: Microhabitat associations of hymenopterous parasitoids that attack house fly pupae at dairy farms in Central New York. Environ. Entomol. 20: 675-684.

Tobin, P.C. & Pitts, C.W. 2002: Geostatistical analysis and the impact of moisture on the spatial and temporal distribution of larval *Musca domestica* (Diptera: Muscidae). Environ. Entomol. 31: 273-280.

Torp, A.M. 2005: The influence of natal host and learning on later host preference of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Master Thesis in Agronomy at The Royal Veterinary and Agricultural University, Copenhagen pp. 46 (*English summary*).

Thomsen, M. 1938: Stuefluen (*Musca domestica*) og stikfluen (*Stomoxys calcitrans*). Forsøgslaboratoriet. 176. beretning (In Danish), pp. 352.

Fungi for control of the poultry red mite, *Dermanyssus gallinae*

Tove Steenberg[1], Ole Kilpinen[1], Dave Moore[2]

[1]Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Danish Institute of Agricultural Sciences, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark; [2]CABI Bioscience, Silwood Park, Ascot, Berk SL5 7TA, United Kingdom

Abstract: The poultry red mite is a major pest in European egg production, and new control methods are needed. Entomopathogenic fungi may have potential for mite control, and this is being studied as part of the EU funded CHIMICO project. The paper is a brief summary of the outcome of the work with entomopathogenic fungi.

Key words: Entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, *Dermanyssus gallinae*

Introduction

The poultry red mite (*Dermanyssus gallinae*) is the number one pest in European egg production, and is currently almost impossible to control during the production cycle with traditional measures. High populations of the haematophagous mite stress the birds and decrease their welfare significantly. Furthermore, it is likely that the mites act as reservoirs for zoonotic bacteria since the mites will hide in the structure and thus be out of reach of the sanitation measures carried out between flocks. The transition in the EU from traditional battery systems to furnished cages is likely to increase the mite problems even further.

In an EU funded project initiated in 2001, partners from the United Kingdom, Spain and Denmark attempt to develop a novel control method based on entomopathogenic fungi and semiochemicals to be combined in a trapping system, in which mites are inoculated with fungus and then disseminate the fungus to conspecifics. As the mites spend most time off the hens, form aggregations in cracks and crevices and will need to be controlled at temperatures (and to a lesser degree also relative humidities) conducive for fungus infection, entomopathogenic fungi seemed good candidates.

This paper provides an overview of the progress of the work on entomopathogenic fungi, which includes their natural occurrence in poultry farms, screening of isolates for virulence against mites and effects on mite fecundity, transmission of infection under laboratory and semi-field conditions as well as on the longevity of fungal conidia under the abiotic conditions prevailing in layer farms.

Material and methods

Mite rearing and fungus isolates

Mites were reared *in vivo* on caged hens. Most tests were performed with adult blood-fed females. Three isolates of *Beauveria bassiana* and two isolates of *Metarhizium anisopliae* were selected early in the project, and the majority of tests were carried out using one isolate of each species. Fungi were cultivated at 25°C for 3 weeks on plates with Sabouraud dextrose agar, air-dried over night and stored over silicagel at 4°C until use.

Bioassays with mites

Bioassays were carried out in glass tubes where mites were held in groups of 10 individuals. Dry conidia were sucked into the glass tubes, and surplus conidia were subsequently removed by transferring the mites to other glass tubes. Mites were incubated at 25°C/85% r.h. and L:D 12:12 for 10 days, during which the mortality was recorded at intervals.

Conidial longevity

Percentage germination in conidial powders was determined on plates with SDA. In the case of dried *M. anisopliae*, the test needed to be prolonged in time in order to allow for full germination to occur. Tests ranged from long-term longevity studies at 25°C/85% r.h. to short term exposure tests where conidia were exposed to different concentrations of gaseous ammonia.

Semi-field experiments

High populations of mites were treated with fungus added to hollow perches. At intervals mites were allowed to feed on a caged hen. Two weeks after treatment populations were harvested by suction and weighed.

Results and discussion

Among the five selected isolates, *B. bassiana* consistently proved to be most virulent. Similarly, the three *B. bassiana* isolates proved be most persistent over time, when dry unformulated conidia were stored at abiotic conditions similar to those found at poultry farms. Even very high concentrations of ammonia did not impair the germination of conidia of either species nor their infectivity to mites. None of the isolates caused a reduction in fecundity of blood-fed females. Transmission rates were high, with up to 80% transmission at a ratio of 5% treated mites. At present, an isolate of *Beauveria bassiana* has been selected and its mass production is being optimised. Semi-field experiments showed that the fungus is capable of reducing the population growth even at very high mite levels. However, control levels were not satisfactory, and further experimentation is needed before the full potential of the fungus is reached. This isolate will be tested in field experiments at a later stage. Future application scenarios could include traps with fungus (with or without an attractant), combination of fungus and diatomaceous earth or surface treatments using oil-formulated conidia.

Acknowledgements

Minna Wernegreen, Nicolai Hansen, Kristian Hansen and Emma Thompson provided technical assistance. The project (acronym CHIMICO) is funded by the EU (contract no. QLRT-2000-01236).

Development of a fungal biocontrol agent based on *Clonostachys rosea* 'IK726'

Dan Funck Jensen, Inge M.B. Knudsen, Mette Lübeck, John Hockenhull, Birgit Jensen

Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: We are working on developing a biological control agent (BCA) based on *Clonostachys rosea* 'IK726'.

Isolation, selection and field performance

In planta screening was used for selection of candidates with multifactor or plant related mechanisms of control and well adapted to the environment where they are to act. A strain 'IK726' with high efficacy against several seed-borne diseases was selected. A bioassay with plants grown in sand has been developed for testing IK726. This assay reflects the performance of IK726 under field conditions and thus, has been a unique tool for developing the BCA further. GEP field testing of efficacy is now being carried out.

Production, formulation and delivery

IK726 can be produced using both wet and dry fermentation. Our experiences are restricted to small scale fermentations although production of *C. rosea* in large scale has been reported. Formulation of IK726 has resulted in a good shelf life of the product. Seed treatments may be adapted in a way that will ensure both optimal conditions for seed germination and for the BCA delivered with the seed. Coating of carrot seeds with IK726 effectively controlled seedling diseases caused by *Alternaria* spp. and, biopriming almost eradicated *Alternaria* spp. on carrot seeds and enhanced seedling establishment. There have also been indications of control of soil-borne diseases using seed treatment with IK726. IK726 can also be incorporated in greenhouse substrates such as sphagnum peat, compost or rockwool for use with protected crops or, it can be used for phyllosphaere application. This is currently under further investigation.

Molecular approaches for understanding the biology of IK726

DNA reporters transformed into IK726 (GUS, GFP, DsRed) are used to study *in situ* interactions. In this way we have observed conidial germination, colonization and conidiogenesis in soil, in sphagnum peat, in vermiculite and on seed, roots and leaves. Moreover *in situ* interactions with *Alternaria* sp. have been studied. Work focusing on enzymatic activities as they relates to biocontrol is in progress.

EU-registration including risk assessment

EU-registration including risk assessment still needs to be addressed although some relevant information has been gathered. This is believed to be done in close collaboration with an industrial partner.

Development and registration of biocontrol products - experiences and perspectives gained from the bacterial seed treatment products Cedomon® and Cerall®

Margareta Hökeberg
BioAgri AB, P.O. Box 914, SE-751 09 Uppsala, Sweden

Abstract: The bacterium *Pseudomonas chlororaphis*, strain MA 342, is the active ingredient of the seed treatment biocontrol products Cedomon and Cerall (BioAgri AB, Uppsala, Sweden). The products have been shown to control a large range of seed-borne diseases in cereals. Cedomon, which is an oil-based formulation, is mainly used for covered seeds as barley, oats and spelt. The water-based formulation Cerall is primarily used for seed-treatment in non-covered seeds like wheat, rye and triticale. Since the first commercial application eight years ago, more than 1,5 million hectares in Europe have been drilled with Cedomon treated barley. The new product Cerall has since 2003 been used on commercial seed lots in Sweden, Finland and Austria. Different aspects of production, formulation and application with relevance for product development will be covered as well as experiences from the challenging EU regulatory process for biological control agents/products.

Test of efficacy of biological control agents

Bent J. Nielsen

Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark

Abstract

General requirement

Requirements for efficacy data in order to obtain a Danish authorization of a biological control agent follows in principle the same requirements as for conventional pesticides and based on Directive 93/71/EEC amending the Commission's Directive 91/414/EEC concerning the placing of plant protection products on the market. The necessary data can come from Danish trials or trials from comparable regions, where the climate (temperature, rain, water balance), length of growing season, soil conditions, agricultural practice, cultivars, yield, etc. do not differ significantly from Danish conditions.

Efficacy requirements for registration in Denmark

The specific requirements for efficacy data for both conventional pesticides and biological products are listed in "Vejledende krav til effektivitetsdata med henblik på dansk godkendelse af plantebeskyttelsesmidler" (Guidance to requirements for efficacy data in order to obtain a Danish authorization).

Specific requirements on number of trials and dose levels

Field trials must have been conducted in at least 2 growing seasons for new active substances and at least one growing season for new formulations of known active substances. In the major crops and for each of the major pathogens a minimum of 6 trials are required of which 3 trials must be dose response trials with several dose rates (1/1, 1/2 and 1/4 x normal dose). In minor crops or crops with various harmful organisms, and for products with new formulations of known active substances less data are required.

Resistance

In case there are reasons to expect development of resistance or in case of documented resistance in other harmful organisms or crops, results from a recent and representative Danish investigation of the sensitivity of the population of harmful organisms must be provided, or investigations from a comparative area. In such cases a management strategy designed to minimize the likelihood of resistance or cross-resistance developing in the target species must be provided.

Phytotoxicity

Information on the phytotoxicity of a product is required when adverse effects have been observed on the host plants in the efficacy trials. The safety of a product to the main cultivars of the main crops for which it is recommended must be demonstrated. As it traditionally is known that seed treatments have a high risk of phytotoxic there will normally be requirements for data on 2/1 * normal dose.

REBECA – a project to review regulation of BCAs

Ralf-Udo Ehlers, Olaf Strauch
Christian-Albrechts-University Kiel, Germany, Raisdorf, Germany

Abstract: Biological control agents (BCAs) are sustainable and environmentally safe tools to manage pest insects, nematodes, weeds and diseases in agriculture, forestry and horticulture. However, registration of BCAs largely follows rules developed for synthetic pesticides, thus many possibly irrelevant investigations, e.g. on the ecotoxicology, are requested. Costly risk assessment studies and long-term evaluation of dossiers keep these products off the market. For example, the average time frame for the EU evaluation of dossiers according to Directive 91/414/EEC for beneficial micro-organisms on Annex 1 is > 82 months compared with 23 months for the same products in the USA. The objective of the Policy Support Action REBECA is to accelerate the regulation process for BCAs and make it more cost-effective without compromises to the level of safety. The action will connect stakeholders from industry, science, regulation authorities, policy and environment to form a network within Europe and bring together the expertise and critical mass necessary to improve regulation procedures for BCAs in Europe. REBECA will provide potential experts, who can assist the EU and member states in the evaluation of risks and regulation of BCAs, and will identify future EU research tasks to develop effective risk assessment procedures and balanced regulation strategies.

Key words: Biological control agents, regulation, registration, EU

Project objectives and structure

Biological control agents (BCAs) are increasingly occupying their place in Integrated Crop Protection Programmes. They are sustainable and environmentally safe tools managing invertebrate pests, weeds and diseases in agriculture, forestry and horticulture. However, registration procedures have been established for microorganisms, semiochemicals and botanicals, which prevent their immediate market introduction. In contrast, macrobials (insects, mites and nematodes) are exempted from registration in most European countries. European SMEs, through the sale of macrobial BCAs, increased their turnover from almost zero to >100 million € within the last two decades.

Registration largely follows rules developed for synthetic pesticides (Council Directive 1991/414/EEC); thus many possibly irrelevant investigations, e.g. on the ecotoxicology of microorgansims, are requested. Costly risk assessment studies and long-term evaluation of dossiers keep these products off the market. EU Annex I inclusion for the microorganism *Pseudomonas chlororaphis* (the active ingredient of the product Cedomon®) took more than 8 years and an investment of over 2.5 million €. This situation cannot attract monetary inputs

into the biocontrol sector and thus no further products will be registered in the future. Compared with the biocontrol market in the USA (59 BCAs registered), the market in Europe is exposed to major restrictions, resulting in only 5 BCAs included in Annex I (Table 1). More striking is the fact that the time frame for the EU evaluation of dossiers is 82 months compared with 23 months for the same products in the USA. Regulation authorities in the USA seem to be able to consent to waivers more easily based on the knowledge they have already acquired. Consequently, less time and money is involved. Registration fees are lower for the applicants because governments pay most of the costs. In the EU, on the contrary, the applicants pay all the costs. Registration boards may be less experienced and thus require more data prolonging the registration process at increasing costs.

Table 1. Microbial products on Annex 1 Dir. 91/414/EEC or in the registration process (- xx) and time frame for processing of dossiers in the EU and the USA (EPA).

Organism	**EU period (month.year)**	**EU time frame (months)**	**EPA time frame (months)**
Paecilomyces fumosoroseus Preferal®	5.94-6.01	**85**	60
Coniothyrium minitans Contans®	11.98-8.03	**57**	15
Pseudomonas chloroaphis Cedomon®	1.96-4.04	**99**	-
Ampelomyces quisqualis AQ10®	2.96-10.04	**104**	?
Spodoptera NPV Spodex®	7.97-xx	> 90	12
Gliocladium catenalatum Prestop®	3.99-10.04	**67**	13
Bacillus subtilis Serenade®	5.00-xx	> 56	14
Pseudomyza flocculosa Sporodex®	3.01-xx	>46	39
Paecilomyces lilacinus Bioact®	10.02-xx	> 27	11
Average time period for completion		**82.4**	23.4

Several European governments have developed strategies to reduce the inputs of chemical pesticides and concomitantly support the use of sustainable, biological control strategies. For instance the UK and NL try to encourage registration of BCAs by reducing the registration fees. Sweden intends to propose strategies and amendments of legislation during the fourth round of the EU review of active ingredients to allow "products of low concern" to remain on the market. Urgent need for harmonisation and reduction of requirements have resulted in the development of more balanced directives (e.g., Directive 2001/36/EC amending Council Directive 1991/414/EEC concerning the placing of plant protection products on the market) and guidelines (e.g. OECD Guidance for Registration Requirements for Microbial Pesticides, 2003). However, this has not yet resulted in an easier access to EU markets.

The low number of BCA products in the EU alarmed the European Commission and a call was published in the Sixth Framework Programme (Priority 8.1, Scientific Support to Policies) for the formation of a network action targeting BCAs: "Developing a balanced system for registration, including Macroorganisms, Microbial Biopesticides, Plant Extracts and Semiochemicals" (Call identifier FP6-2004-SSP-4). According to that call the Scientific Support Action REBECA was proposed.

The major objective of REBECA is to accelerate the registration process of BCAs to reduce costs and at the same time maintain the level of safety to producers and users of these compounds and to consumers of agricultural products. The project will yield proposals on how regulation of BCAs can be balanced according to their potential hazards. REBECA will review the current legislation requirements for BCAs at EU and member states level and compare the rules with those applied in other countries like the United States, Canada and Switzerland, where BCAs have an easier access to the market. The risks related to the use of BCAs will be reviewed, and appropriate risk assessment strategies to estimate risks to human health and the environment related with the use of BCAs will proposed. The variability of the different BCAs makes necessary a specific analysis of related risks according to their nature. Risks related to the use of semio-chemicals, for instance, differ from those related to the use of microbials. The risk assessment thus needs to consider specific characters of the different groups of BCAs. Potential risks will be evaluated and a cost-benefit analysis of regulation will be performed. In this analysis risks to humans and the environment will be compared. Costs, necessary to perform the investigations to estimate potential risks will be estimated. A comparative analysis will weigh the benefits and risks of regulation and will compare these among different groups of plant protection products.

Based on the review of current regulation practices and the evaluation of potential risks, proposals will be developed and road maps designed for appropriate and balanced regulatory testing of microbial BCAs (e.g. insect viruses, bacteria, fungi), for macrobial organisms (e.g. mites, insects, nematodes) for semiochemicals (insect pheromones) and botanicals (extracts of plants and microorganisms). Costs and benefits related to different levels of regulation will be reviewed and trade-offs evaluated. REBECA will also contribute to the definition of low-risk products, which may be exempted from registration and develop proposals for alternative regulation systems. REBECA will bring together stakeholders from industry, science, regulation authorities, policy and environment to spread knowledge and experience in regulation and safety of BCAs and to identify those fields that need further research to assist regulation. A major objective of this action is to form a network within Europe bringing together the expertise and critical mass necessary to improve regulation procedures for BCAs and to disseminate relevant information among companies developing BCAs and regulatory authorities on EU and national level and other interested stakeholders. The action will provide potential experts, who can assist the EU and member states in the evaluation of risks and regulation of BCAs and identify future research tools to support the development of balanced regulation strategies.

Table 2. Project partners.

Partner	Abbr.	Country
Ralf-Udo Ehlers, Christian-Albrechts-University Kiel	CAU	Germany
Herrmann Strasser, Leopold-Franzens-University	LFU	Austria
Lucius Tamm, Research Institute of Organic Agriculture	FIBL	Switzerland
Jeffrey Bale, University of Birmingham	UOB	United Kingdom
Heikki Hokkanen, University of Helsinki	UHEL	Finland
Anita Fjelsted, Danish Environmental Protection Agency	DEPA	Denmark
Dr. Wolfang Oellrich, Chemical and Agrochemical Services	GAB	Germany
Dr. Ulrich Kuhlmann, CABI Bioscience	CABI	Switzerland

REBECA will be launched on 1st January, 2006. The action will be organised by eight project partners (Table 2), which will be responsible for 7 work packages (Table 3). So far nearly 200 experts in the fields of science, regulation, industry and policy from all over Europe have indicated their interest to make contributions to the action. All national and community authorities responsible for regulation and registration of pesticides in the EU will be identified and affiliated into the list of external experts and will be informed about all relevant issues of the Action by the coordinator. External experts will have a strong influence on the outcome of the Action via the workshops, conferences, the Internet page and by contact with the Action co-ordinator and partners. In this way all experts will be able to discuss the outcome of the action with the project partners and with each other. The project partners will be responsible for the implementation of valuable contributions from the external experts within the action. Experts in the field, who are interested in the Action should contact the co-ordinator (ehlers@biotec.uni-kiel.de). Further information will be available from 1st February, 2006 at www.rebeca.eu.

Table 3. Work package list.

Work package title	Responsible Project Partners
Project management and dissemination of results	CAU
Inventory of current legislation and guidance documents	GAB, CABI, DEPA, CAU
Risk assessment Microbials	**LFU, CAU**
Risk assessment Botanicals & Semiochemicals	FIBL
Risks assessment Macrobials	UOB, CAU
Risk-benefit analysis of regulation	UHEL
Measures to accelerate regulation processes	DEPA

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Natural enemies to control stored-product pests in grain stores and retail stores

Matthias Schöller, Sabine Prozell
Biological Consultance, Hosemannstr. 8, D-10409 Berlin, Germany

Abstract: In temperate regions, durable stored products become infested mainly by insects and mites. Rodents and birds are of minor importance. There are approximately 120 species of beetles, moths, psocids and mites which are common. Natural enemies against almost all of them are known. However, only few have been studied in detail and applied in practice for biological control. The biology of the most important natural enemies is illustrated, and selection criteria for natural enemies in the stored-product environment discussed. Information on simulation models improving our understanding of populations dynamics is given. Current commercial application of beneficials in grain stores, food processing industry and retail stores is described. Biological control of stored-product pests is always a component of an integrated control approach. Early detection of pests and good sanitation is crucial to achieve effective control. Strategies for successful combination of physical, chemical and biological control are outlined.

Key words: Biological control, predators, parasitoids, simulation models, integrated control

Introduction

Stored product pests attack dry plant products which are stored for a longer period. Most of these organisms are insects, namely beetles and moths, and there are also a number of psocid, mite and rodent species which are regularly occurring in stores. Natural enemies against almost all of them are known (Schöller, 1998). Stores are usually closed structures, which offer promising prerequisites for the application of natural enemies for biological control.

Helbig (1996) speculated about future application of biological control of stored-product pests in Europe (Table 1). He thought that applications related to food are unlikely, that the application in large stores has low potential and that there will be little acceptance by consumers in private households. Ten years later, we see commercial applications in these fields thought to have low potential (Table 1). Parasitoids are applied by pest control companies in Germany, Austria and Switzerland, and many people use egg parasitoids in their private households to control stored-product moths.

In the USA, the Federal Register (Anonymous, 1992) published a rule that allows the release of parasitoids and predators into stored grain, stored legumes, and warehouses in the US. The rule makes the use of beneficial insects subject to regulation by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) and exempt from the requirement of a

tolerance in food products. In Europe there are no adhered-to regulations to releases of beneficials in stored-product environments. Recently, in Germany a DIN Norm was developed for Pest Control where biocontrol is included. As long as beneficials do not become part of food, there are many opportunities to apply beneficials in grain stores and retail stores. In the following, research and application of natural enemies in these environments in the temperate regions of Europe and the USA is reviewed.

Table 1. Criteria for the use of biological control of stored-product pests. Estimation for the potential of application by Helbig (1996) and current commercial application; + = high potential, 0 = intermediate potential, - = low potential nicer.

Criterion	Category	Potential according to Helbig, 1996	Current commercial application
Pest species	Lepidoptera	+	+
	Coleoptera	0	+
	Mites	-	0
Beneficial	Parasitoid	+	+
	Predator	+	+
	Pathogen	-	-
Type of product	Fodder	+	-
	Raw material for industrial processing	+	+
	Raw material for production of foods	0	+
	Foods	-	+
Type of store	Large scale store	-	+
	Small scale farmer store	+	+
	Processing industry	0	+
	Food wholesale trade	0	+
	Food retail trade	+	+
	Private household	-	+
Type of storage	Bulk	+	+
	Packed products	+	+

Release strategies

Classical biological control

Classical biological control usually involves searching for natural enemies of an exotic pest insect in its country of origin and importing them. There is no case of classical biological control of stored-product pests in temperate regions. As typical stored-product pests are almost cosmopolitan in distribution and do not develop in natural habitats in temperate climates, it is unlikely that classical biological control will be applied here.

Augmentative control

Augmentative control involves using mass-reared beneficial organisms and releasing them before the pest population reaches high numbers. In stored-product protection, generally the pest organisms have a high intrinsic rate of increase, and the pest population build-up has to be prevented; therefore, inundative releases, using mass-reared predators or parasitoids, have been used in the majority of cases. The timing of the releases has to be synchronized with the growth of the pest population. Monitoring with traps can help to determine the best time to release mass-reared beneficial insects. Generally, low numbers of insects initially infest commodities; therefore, parasitoids or predators need to be released early before pests reach high numbers. Inundative releases are most effective when there are more parasitoids released than hosts.

Grain stores

Freshly harvested grain comes in from the field with zero stored-grain insect contamination. This is true for grain that is grown in the northern parts of Europe and the USA. After the grain has been deposited into bins, stored-grain insects that are in the local area may be attracted to the grain. Normally, stored-grain insects immigrate into the grain mass at low numbers during the storage period. In one study, it was estimated that approximately 4-6 insects moved into the bin per day in a bin of wheat in central Kansas (Hagstrum, 2001). During the summer, grain temperatures favour insect development (20-25ºC). Under these conditions, stored-grain beetles will go through one generation in about 30 days, and every 30 days they will increase about ten-fold in number.

Predators

The best studied stored-grain insect predator is the warehouse pirate bug, *Xylocoris flavipes*. This insect is known as an efficient predator of eggs, larvae and pupae of beetles and moths. Some interesting biological characteristics of the warehouse pirate bug are that it requires a low number of prey to complete development and uses cannibalism to survive when prey is absent. It is not effective against large larvae and adult pests as well as internal feeders. Optimum temperature range for development is 30 to 35ºC. They do not develop below 19°C. Adult longevity is from 23 to 50 days for females at 24°C to 32°C, and 30 days at 21°C. Prey are located by intense random searching activity. An attack is successfully completed when the predator is able to insert its stylet into the softer areas of the body of the prey, after which

death usually follows within 30 to 60 s. The victim is held by the predator until the body contents are completely consumed. These predators are larger than most parasitoids, and cannot penetrate as deeply into the grain mass as the parasitoids (Arbogast, 1975; Keever *et al*., 1986).

The predatory mite *Cheyletus eruditus* is approximately 0.5 mm long, with two large pedipalps in front of the body (Figure 1). It reproduces parthenogenetically, i.e. without males. Females lay their eggs in clusters and guard them until the young hatch. All developmental stages are predators and thus they do not damage stored products. The predatory mites can also be cannibalistic, when there is a lack of other food. The predator is resistant to low temperature and does not develop at temperatures below 12°C. They are also resistant to organophosphate insecticides. *C. eruditus* prefers slowly moving prey species to fast moving ones and has a voracious appetite. It is relatively easy to mass rear, and space requirements are well known. The predatory mites can complete their life cycle at temperatures and humidities ranging from 12 to 35°C and 60 to 90% RH, respectively. The higher the temperature and humidity, the faster is their development. Their life cycle lasts from 18 to 164 days depending on temperature (Žďárková and Horák, 1999). Žďárková and Pulpán (1973) tested the survival of the predators at low temperatures ranging from -1.7 to + 2.0°C and relative humidities of 80 to 90%. The best results were obtained at + 2.0°C. Fifty per cent of population of the predatory mites survived for 62 days, and 8% for 200 days without losing their ability to reproduce when transferred back to favourable conditions. Individual stages of mites prefer prey sizes similar to their own size. All stages of mites prefer to consume larvae, while adults were consumed least frequently. The eggs were also eaten in spite of the fact that the predators preferred moving prey (Žďárková and Horák, 2000).

Figure 1. *Cheyletus eruditus* consuming prey.

Parasitoids

Most of the parasitoids that attack the primary beetle pests of stored wheat are in the families Pteromalidae and Bethylidae. These parasitoids are typically small (1 to 2 mm) and do not feed on the grain. They will normally die within 5 to 10 days if no beetles are present in the

grain. These parasitoids are found naturally in the grain, which suggests that after they are released they may continue to suppress pests for many years (Sinha *et al.*, 1979).

Wasps of the genus *Cephalonomia* search for larvae of beetles that develop outside the grain kernel. *Cephalonomia tarsalis* (see Lukas, this volume) and *C. waterstoni* will parasitise the saw toothed and rusty grain beetles, respectively. *Cephalonomia waterstoni* lays a mean of 2.3 eggs per day on the last instar larvae of the rusty grain beetle. Development takes 12 to 22 days at temperatures between 25 and 30°C. Adult wasps live 5 to 20 days depending on temperature. They will search within the entire grain mass and in cracks and crevices to find pest larvae. Both species are naturally occuring in Europe and the USA.

The parasitoid wasps *Anisopteromalus calandrae*, *Theocolax elegans* and *Lariophagus distinguendus* lay their eggs on host larvae or pupae inside grains; because of this, the ovipositor is inserted into the grain kernel. Females are able to distinguish between uninfested and infested grain kernels. The host larva is paralysed prior to oviposition. After emergence from the egg, the parasitoid larva feeds on the host larvae from the outside, thereby killing it. Pupation of the parasitoid takes place inside the grain. Developmental time of *L. distinguendus* (Figure 2) at 26°C is 20 days; adults live for about 12 days.

Figure 2. *Lariophagus distinguendus* foraging for hosts.

Depending on temperature and pest species, the optimal time to release parasitoids into stored grain is about three to five weeks from when the freshly harvested grain was put into a bin. At this time, the later larval stages are starting to develop, and it is these stages that the parasitic wasps find most suitable for stinging and depositing eggs on. If the wasps are released too early, they will not find enough older beetle larvae to parasitise, and they will either leave the bin or die before they have had a chance to parasitise. If the wasps are released too late, then the beetle larvae will have escaped parasitisation by developing into adults. Releasing the sufficient number of parasitoids is also important. Previous studies have shown that releasing about twice as many wasps as adult beetles that are present in the grain works well. Releasing too many parasitoids is not cost effective. In stored grain, two or three properly timed releases

are thought to be adequate because the wasps will reproduce in the grain, continuing to suppress the beetle populations.

Field studies

Only a few large-scale field studies have been conducted on the use of beneficial insects to suppress beetle pests in stored grain. Flinn *et al.* (1996) showed that augmentative releases of the parasitoid wasp *T. elegans* were very effective in suppressing lesser grain borer populations in large bins of stored wheat. After 198 days from initial beetle release in 1993 and 131 days from initial beetle release in 1994, the beetle population in bins treated with parasitoids was suppressed by 98 and 91% in comparison with control bins. In the USA, the Federal Grain Inspection Service (FGIS) standard for insect-infested wheat is two or more live insects per kg of wheat. After 131 days from initial beetle release in 1993, lesser grain borer densities in the treatment and control bins were 0.05 and 2.06 insects/kg, respectively. Thus, the beetle density in the treatment bin is well below the FGIS threshold. Because of the high lesser grain borer release rates in 1994, both the control and treatment bins were above the FGIS threshold (81.03 and 6.94 beetles/kg). Although a very high beetle release rate was used in 1994, the wasps still managed to suppress lesser grain borers by 91%, indicating the same level of effectiveness as in the 1993 study.

This study showed that the parasitoid wasp *T. elegans* effectively suppressed lesser grain borer populations below economic levels for up to 198 days of storage. Cool temperatures in the fall helped suppress the beetle population in the first experiment. Grain naturally cools in the fall, and this cooling can occur earlier if aeration is used. Under U.S. wheat storage conditions, parasitoids would need to suppress beetle populations for about 60 to 90 days until the grain begins to cool in the fall. The wasps used in this study often occur naturally in stored grain (Hagstrum, 1987). Grain managers need to be aware of the benefits these wasps have in suppressing beetle infestations and not confuse them with pest insects. These parasitoid wasps will continue to parasitise beetle larvae and develop into adult wasps until the grain temperature falls below 20°C in the winter. At this temperature, beetle development and reproduction will also cease.

Data from Flinn and Hagstrum (2001) indicated that insect fragments were greatly reduced in grain treated with parasitoid wasps. In this field study, *Theocolax elegans* was released for suppressing populations of *Rhyzopertha dominica* in six bins, each containing 27 tonnes of wheat. Beetles were released into all six bins at monthly intervals for 3 months. Parasitoid wasps were released into three of the bins, 21 days after the first beetle release. After 131 days of storage, wheat samples from the bins were milled to determine the effects of parasitoid releases on insect fragment counts in flour. Fragment counts averaged 56 and 487/50 g in the treatment and control bins, respectively; a reduction in the former of 89%.

In Germany, a number of studies have been conducted on the use of biological control agents to suppress insects in stored grain. These studies have shown that parasitoids must be able to find pests over large distances and at considerable depths within bulk grain to be effective. The host-finding ability of *L. distinguendus* was examined under field conditions in actual grain bins. In a grain bin and a flat storage, adult parasitoids were released on the grain

surface. The parasitoids were able to find and parasitize hosts located up to 4 m vertically and horizontally from the release point within one week; grain depths greater than 4 m were not tested (Steidle & Schöller, 2002). It was hypothesized that a prophylactic release of wasps at the beginning of the grain storage could result in an effective suppression of pest beetles. Thus, the ability of *L. distinguendus* to suppress *S. granarius* was examined in the laboratory in 3 l jars with wheat over a period of 7 months. The following host-parasitoid ratios were studied: 1:0.2, 1:0.5, 1:1, 1:2, 1:5. Regardless of ratios, the population size of *S. granarius* was reduced in the presence of *L. distinguendus* by 75-90% over the whole period (Reppchen *et al.*, 2003). This indicated that *L. distinguendus* is highly suitable for the biological control of stored product pest beetles in bulk grain. In 2003, the laboratory experiments were scaled up and *L. distinguendus* was released under field conditions. The suppressive effect was shown after 3 months even at a high initial beetle population of 3000 beetles per 75 kg wheat. Field releases included silos, flat stores and an in-transit ship treatment.

The laboratory rearing of *L. distinguendus* started in Germany in 1995. The developmental period until the commercial application took almost 10 years. During this time, many aspects of the biology of this parasitoid were investigated in the laboratory. Important in the context of biological control was the finding that the females use larval faeces as kairomones for long-range host finding (Steidle and Schöller, 1997) and that *L. distinguendus* has a preference for environments with low relative humidity (Steidle and Reinhard, 2003).

L. distinguendus was recorded in Germany before 1950 (e.g. Hase, 1920) and was consequently listed in the Hymenoptera volume of the Entomofauna Germanica (Dathe *et al.*, 2001) as native to Germany, but without collection localities. Between 1995 and 2005, six records of natural occurrence of *L. distinguendus* were found (Figure 3). The most northern record is from Schleswig-Holstein near the border with Denmark, the most southern from Switzerland, St. Gallen close to the border with Germany and four records in between from Hamburg, Berlin, Nordrhein-Westfalen and Sachsen-Anhalt. Four records are from private households, where it was found together with the warehouse beetle *Stegobium paniceum*. One of the records from Hamburg is from a pigeon's nest, indicating that this species is able to develop outside buildings, too. For Southern Europe, *L. distinguendus* was recorded from Greece (Papadopoulou and Athanassiou, 2004), Spain (Lucas and Riudavets, 2002), Portugal (Carvalho *et al.*, 2000) and outside Europe it was recorded e.g. from Korea (Yoo *et al.*, 1989), China (Zhaohui *et al.*, 1998), Japan (Kamijo, 1981) and Russia (Smirnov and Polejaeff, 1937).

For the knowledge of the natural distribution of species applied for biological control, it is important to document records prior to mass releases. Figure 3 shows the localities where *L. distinguendus* was released for biological control in 2005, the first year of a wider application of this beneficial. There were releases in all countries except for Saarland and Mecklenburg-Vorpommern, as *L. distinguendus* was released in Hamburg and Berlin against other beetles as well.

In the Czech Republic research has been conduced on the use of predatory mites to control pest mites in stored grain. These studies have shown that biological control of mites can be used as a preventive measure in empty grain bins and to prevent or suppress mite pest

populations in stored grain. Initially, populations obtained from natural reservoirs and preserved under low temperatures were used for biological control. Later on, a technology for mass rearing was developed in order to use this method on a large scale. The predators were sold in paper bags, each containing 2,000 to 3,000 live specimens of *Cheyletus eruditus* under the commercial name of Cheyletin.

Preventative applications of predatory mites can be used in empty grain stores. One cause of infestation of stored food materials by mites is insufficient cleaning of empty bins. Particles of seeds and other residues provide food and shelter for the stored food mites. Hence the disinfestations of empty bins are very important for post-harvest protection. If this is not done, the residual pest population surviving in the bins from the previous season infests the freshly loaded grain. Chemical treatment, especially with synthetic pyrethroids, does not work well for controlling mites. The pesticides do not penetrate all small cracks and crevices of the floor, where the mites may find a shelter. Therefore a biological agent is recommended. The predatory mites should be released by distributing the contents of one bag of Cheyletin evenly over an area of 100 m^2. Higher effectiveness is achieved if the bin is mechanically cleaned before application of Cheyletin. The predatory mites can be applied one week after chemical treatment.

Figure 3. Natural occurrence of the store chalcid *Lariophagus distinguendus* in Germany (white triangles) and commercial releases in 2005 (black squares; several close sites: white squares).

Preventative applications of predatory mites can be made in bulk grain, too. Ten predators per 100 kg of grain can be applied as a preventive measure if the following conditions are met: 1) The moisture of the stored material is higher than 14% and the infestation by mites is likely; 2) the material will be stored for at least three months. The predators are released evenly on the grain surface (Žďárková & Horák, 1990).

Simulations

A computer model was developed for *C. waterstoni* parasitizing the rusty grain beetle (Flinn and Hagstrum, 1995). This model was instrumental in determining the optimal time to release parasitoids and the correct number to release in the field study that Flinn *et al.* (1996) conducted. Parasitoids are most effective when releases are timed so that parasitoids find the 4th instar larvae before they become adults. Once the beetles enter the adult stage, the parasitoids cannot attack them. The simulation study showed that changing the timing of parasitoid release had a greater effect than releasing more parasitoids. Releasing parasitoids at 20 days after initial storage, instead of after 40 days, resulted in a 75% greater reduction in host population. To achieve the same level of control, the model predicted that 20 times more parasitoids would need to be added if wasps were added at 40 days compared with 20 days.

The simulation model SITOPHEX (Figure 4) was developed to optimise timing of releases and number of *L. distinguendus* to be released in control situations (Prozell *et al.*, 2004). The simulation study showed that releasing the parasitoids twice, in autumn, 4 weeks after harvest, and in spring can be sufficient to prevent population build-up in the following summer. This is also the current recommendation for preventative application (Figure 5).

Integration

Combination of chemical and biological control using *Cheyletus eruditus* is possible. When the infestation of acaroid mites is higher than 1,000 mites per kg of material, or when other insect pests are also present, it is necessary to suppress the population of mites using insecticides. Both fumigation and contact insecticides can be used. After the treatment, sampling needs to be done, because the mortality of the pest mites may not be 100%. Predatory mites can be applied as early as one week after the chemical treatment. Three organophosphates (pirimiphos-methyl, chlorpyrifos-methyl, chlorpyrifos) were tested on two species of acaroid mites, *Acarus siro* and *Tyrophagus putrescentiae*, and on six strains of *C. eruditus* originating from laboratory and field populations of different sources, such as grain stores, a chaff pile and pheasant feed. The organophosphates were almost equally effective on both acaroid mite species. It was found that chlorpyrifos-methyl was most toxic against *C. eruditus*. The effectiveness of pirimiphos-methyl and chlorpyrifos was approximately equal and was three times lower than the effectiveness of chlorpyrifos-methyl. The strain least susceptible to all the organophosphates tested was the one that survived fumigation by phosphine. Obviously, it is better to use the most resistant strain of *C. eruditus* to manage acaroid mites in situations where these chemical insecticides are being used (Žďárková, 1997).

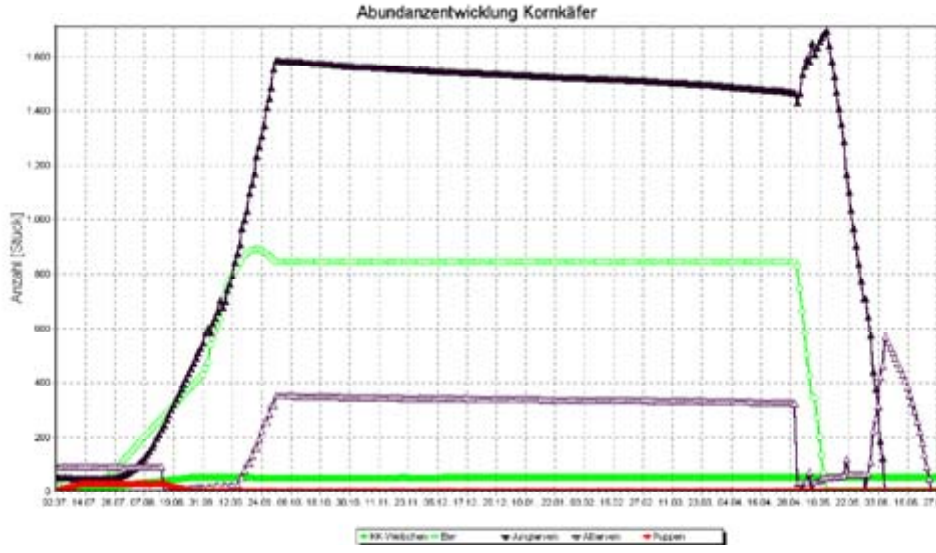

Figure 4. Surface of the simulation programme SITOPHEX for the granary weevil *Sitophilus granarius* and the store chalcid *Lariophagus distinguendus* (Prozell *et al*., 2004), showing the numbers as a function of time of young larvae (▲, upper curve), eggs (○, light grey curve, second from top), old larvae (Δ), females (thick grey line at the bottom), and pupae (thick line at the bottom, only visible from 02.07 to 31.08).

The release of parasitoids in grain is compatible with aeration and cooling of the grain. None of registered grain protectants, including diatomaceous earths, seem to be compatible with macroorganisms for biological control. Protein-enriched pea flour is toxic and repellent to several major stored-product pests but not to the parasitoids *Anisopteromalus calandrae* and *Cephalonomia waterstoni* (Hou *et al*., 2003). Large-scale tests showed a reduction of the rice weevil population by 99.8% when pea flour at 0.1% and *A. calandrae* were combined (Hou *et al*., 2003).

Commercial application

CHEYLETIN was used in the past in the Czech Republic, but there seems to be very limited application at present. The cost of the application of CHEYLETIN as prevention in empty stores is 2 € for 100 square metres. The cost of the control of acaroid mites in grain is 4.60-11 € per tonne, depending on the degree of infestation.

In Europe, commercial application of benefials in grain has started, but in the United States, predators and parasitoids are not frequently used to control insect pests in stored grain. However, there are a few biological control companies that produce insects that can be used to suppress insects in stored grain. The majority of the early adopters of storedproduct

biological control are in the organic foods business. The cost of one release unit of *Lariophagus distinguendus* or *Anisopteromalus calandrae* containing 30 females is 10.25 €. For preventative treatment, one release unit with a mixture of *Lariophagus distinguendus* and *Anisopteromalus calandrae* per 100 square metres in empty stores and one release unit of *Lariophagus distinguendus* for 15 tonnes in bulk grain is recommended.

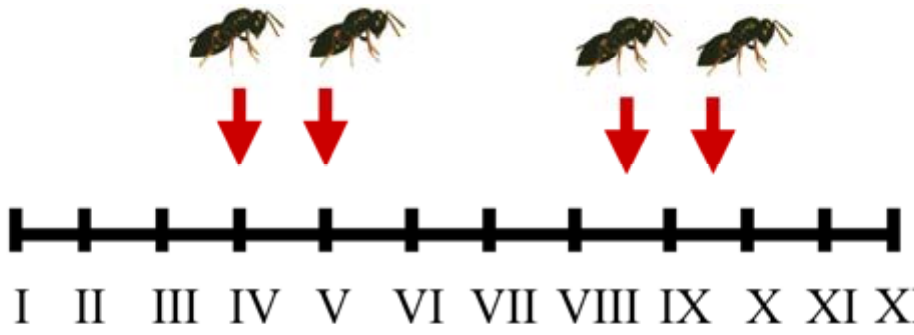

Figure 5. Recommended timing for the release of *Lariophagus distinguendus* against *Sitophilus granarius* in bulk grain.

The estimated price for a treatment of a grain store with 3,000 tonnes, which is slightly infested with *Ephestia elutella*, by releasing *Habrobracon hebetor* would range between 210 € (30 cent/tonne) and 3,000 € (1.26 cent/tonne), depending on the level of infestation (Prozell & Schöller, 2000). The releases of *Habrobracon hebetor* and *Trichogramma evanescens* can be combined and timed as in Figure 6.

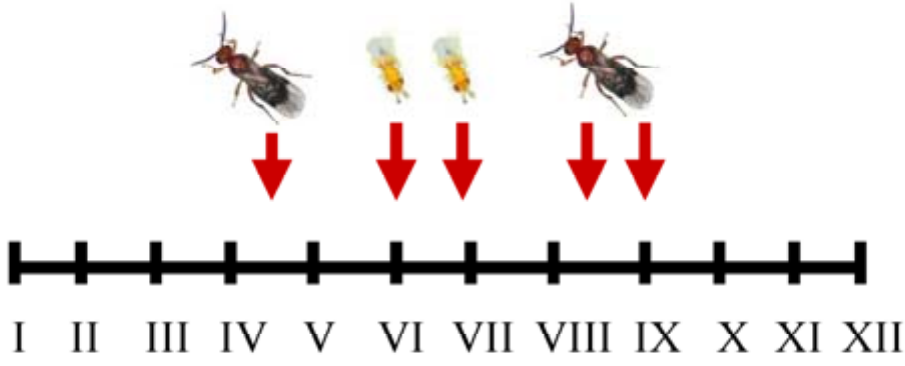

Figure 6. Recommended timing for the release of *Habrobracon hebetor* and *Trichogramma evanescens* in bulk grain against the warehouse moth *Ephestia elutella*.

Warehouses and retail stores

Warehouses harbour a wide variety of storedproduct pests. The presence of bag storages, in addition to processing areas, provides a wealth of microhabitats and a comparatively high species richness.

The pest species complex of milling areas consists of insects that feed on broken or milled grain. In both Europe and the USA these include: the Mediterranean flour moth *Ephestia kuehniella,* the red flour beetle *Tribolium castaneum*, the confused flour beetle *Tribolium confusum*, the broad-horned flour beetle *Gnathocerus cornutus* and the cadelle *Tenebroides mauritanicus*. Additionally, in small bakeries the Indianmeal moth *Plodia interpunctella* can be a dominant pest developing on flour. Moreover, the Mediterranean flour moth can be the primary pest in bakeries utilizing refrigerated storage (e.g. 15°C), due to its low developmental threshold of 8-10°C. In bakeries, high temperature and relative humidity conditions allow the mass development of hygienic pests including: the German cockroach *Blatella germanica* and the house cricket *Acheta domesticus*.

The layout and environmental conditions of warehouses and retail stores is perhaps only slightly less varied than the products contained within them. Not surprisingly, a great diversity of arthropods is found in both warehouses and retail stores including many of the moths and beetles associated with bulk stored products as well as more specialized pests such as the dermestid beetle *Anthrenus verbasci* in Europe or the warehouse beetle *Trogoderma variabile* in the USA, the cigarette beetle *Lasioderma serricorne* and the drugstore beetle *Stegobium panecium*. The Pyralid storedproduct moths such as the Indianmeal moth and the Mediterranean flour moth are perhaps the most important pests of these systems and can be difficult to manage due to their broad dietary range, relatively short life cycle, late larval ability to penetrate packaging materials as well as the adult moths' efficient dispersal ability.

Biological control in warehouse and retail store facilities present some interesting challenges for the manager or pest control professional. First, a great diversity of products from a variety of sources may be present in warehouses and retail stores, each with its own history of pest exposure and management. Second, the stock will be rotated in or out on a sometimes unpredictable basis that can result in overlapping generations of product and therefore pests. Finally, because of their relative proximity to the consumer there is little if any chance detecting and removing infested products prior to their sale.

Predators

Blattisocius tarsalis is a polyphagous predatory mite found frequently in food processing facilities in association with several pest species, mainly phycitine moths (Graham, 1970). It was shown to prey on eggs of the mold mite *Tyrophagus putrescentiae*, the Indianmeal moth, the Mediterranean flour moth, the tropical warehouse moth, the cigarette beetle, the rusty grain beetle *Cryptolestes ferrugineus*, the red flour beetle, the bean weevil *Acanthoscelides obtectus*, first instar of the booklouse *Liposcelis bostrychophila* (Riudavets *et al.*, 2002a) and the confused flour beetle (Riudavets and Quero, 2003). In a choice experiment, *B. tarsalis* showed a clear preference for eggs of the Indianmeal moth over those of the confused flour

beetle and the Mediterranean flour moth (Riudavets & Quero, 2003) as well as for eggs of the tropical warehouse moth over those of the red flour beetle (Haines, 1981).

Parasitoids

Augmentative biological control consisting of mass releases of insect parasitoids or predators is the most promising type of biological control for warehouses, and retail stores. This is primarily because of the combination of product diversity, temporal and spatial heterogeneity, and product proximity to the final customer. Both egg and larval parasitoids have been applied for the management of storedproduct moths in the above situations.

Trichogramma spp. is small endoparasites of Lepidopteran eggs. The major advantage of *Trichogramma* species as biological control agents is their extremely small size; with adult egg parasitoids measuring only 0.3 mm in length, making them virtually invisible to the casual observer. *Trichogramma* spp. is probably best suited to use as a preventative treatment on uninfested, packaged products. *Trichogramma* spp. lay their eggs in Lepidopteran eggs (Figure 7), killing the developing moth embryo prior to hatching and therefore preventing the damaging larval stage. The parasitoid larva consumes the contents of the moth egg, pupates and emerges as an adult wasp in 7 to 14 days. Adult parasitoids mate shortly after emergence, and a single female wasp is capable of parasitizing up to 50 eggs in her adult lifespan of 3 to 14 days. *Trichogramma* spp. generally forage while walking on a substrate. Typically, parasitized eggs are fixed to a card and can be stored at 8 to 12°C for up to seven days.

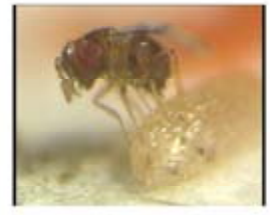

Figure 7. The egg parasitoid *Trichogramma evanescens*.

No allergic or other adverse reactions have been reported from the use of *Trichogramma* spp. in glasshouse or storedproduct conditions. Several species of the genus *Trichogramma* have been released to manage the Indianmeal moth, the Mediterranean flour moth as well as the warehouse moth in warehouses in Europe, North America and Australia as well as in retail stores in Germany, Austria, Canada and the USA. In Europe, *T. evanescens* is currently released commercially in retail stores and warehouses (Prozell & Schöller, 2000). In the USA, *T pretiosum* and *T. parkeri* were found to occur naturally in peanut warehouses (Brower, 1984). *Trichogramma* species typically have a fairly broad host range with many strains reared on the eggs of storedproduct moths. However, various species and strains of *Trichogramma* may differ significantly in the acceptance of eggs of storedproduct moths, especially those of the Indianmeal moth. Schöller and Fields (2003) evaluated a number of different Nearctic species

in the laboratory and found *T. deion* and *T. pretiosum* to be most suitable to control the Indianmeal moth. The eggs of all species of storedproduct moths would most likely be parasitized by the previously mentioned *Trichogramma* species.

Trichogramma are usually released as pupae glued to egg cards at the rate of at least 500 females per card, and one card per linear metre of shelving. Higher release rates may be needed for situations where shelving is more than 2 m in height (Prozell *et al.*, 2004). *Trichogramma* spp. do not establish (Hansen & Jensen, 2002), and they are short-lived (2-7 days); thus, release cards need to be replaced every 3 weeks.

Habrobracon hebetor is a gregarious ectoparasitoid of moth larvae. Female *H. hebetor* parasitise the larvae of several species of storedproduct moths, including Indianmeal moth, Mediterranean flour moth, warehouse moth (*Ephestia elutella*) and the tropical warehouse moth (*Cadra cautella*) (Figure 8).

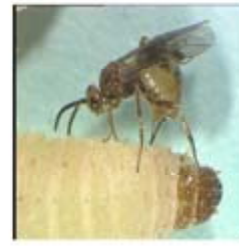

Figure 8. The larval parasitoid *Habrobracon hebetor*.

Contrary to *Trichogramma* species, *H. hebetor* are strong flyers with good long-range searching ability and a longer lifespan. This allows wider dispersal within the facility and the potential of establishing a population, making inoculative releases an option (Schöller and Prozell, 2001). Female parasitoids paralyse their hosts prior to oviposition, with paralysis always leading to the death of the host. *H. hebetor* will paralyse significantly more larvae than they can parasitise, making them well suited for control of wandering larva. Long-range host finding is mediated by kairomones, volatile components of the webbing of the larvae (Strand *et al.*, 1989). Activity starts at 12°C; females can lay up to 100 eggs, and oviposition starts at 16°C, reaches a peak at 30°C and ends at 44°C (Genieys, 1924). Total life cycle at 30°C ranges from 9-10 days with adult females living 7 to 23 days. Furthermore, in unheated stores, pupae and adults are capable of overwintering (Franqui Rivera, 1995). Adults and pupae can be stored at 8 to 12°C for seven days. No allergic or other adverse reactions have been noted with workers in the field. *H. hebetor* is released as pupae attached to cards at a rate of 25-50 females per card.

Field studies in warehouses and retail stores

In Germany, the foraging behaviour of *Trichogramma evanescens* was tested on wooden shelves. 20 batches of 10 sentinel eggs each were placed on the shelf. The shelf was 160 cm

high, 66 cm in width and 41 cm in depth. Pupae of *T. evanescens* shortly before emergence were placed 8 cm (release point 1) and/or 150 cm (release point 2) from the floor on the shelf. The number of *T. evanescens* per release point was 200, 500, 1,000, 2,000 or 4,000, respectively. With each parasitoid release, every sentinel egg patch was found at least once. Releasing 200 *T. evanescens* was less effective compared to releasing more than 200. The effectiveness of releasing 200, 500, 1,000, 2,000 and 4,000 *T. evanescens* was 65.2, 91.9, 94.6, 93.8 and 100%, respectively. In a second experiment, sentinel eggs were placed in comparable positions on both wooden and metal shelves in retail stores. Despite the larger surface area due to food packages, host-finding by *T. evanescens* was as good as in the laboratory experiment (Prozell *et al.*, 1995). In a third experiment, the oviposition site selection of Indianmeal moth was investigated. Most eggs, i.e. 75%, were deposited on shelves, and 25% directly onto the packages. Consequently, studying the foraging behaviour on shelves is a realistic scenario. Packaging itself was shown to effectively protect food packages from *T. evanescens*. Flour packaged in paper bags is not moth-proof, but no *T. evanescens* were found inside these packages when the wasps were foraging on experimental shelves (Ambrosius, 2003).

To evaluate the consumer acceptance of biological control in retail stores, a questionnaire was distributed in organic food shops. Customers were asked for their opinion about releasing parasitic wasps in retail stores (Prozell *et al.*, 1995). Although no detailed information was available at that time about the usage of biological control of moths in private appartments, almost 50% claimed to release parasitoids. Out of the group that decided not to release, 50% said that they did not dislike all insects, but did dislike those that are stinging and dangerous and that more information would be appreciated. A microbiological study (Ambrosius, 2003) showed that bacteria were suppressed on moth eggs used for rearing *T. evanescens* by the use of UV radiation to sterilize the moth eggs. This technique has become standard for the production of *T. evanescens*.

Encouraged by these results, commercialisation started in 1997. Since then, no complaints due to the release of *T. evanescens* and *H. hebetor* have been recorded. At least 180 retail shops are currently using *T. evanescens*. The duration of release ranges from 8 to 12 months, depending on the moth species. The retailers evaluated the success of the biological control programme by the number of complaints by customers due to moth infestation and by the detection of infested packages in the stores. In private households, *T. evanescens* is frequently released, too, and for many people this was the first personal experience with biological control (Prozell & Schöller, 2003).

In the USA and Canada, currently only a limited number of field studies have been conducted exploring the potential of various *Trichogramma* species and *H. hebetor* for the management of storedproduct moths under warehouse and retail conditions. Cline *et al.* (1984) explored the potential of *H. hebetor* to protect uninfested corn meal packaged in paper bags, adjacent to food debris infested with wandering larva of the almond moth under simulated warehouse conditions. Their findings indicated that *H. hebetor* significantly reduced the number of larvae that penetrated paper packages although the parasitoid by itself did not completely mitigate damage.

In the USA, three species of *Trichogramma*, namely *Trichogramma deion* Pinto and Oatman, *T. ostriniae* Pang et Chen and T. *pretiosum* Riley, were evaluated as potential biological control agents for the Indianmeal moth in retail stores. A single shelving unit was used in each trial and foraging success was tracked via a grid of sentinel egg patches. The shelving units were either bare or were stocked with simulated packages and consisted of pallet units with five shelves measuring 1.83 m high by 1.22 m wide by 0.61 m deep. 15 sentinel egg patches were placed in identical arrangements on each shelf as well as on the floor beneath the unit. In trials with packaging, 24 boxes totalling 3.97 m^2 surface area/shelf were placed on the four lower shelves. Approximately 500 female *Trichogramma* sp. were released at the centre of the shelving unit and allowed to forage for 48 hours and per cent parasitism was recorded after seven days. Foraging success as well as the spatial pattern of parasitism differed significantly among the three *Trichogramma* species with *T. deion* performing the best of the three species. Additionally, packaging affected the foraging efficiency of *T. ostriniae* and *T. pretiosum* but did not appear to affect *T. deion* (Grieshop *et al.*, 2004).

Integration

Augmentative biological control programmes are compatible with a variety of other management tactics including insect-resistant packaging, cool temperatures, heat treatments and some insecticides and fumigants (Schöller, 1998). The release of parasitoids in a biological control programme needs to be integrated with pest monitoring and sanitation programmes. Localized infestations typically occur in treated buildings, and these can be targeted by trapping and visual inspection of the storage facilities. Pyralid moths are monitored with the help of pheromone-baited traps. Natural enemies should be released when the susceptible stages of the pests are present. *Trichogramma* spp. and *Blattisocius tarsalis* should be applied when adult moths are active, ideally beginning with the first appearance of moths. The monitoring of pyralid moths with pheromone-baited traps is compatible with the release of *T. evanescens*, *H. hebetor* and *V. canescens*. If coloured funnel traps are used, they should be green or transparent rather than yellow or white, because the parasitoids are attracted by these colours (Schöller and Prozell, 2003).

Hygienic and sanitary measures in storage are a prerequisite to avoid the mass development of a pest species. As natural enemies were shown to be most effective at low pest densities (Žďárková, 1996), the development of proper hygiene programmes is therefore critical for the successful application of beneficial insects. Similarly, augmentative releases made after a heat treatment might be used in an attempt to prolong control efficacy by catching pests that escape lethal temperatures as well as new immigrants into the system. Finally, insecticides that leave a minimum of residual, such as fumigants, may be compatible with augmentative releases, provided releases are made after fumigant residues have dissipated. Bait-based formulations have been shown to be compatible with the release of *Trichogramma evanescens*. If cockroaches like the German cockroach *B. germanica* or the Oriental cockroach *B. orientalis* are present in bakeries, they should be controlled before releases are made. The side effects of three compounds formulated as gels, Fenitrothione, Hydramethylnone and Fipronil on *T. evanescens* have been tested. All three insecticides were found to have no side effects on

T. evanescens, i.e. parasitism and emergence of progeny was not affected. The simultaneous release of *T. evanescens* and the application of gels were tested in practice (Schöller *et al.*, 2002). Generally, sprayed residual insecticides are toxic to beneficials. However, certain pyrethroids are 2 to 145 times more toxic *to E. kuehniella* than to *V. canescens* (Elliott *et al.*, 1983). A review of integration of biological and non-biological methods to control storedproduct pests can be found in Schöller and Flinn (2000).

Commercial application

The cost of a treatment in private households with *Trichogramma evanescens* is usually 23 € plus postage. In Germany, the release of natural enemies in warehouses and the retail trade is part of an integrated pest management. The release of beneficial insects without a consultation is not recommended, and prices for the whole programme depend largely on the pest control situation (Prozell & Schöller, 2000). Examples would be between 500 and 3,000 € per year per facility, depending on the size of the facility. However, as the surface and nature of the stored products determine the number of *T. evanescens* to be released, cost can not be given in €/m^2. Against the Indianmeal moth, nine releases are recommended from mid-April to mid-October (Figur 9); of course this has to be adapted to the local situation if adult moths are present outside this period.

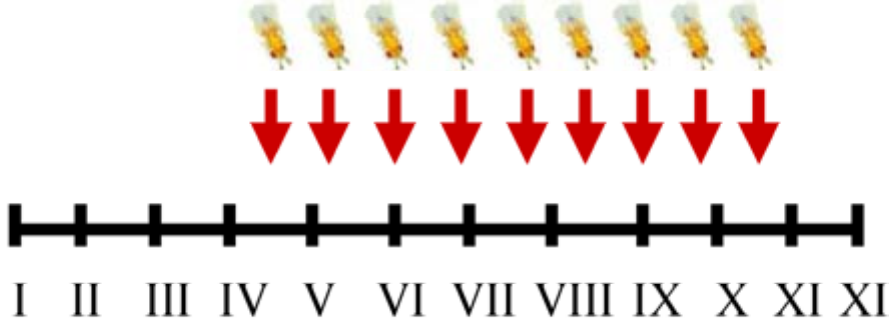

Figure 9. Recommended timing for the release of *Trichogramma evanescens* against the Indianmeal moth in warehouses with packed products and the retail trade.

Cost of application is low, as biological control "formulations" such as pupal cards containing *Trichogramma* sp. or *H. hebetor* can be fairly easily distributed in the warehouse by untrained workers. Unlike traditional insecticides, there are no applicator or customer safety concerns. There are only some potential problems in releasing biological organisms close to foods that are in the final stages of packaging. For example, in retail shops a possible mis-use would be to place the release units close to meat or other food of animal origin. Currently, release cards

containing 3,000 *Trichogramma evanescens* can be purchased for € 1.15 per card, while a release card containing 25 *H. hebetor* can be purchased for € 3.60 per card.

Research needs

Progress has been made on the way to integrated control of storedproduct pests based on biological control. However, a lot of knowledge is still missing. With every previously unexperienced storedproduct situation, due to e.g. new products or storage types, new questions arise. This is mostly due to unknown effects of habitat complexity on parasitoid or predator foraging efficiency. Knowledge on these factors would aid the development of effective release guidelines for natural enemies. Beside bulk grain and warehouses, biological control in only few other storage situations was investigated to some extent, e.g. bakeries and mills (Schöller, 2004). A lot of information on the biology of natural enemies of storedproduct pests is available, due to their role as model organisms. However, mass rearing techniques for many beneficials are not known yet. Further research on the integration of biological control with other pest control methods would be needed, especially on aeration and cleaning of the grain in bulk grain storage and on the combination of specific sanitation measures and heat treatments in warehouses and retail stores.

References

Ambrosius, F. 2003: Zum Eindringverhalten des Eiparasitoiden *Trichogramma evanescens* Westwood (Hym.: Trichogrammatidae) als Gegenspieler der Dörrobstmotte *Plodia interpunctella* Hübner (Lep.: Pyralidae) im Rahmen einer biologischen Bekämpfung an Lebensmittelverpackungen – auch im Hinblick auf die Lebensmittelsicherheit. Diploma-Thesis, Free University Berlin.

Anonymous. 1992: Parasitic and predaceous insects used to control insect pests; exemption from a tolerance. Federal Register, 57: 14644-14646.

Arbogast, R.T. 1975: Population growth of *Xylocoris flavipes* (Hemiptera: Anthocoridae): Influence of temperature and humidity. Environ. Entomol. 4, 825-831.

Brower, J.H. 1984: The natural occurence of the egg parasite, *Trichogramma*, on almond moth eggs in peanut storages in Georgia. J. Georgia Ent. Soc. 19, 285-290.

Carvalho, M.O., Pereira, A.P. & Mexia, A. 2000: Occurrence of *Lasioderma serricorne* F. and *Ephestia elutella* (Hb.) in tobacco Virginia fields and curing barns. In: Integrated protection of stored products, eds. Adler, C. & Schöller, M., IOBC Bulletin 23(10): 91-101.

Cline, L.D., Press, J.W. & Flaherty, B.R. 1984: Preventing the spread of the almond moth (Lepidoptera: Pyralidae) from infested food debris to adjacent uninfested packages, using the parasite *Bracon hebetor* (Hymenoptera: Braconidae). J. Econ. Entomol. 77: 331-333.

Dathe, H.H., Taeger, A. & Blank, S.M. (eds.) 2001: Verzeichnis der Hautflügler Deutschlands (Entomofauna Germanica 4). Entomologische Nachrichten und Berichte, Beiheft 7, 1-178.

Elliot, M., Janes, N.F., Stevenson, J.H., & Walters, J.H.H. 1983: Insecticidal activity of the pyrethrins and related compounds. Part XIV: Selectivity of pyrethroid insecticides between *Ephestia kuehniella* and its parasite *Venturia canescens*. Pestic. Sci. 14: 423-426.

Flinn, P.W. & Hagstrum, D.W. 1995: Simulation model of *Cephalonomia waterstoni* (Hymenoptera: Bethylidae) parasitizing the rusty grain beetle (Coleoptera: Cucujidae). Environ. Entomol. 24: 1608-1615.

Flinn, P.W. & Hagstrum, D.W. 2001: Augmentative releases of parasitoid wasps in stored wheat reduces insect fragments in flour. J. Stored Prod. Res. 37, 179-186.

Flinn, P.W., Hagstrum, D.W. & McGaughey, W.H. 1996: Suppression of beetles in stored wheat by augmentative release of parasitic wasps. Environ. Entomol. 25: 505-511.

Franqui Rivera, R.A. 1995: Behavior, patterns of seasonal field activity and cold tolerance in *Bracon hebetor* Say (Hymenoptera: Braconidae). Dissertation, University of Wisconsin-Madison, 143 S.

Genieys, P. 1924: *Habrobracon brevicornis* Wesm.: the effects of the environment and the variation which it produces Ann. Ent. Soc. Am. 18: 143-202.

Graham, W.M. 1970: Warehouse ecology studies of bagged maize in Kenya - II Ecological observations of an infestation by *Ephestia* (*Cadra*) *cautella* (Walker) (Lepidoptera, Phycitidae). J. Stored Prod. Res. 6: 157-167.

Grieshop, M.J., Flinn, P.W. & Nechols, J.R. 2004: Foraging success of three species of *Trichogramma* in a simulated retail environment. National Entomological Society of America Annual Meeting, Salt Lake City, Ut.

Hagstrum, D.W. 1987: Seasonal variation of stored wheat environment and insect populations. Environ. Entomol. 16: 77-83.

Hagstrum, D.W. 2001: Immigration of insects into bins storing newly harvested wheat on 12 Kansas farms. J. Stored Prod. Res. 37: 221-229.

Haines, C.P. 1981: Laboratory studies on the role of an egg predator, *Blattisocius tarsalis* (Berlese) (Acari: Ascidae), in relation to the natural control of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) in warehouses. Bulletin of Entomological Research 71: 555-574.

Hansen, L. Stengård & Jensen, K.-M.V. 2002: *Trichogramma turkestanica* against *Ephestia kuehniella* in flour mills: extent of host-feeding and initial results of a field trial. In: Integrated protection in stored products, eds. Adler, C., Navarro, S., Schöller, M. & Stengard-Hansen, L., IOBC wprs Bulletin 25 (3): 105-108.

Hase, A. 1920: Über den Putzvorgang bei der Schlupfwespe *Lariophagus distinguendus* (Först.). Naturwissenschaftliche Wochenschrift 6: 81-87.

Helbig, J. 1996: Biological control of post-harvest pests – a realistic alternative? In: Stored product protection and post-harvest treatment of plant products. Proceedings International forum Strasbourg (France), 7-8 November 1995, ed. Council of Europe Publishing: 189-198.

Hou, X., Fields, P., Flinn, P., Perez-Mendoza, J. & Baker, J. 2003: Efficacy of pea protein and combination of pea protein and wasps against stored-grain insects in large-scale tests. In: Advances in Stored Product Protection. Proceedings of the 8th International Working Conference on Stored Product Protection, 22.-26. July 2002, York, United Kingdom, eds. Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M. & Highley, E.: 603-607.

Kamijo, K. 1981: Two New Species of *Lariophagus* (Hymenoptera, Pteromalidae) from Japan, with a note on a known Species. Kontyû, Tokyo 49: 81-85.

Keever, D.W., Mullen, M.A., Press, J.W. & Arbogast, R.T. 1986: Augmentation of natural enemies for suppressing two major insect pests in stored farmers stock peanuts. Env. Ent. 15: 767-770.

Lucas, E. & Riudavets, J. 2002: Biological and mechanical control of *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice. J. Stored Prod. Res. 38: 293-304.

Papadopoulou, S.C. & Athanassiou, C.G. 2004: *Lariophagus distinguendus* (F.) (Hyme., Chalcidoidea, Pteromalidae), an ectoparasitoid of *Lasioderma serricorne* (F.) (Col., Anobiidae), found for the first time in tobacco stores in Greece. J. of Pest Science 77: 183-184.

Prozell, S., Reichmuth, Ch., Roßberg, D., Schöller, M. & Steidle, J.L.M. 2004: Vorratsschutz im ökologischen Landbau. Entscheidungshilfe, Lexikon, Expertise. Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), CD-ROM, ISBN 3-930037-09-2.

Prozell, S. & Schöller, M. 2000: Commercial application of parasitoids and predators of stored-product pest insects. In: Integrated protection in stored products, eds. Adler, C. & Schöller, M, IOBC wprs Bulletin 23(10), 165-168.

Prozell, S. & Schöller, M. 2003: Five years of biological control of stored-product moths in Germany. In: Advances in Stored Product Protection. Proceedings of the 8th International Working Conference on Stored Product Protection, 22.-26. July 2002, York, United Kingdom, eds. Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M. & Highley, E.: 322-324.

Prozell, S., Scholler, M., Reichmuth, Ch., Wuhrer, B. & Hassan, S.A. 1995: Akzeptanz von *Trichogramma*-Freilassungen im Einzelhandel - Monitoring und Erfolgskontrolle. Dtsch. Ges. Allg. Angew. Ent.-Nachrichten, 121.

Reppchen, A., Schöller, M., Prozell, S., Adler, C., Reichmuth, Ch. & Steidle, J.L.M. 2003: The granary weevil *Sitophilus granarius* is suppressed by the parasitoid *Lariophagus distinguendus*. In: Advances in Stored Product Protection. Proceedings of the 8th International Working Conference on Stored Product Protection, 22.-26. July 2002, York, United Kingdom, eds. Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M. & Highley, E.: 230-232.

Riudavets, J., Maya, M. & Monserrat, M. 2002: Predation by *Blattisocius tarsalis* (Acari: Ascidae) on stored product pests. In: Integrated protection in stored products, eds. Adler, C., Navarro, S., Schöller, M. & Stengard Hansen, L., IOBC wprs Bulletin 25 (3): 121-126.

Riudavets, J. & Quero, R. 2003: Prey preference of the predatory mite *Blattisocius tarsalis* (Acari: Ascidae). In: Proceedings of the 8th International Working Conference on Stored-Product Protection, York, UK, 2002, eds. Credland, P. F., Armitage, D. M., Bell, C. H., Cogan, P. M. & Highley, E.: 297-299.

Schöller, M. 1998: Biologische Bekämpfung vorratschädlicher Arthropoden mit Räubern und Parasitoiden - Sammelbericht und Bibliographie. In: 100 Jahre Pflanzenschutzforschung. Wichtige Arbeitsschwerpunkte im Vorratsschutz, Reichmuth, Ch. (ed.). Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Heft 342, 85-189. Parey, Berlin.

Schöller, M. 2004. Potential use of biological control of stored-product pests in bakeries and mills. In: Stengård Hansen, L., Wakefield, M., Lukáš, J. & Stejskal, V. (eds.) Proceedings of the third meeting of working group 4 of COST Action 842, RICP, Prague, 31-37.

Schöller, M. & Fields, P. 2003: Evaluation of North American species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) for control of the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). In: Advances in Stored Product Protection. Proceedings of the 8th International Working Conference on Stored Product Protection, 22.-26. July 2002, York, United Kingdom, eds. Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M. & Highley, E.: 233-237.

Schöller, M. & Flinn, P.W. 2000: Parasitoids and Predators. In: Alternatives to Pesticides in Stored-Product IPM, eds. Subramanyam, B. and Hagstrum, D.W. Boston /Dordrecht/ London: Kluwer Academic Publishers.

Schöller, M. & Prozell, S. 2001: Die Mehlmottenschlupfwespe *Habrobracon hebetor* (Hymenoptera: Braconidae) als Antagonist vorratsschädlicher Motten. Gesunde Pflanzen 53 (3): 82-89.

Schöller, M., Reppchen, A., Prozell, S. & Beckmann, A. 2002: Integration of chemical control of cockroaches and biological control of stored-product moths. In: Integrated protection in stored products, Adler, C., Navarro, S., Schöller, M. & Stengard Hansen, L. (eds.). IOBC wprs Bulletin 25 (3): 21-25.

Sinha, R.N., Wallace, H.A.H., Reiser, B. & Lefkovitch, L.P. 1979: Interrelations of arthropods microorganisms in damp bulk stored wheat - a multivariate study. Res. Popul. Ecol. 21: 40-67.

Smirnov, E. & Polejaeff, W. 1937: On the behavior of *Lariophagus distinguendus* Först. a parasite of the granary weevil *Calandra granaria* L. Zoologitschesky Zjurnal 16: 999-1012.

Steidle, J.L.M. & Reinhard, J. 2003: Low humidity as a cue for habitat preference in the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). Biological Control: 169-175.

Steidle, J.L.M. & Schöller, M. 2002: Fecundity and ability of the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) to parasitize larvae of the granary weevil *Sitophilus granarius* (Coleoptera: Curculionidae) in bulk grain. J. Stored Prod. Res. 38: 43-53.

Yoo, Ch.K. & Ryoo, M.I. 1989: Host preference of *Lariophagus distinguendus* Foerster (Hymenoptera: Pteromalidae) for the instars of rice weevil (*Sitophilus oryzae* (L.)) (Coleoptera: Curculionidae) and sex ratio of the parasitoid in relation to the host. Korean J. Appl. Entomol. 28: 28-31.

Žďárková, E. 1996: Control of stored food mites by non-chemical methods. In: Stored product protection and post-harvest treatment of plant products. Proceedings International forum Strasbourg (France), 7-8 November 1995, ed. Council of Europe Publishing: 165-169.

Žďárková, E. 1997: The susceptibility of different strains of *Cheyletus eruditus* (Acarina: Cheyletidae) to organophosphate acaricides. Exp. Appl. Acarol. 21: 259-264.

Žďárková, E. & Horák, E. 1990: Preventive biological control of stored food mites in empty stores using *Cheyletus eruditus* (Schrank). Crop Protection 9, 378-382.

Žďárková, E. & Horák, P. 1999: Development of *Cheyletus eruditus* (Schrank) at low temperatures. Plant. Prot. Science 35 (1): 14-16.

Žďárková, E. & Horák, P. 2000: Number of prey necessary for completing development of *Cheyletus eruditus* (Acarina: Cheyletidae). XXI. Inter.Congress of Entomology, Brazil 2000, Abstract, Book I: 19.

Žďárková, E. & Pulpán, J. 1973: Low temperature storage of the predatory mite *Cheyletus eruditus* (Schrank) for future use in biological control. J. stored Prod. Res., 9, 217-220.

Zhaohui, L., Fangqiang, Z., Baohua, Y., Guilin, L. & Haiping, L. 1998: Bionomics of *Lariophagus distinguensis* Foerster [Hym.: Pteromalidae] and its control effect on maize weevil, *Sitophilus zeamais* Motschulsky [Col.: Curculionidae]. 7th International Working Conference on Stored Product Protection, Beijing, 118-119.

Biological control of post harvest diseases

Birgit Jensen, Inge M.B. Knudsen, Dan Funck Jensen

Plant Pathology Section, Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: Postharvest infections represent multi-million Euro damage to the fruit and vegetable producing industry. Fungicide treatments have been the main method for controlling postharvest diseases of fruits and vegetables. However, alternative methods such as biocontrol are needed due to a generally restricted pesticide use and the progressive loss of effectiveness due to development of fungicide-resistant pathogen populations. In the postharvest environment, biological control of wound invading necrotrophic pathogens by means of antagonistic microorganisms is a very promising alternative as (1) exact environmental conditions can be established and maintained, (2) the target area is well defined and accessible - directly onto the harvested product – and the BCA often encounter minimal competition from indigenous microorganisms on the product, and (3) expensive control procedures are cost-effective due to the relative high value of harvested fruit and vegetables. However, strict food safety considerations regarding direct application of microorganisms to food products as well as consumers reluctant acceptance of deliberate BCA application to fruit and vegetables must be carefully addressed. Some postharvest rots result from preharvest latent infections and may therefore be difficult to control with postharvest application of BCAs.

At least four products are available for postharvest biocontrol of wound pathogens: Aspire, based on the yeast *Candida oleophila* (Ecogen Inc. Langhorn PA, USA), Yield plus based on the yeast *Cryptococcus albidus* (Anchor yeast, Cape Town South Africa) and Bio-Save 110 and Bio-Save 111 based on the bacterium *Pseudomonas syringae* (EcoScience, Orlando, FL, USA). These products are mainly developed for control of postharvest rots of pome and citrus fruit. Antagonists alone do not always provide commercially acceptable control of decay, but their activity can be enhanced by manipulation of the environment or the formulation of the BCA, by the use of mixtures of niche overlapping antagonists or by physiological or genetic manipulation of the biocontrol mechanism. Furthermore integration of BCAs with other methods such as heat treatment, UV-irradiation, chitosan or $CaCl_2$ application, low doses of fungicides or controlled atmosphere (CA) storage has improved control efficacy. Aspects of postharvest biocontrol of wound pathogens and latent infections will be discussed based on literature review and research at our department.

Invasion of *Trichogramma evanescens* into food packages and the risk of food contamination

F. Ambrosius[1], C. Adler[1], Ch. Reichmuth[1], J.L.M. Steidle[2]

[1]*Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Vorratsschutz, Königin-Luise-Str. 19, D-14195 Berlin, Germany;* [2]*Universität Hohenheim, Fachgebiet Tierökologie 220c, 70593 Stuttgart, Germany*

Abstract: Consumer packages were emptied and tested with a solution of Rhodamin red for leaks that could allow the invasion of stored product pests or parasitoids. Package types for bird food (cardboard boxes), ground hazelnuts (bags of polyethylene foil), flour (paper bags), baby food (cardboard boxes with 2 aluminium foil bags inside) and maize flour (Polenta, paper bags) found to be not insect-proof were tested for the invasion of *Trichogramma evanescens* adults by placing 2 sticky tapes with 100 *Sitotroga cerealella* eggs each inside the empty packages. This test was carried out under laboratory conditions both in a plastic box with a volume of 55.4 l and under semi-field conditions with packages displayed on an industrial shelf. Under extreme conditions with approximately 400 *T. evanescens* adults hatching on top of each tested package in the plastic box, 12% of the packages were found to be invaded compared with only 2% of the packages displayed on an industrial shelf. In order to define minimum pore sizes for the invasion of adult *T. evanescens*, additional tests were carried out by placing parasitized egg cards with hatching adults in a set of wire mesh screens with apertures between 0.5 and 0.063 mm. *T. evanescens* was found to be able to invade apertures as small as 0.2 mm but was retained by apertures of 0.15 mm or smaller. Under practical conditions in a shop, the chance of invasion and the risk of food contamination with pathogenic germs by invading *T. evanescens* is regarded as rather low.

Key words: Package, pore size, oviposition

Introduction

Food packages made of paper, cardboard or polyethylene foil are frequently attacked by stored product insects. Packages sent to the Institute for Stored Product Protection for determination of pest species have mostly been infested by moths, with the Indian meal moth *Plodia interpunctella* HÜBNER being by far the most important one. *P. interpunctella* attacks a broad variety of products such as grain and grain products, nuts, chocolates and dried fruits. If adult females cannot invade the product directly, they lay their eggs in the vicinity of openings, and the neonate larvae hatching from these eggs find their way into the food and feed products. They cannot penetrate packaging material by chewing due to the fact that their mouthparts are still too weak, in contrast to the grown larvae, which may penetrate packaging

material in their search for a spot for pupation. Detailed studies on the quality of packaging materials to resist stored product pests and the effects of ionising radiation of food rations were carried out for the U.S. Army (Proctor *et al.*, 1954). Khan (1982, 1983a, 1983 b) investigated the resistance of various mono and compound foils against the attack of various stored product pest species and studied the invasion through tiny openings in packages. A review on stored product protection in the context of food packaging is given by Wohlgemuth & Reichmuth (1998).

The egg parasitoid *Trichogramma evanescens* WESTWOOD is a natural antagonist of stored product moths and is used commercially in German pet food shops and whole food stores to control stored product pyralids (Prozell *et al.*, 1995, 1996; Schöller *et al.*, 1997).

A potential risk in applying parasitoids around consumer packages is the risk of food contamination by individuals entering into the packages. The following study was carried out to investigate if *T. evanescens* invades packages and how frequently this might occur.

Material and methods

Insect rearing

T. evanescens was cultivated at 25°C on eggs of the Angoumois grain moth *Sitotroga cerealella* OLIVIER, which had been glued onto a piece of cardboard and sterilized by UV light (1 h) prior to parasitisation. The *T. evanescens* individuals hatching from the egg cards used in this experiment all had approximately the same age and it was observed that males hatched earlier than females and searched around for mating partners.

P. interpunctella was reared on wheat bran and broken almonds at 25±1°C and 65±5% rh. Eggs were obtained by placing fertile adults in an oviposition container, where females laid their eggs through a fine wire mesh sieve (aperture 0.2 mm) into a bowl. Eggs were then counted and placed on sticky tape in batches of 100. Preliminary tests had shown that eggs fixed on this tape were readily parasitised by *T. evanescens* and that adult parasitoids did not become stuck on the surface of this tape. Two pieces of tape with 100 eggs each were placed inside of a package that was subsequently closed with a sealing tape and tested for invasion by young adult *T. evanescens*. Parasitised moth eggs were the criteria for successful invasion.

Testing food packages

Food packages were chosen to comprise the most frequently used package types. Therefore, paper bags containing wheat flour or corn meal ("Polenta"), respectively, bags made of polyethylene (PE) foil containing ground hazelnuts, cardboard boxes containing bird food and cardboard boxes containing sealed aluminium bags with baby food were tested. Packages were emptied and cut into halves. Openings in the package were made visible by pouring a solution containing Rhodamin red into each half, thus staining a test package in the area where liquid ran out. Tests with Rhodamin red have been described in earlier publications (Wohlgemuth & Reichmuth, 1998).

Laboratory test in plastic boxes

The package types found leaky in the Rhodamin red staining test were also tested for invasion by *T. evanescens*. For this purpose packages were opened in a central portion which could easily be sealed again, and emptied of all products. Two pieces of tape each containing 100 *P. interpunctella* eggs were placed inside and the packages were sealed again. The packages were then exposed to young adult *T. evanescens* in a square translucent plastic box and an egg card with approx. 400 *T. evanescens* was placed on top of each tested package (Figure 1). Each box was 63 cm long, 44 cm wide and 20 cm high (volume: 55.4 l). The box was covered with a plastic liner fixed to the upper rim of the box with adhesive tape, sticky on both sides. Preliminary tests had shown that both the box and the covering plastic foil were antistatic and did not adversely affect the locomotion behaviour of *T. evanescens*. Experiments were performed at temperatures ranging from 20 to 29°C following diurnal changes, at a relative humidity of 43% to 53% and 16:8 h (L:D) light regime. Each package type was tested in 15 replicates. After an experimenal period of 7 d the tapes with *P. interpunctella* eggs were removed from the package and kept in a Petri dish for another two days before examining them for parasitation. Eggs found dark in color were regarded as parasitised.

Figure 1. Package type for ground hazelnuts equipped with moth eggs and an egg card with *T. evanescens* in a plastic box during the invasion test.

Semi-field test on an industrial shelf

In order to simulate a practical application of *T. evanescens*, 5 empty food packages of each type equipped with moth eggs as described above were displayed on an industrial shelf (2.4 m wide, 1.6 m high, 0.4 m deep) in the same room where the plastic boxes had been kept (size approx. 40 m^2). A total of four egg cards of *T. evanescens* each containing approx. 3,200

adults was added by sticking a card in the middle of both the top and the bottom shelf and one at medium height both on the left and right side of the shelf (Figure 2). This resembles the standard release method of *T. evanescens* in control measures against stored product moths. Temperatures were kept above 24°C by heaters to avoid lower temperatures due to winter conditions outside. The relative humidity was kept between 45 and 50% with the help of humidifiers (Defensor). This experiment was carried out under a similar light/dark regime as the previous test in three replicates for an experimental period of 9 d each.

Figure 2. Industrial shelf with empty test packages equipped with moth eggs for invasion by *T. evanescens*. Egg cards with *T. evanescens* are marked with white arrows.

Laboratory test in a set of wire mesh sieves

A set of 6 stainless steel wire mesh sieves (Retsch Co., Haan, Germany) was assembled in the way that the largest aperture was at the bottom, the smallest at the top. Apertures (open distance between two parallel metal threads) were 0.5 mm, 0.4 mm, 0.3 mm, 0.2 mm, 0.135 mm, 0.1 mm and 0.063 mm, respectively. The inner diameter of each sieve was 200 mm. Two commercially available egg cards with approx. 3,200 parasitized eggs of *S. cerealella* were placed in the dish below the sieve with the largest apertures. The finest sieve was covered with a translucent plastic sheet that was fixed with adhesive tape. Light conditions were natural daylight in a laboratory room without direct sunlight in late fall (approx. 12 h daylight: 12 h dark). This design was chosen to separate *T. evanescens* adults by

size due to their negative geotaxis and positive phototaxis. The experimental period was 6 days. At the end of the experiment, the set of sieves was turned onto the side and flushed with CO_2 to narcotisise *T. evanescens* and to prevent narcotised individuals from falling back through larger sieves. Subsequently, the set was turned upside down and disassembled while continuously flushing with CO_2 from below. All individuals found in the various layers were collected into small glass vials, frozen over night at -20°C and counted under a microscope. The experiment was carried out in two replicates.

Results and discussion

Testing food packages for leaks

The Rhodamin red staining test showed that cardboard boxes for bird food were leaky in all corners and seams where two layers of cardboard were connected. Plastic bags for ground hazel nuts were found leaky at both bottom and top seals that are produced by a rapid welding process prior to and after filling in the product. The vertical seam in the middle of the back of the package, however, was found well sealed. Wheat flour bags were insect proof with one exception, where a leak was found in the bottom region. The good seal may be attributed to the additional tag glued on top of the bottom seal covering the central part of the paper folds, a good and even seal of the vertical seam and a sufficiently strong seal of the tightly rolled top end of the paper bag. In contrast, the cardboard boxes used for baby food were found leaky both at the top and bottom due to the fact that lashes were glued together at just a few points

Figure 3. Set of wire mesh sieves for testing the size distribution of *T. evanescens*.

and some additional perforation at the top end of the box. The two aluminium bags with baby food inside the cardboard box, however, were found completely leak-proof (N = 30). Of the 15 tested corn meal bags (Polenta), all top ends were found leaky as well as one bottom seal. No leak could be discovered in the vertical seam. The percentage of packages with leaks is given in Table 1.

Table 1. Percentage of leaky packages detected with the Rhodamin red staining test (n = 15).

Package / Leak type / location	Cardboard box, bird food, 120g	Plastic bag hazelnuts, 200 g	Paper bag wheat flour, 1 kg	Cardboard box, baby food, 500 g	Alumin. bags, baby food, 250 g	Paper bag, Polenta, 500 g
Bag top seam	-	87	0	-	0	100
Top perforation	-	-	-	60	-	-
Top left	33	-	-	100	-	-
Top centre	27	-	-	0	-	-
Top right	100	-	-	100	-	-
Bag vertical seam		0	0	-	0	0
Bottom left	73	-	-	100	-	-
Bottom ctr.	67	-	-	0	-	-
Btm. right	100	-	-	100	-	-
Bag bottom seam	-	73	7	-	0	7

Laboratory test in plastic boxes

Of the total of 75 packages tested, parasitised moth eggs were found in 9 packages, four of these packages were the cardboard boxes for baby food, three paper bags for Polenta (Table 2). This proves that *T. evanescens* may accidentally invade a not sufficiently insectproof package on its random search for host eggs, though the frequency of this incidence should be much lower under practical conditions than the ones tested here, where up to 400 *T. evanescens* were exploring one package. The flour bags that had proven quite well sealed in the Rhodamin red leakage test were not found invaded by *T. evanescens*.

Semi-field test on an industrial shelf

The number of packages found invaded under semi-practical conditions on a shelf with various package types was considerably smaller than that in the plastic box, even though a -test revealed that these differences were not significant (Table 2).

Table 2. Amount of packages found invaded by *T. evanescens* in the experiments in a plastic box and on an industrial shelf

	Plastic boxes	Shelf	χ^2	*P*
Cardboard box, bird food, 120g	14:1*	15:0	1.03	0.3091 n.s.
Plastic bag, hazelnuts, 200g	14:1	15:0	1.03	0.3091 n.s.
Paper bag, wheat flour, 1kg	15:0	15:0	-	-
Cardboard box, baby food, 500g	11:4	13:2	0.83	0.3613 n.s.
Paper bag, Polenta, 500g	12:3	15:0	3.33	0.0679 n.s.

*number of non-invaded packages : number of invaded packages.
n.s.: not significant

Laboratory test in a set of wire mesh sieves

The test conducted in a set of laboratory sieves showed that at least a few *T. evanescens* can invade through openings as small as 0.2 mm while none of the tested more than 4,000 individuals crawled through an aperture of 0.15 mm (Table 3). That the majority of wasps was found remaining in the bottom dish may be due to the fact that *T. evanescens* hatches from these egg cards over a period of two weeks and at the end of the experiment just one week had passed. Perhaps prolonged experimental times could have prompted a more pronounced upward movement of *T. evanescens*. Another reason may be that the steep metal walls of the sieves were not easy to climb or that the wire meshes, forming a horizontal barrier, made upward movement more difficult.

Probability of invasion and risk of contamination

According to the results of this study, the risk that single specimens of *T. evanescens* used for stored product protection around packed goods enter food packages and contaminate food cannot be ruled out. However, the fact that only in very few experiments wasps have been found to invade the packages strongly indicates that the probability of invasion is rather low.

Another important aspect to consider is the probability that *T. evanescens* could become a vector of pathogenic germs. One possibility could be that mass cultures are contaminated with pathogenic germs. This risk can be regarded as rather low due to the fact that the entire juvenile development of *T. evanescens* takes place inside a moth egg. Individuals used for biological control even develop in UV-sterilised eggs. In an earlier study sterilised and non-sterilised *S. cerealella* eggs had been compared for bacterial growth by stirring samples for 30 min in aqua bidest with addition of 0.01% Tween 80 (polyethylenglycolsorbitanoelate) solution and subse-

quently transferring them onto Agar plates. After 7 d in darkness at 21°C or 37°C, respectively, the plates were checked for the presence of bacteria, fungi and yeasts. While non-sterilised eggs contained about 40 germs per egg on average, in sterilised eggs a maximum of 4 germs was found (Schöller & Koch, unpublished data).

Table 3. Distribution of adult *T. evanescens* in a sieve after 6 d.

	Experiment 1		**Experiment 2**	
Sieve aperture	**Absolute nos**	**% of total**	**Absolute nos**	**% of total**
> 0.063 mm	0	0	0	0
> 0.1 mm	0	0	0	0
> 0.15 mm	25	1.5	57	2.3
> 0.2 mm	296	17.2	50	2.0
> 0.3 mm	168	9.8	129	5.1
> 0.4 mm	110	6.4	140	5.5
> 0.5 mm (bottom dish)	1119	65.1	2154	85.1
Total no. of adult *T. evan.*	1718	100	2530	100

Another risk could be the uptake of germs by adult *T. evanescens*, either orally or by the body surface. In nature, individuals may feed on plant juices. In the environment of a shop, the presence of accessible liquids is rather improbable. Also the transport of germs on the body surface can be ruled out because pools of pathogenic germs are not to be expected within reach of *T. evanescens* in the environment of a shop. In conclusion, the authors judge the risk of food contamination due to *T. evanescens* released around packed products as rather low.

Acknowledgements

This paper contains parts of the "Diplom" thesis of Felicitas Ambrosius, supervised by Johannes Steidle and Christoph Reichmuth. The part on size distribution in a set of wire mesh sieves was added by Cornel Adler who also translated and compiled the manuscript. We thank Matthias Schöller and Sabine Prozell (BiP Co. Berlin) for providing *T. evanescens*, information on this parasitoid species and valuable comments regarding the experimental design and this manuscript. Sabine Berger is thanked for providing the cultures of *P. interpunctella* and we thank Heidi Anders for assistance in the experiments with the wire mesh sieves.

References

Khan, M.A. 1982: Die Widerstandsfähigkeit von Mono- und Verbundfolien gegen Vorratsschädlinge. Zeitschrift für angewandte Entomologie, 94: 127-133.

Khan, M.A. 1983a: Invasion von Vorratsschädlingen durch Verschlüsse. Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz 56: 91-94.

Khan, M.A., 1983 b: Untersuchungen über die Invasion von Eilarven von vorratsschädlichen Insekten durch verschieden große Poren des Verpackungsmaterials. Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz 56, 25-29.

Proctor, B.E., Lockhard, E.E., Goldblith, S.A., Grundy, A.V., Tripp, G.E., Karel, M. & Brogle, R.C. 1954: The use of ionizing radiations in the irradication of insects in packaged military rations. Food Technology 8: 535-540.

Prozell, S., Schöller, M., Reichmuth, Ch., Hassan, S.A. & Wührer, B. 1995: Akzeptanz von *Trichogramma*-Freilassungen im Einzelhandel – Monitoring und Erfolgskontrolle. DgaaE Nachrichten 9, 121.

Prozell, S., Schöller, M., Reichmuth, Ch. & Hassan, S.A., 1996: Release of *Trichogramma evanescens* as a component of an integrated pest management programme in organic food bakeries and stores. Proc. 20th Intern. Congr. Entomology, Firenze, Italy, 555.

Schöller, M., Prozell, S., Al-Kirshi, A.G. & Reichmuth, Ch. 1997: Towards biological control as a major component in integrated pest management in stored product protection. Journal of Stored Product Research 33: 81-97.

Wohlgemuth, R. & Reichmuth, Ch. 1998: Verpackung zum Schutz von Vorräten gegen Insekten. In: 100 Jahre Pflanzenschutzforschung – Wichtige Arbeitsschwerpunkte im Vorratsschutz, Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft 342, Parey Buchverlag Berlin: 325-336.

Potential of biocontrol of pests in grain stores and flour mills

Lise Stengård Hansen, Tove Steenberg

Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Danish Institute of Agricultural Sciences, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark

Abstract: Two recent projects have dealt with the potential of biocontrol of pests in grain stores and flour mills in Denmark. In grain stores the most important pest is the granary weevil *Sitophilus granarius*. An investigation is being carried out to elucidate the effect of combining parasitoids against the larvae with entomopathogenic fungi against the adult weevils. Two pteromalid parasitoids are being used in the study: *Lariophagus distinguendus* and *Anisopteromalus calandrae*. As the result of a screening programme a strain of *Beauveria bassiana* was selected for the investigations. A "semi-field" investigation is being conducted at present using containers with 9 kg of grain infested with *S. granarius*. The trial involves the following: i) addition of *L. distinguendus*, ii) addition of *A. calandrae*, iii) addition of *B. bassiana*, and different combinations of the natural enemies. The trial will run for a total of 24 weeks. Preliminary results are presented.

Another project has elucidated the potential of using the egg parasitoid *Trichogramma turkestanica* or the egg predator *Blattisocius tarsalis* for control of the Mediterranean flour moth *Ephestia kuehniella* in Danish flour mills. Biological studies revealed that both natural enemies were active and able to reproduce under the temperature conditions found in flour mills. Two field trials were conducted; the results are discussed.

Key words: Grain stores, flour mills, *Sitophilus granarius, Lariophagus distinguendus, Anisopteromalus calandrae, Beauveria bassiana, Ephestia kuehniella, Trichogramma turkestanica, Blattisocius tarsalis*

Introduction

In grain stores in Denmark and northern temperate regions the most important pest is the granary weevil *Sitophilus granarius* L. (Coleoptera: Cucurlionidae). Several species of parasitoids have potential as biological control agents of *S. granarius* larvae (Schöller & Flinn, 2000). However, the long oviposition period of *S. granarius* makes it difficult to rely on larval parasitoids alone for satisfactory control. Entomopathogenic fungi may have potential for control of the adult weevils, as they infect related species (Adane *et al*., 1996) and also can kill significant proportions of weevils when applied in high dosages (Athanassiou *et al*., 2005). An investigation is being carried out to elucidate the effect of combining parasitoids against the larvae with entomopathogenic fungi against the adult weevils. Two species of larval parasitoids are being used in the study: *Lariophagus distinguendus* Förster and *Anisopteromalus calandrae* (Howard) (both Hymenoptera: Pteromalidae). As the result of a screening programme a strain of *Beauveria bassiana* (Hyphomycetes) was selected for the investigations. A "semi-field"

investigation is being conducted. The trial involves different combinations of the natural enemies. Preliminary results are presented below.

Another project has elucidated the potential of using the egg parasitoid *Trichogramma turkestanica* Meyer (*T. evanescens* Westwood) (Hymenoptera: Trichogrammatidae) or the egg predator *Blattisocius tarsalis* (Berlese) (Acari: Ascidae) for control of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) in flour mills. Biological studies revealed that both natural enemies were active and able to reproduce under the temperature conditions in flour mills when the first flour moth eggs occur in spring in Denmark (Hansen, 2000; Hansen and Jensen, 2002; Nielsen, 2001; Nielsen, 2003). Two field trials were conducted; the results will be discussed.

Biocontrol of *Sitophilus granarius* in grain stores

Materials and methods

A semi-field experiment was conducted using 60 plastic containers each with 9 kg of wheat grain infested with *S. granarius*. The infestation was initiated by adding two adult *S. granarius* per kg grain. After the pest population was established, two species of parasitoids, *L. distinguendus* and *A. calandrae* (1.5 per kg grain) and the entomopathogenic fungus *B. bassiana* (2 x 10^6 spores per g grain, isolate no. 678 originately isolated from *Musca domestica*) were added, either alone or in combination. The units were placed at 20°C, 70% RH. After varying periods of time (7-26 weeks), densities of pests and natural enemies were determined. Mortality in weevils and parasitoids caused by the fungus was determined as well.

Results

After 7 weeks 65% of the initial adult *S. granarius* had died in units treated with the fungus, compared with 5% in the untreated units. After 16 and 20 weeks the reduction in the pest population related to the untreated control was 50-92%. The greatest reduction was seen in units with *L. distinguendus* or *A. calandrae*. The pest density was intermediate in units with a combination of parasitoids and fungus. The investigation is ongoing as present; the population development will be determined one more time.

Biocontrol of *Ephestia kuehniella* in flour mills

Materials and methods

Field trials were conducted in two industrial flour mills from April until mid-October. In mill A the egg parasitoid *T. turkestanica* was released in the galleries above and below the grain and flour silos, a total of four rooms. The beneficials were released weekly as pupae in rates ranging from 300 to 600 per m^2, starting in April.

In mill B a trial was conducted with the egg predator *B. tarsalis* in a 5-storey room containing a bran silo and treatment plant, total volume of the room: 1,100 m^3. 6,400 predators were released weekly.

In both trials the pest population was monitored weekly by the means of pheromone traps (funnel trap, AgriSense-BCS Ldt., U.K.). Pheromone trap data from the previous years were obtained from the mills' files for comparison. In both locations chemical control (aerosol treatment with pyrethrum) had been conducted on a regular basis; in mill A once a week and in mill B twice daily. This was stopped during the trials.

Results

In mill A daily trap catches were generally below 10 moths per day, with occasional peaks reaching 25 moths per day, during the three years with chemical control prior to the trial. During the trial with *T. turkestanica* trap catches were below 15 moths per day for most of the trial in three rooms; in mid-September trap catches increased to higher levels in two of these rooms. In the fourth room trap catches passed 20 per day in June and remained high for the remaining period. The overall result is that moth levels were similar to the previous years in two of the rooms, with an increase late in the season in one of these two. In two other rooms moth trap catches were higher than during the previous years. However, it must be stressed that these results are compared with a chemical treatment; it is practically impossible to obtain an untreated control unit for comparison in an industrial flour mill.

In mill B the maximal trap catch during the previous four years with chemical control was 12 moths per day. During the trial with *B. tarsalis* daily trap catches were below 2 moths per day (P.S. Nielsen, pers. comm.).

Discussion

The potential for using biological control in durable stored products is considered to be good. Time is generally not a limiting factor although there are seasonal variations in temperatures. Grain, e.g., enters storage at harvest with high temperatures and in some cases high moisture content. When possible, the grain is aerated with ambient air to cool the grain and remove moisture. In Denmark grain temperatures usually reach levels below 15°C in October and are as low as 5°C during winter. This means that the parasitoids must be established in the pest population and kill a large proportion of the larvae before their activity stops due to low temperature. Steidle & Schöller (2002) found that *Lariophagus distinguendus* were active in experiments in silo bins with temperatures ranging between 16 and 20°C at the end of the trial. If, in addition to this, the lifespan of the adult *S. granarius* is reduced during the first weeks of storage by infections of *B. bassiana*, the proportion of pests surviving the winter is estimated to decrease. The present investigation is being conducted at 20°C; at this temperature the initial results indicate that the parasitoids alone lead to the greatest reduction in the pest population. The results are similar to those obtained by Reppchen *et al.* (2002), but the present investigations involved far fewer parasitoids in relation to pests. The reduction is smaller in units with both *B. bassiana* and parasitoids; it seems that the fungus affected the parasitoids negatively. However, the fungal dose was relatively high in the present case. It is encouraging to see that the fungus led to high mortality in the pest population and obviously continues to do so, even after 20 weeks. In the future targeted application of the fungus should be devel-

oped, e.g. by combining fungus with a potent attractant; this could maybe overcome the constraints of the fungus killing parasitoids.

The trial with *B. tarsalis* in flour mills resulted in very low pest levels during a whole season; this was very encouraging, but it must be stressed that only one area was treated. Further trials must be conducted with this species. The results with the egg parasitoid *T. turkestanica* were more variable. Differences may be due to the differing value of grain and flour, respectively, as food for flour moths. This may have affected their population development. Different temperatures in the rooms may also have affected the results. Though less encouraging, the potential of this species must be further studied.

Acknowledgements

The technical assistance of Lars Damberg, Bodil M. Pedersen, Minna Wernegreen, Claus Dahl and Ulrik Cold is gratefully acknowledged. The projects were supported by the Ministry of Food, Agriculture and Fisheries.

References

Adane, K., Moore, D. & Archer, S.A. 1996: Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae). Journal of Stored Products Research 32: 105-113.

Athanassiou, C.G., Steenberg, T. & Kavallieratos, N.G. 2005: Insecticidal effect of diatomaceous earth applied alone or in combination with *Beauveria bassiana* and beta cyfluthrin against *Sitophilus granarius* on stored wheat. Integrated Protection of Stored Products, Bulletin IOBC wprs (in press)

Hansen, L. Stengård. 2000: Development time and activity threshold of *Trichogramma turkestanica* on *Ephestia kuehniella* in relation to temperature. Entomologia Experimentalis et Applicata 96: 185-188.

Hansen, L. Stengård & Jensen, K.-M. V. 2002: Effect of temperature on parasitism and host-feeding of *Trichogramma turkestanica* (Hymenoptera: Trichogrammatidae) on *Ephestia kuehniella* (Lepidoptera: Pyralidae). Journal of Economic Entomology 95: 50-56.

Nielsen, P.S. 2001: Developmental time of *Blattisocius tarsalis* (Acari: Ascidae) at different temperatures. Experimental and Applied Acarology 25: 605-608.

Nielsen, P.S. 2003: Predation by *Blattisocius tarsalis* (Berlese) (Acari: Ascidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). Journal of Stored Products Research 39: 395-400.

Reppchen, A., Schöller, M., Prozell, S., Adler, C., Reichmuth, C. & Steidle, J. 2002: The granary weevil *Sitophilus granarius* is suppressed by the parasitoid *Lariophagus distinguendus* Förster (Hymenoptera: Pteromalidae). *In* Credland, P.F., D.M. Armitage, C.H. Bell, P.M. Cogan and E. Highly (eds): Advances in Stored Product Protection. Proceedings of the 8th International Working Conference on Stored Product Protection, 22-26 July 2002, York, UK. CAB International, Oxon UK. 230-232.

Schöller, M. & Flinn, P.W. 2000: Parasitoids and predators. *In*: Subramanyam, B. & D.W. Hagstrum (eds.): Alternatives to pesticides in Stored-Product IPM. Kluwer Academic Press. Boston. Pp 229-272.

Steidle, J. & Schöller, M. 2002: Fecundity and ability of the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) to find larvae of the granary weevil *Sitophilus granarius* (Coleoptera: Cucurlionidae) in bulk grain. Journal of Stored Product Research 38, 43-53.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Is *Cephalonomia tarsalis* suitable for biological control of *Oryzaephilus surinamensis*?

Jan Lukáš
Research Institute of Crop Production, Department of Stored Product Pest Control, Drnovská 507, Prague 6, Czech Republic

Abstract: The saw-toothed grain beetle, *Oryzaephilus surinamensis* (Linaeus) (Coleoptera: Silvanidae), is one of the most abundant pests of stored and processed grain in the Czech Republic. Both adults and larvae cause damage and are usually found on grain damaged by other insects. Biological control using *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) is a possible alternative to currently applied chemical control against this pest. An overview of currently obtained results on: 1) temperature dependent development, 2) temperature dependent functional response, 3) susceptibility of *C. tarsalis* adults to deltamethrin, 4) compatibility of *Cheyletus eruditus* (Schrank) (Acari: Cheyletidae) and *C. tarsalis* in biological control of stored grain pests and 5) temperature and time dependent survival and fecundity of *C. tarsalis* is presented and discussed.

Key words: *Cephalonomia tarsalis*, *Oryzaephilus surinamensis*, biological control

Introduction

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (Linaeus) (Coleoptera: Silvanidae), is a cosmopolitan stored-product secondary pest whose adults and larvae cause economic damage. This pest is considered as a key pest of stored and processed grain in the Czech Republic. Fumigants and contact biocides are recommended and broadly used in control programmes of this pest. Nevertheless, because of a ban of methyl bromide, resistance, toxicity and residues of biocides, there is a need for alternative control means. One possible alternative is use of natural enemies.

Cephalonomia is a genus of parasitoids of the larvae and pupae of small cryptic beetles that feed upon stored products, bark and fungi. *C. tarsalis* was found to parasitise *O. surinamensis* by Powell (1938). Although *C. tarsalis* reportedly uses several different stored product beetle hosts, it appears to be primarily associated with *O. surinamensis* (Howard *et al*., 1998). This parasitoid is naturally present in stored products in the Czech Republic (Lukáš, 2002). The basic biology of the parasitoid was described by Powell (1938). Some aspects of behavioural traits of this parasitoid were studied by (Howard *et al*., 1998; Cheng *et al*., 2003, 2004). Lord (2001) conducted experiments on compatibility of this parasitoid with an entomopathogenic fungus *Beauveria bassiana*. Lukáš (2005) described its temperature dependent functional response.

C. tarsalis is studied in our laboratory with an aim to assess its suitability for biological control program with regard to temperature requirements. This study presents an overview of currently obtained results on: 1) temperature dependent development (Lukáš & Stejskal, 2005), 2) temperature dependent functional response (Lukáš, 2005), 3) a susceptibility of *C. tarsalis* adults to deltamethrin (Lukáš & Šambergerová, 2006), 4) Compatibility of *Cheyletus eruditus* (Schrank) (Acari: Cheyletidae) and *C. tarsalis* in biological control of stored grain pests (Žďárková *et al.*), and temperature and time dependent survival and fecundity of *C. tarsalis* (Lukáš, 2006).

Material and methods

Insect rearing

Cultures of *Cephalonomia tarsalis* and *Oryzaephilus surinamensis* originated from stored wheat samples obtained from a warehouse near to Prague in 2002. *O. surinamensis* was reared on rolled oats and *C. tarsalis* on 4th instar larvae of *O. surinamensis* in wheat. Both cultures were maintained in climatic chambers in a constant temperature of 30°C, relative humidity of 75-80% at a photoperiod of 16:8 (L:D).

Susceptibility of* Cephalonomia tarsalis *adults to deltamethrin

The dipping technique was adopted for the bioassay with adult males and females of *C. tarsalis*. The test solutions of the insecticide were prepared by dilution of appropriate amounts of K-Othrine 25WP with distilled water containing 5% Tween 80 to make desired concentrations of 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5% and 2%. For control tests, insects were dipped in distilled water containing Tween 80 only. Experiments were done with 120 males and 200 females per test data of LC_{50}. The definition of death was that the adults did not respond to prodding with tweezers. No control mortality was observed during experiments. All bioassay tests were conducted in an environmental chamber at 25±1°C at a photoperiod of 16:8 (L:D).

The analysis of the influence of deltamethrin on the mortality of *Cephalonomia tarsalis* was done by means of GLM logit analysis using the R freeware statistical package (http://cran.at.r-project.org) assuming quasibinomial distributed residuals to adjust overdispersion and asymmetries in the distributions (Crawley, 2002). Examination of the residuals confirmed the fit of the models to the data.

***Temperature dependent developmental characteristics of* Cephalonomia tarsalis**

The 4th instar of *O. surinamensis* larvae served as hosts. The experiments were conducted in parallel in four temperature-controlled chambers at four constant temperatures of 21°C, 24°C, 27°C and 30°C at L16:D8 photoperiod and RH 75%. Eggs of *C. tarsalis* 0-12 hours after being laid on larvae of *O. surinamensis* were placed at each temperature. The hatching of larvae, pupae and adults were checked at 12-hour intervals. For each temperature, rate of development (rD: day^{-1}), a reciprocal of the development duration, was calculated for the overall development of *C. tarsalis*. The relationships between developmental rate (rD) and temperature (T) were described by a linear model:

$$rD = a + b.T$$

from which the lower developmental threshold (LDT = $-a/b$) and the sum of effective temperatures (SET =1/b) were calculated. Slopes and intercepts of the linear models were estimated by the linear procedure of QCexpert 2.5 statistical program.

Age specific fecundity and survivorship of* Cephalonomia tarsalis *in different temperatures

Age specific fecundity of mated females of *C. tarsalis* and survivorship of both males and mated females of *C. tarsalis* were studied at constant temperatures of 21°C, 24°C, 27°C, 30°C and 33°C in temperature-controlled chambers. The preoviposition period, number of laid eggs and survivorship of males and females of *C. tarsalis* were recorded daily until the parasitoid had died. Hosts were renewed (replacement of parasitized hosts) daily until the female died.

The analysis of the influence of temperature and age on the preoviposition period, number of laid eggs and oviposition period of *C. tarsalis* was done by means of GLM logit analysis and survival analysis using the R freeware statistical package. Examination of the residuals confirmed the fit of the models to the data.

Compatibility of* Cheyletus eruditus *and* Cephalonomia tarsalis *in biological control of stored grain pests

Twelve cardboard barrels (22 cm in diameter, height 50 cm), each loaded with 7 kg of clean sterile wheat and 0.5 kg of oat flakes, were used to simulate a stored grain environment. The barrels were covered with cloth and left at 22°C and 75% RH for a week to balance the moisture content of the wheat. Pairs of barrels were infested with mites and beetles to give the following six combinations:

I a, b – 1,000 mites *A. siro*
II a, b – 100 beetles *O. surinamensis*
III a, b – 1,000 mites *A. siro* + 100 beetles *O. surinamensis*
IV a, b – 1,000 mites *A. siro* + 50 predators *C. eruditus*
V a, b – 100 beetles *O. surinamensis* + 4 parasitoids *Cephalonomia tarsalis*
VI a, b – 1,000 mites *A. siro* + 50 predators *C. eruditus* + 100 beetles *O. surinamensis* + 4 parasitoids *Cephalonomia tarsalis*.

The mites and their predator came from cultures reared in the laboratory at 25°C and 75% RH on wheat germs, the predators were reared on lettuce seed with *A. siro* as prey. The experiment lasted 3 months; samples (100–200 g) were taken at the end of each month. Berlese funnels were used to extract the mites, and the beetles were sifted out and counted. At the end of the experiment all wheat from the barrels was sifted out and beetles and parasitoids were counted.

Functional response

Functional response of mated experienced 5-day-old females of *C. tarsalis* paralyzing *O. surinamensis* larvae was studied at constant temperatures of 21°C, 24°C, 27°C, 30°C in temperature-controlled chambers. Experienced *C. tarsalis* females (96 hours old) were placed for 24 hours in a plastic jar containing 10 g of wheat and provided with 10 4th instar *O. surinamensis*. A factorial arrangement of all treatments replicated as complete blocks was used. Treatments included four constant temperatures (see above) and targeted host densities of 0, 1, 2, 4, 8, 16 or 32 4th instar larvae of *O. surinamensis*. Experimental blocks were replicated

10 times. After 24 hours, the female parasitoids were removed from the experimental jars and the number of paralyzed host larvae was recorded.

A type II disk equation for parasitoids (Royama, 1971) was fitted to the obtained data:

$$N_p = N_t\left(1-\exp\left(-\frac{aTP_t}{1+aT_hN_t}\right)\right)$$

where *Np* is the number of hosts attacked, *Nt* are the number of hosts available, *a* is the instantaneous search rate, *T* is the total time of the experiment, *Pt* is the number of parasitoids and *Th* is the parasitoid handling time. A nonlinear analysis of the R freeware statistical package was used to estimate the coefficients *a* and *Th*.

A model capable of predicting functional response over a range of temperature was used (Flinn, 1991):

$$N_p = N_t\left(1-\exp\left(-\frac{aTP_t}{1+a\left(\beta_0+\beta_1X+\beta_2X^2\right)N_t}\right)\right)$$

where *X* is the temperature (°C) and the other parameters are as previously described. A nonlinear analysis of the R freeware statistical package was used to estimate the coefficients $a, \beta_0, \beta_1, \beta_2$.

Results and discussion

Susceptibility of* Cephalonomia tarsalis *adults to deltamethrin

Males and females of *C. tarsalis* were unequally sensitive to deltamethrin ($F=6.1$, $df=1,13$, $p<0.05$); males were more sensitive than females. Lethal concentrations for males and females of *C. tarsalis* are summarized in Table 1.

Table 1. Estimates of lethal concentrations (LC) of deltamethrin for *Cephalonomia tarsalis* males and females. The LC_{50} values were calculated based on the mortality at 24h.

	MALES		FEMALES	
Deltamethrin	**conc. %**	**SE**	**conc. %**	**SE**
LD10	0.074	0.103	0.290	0.076
LD25	0.374	0.074	0.685	0.059
LD50	0.758	0.069	1.201	0.059
LD75	1.249	0.091	1.875	0.075
LD90	1.878	0.129	2.754	0.099
LD99	3.930	0.233	5.724	0.169

The sex of the insects was found to important for explaining the mortality of *C. tarsalis* exposed to deltamethrin. A possible explanation could be differences in size between males and females (males 1.0805 ± 0.009 mm, females 1.268 ± 0.005 mm; Cheng *et al.*, 2003). Nevertheless, Baker and Weaver (1993) did not find any differences between males and females of *Anisopteromalus calandrae* exposed to malathion despite the fact that males were significantly smaller than females. The fact that females of *C. tarsalis* are less sensitive to deltamethrin than males would also indicate a potential for breeding a resistant strain of this parasitoid under laboratory conditions.

***Temperature dependent developmental characteristics of* Cephalonomia tarsalis**

The temperature dependent effects on the rate of development (RD) of *C. tarsalis* are summarized in Table 2. In all stages the developmental times were shortest at 27 and 30°C. Egg development was shortest at 30°C and lasted on average 1.1 days. Likewise, the shortest larval development time of *C. tarsalis* was 5.2 days at 30°C and the shortest pupal development time was 5.2 days at 27°C.

Table 2. Temperature dependent developmental constants of *Cephalonomia tarsalis.*

	Egg	Larvae	Pupae	Overall
LDT	18.6°C	16.8°C	17.9°C	15.5°C
SETk	7.5 DD	41.3 DD	49.3 DD	164.8 DD

Age-specific fecundity and survivorship of* Cephalonomia tarsalis *in different temperatures

The preoviposition period was nonlinearly temperature dependent. Females that oviposited first did so at 30°C after 1-2 days. At 21°C females started to oviposit at the age of 2-8 days. An increased preoviposition period of 1-6 days of age was recorded at 33°C. Adult longevity was temperature and sex dependent. Adults lived longer at lower temperature, and females lived longer than males. The overall mean longevity of males was 4-8 days at temperatures ranging between 21 and 33°C. At 21°C males lived for up to 17 days. Mean longevity of females varied between 43 days at 30°C and 82 days at 21°C. An individual female lived more than 90 days at 21°C. Oviposition period was negatively linearly temperature dependent. Females oviposited on average for 54 days at 21°C but for 33 days at 30°C. Egg production was nonlinearly temperature dependent. Up to 198 eggs were laid by an individual female during its lifetime at 27°C. A maximal mean fecundity of 110 eggs per female was recorded at 27°C but only 32 eggs per female (mean) were laid at 21°C. Figure 1 shows the mean age specific fecundity at 21°C, 24°C, 27°C and 30°C. The rate of egg laying was highest at 30°C and lowest at 21°C.

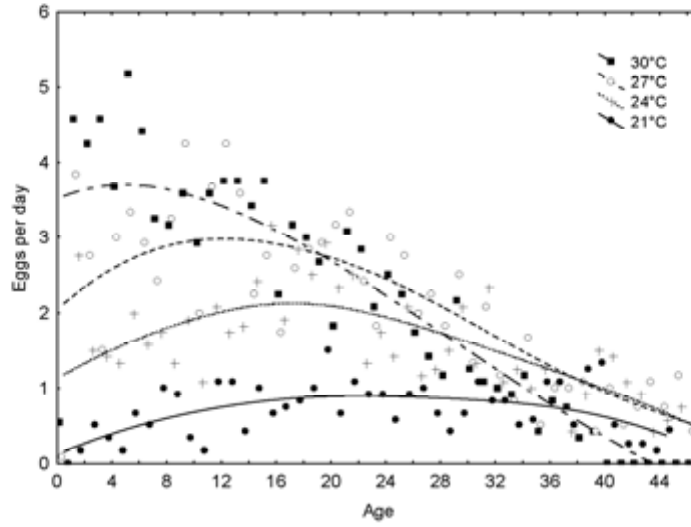

Figure 1. Mean age specific fecundity of *Cephalonomia tarsalis* at different constant temperatures (21°C, 24°C, 27°C, 30°C; n=12 for each treatment). The experiment is ongoing.

The results show that temperature strongly influenced survivorship and fecundity of *C. tarsalis*. The optimal temperature range for reproduction is between 24 and 30°C. Preliminary results (not shown) suggest no reproduction at 18°C and very limited reproduction at 36°C. These findings are in accordance with lower and higher developmental thresholds (LDT and HDT) for development of *C. tarsalis*. LDT for eggs of *C. tarsalis* is 18.6°C (Lukáš & Stejskal, 2005) and HDT for overall development of *C. tarsalis* is slightly above 36°C (unpublished). Nevertheless, outside of these temperature boundaries *C. tarsalis* paralyze and host feed on larvae of *O. surinamensis* (unpublished). Flinn & Hagstrum (1995) found the highest age-specific fecundity of *Cephalonomia waterstoni* at 35°C, no fecundity at 40°C and very low fecundity at 21°C. *C. tarsalis* seems to be about 5°C more cold tolerant and less high temperature-adapted than *C. waterstoni*. Moreover, females of *C. tarsalis* survived longer than females of *C. waterstoni* at similar constant temperatures.

Compatibility of* Cheyletus eruditus *and* Cephalonomia tarsalis *in biological control of stored grain pests

Synchronous application of both natural enemies resulted in more effective control of *O. surinamensis* than treatment with either biological agent alone; the number of beetles was only 1/40th of that in the control. The predator reduced *A. siro* to 7 specimens compared with 5000 in the control. The parasitoid multiplied from 4 to 21 specimens. The predatory mites were observed to prey on the eggs of beetles.

It was found that *C. eruditus* enhances the control of *O. surinamensis*. Eggs of the beetle serve as an alternative food source for *C. eruditus* when the main prey *A. siro* is scarce. Fur-

ther research is needed to quantify this effect. Nevertheless, this finding leads to predicting a higher stability of a biological control programme based on *C. eruditus*.

Functional response

Handling time (*Th*) was inversely proportional to temperature and ranged from 0.167 at 21°C to 0.024 at 30°C. Instantaneous search rate (*a*) also changed with temperature. It was lowest at 30°C and highest at 27°C. The predicted maximum number of paralyzed larvae in 1 day (1/*Th*) was highest at 30°C (41.7 larvae/day) and 27°C (21.3 larvae/day). But the value of *Th* at 30°C was on the board of significance (p=0.051). A temperature-mediated functional response equation explained 77% of the variance in the paralyzation rate. The temperature dependent functional response equation fitted to the data set ($r^2 = 0.77$, $p<0.0001$) is shown in Figure 2. The optimal temperature for paralyzing the maximum number of host larvae is about 28°C.

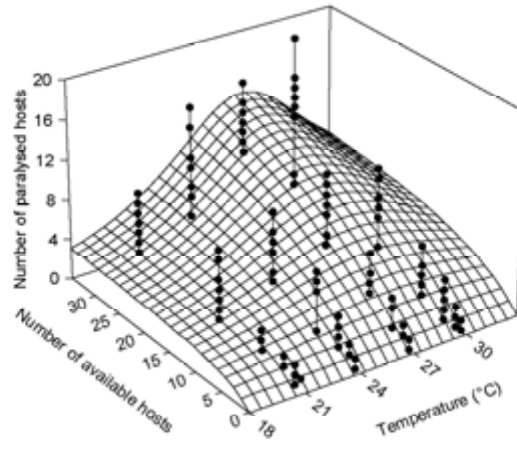

Figure 2. Temperature dependent functional response of *Cephalonomia tarsalis* paralyzing *Cephalonomia tarsalis* larvae.

Functional response analysis is commonly used to help predict the potential of parasitoids to regulate a host population (Oaten and Murdoch, 1975). Estimated instantaneous search rates for *C. tarsalis* were relatively high at all temperatures. The highest search rate was found at 27°C, lower at 21°C and 30°C and not significant at 24°C (p>0.05). *C. tarsalis* was able to attack and paralyze up to 21.3 larvae at 27°C. Flinn (1991) found that *C. waterstoni* attacked 7.5 larvae in 12 hours at 25°C.

Considering the suitability of *C. tarsalis* for biological control programs of *O. surinamensis*, it is necessary to take into account that not all paralyzed hosts are used for oviposi-

tion. Some of them serve as a source of nutrient (host feeding) for maturation of eggs and some are abandoned. Thus, host feeding is an important mortality factor which is welcomed for parasitoid. Moreover, paralyzed host larvae are not able to recover. The parasitation rate indicates only the number of hosts used for reproduction of parasitoid but the paralyzation rate is of greater importance, as it is shows the real capacity of the parasitoid to suppress the pest population. *C. tarsalis* is highly host specific as it seems that *O. surinamensis* is its exclusive host (Howard *et al.*, 1998; unpublished data). Developmental time of *C. tarsalis* is shorter Lukáš & Stejskal (2005) than of its host (Hagstrum and Milliken, 1988). Results of all the presented studies suggest that this parasitoid could be an effective biological control agent against the population of *Oryzaephilus surinamensis*.

Acknowledgements

The study was supported by the grant GACR No. 522/04/P169.

References

Baker, J.E. & Weaver, D.K. 1993: Resistance in field strains of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) and its host, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), to malathion, chlorpyrifos-methyl, and pirimiphos-methyl. Biological Control. 3: 233-242.

Cheng, L., Howard, R.W., Campbell, J.F., Charlton, R.E., Nechols, J.R. & Ramaswamy, S. 2003: Behavioral interactions between males of *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) competing for females. J. Insect Behav. 16: 625-645.

Cheng, L., Howard, R.W., Campbell, J.F., Charlton, R.E., Nechols, J.R. & Ramaswamy, S. 2004: Mating behavior of *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) and the effect of female mating frequency on offspring production. J. Insect Behav. 16: 227-245.

Crawley, M.J. 2002: Statistical computing – an introduction to data analysis using S-Plus. John Wiley, Chichester: 761 pp.

Flinn, P.W. 1991: Temperature dependent functional response of the parasitoid *Cephalonomia waterstoni* (Gahan) Hymenoptera: Bethylidae) attacking rusty grain beetle larvae (Coleoptera: Cucujidae). Environ. Entomol. 20: 872-876.

Flinn, P.W. & Hagstrum, D.W. 1995: Simulation model of *Cephalonomia waterstoni* (Gahan) Hymenoptera: Bethylidae) parasitizing the rusty grain beetle (Coleoptera: Cucujidae). Environ. Entomol. 24: 1608-1615.

Hagstrum, D.W. & Milliken, G.A. 1988: Quantitative analysis of temperature, moisture, and diet factors affecting insect development. Ann. Entomol. Soc. Am. 81: 539-546.

Howard, R.W., Charlton, M. & Charlteon, R.E. 1998: Host-finding, host-recognition, and host-acceptance behaviour of *Cephalonomia tarsalis* (Hymenoptera: Bethylidae). Ann. Entomol. Soc. Am. 91: 879-889.

Lord, J.C. 2001: Response of the wasp *Cephalonomia tarsalis* (Hymenoptera: Bethylidae) to *Beauveria bassiana* (Hyphomycetes: Moniliales) as free conidia or infection in its host, the saw-toothed grain beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). Biological control. 21: 300-304.

Lukáš, J. 2002: Parasitoids occurring in food-processing factories and grain stores. *In:* Žďárková, E., Wakefield, M., Lukáš J. & Hubert, J. (eds): Proceedings of the 2nd Meeting of Working Group 4 "Biocontrol of arthropod pests in stored products", May 30-31, 2002, Prague, Czech Republic, 83-86.

Lukáš, J. & Stejskal, V. 2005: *Cephalonomia tarsalis* – egg, larval and pupal development in dependence on temperature. *In*: Hansen, L.S., Wakefield, M., Lukáš, J., & Stejskal, V. (eds.): Proceedings of 5th meeting of COST 842 Working Group 4 "Biocontrol of arthropod pests in stored products", Barcelona, 27-31 October 2004: 20-21.

Lukáš, J. 2006: Temperature dependent functional response of *Cephalonomia tarsalis*. *In*: Hansen, L.S., & Wakefield, M. (eds): Proceedings of the 6th meeting of Cost Action 842, Working Group 4 "Biocontrol of arthropod pests in stored products", Locorotondo 10-11th June 2005 (*in press*).

Lukáš, J. 2006: Age specific fecundity and survivorship of *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) in different temperatures. *In:* The book of abstracts from the "Conference of the IOBC/OILB WPRS/SROP working group", Prague, Czech Republic, September 20-23 (*in press*).

Lukáš, J. & Šambergerová, V. 2006: An influence of deltamethrin on *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae). - *In:* The book of abstracts from the "Conference of the IOBC/OILB WPRS/SROP working group", Prague, Czech Republic, September 20-23 (*in press*).

Oaten, A. & Murdoch, W.W. 1975: Functional response and stability in predator – prey systems. Am. Natur. 109: 289-298.

Powel, D. 1938: The biology of *Cephalonomia tarsalis* (Ash.), a vespoid wasp (Bethylidae: Hymenoptera) parasitic on the saw-toothed grain beetle. Ann. Entomol. Soc. Am. 31: 44-48.

R Development Core Team 2004. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Žďárková, E., Lukáš J. & Horák, P. 2003: Compatibility of *Cheyletus eruditus* (Schrank) (*Acari: Cheyletidae*) and *Cephalonomia tarsalis* (Ashmead) (*Hymenoptera: Bethylidae*) in Biological Control of Stored Grain Pests. Plant Prot. Sci. 39: 29-34.

Biological control of storage fungi on acorns (*Quercus robur*)

Inge M.B. Knudsen[1], Kirsten A. Thomsen[2], Birgit Jensen[1], Dan Funck Jensen[1]

[1]Plant Pathology Section, Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark; [2]The State Forest Tree Improvement Station, Krogerupvej 21, DK-3050 Humlebaek, Denmark

Abstract: English oak (*Quercus robur*) is a very important hardwood in Denmark. However, poor storage potential of acorns resulting from recalcitrant behaviour combined with irregular fruiting, hampers planting of Danish seed sources. In order to maximize survival of acorns, they are stored at low temperatures (-3° to 0°C). Acorn does not tolerate drying and therefore the moisture content is kept high, approximately at 38%, during the whole storage period. However, the humid conditions favour growth of harmful indigenous microorganisms. The pathogen *Ciboria batschiana* (Zopf) Buchwald is the most serious problem affecting acorn storage (Delatour & Morelet, 1979). Thermotherapy (hot water treatment) is often used to eliminate this fungus, but it has been observed that the treatment favours growth of "moulds" during the subsequent storage. Mould fungi surviving the treatment may eventually invade the seeds which are more vulnerable after hot water treatment (Grondeau & Samson, 1994). Thus changes in microflora related to treatments with thermotherapy may reduce acorn storability (Kehr & Schroeder, 1996). Therefore, thermotherapy has typically been followed by a fungicide treatment, because more environmentally friendly control measures are not available. The aim of the work -summarized in the following - was to test the impact of different alternative control measures on English oak acorn storability and control of *Ciboria* and other fungi affecting viability during low temperature storage. For details in materials and methods, results and discussion, see Knudsen *et al.* (2004).

C. rosea isolate IK726 and commercial BCAs: Supresivit™ (*Trichoderma harzianum*) and Binab TF™ (*T. harzianum+T. polysporum*), Rotstop™ (*Phlebiopsis gigantea*), FZB24WG™ (*Bacillus subtilis*), Cedomon™ (*Pseudomonas chlororaphis*) and Mycostop™ (*Streptomyces griseoviridis*)) were tested on *Ciboria batschiana*-infected English oak acorns during cold humid storage. The BCAs were applied to acorns with and without hot water treatment and compared with the fungicide (Prochloras-Mangan™).

The hot water treatment (2.5 hours at 41°C) was effective in eliminating *Ciboria*. After 4 months of storage, 16% of the acorns were infected in the untreated control compared with 0% in the hot water-treated control. All hot water-treated acorns germinated much better than treatments based on skimmed acorns. After storage for four months, all treatments except control + sticker (Sepiret) had a positive effect on germination. When applied without hot water treatment, all BCAs had a positive effect on survival of acorns and reduced development of *Ciboria*.

Hot water treatment reduced the abundance of *Cladosporium* spp. and *Papulaspora* spp. but enhanced the growth of *Alternaria* spp., Mucoracea and *Penicillium* spp. In contrast, several BCAs including *C. rosea* IK726 significantly reduced the growth of these fungi when combined with hot water treatment. Moreover *C. rosea* IK726 reduced the growth of *Fusarium* spp. and *Acremoniella*

atra; fungi which might be pathogenic to acorns. Also a scarcely recorded taxon as *Acrospeira mirabilis* was inhibited by *C. rosea* IK726 (Knudsen *et al.*, 2001).

Therefore, there are promising perspectives in using biological control for improving seed quality during storage of recalcitrant seeds.

References

Delatour, C. & Morelet, M. 1979: La pourriture noire des glands. Revue Forestiéres Francaise 31: 101-115.

Grondeau, C. & Samson, R. 1994: A review of thermotherapy to free plant material from pathogens, especially seeds from bacteria. Critical Reviews in Plant Sciences 13: 57-75.

Kehr, R.D & Schroeder, T. 1996: Long-term storage of oak seeds – new methods and mycological aspects. Proceedings Tree Seed Pathology Meeting, Opocno, Czech Republic, October 9-11, 1996. Ed. by: Procházkova Z. & Sutherland, J.R.: 50-61.

Knudsen, I.M.B., Thomsen, K.A., Jensen, B., Poulsen, K.M. & Jensen, D.F. 2001: Thermotherapy and microbiological control of storage fungi on acorns (Quercus robur). Proceedings of the Phytopathogens WG meeting: Biocontrol agents: Mode of action and interaction with other means of control. Eds Y. Elad, S. Freeman and E. Monte. IOBC wprs Bulletin 24 (3): 313-316.

Knudsen, I.M.B., Thomsen, K.A., Jensen, B. & Poulsen K.M. 2004: Effects of hot water treatment, biocontrol agents, disinfectants and a fungicide on storability and control of the pathogen *Ciboria batschiana* on English oak acorns. Forest Pathology 33: 47-64.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biocontrol in the mycorrhizosphere

Robert G. Linderman
USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, Oregon USA 97330

Abstract: The mycorrhizal association of fungi with the roots of land plants in natural, undisturbed ecosystems logically includes associations with other functional groups of soil microbes, including a myriad of rhizobacteria, other rhizosphere fungi, and diverse fauna that contribute to successful plant growth and health. We have attempted to characterize qualitative changes in populations of rhizobacteria associated with plants with mycorrhizae in what is called the "mycorrhizosphere". Microbial populations in the mycorrhizosphere can change dynamically over time and are influenced by what microbes are present in the background soil or growth medium and by the process of selective enrichment of specific functional groups of microbes from that medium due to root exudation and arbuscular mycorrhizal (AM) fungus hyphal exudates. The mycorrhizosphere phenomenon includes specific roles that some rhizobacteria might play in combination with mycorrhizal fungi, especially in relation to plant growth enhancement and increased antagonism against soilborne pathogens. Plant diseases are rare in undisturbed ecosystems compared to disturbed agroecosystems where they often cause serious economic loss. Disease suppressive soils occur naturally or due to specific management practices, and are thought to involve soil type and specific bacteria, fungi, or actinomycetes. However, I believe that mycorrhizae play a significant role as well. In our research, we have explored factors that affect AM formation and have determined that AM formation causes an increase in levels of antagonistic bacteria, provided the background soil contains effective antagonists to be selectively increased. This has led me to describe a new mycorrhizosphere paradigm that is a microbial hierarchy wherein roots attract mycorrhizal fungi and the latter attract bacterial associates. The result is a "team" system that functions to support plant growth and health. The microbial components of the system must come from inoculation (mycorrhizae) or selection of bacterial associates from the bulk soil or potting medium. Optimization of any production system comes from having microbes, such as bacterial antagonists, selected from a medium with high microbial diversity, that are efficacious and compatible and therefore can function in tandem. This mycorrhizosphere paradigm involving plants forming AM that select specific bacterial associates can explain the success of the AM symbiosis in supporting plants for some 460 million years.

Key words: Mycorrhiza, rhizosphere, hyphosphere, mycosphere, plant growth-promoting rhizobacteria (PGPR), biological control, antagonistic potential, arbuscular mycorrhizal fungi (AMF)

Introduction

My interest in understanding the rhizosphere has always been from the perspective of controlling soilborne diseases through some manipulation of the microbial populations

therein. My assumptions or beliefs are that root health is the product of microbial activities in the rhizosphere, and that above-ground plant growth is a reflection of the health of the root system. A parallel assumption, based on my observations and those of others, is that root disease is rare in natural ecosystems, due to microbial support systems in the rhizosphere soil associated with plant roots. My goal has always been to characterize the microbial systems involved in normal healthy growth of plants and to incorporate that knowledge into agricultural systems as a means of improving crop productivity and health. This has led me to believe that among the rhizosphere microbial populations with the greatest influence, arbuscular mycorrhizal (AM) fungi are the most important, but only in combination with bacterial associates in what we now call the "mycorrhizosphere".

A number of mechanisms have been proposed whereby mycorrhizae can reduce the incidence and/or severity of diseases. Such proposed mechanisms are based on research using experimental systems that vary between researchers, and the results do not allow for comparisons or final conclusions. Thus, it may be that multiple mechanisms are involved overall, and that it might not be impossible to point to any one as the most likely. However, among those proposed, including mycorrhiza-enhanced nutrition, competition for nutrients and infection sites, morphological changes, changes in chemical constituents of plant tissues, alleviation of abiotic stress, and microbial changes in the mycorrhizosphere (Linderman, 1994, 2000), the latter seems to be the most likely and has been the focus of my research.

The mycorrhizosphere concept

The rhizosphere phenomenon, as described by Hiltner (1904), described the increase in microbial populations in the soil next to roots, induced initially by nutrients released from the roots. The realization that mycorrhizae altered the microflora in the rhizosphere led to the expanded concept of the mycorrhizosphere (Linderman, 1988) in which mycorrhizae significantly influence, qualitatively and quantitatively, the microflora due to altered root physiology and exudation (Ames *et al*., 1984; Bagyaraj, 1984; Fitter and Garbaye, 1994; Meyer and Linderman, 1986; Secilia and Bagyaraj, 1987; Gryndler, 2000). But the paradigm of the mycorrhizosphere, as initially described (Oswald and Ferchau, 1968; Rambelli, 1973; Linderman, 1988), is not complete, both temporally and spacially, and in terms of the dynamic processes that occur. Following the initial enrichment by root products that are specific to the plant species, the dynamic process is influenced by the age of the plant, the nature and treatment of the soil, foliar applications, environmental factors, fertilizer applications and host nutrition, and last, but not least, by the microbial interactions that occur therein. Because they establish a persistent interface between the host root and the soil, mycorrhizae become perhaps the only stable microbial system in the rhizosphere. While increases and decreases in the abundance of certain types of microorganisms have been reported, how and when those changes occur has not been determined fully. Further, descriptions of qualitative changes in microbial populations with potential functional activity have only inferred that such activity would occur because of the increased numbers of microbes with that potential. Measurement

of actual *in situ* activity, such as antagonistic activity against a specific pathogen, has not been documented.

Consideration of the microbial shifts that can be induced by the formation of mycorrhizae requires examination of the sources of nutrient enrichment within the mycorrhizosphere: (a) root tissue exudates and sloughed cells, and (b) AM fungal hyphal exudates (Figure 1). Both can have qualitatively specific chemical components that favor some microbes and not others (Andrade *et al.*, 1997, 1998a, b; Olsson *et al.*, 1996; Vancura *et al.*, 1989). When considering the microbial composition of the mycorrhizosphere, the sum of the two sources must be included. **Thus, rhizosphere soil is soil adjacent to roots and influenced by root exudates, while mycorrhizosphere soil is soil adjacent to mycorrhizae and influenced by exudates from both the root tissue and the fungal hyphae. Both have increased populations of specific microbes selected from the bulk soil.**

Figure 1. Bacterial growth on the hyphal surface of an arbuscular mycorrhizal fungus on agar in response to nutrients exuded from the hypha. (Photo from R. P. Schreiner).

Recent studies have physically separated AM fungal (AMF) hyphae from roots or roots + AMF hyphae by means of mesh that restricts root growth but allows AMF hyphae to pass through, and have distinguished microbial changes induced directly by the hyphae due to their specific exudates (Andrade *et al.*, 1997, 1998a, b; Filion *et al.*, 1999; Vancura *et al.*, 1989). Others have examined the interactions of the AMF hyphae with other microbes in a two-compartment *in vitro* system that also separates hyphae from host roots (Fortin *et al.*, 2002). The *in vitro* system, of course, eliminates the dynamic interactions that occur from having different hosts, different AMF symbionts, changing environmental conditions, and from having a myriad of other microbes that would be present in a soil system. Nonetheless, there is information derived from both culture-based and non-culture-based systems that sheds light on what the mycorrhizosphere phenomenon is and how it relates to microbial shifts that could affect plants. Our research has been strictly culture-based.

Rhizosphere/mycorrhizosphere microbial composition

A myriad of microbes can be present and functioning in the rhizosphere of plants, including rhizobacteria, rhizosphere fungi, fauna, and mycorrhizal fungi. How these microbes may interact and function in relation to plant growth and health is of great interest and relevance. Our research focus, however, has been limited to bacteria-mycorrhiza interactions.

Rhizobacteria. Bacteria that occupy the rhizosphere/mycorrhizosphere soil can have various functions in relation to plant growth and health. We know that some of those bacteria can be antagonistic to soilborne pathogens, based on *in vitro* tests showing inhibition due to the production of antibiotics or other inhibitors. What is often not appreciated, however, is that many, if not most, of the antagonists are also plant growth-promoting rhizobacteria (PGPR) (Mahaffee and Kloepper, 1994; Pieterse *et al.*, 2003). We have confirmed this in tests with petunia using a range of bacterial or actinomycete antagonists to inoculate young seedlings. All of the antagonists stimulated plant growth and flowering, and thus would be classified as PGPR (Linderman, 1993) (Figure 2). Of course, other bacteria, such as symbiotic or free-living nitrogen fixing bacteria, can also be considered as PGPR (Bashan *et al.*, 2004). We should not forget, too, that some of the rhizobacteria might have deleterious effects on plant growth (deleterious rhizobacteria, DRB), presumably due to the production of toxic materials that retard plant growth (Nehl *et al.*, 1997; Suslow and Schroth, 1982).

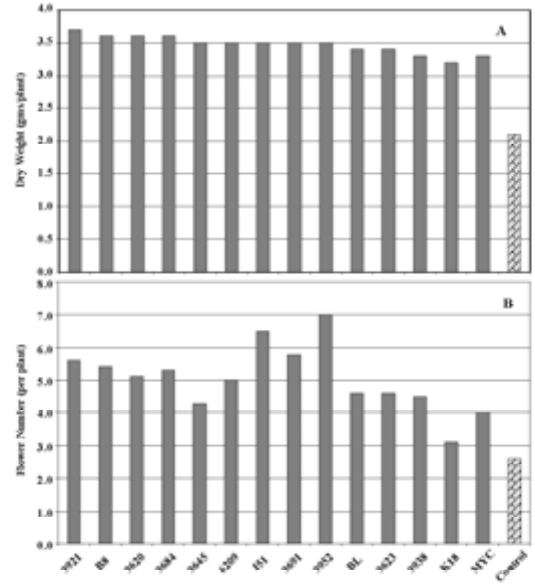

Figure 2. Experimental data showing that rhizobacteria antagonistic toward soilborne pathogens can function as plant growth-promoting rhizobacteria (PGPR) in enhancing the growth (A) and flowering (B) of inoculated petunia plants compared to the water control (Linderman, 1993).

Arbuscular mycorrhizae (AM). We know of many benefits of AM to plant growth and health, due to the unique capacity of AMF to colonize host plant roots internally as well as externally into the surrounding soil. The soil hyphae and spores provide a source of inoculum for new infections as well as uptake of water and nutrients from the soil (Smith and Read, 1997). Exchange of materials within the root takes place by means of the arbuscules. The symbiotic relationship that is established is reported in many publications documenting many benefits to plants. Those benefits include improved plant growth under nutrient (especially P) deficient conditions (Figure 3), improved tolerance to soil toxicity from heavy metals and salinity, improved transplant success, improved crop uniformity, improved root development on cuttings and transplants, improved drought tolerance, and improved disease tolerance. Benefits to plant growth can also be the result of improved soil structure by means of enhanced formation of water-stable aggregates resulting from the entanglement and binding of microaggregates into macroaggregates (Tisdale *et al.*, 1997; Wright and Upadhyaya, 1996). Such aggregates are significant sites within the mycorrhizosphere, providing conditions for microbial activity within the aggregates, such as phosphate solubilization (Andrade, 1998b) as well as the production of other bacterial metabolites and substances that hold the aggregates together. The point to remember, however, is that the microbial products within the aggregates would be immediately available for uptake by the AMF hyphae and translocation to the plant root. Those microbial products may contribute significantly to the overall effects of the mycorrhizae on plant growth and health (Bethlenfalvay and Linderman, 1992).

Figure 3. Growth enhancement of redwood seedlings (left) and lavender (right) inoculated or not with *Glomus intraradices* and grown in P-deficient growth media.

Effects of AM on diseases

Root disease management strategies: Among the strategies for managing soilborne diseases (including chemical, cultural, and biological strategies), I have always favored the biological

approaches, including microbial antagonism and organic amendments. I have a keen interest in understanding the basis for natural disease suppressive systems, with the hope of transferring the key components into agricultural production systems. In analyzing effective suppressive soil systems, such as the Ashburner system for controlling Phytophthora root rot of avocado in Australia, I accept the involvement of bacteria or actinomyces, increased in population due to the build-up of an organic layer on the soil, in the disease suppression, but reason too that the roots had to be colonized by AM fungi as well. So, understanding the factors that affect AM formation and how they could contribute to the disease suppression has been a personal goal.

AM formation and benefits: Arbuscular mycorrhizae are best known for their effects on plant growth due to increased capacity of colonized roots to absorb water and nutrients from the soil, especially under P-limiting conditions. Other benefits as mentioned above, however, are not well understood. An underlying need in all cases, however, is to determine how to incorporate mycorrhizae into the production systems in order to realize the benefits, especially under environmental stress conditions.

The general consensus of mycorrhiza researchers has been that mycorrhizae function primarily as scavengers of nutrients from the soil, but they also induce significant physiological changes in their host plant, one of which is to alter the quantity and quality of root exudates (Graham *et al.*, 1981). The result of those changes is a shift in the microbial composition in the mycorrhizosphere soil. In defining the mycorrhizosphere, however, one must consider the processes and components that are involved in establishing mycorrhizae in the first place, including the soil or substrate; the microbial dynamics in the rhizosphere over time; and inputs of fertilizers as well as organic matter amendments to soil or to soilless potting media. A myriad of microbes occur in the bulk soil, and every soil or soilless medium has a different composition of microbes and is physically and chemically different, depending on the parent material, geographic origin, and cropping history or plant cover. In artificial substrates or other soilless media, these traits are generally very distinct from those of soil. The substrate variability can, in my opinion, significantly affect the formation and function of AM, thus explaining in part why different studies under different conditions yield different results.

We have investigated the effect of different components of soilless plant growth media used in the nursery industry on the establishment and function of the AM symbiosis. If we hope to employ AM on plants to suppress soilborne plant diseases, or any other beneficial function for that matter, we must first evaluate the most commonly used materials in soilless media to determine which favor and which suppress AM formation. Our work has been a continuation of the work of Menge *et al.* (1982), who showed that organic matter in soilless nursery media inhibited the establishment of AM. We investigated different peat mosses to determine if they were responsible for the inhibition and found that some inhibited but did not completely suppress AM formation (Linderman and Davis, 2003a). We examined the use of coconut fiber (coir) as a soilless medium component and found that it did not adversely affect AM formation (Linderman and Davis, 2003b). We then examined the use of different com-

mercial organic and inorganic fertilizers to determine which were more compatible with AM. In general, we found that organic fertilizers were more compatible with AM formation, presumably because they require microbial breakdown and thus more slowly release bound nutrients. However, inorganic fertilizers were compatible if the P content was kept low (Linderman and Davis, 2004). Currently we are investigating the amendment of soilless media with different composts to determine their influence on AM formation. In general, different composts inhibit AM formation in soilless media, presumably due to their high P content. Composts do not inhibit AM formation in soil, however (Linderman *et al.*, 2003). Nonetheless, composts in general add to soilless media a more diverse microbial community, some of which could have significant effects on AM formation and function, both negative (Hetrick *et al.*, 1986) and positive. Some may provide microbes that are "helpers" in the formation of AM (Garbaye, 1994).

Disease suppressive systems: There are numerous examples of disease suppressive soils, such as the Ashburner system for controlling root rot of avacado caused by *Phytophthora cinnamomi* (Linderman *et al.*, 1983). Ashburner was a farmer who sought to transfer what appeared to be natural pathogen suppression in the adjacent rain forest into his avocado orchard. He deduced that the key was to create a layer of organic matter around the trees that would simulate the accumulated litter layer in the forest. The intense microbial activity that occurred in the decomposition of the organic matter appeared to be responsible for the disease suppression that he observed. The roots that grew into the decomposing organic matter were free of the pathogen and thus were able to support normal growth of the trees. Work by Australian scientists showed that heat-tolerant bacteria or actinomycetes were involved in the observed pathogen suppression (Figure 4). The component of the microbial community that was not considered by them, however, was the AM fungi that surely had colonized those roots.

Many reviews on the subject of plant disease suppression by mycorrhizae (Azcon-Aguilar and Barea, 1996; Caron, 1989; Dehne, 1982; Filion *et al.*, 1999; Hooker *et al.*, 1994; Jalali and Jalali, 1991; Linderman, 1992, 1994, 2000; Linderman and Paulitz, 1990; Zak, 1964) have focused on the mechanisms of interaction such as (a) enhanced nutrition, (b) competition for nutrients and infection sites, (c) morphological changes, (d) changes in chemical constituents in plant tissues, (e) alleviation of abiotic stress, and (f) microbial changes in the mycorrhizosphere. Depending on the disease and the environmental situation, any or all mechanisms could be involved, but change in microbial populations in the mycorrhizosphere seems to be the best explanation, yet the least studied.

We believe that mycorrhiza formation establishes a selective pressure on microbes in the background soil, and that the greater the diversity of microbes, the greater the chance that antagonists would be increased. Our hypothesis is that changes in antagonist populations induced in the mycorrhizosphere can influence the incidence and severity of plant diseases (Figure 5). We developed an *in vitro* method of assessing the antagonistic potential of bacterial populations that occur in the rhizosphere soil of plants with or without AM against a range of soilborne, root pathogens. We define the antagonistic potential as the sum of the potential of bacteria to suppress any specific pathogen, and the antagonistic potential index

Figure 4. Biological suppression of *Phytophthora cinnamomi* due to activity of specific microbes from Ashburner's avocado orchard soil, demonstrated by means of heat treatment using aerated steam to establish specific temperatures at (left to right) ambient, 120°F, and 212°F for 30 min. Each flat was inoculated with the pathogen and seeded to susceptible jacaranda. Heat tolerant microbes, such as spore-forming bacteria or actinomycetes, were shown to be responsible for the suppression. Photo by P. Broadbent as presented in Linderman *et al.*, 1983.

Figure 5. Pythium root rot on snapdragon plants not inoculated with AM fungi. Inoculated plants were disease free.

(API) as the number generated by summing the widths of the *in vitro* zones of inhibition against a pathogen by all the bacterial antagonists isolated. Bacteria are isolated from dilution plates of rhizosphere or mycorrhizosphere soil extracts. Our results show that, in general, when AM are formed, there is an increase in the number and proportion of bacteria from the mycorrhizosphere soil that can inhibit specific pathogens *in vitro*, compared to those from rhizosphere soil from non-mycorrhizal plants (Figure 6).

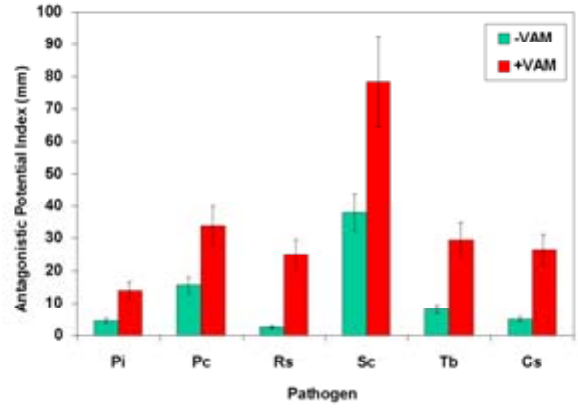

Figure 6. Antagonistic potential index (API) of rhizobacteria from rhizosphere soil around roots of plants with or without AM (VAM) against the soilborne pathogens *Pythium irregulare* (Pi), *Phytophthora cinnamomi* (Pc), *Rhizoctonia solani* (Rs), *Sclerotium cepivorum* (Sc), *Thielaviopsis basicola* (Tb), and *Cylindrocladium scoparium* (Cs) (Linderman, 2000).

A number of factors can influence the potential and magnitude of disease suppression due to mycorrhizosphere microbial populations. One significant factor is the microbial diversity as affected by the amendment of soil or potting mix with composted materials (Figure 7). The host species or genotype within the species can also affect the nature of root exudation and the specifics of the AM association. Any change in the combination of host and fungal endophyte can alter the energy supply to the microbial associates in the mycorrhizosphere. As mentioned before, the soil or growth medium can provide different numbers and kinds of microbes that become AM associates, and different soils have different AMF to form the AM association. It is also important to consider the temporal aspects of AM formation in relation to infection by pathogens: time to establish the mycorrhizal association, to effect physiological change, and to establish a fully functional extraradical mycelial network will affect the effectiveness of the mycorrhizosphere microbial community to suppress root pathogens. For many annual crop plants, time required for disease onset is often too short for AM to become established. This

fact strongly suggests the need for establishing AM and their antagonistic associates as early in the production cycle as possible, even by preinoculating transplants before outplanting into the field.

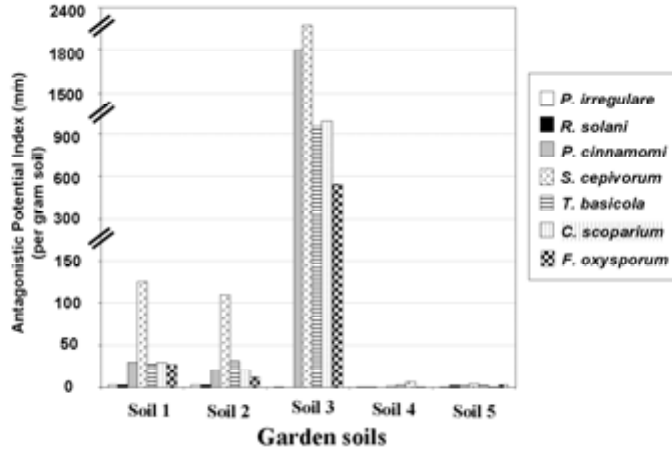

Figure 7. Antagonistic potential of garden soils amended with composts for 1 year (soils 1 and 2), 3 years (soil 3), or non-amended (soils 4 and 5). The antagonistic potential index (API) was determined against a series of soilborne pathogens: *Pythium irregulare*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, *Sclerotium cepivorum*, *Thielaviopsis basicola*, *Cylindrocladium scoparium*, and *Fusarium oxysporum*.

In our studies, inoculating marigold seedlings with the AMF *Glomus intraradices* and transplanting them into soil, amended or not with compost, increased the API dramatically only on plants with AM (Figure 8).

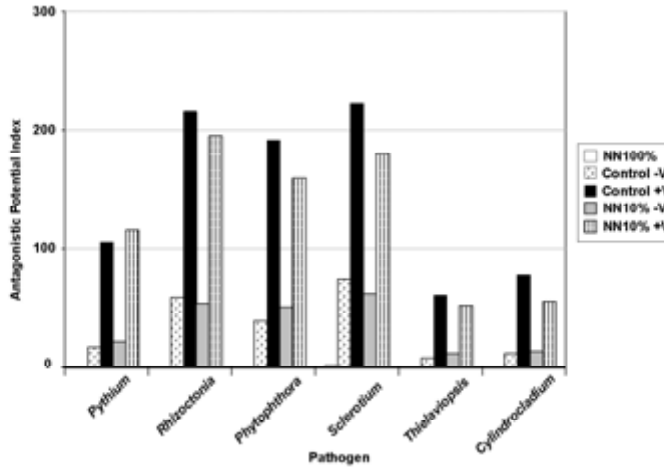

Figure 8. Antagonistic potential index (API) of soil amended or not with compost (10% Natures Needs Compost (NN) or non-amended control) and inoculated or not with the AMF *Glomus intraradices* (V). The data indicate that the API increases dramatically against all pathogens in mycorrhizosphere soil compared to rhizosphere soil from non-mycorrhizal marigold plants. Pathogens used were: *Pythium irregulare*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, *Sclerotium cepivorum*, *Thielaviopsis basicola*, and *Cylindrocladium scoparium*.

Other roles of AM bacterial associates

While our studies have focused on antagonistic bacterial associates of AM in the mycorrhizosphere, we should consider other possible roles that bacterial associates may play in plant health. Bacterial associates of mycorrhizae could also alter the normal life cycle stages of the pathogens involved. For example, root infections by species of *Phytophthora* normally produce sporangia that release zoospores that initiate root infections. Sporangia production is stimulated by metabolites of soil bacteria, but some bacteria can also inhibit sporangia production. We are currently characterizing populations of rhizobacteria for their capacity to inhibit sporangia production. This is a follow-up of earlier work (Meyer and Linderman, 1986) where we demonstrated that an extract of rhizosphere soil from the roots of mycorrhizal plants inhibited sporangia production of *P. cinnamomi*, compared to an extract of rhizosphere soil from a non-mycorrhizal plant. If we could increase populations of sporangia inhibitors in soil, we could prevent root infections from being initiated, much as was demonstrated in the Ashburner system in Australia, and has been demonstrated recently (Sultana *et al.*, unpublished results) by testing sporangia production in response to bacteria isolated from the roots of Phytophthora-infected avocado roots. They found a high population of sporangia stimula-

tors and fewer sporangia inhibitors. We also have developed some preliminary results indicating that antagonistic bacteria isolated from compost can suppress sporangia production by *Phytophthora ramorum* in a bioassay.

Another approach to creating suppressive soils would be to increase populations of cellulose-producing microbes that could lyse the mycelium of *Phytophthora* as was demonstrated by Downer *et al.* (2001). Mulching avocado trees with organic matter increased populations of cellulase-producing microbes that decreased the population density of the pathogen, leading to improved tree growth and health.

Summary, conclusions, and future prospects for disease management

Formation of an effective AM symbiosis in production agriculture can be important under a number of stressful situations, including the growth-limiting effect of P deficiency, soil salinity, drought stress, and disease pressure. Several management strategies must be considered in order to assure AM formation and the prospect of having any effect on plant performance in early growth stages or after transplanting. Preinoculation of transplants seems to be a logical approach in order for AM to effectively address any future stresses. Nursery practices for production of transplants with AM should include organic fertilizers or inorganic fertilizers with low P, could include peat or coir as an amendment to the soilless growth media commonly used, and could include the use of compost to increase the microbial diversity of the medium that could contribute to potential disease suppression. Without that diversity, there might be too few of the needed bacterial associates to complete the "team", the members of which function in tandem to support or enhance plant growth and health. This means that the mycorrhizosphere paradigm is actually a hierarchy wherein the plant roots select and allow formation of AM, and the extraradical hyphae, along with modified host root exudates changes (Graham *et al.*, 1981; Lynch and Whipps, 1990), select specific bacterial associates and sustain them, in part, by means of specific hyphal exudates (Bago *et al.*, 1996; Bansal amd Mukerji, 1994). The specificity of AM function that we see could be explained in terms of quality and completeness of the mycorrhizosphere team that can vary with different AM fungi and the soil/growth medium and the microbial populations contained therein. I believe that all soils contain microbial components capable of performing needed functions that aid "normal" plant growth. This mycorrhizosphere paradigm could explain the success of the AM system for some 460 million years (Remy *et al.*, 1994; Smith and Read, 1997; Taylor *et al.*, 1995; Simone *et al.*, 1993).

References

Ames, R.N., Reid, C.P.P. & Ingham, E.R. 1984: Rhizosphere bacterial population responses to root colonization by a vesicular-arbuscular mycorrhizal fungus. New Phytologist 96: 555-563.

Andrade, G., Mihara, K.L., Linderman, R.G., & Bethlenfalvay, G.J. 1997: Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant and Soil 192: 71-79.

Andrade, G., Linderman, R.G. & Bethlenfalvay, G.J. 1998a: Bacterial associations with the mycorrhizosphere and hyphosphere of the arbuscular mycorrhizal fungus, *Glomus mosseae*. Plant and Soil 202: 79-87.

Andrade, G., Mihara, K.L., Linderman, R.G. & Bethlenfalvay, G.J. 1998b: Soil aggregation status and rhizobacteria in the mycorrhizosphere. Plant and Soil 202: 89-96.

Azcon-Aguilar, C. & Barea, J.M. 1996: Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - An overview of the mechanisms involved. Mycorrhiza 6: 457-464.

Bago, B., Vierheilig, H., Piche, Y. & Azcón-Aguilar, C. 1996: Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. New Phytologist 133: 273-280.

Bagyaraj, D.J. 1984: Biological interactions with VA mycorrhizal fungi. In: VA Mycorrhiza. eds. L.L. Conway Powell and D.J. Bagyaraj. CRC Press, Boca Raton, FL. 131-153.

Bansal, M. & Mukerji, K.G. 1994: Positive correlation between AM-induced changes in root exudation and mycorrhizosphere mycoflora. Mycorrhiza 5: 39-44.

Bashan, Y., Holguin, G. & de-Bashan, L.E. 2004: Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Canadian Journal of Microbiology 50: 521-577.

Bethlenfalvay, G.J. & Linderman, R.G. eds. 1992: Mycorrhizae in Sustainable Agriculture, ASA Spec. Publ. No. 54., Amer. Soc. Agronomy Press, Madison, WI.

Caron, M. 1989: Potential use of mycorrhizae in control of soil-borne diseases. Canadian Journal of Plant Pathology 11: 177-179.

Dehne, H-W. 1982: Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72: 1115-1119.

Downer, A.J., Menge, J.A. & Pond, E. 2001: Association of cellulytic enzyme activities in eucalyptus mulches with biological control of *Phytophthora cinnamomi*. 91: 847-855.

Filion, M., St-Arnaud, M. & Fortin, J.A. 1999: Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytologist 141: 525-533.

Fitter, A.H. & Garbaye, J. 1994: Interactions between mycorrhizal fungi and other soil organisms. Plant and Soil 159: 123-132.

Fortin, J.A., Becard, G., Declerck, S., Dalpe, Y., St-Arnaud, M., Coughlan, A. & Piche, Y. 2002: Arbuscular mycorrhiza on root-organ cultures. Canadian Journal of Botany 80: 1-20.

Garbaye, J. 1994: Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist 128: 197-210.

Graham, J.H., Leonard, R.T. & Menge, J.A. 1981: Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhizae formation. Plant Physiology 68: 548-552.

Gryndler, M. 2000: Interactions of arbuscular mycorrhizal fungi with other soil organisms. In: Arbuscular Mycorrhizas: Molecular Biology and Physiology. Eds Kapulnik, Y. and Douds, D.D., Kluwer Press. 239-262.

Hetrick, B.A.D., Kitt, D.G. & Wilson, G.T. 1986: The influence of phosphorus fertilization, drought, fungal species, and nonsterile soil on mycorrhizal growth response in tall grass prairie plants. Canadian Journal of Botany 64: 1199-1203.

Hiltner, L. 1904: Über neuere Erlahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngung und Brache (On recent insights and problems in the area of soil bacteriology under special consideration of the use of green manure and fallowing). Arb. Dtsch. Landwirt. Ges. 98: 59-78.

Hooker, J.E., Jaizme-Vega, M. & Atkinson, D. 1994: Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In: Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Eds. Gianinazzi S. and Schuepp. H., Basel: Birkhauser Verlag.191-200.

Jalali, B.L. & Jalali, I. 1991: Mycorrhiza in plant disease control. In: Handbook of Applied Mycology. Soil and Plants. Vol. 1, Eds. Arora, D.K., Rai, B., Mukerji, K.G. and Knudsen, G.R., Marcel Dekker, New York, NY. 131-154.

Linderman, R.G. 1988: Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. Phytopathology 78: 366-371.

Linderman, R.G. 1992: Vesicular-arbuscular mycorrhizae and soil microbial interactions. In: Mycorrhizae in Sustainable Agriculture. Eds. Bethlenfalvay, G.J. and Linderman, R.G., ASA Special Publication No. 54, Madison, WI. 45-70.

Linderman, R.G. 1993: Effects of biocontrol agents on plant growth. Combined Proceedings of the International Plant Propagator's Society 43:249-252.

Linderman, R.G. 1994: Role of VAM fungi in biocontrol. In: Mycorrhizae and Plant Health. eds.Pfleger, F.L. and Linderman, R.G., APS Press, St. Paul, MN. 1-26.

Linderman, R.G. 2000: Effects of mycorrhizas on plant tolerance to diseases. In: Arbuscular Mycorrhizas: Molecular Biology and Physiology. Eds. Kapulnik, Y. and Douds, D.D., Kluwer Press. 345-365.

Linderman. R.G., Moore, L.W., Baker, K.F. & Cooksey, D.A. 1983: Strategies for detesting and characterizing systems for biological control of soilborne plant pathogens. Plant Disease 67: 1058-1064.

Linderman, R.G. & Paulitz, T.C. 1990: Mycorrhizal-rhizobacterial interactions. In: Biological control of soil-borne plant pathogens. Eds. Hornby, D. *et al*., Wallingford: CAB International. 261-283.

Linderman, R.G. & Davis, E.A. 2003a: Soil amendment with different peatmosses affects mycorrhizae of onion. HortTechnology 13(2): 285-289.

Linderman, R.G. & Davis, E.A. 2003b: Arbuscular mycorrhiza and growth responses of several ornamental plants grown in soilless peat-based medium amended with coconut dust (coir). HortTechnology 13(3): 482-487.

Linderman, R.G., Davis, E.A. & Marlow, J.L. 2003: Effects of organic amendments to soil and soilless potting media on arbuscular mycorrhizae and their microbial associates. Proceedings 4th International Conference on Mycorrhizae. Montreal, Canada

Linderman, R.G. & Davis, E.A. 2004: Evaluation of commercial inorganic and organic fertilizer effects on arbuscular mycorrhizae formed by *Glomus intraradices*. HortTechnology 14(2): 196-202.

Lynch, J.M. & Whipp, J.M. 1990: Substrate flow in the rhizosphere. Plant and Soil 129:1-10.

Mahaffee, W.F. & Kloepper, J.W. 1994: Application of plant growth-promoting rhizobacteria in sustainable agriculture. In: Soil Biota Management in Sustainable Farming Systems. Eds. Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., and Grace, P.R., Melbourne, Australia. 23-31.

Menge, J.A., Jarrell, W.M., Labanauskas, C.K., Ojala, J.C., Hiesar, C., Johnson, E.L.V. & Sibert, D. 1982: Predicting mycorrhizal dependency on Troyer citrange on *Glomus fasciculatus*, in California citrus soils and nursery mixes. Soil Science Society of America Journal 46: 762-768.

Meyer, J.R. & Linderman, R.G. 1986: Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Biology and Biochemistry 18: 191-196.

Nehl, D.B., Allen, S.J. & Brown, J.F. 1996: Deleterious rhizosphere bacteria: an integrating perspective. Applied Soil Ecology 5: 1-20.

Olsson, P.A., Baath, E., Jakobsen, I. & Soderstrom, B. 1996: Soil bacteria respond to presence of roots but not to mycelium of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 28: 463-470.

Oswald, E.T. & Ferchau, H.A. 1968: Bacterial associations of coniferous mycorrhizae. Plant and Soil 28: 187-192.

Pieterse, C.M.J., Van Pelt, J.A., Verhagen, B.W.M., Ton, J., Van Wees, S.C.M., Leon-Kloosterziel, K.M. & Van Loon, L.C. 2003: Induced systemic resistance by plant growth-promoting rhizobacteria. Symbiosis 35: 39-54.

Rambelli, A. 1973: The rhizosphere of mycorrhizae. In: Ectomycorrhizae. Eds. Marks, G.L. and Kozlowski, T.T., Academic Press, New York, NY. 299-343.

Remy, W., Taylor, T.N., Hass, H. & Kerp, H. 1994: Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proceedings of the National Academy of Science, USA 91: 11841-11843.

Secilia, J. & Bagyaraj, D.J. 1987: Bacteria and actinomycetes associated with pot cultures of vesicular-arbuscular mycorrhizas. Canadian Journal of Microbiology 33: 1069-1073.

Simon, L., Bousquet, J., Levesque, R.C. & Lalonde, M. 1993: Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363: 67-69.

Smith, S.E. & Read, D.J. 1997: Mycorrhizal Symbiosis. Cambridge: Academic Press.

Suslow, T.V. & Schroth, M.N. 1982: Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72: 111-115.

Taylor, T.N., Ramy, W., Hass, H. & Kerp, H. 1995: Fossil arbuscular mycorrhizae from the early Devonian. Mycologia 87: 560-573.

Tisdall, J.M., Smith, S.E. & Rengasamy, P. 1997: Aggregation of soil by fungal hyphae. Australian Journal of Soil Research 35: 55-60.

Vancura, V., Orozco, M.O., Grauova, O. & Prikryl, Z. 1989: Properties of bacteria in the hyphosphere of a vesicular-arbuscular mycorrhizal fungus. Agriculture Ecosystems Environment 29: 421-427.

Wright, S.F. & Upadhyaya, A. 1996: Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science 161: 575-586.

Zak, B. 1964: Role of mycorrhizae in root disease. Annual Review of Phytopathology 2: 377-392.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biological control of arthropod pests in outdoor crops – the new challenge

Lene Sigsgaard
Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: Natural regulation of insect pests can be improved by conservation biological control. However, these efforts are not always sufficient to keep pest infestations at an economically acceptable level. In some years or some locations natural enemies may occur in the field too late or in insufficient numbers. Inundative and inoculative biological control can provide a supplement to natural control and conservation biological control. Both methods generally involve mass-rearing and mass release of naturally occurring beneficials. Inundative/inoculative biological control is still little used in outdoor crops in temperate regions and results are variable. Some current successful practices will be presented together with a preliminary overview of augmentative biological control in Europe. Research publications will be used to discuss challenges for developing biological control. Analysis will include methods used in selecting biological control agents, release methods, and the ecological factors, which may limit or enhance the efficacy of releases. Strategies for future inundative/inoculative biological control in outdoor crops will be discussed.

Key words: Inoculation, inundation, augmentation, arthropod, predator, parasitoids

Introduction

In outdoor crops the use of naturally occurring species is the first option to consider when reviewing possibilities for biological control. However, natural biological control and alterations of the agricultural system aimed at increasing functional biodiversity to support natural enemies – conservation biological control – are not always sufficient to keep insect pest attacks at an acceptable level. Inoculative or inundative biological control may provide the additional control needed. In view of further restrictions in pesticide use and increasing interest in organic and IP products biological control will probably become more attractive.

This paper covers inoculative and inundative biological control by predators and parasitoids. Following the definition by Eilenberg *et al.* (2001) inoculation biological control is biological control in which periodic releases of natural enemies are made, and control is mostly expected from the offspring of the released beneficials, while in inundative biological control in general a higher number of organisms are released and immediate control is intended to be obtained from the released organisms themselves.

The potential of inoculative or inundative biological control to suppress arthropod pests is recognised but is yet only used commercially in relatively few outdoor crops. Currently natural enemies are used on perhaps 100,000 ha in Europe out of an estimated total world area of 16 mill ha (van Lenteren, 2003a). The number of species for sale has increased from less than 10 species in 1970 to over 125 species in 2000. The worldwide turnover of natural enemies was estimated to be 50 mill USD in 2000 with an estimated annual growth rate of 15-20% with 75% of the commercial augmentative biocontrol taking place in northern Europe and north America (van Lenteren, 2003b). Data for use of predators and parasitoids in greenhouses are available, but not for field applications. However, an estimated 20% of the commercial natural enemies are used for field applications (van Lenteren, 2003a). Excellent overviews on the use, extent and challenges of augmentative biological control exist, though mostly with a focus on greenhouse biological control (see for example van Lenteren (2000) and others by this author) and will not be attempted here.

The most widespread current practice in outdoor crops in Europe is inundative releases in maize of *Trichogramma brassica* Bezdenko (Hymenoptera: Trichogrammatidae) against *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) (approx. 100,000 ha); in vineyards of predatory mites against mites (approx. 40,000 ha); in orchards control of spider mites and rust mites by predatory mites is most widespread (*Typhlodromus pyri* Scheut. and *Amblyseius andersoni* Chant (Acari: Phytoseiidae)) but biocontrol agents are also released against Lepidopteran pests (*Trichogramma sp.*) and Homopteran pests (*Anthocoris nemoralis* (Fabr.) (Heteroptera: Anthocoridae) against pear psyllids) (approx. 30,000 ha orchards under biological control). Finally *Phytoseiulus persimilis* Athias-Henriot and other phytoseiid predatory mites are used against spider mites in strawberries (less than 20,000 ha). Table 1 is a preliminary summary of current practices in Europe based on communication with biocontrol companies and their homepages, individual researchers, and other published sources. Species in production are listed by van Lenteren (2003a).

Published results of experiments with inoculative or inundative biological control in temperate regions show variable degrees of success in suppressing target pests to an acceptable level. Current practices with a focus on selected case-studies from pome fruit, vegetables and maize will be treated in more detail and used in an analysis of which factors may yield success. Central ecological factors of importance to successful biological control will be discussed including unfavourable environmental conditions, mortality, dispersal of the natural enemy, host refuges from natural enemies, and predation of released natural enemies. Finally economical limitations to inoculative or inundative biological control will be discussed.

Current practices

Predatory insects

Several predatory insects are available for biological control in outdoor crops in northern Europe. These include Heteroptera (*A. nemoralis*), Diptera (*Aphidoletes aphidimyza*

Table 1. Some current practices in Western Europe involving releases of arthropod natural enemies in outdoor crops. The table is preliminary. Natural enemies are at least used in the countries listed.

Crop	Pest group	Species	Insect released	Extent	Countries
Apple	Mite	*Panonychus ulmi*	*Typhlodromus* sp.	30,000 ha	Denmark, UK, widespread
	Lepidoptera	*Cydia pomonella*	*Trichogramma cacaeciae* *T. dendrolimi*	Small growers	Denmark, Germany
	Aphid	*Eriosoma lanigerum*	*Aphelinus mali*		Netherlands
	Aphid	*Aphis pomi*	*Adalia bipunctata*		France
Plum	Lepidoptera	*Grapholita funebrana*	*Trichogramma cacaeciae* *T. dendrolimi*	Small growers	Germany
Sweet cherry ao	Aphid	*Myzus cerasi, Myzus persicae*	*Adalia bipunctata*	Limited area	France, Switzerland
Vineyard	Lepidoptera (Vine moths)	*Lobesia botrana, Eupoecilia ambiguella*	*Trichogramma*	Small growers	Germany
	Mite	*Tetranchus urticae* ao	*Kampimodromus aberrans* *Typhlodromus pyri*	40,000 ha	Widespread
Artichoke ao	Diptera	*Delia spp.*	*Aleochara* spp.		N. France
Bean	Mite	*Spider mite*	*Phytoseiulus persimilis*		Italy
Vegetables	Lepidoptera	Various noctuids	*Trichogramma evanescens*		France
Citrus	Scale	Coccidae, pseudococcidae	*Leptomastix abnormis*		Italy
		California red scale	*Cryptolaemus montrouzieri*		Italy
Fruit bushes	Thrips				Denmark
Raspberries	Mites	*Panonychus ulmi*	*Phytoseiulus persimulis*		Denmark
Maize	Lepidoptera	*Ostrinia nubilalis*	*Trichogramma brassicae*	100,000 ha	France, Germany, Switzerl.
Nursery crops	Aphid	*Aphidoletes aphidimyza*, parasitoids		Few nurseries	Denmark
Gardens,ornametals	Aphid	Various	*Harmonia axyridis* (apterous)		France
Pear	Psyllid	*Pear psylla*	*Anthocoris nemoralis*	100dreds of ha's	Italy, France, Netherlands, Belgium
Strawberry	Mite	*Tetranychus urticae*	*Phytoseiulus persimilis*	Widespread –20,000 ha	Italy, Spain, Denmark, NL etc
	Mites	*Phytonemus pallidus*	*Amblyseius cucumeris* &		Denmark
	Thrips	Various	*A. barkeri*		Italy, Denmark
Tomato and other vegetables	Mite	Spider mite	*Phytoseiulus persimilis*	Widespread but decreasing	Italy
	Whitefly	*Bemisia tabaci*	*Eretmocerus mundus* *Nesidiocoris tenuis* *Macrolophus caliginosus*	National initiative to control vectors of tomato viruses. In two provinces outdoor releases between crops and in gardens	Spain (Valencia)
	Whitefly	*Bemisia tabaci*	*Eretmocerus mundus*		Spain (Murcia)
	Thrips and other pests	*Franklinella occidentalis*	*Orius laevigatus* *Amblyseius cucumeris*		

(Rondani) and *Episyrphus balteatus* (De Geer)), Coleoptera (*Adalia bipunctata* (L.), *Aleochara bilineata* (Gyllenhal)) and Neuroptera (*Chrysoperla carnea* (Stephens)).

Anthocoris nemoralis is used against pear psyllids (*Cacopsylla pyri* (L.) and *C. pyricola* (Foerster) Homoptera: Psyllidae)). In Italy the area treated may be over 100 ha. In the Netherlands it is an important product for outdoor releases. Pear psyllids overwinter as adults and are ovipositing in pear from early spring. If infestation is heavy, considerable damage can be caused by nymphal feeding on the flower stalks and leaves, which diminishes plant growth by withdrawal of plant sap while the secreted honeydew favours the growth of sooty mould. Outbreaks are associated with climate in the given year and previous insecticide use (Bues *et al.*, 1999; Horton *et al.*, 1992).

Improved natural control can be achieved by avoiding or strongly reducing insecticide use and by conservation of natural enemies in orchards and surrounding hedgerows (Fitzgerald & Solomon, 2004). For the last five years or more the Danish advisory service has advocated a no spray strategy to pear growers with pear psyllid problems. However, these efforts are not always sufficient to keep the infestation at an economically acceptable level.

An alternative to chemical control of outbreaks of pear psyllids may be early release of mass-reared anthocorids. Biocontrol companies suggest spring release of 1,000-1,500 adults/ha of pear orchard at 5-6 release points, and possibly repeated releases. However, published results are variable. In an Italian experiment releases of adults were not different from control (Beninato & la Morella, 2000). In a preliminary release trial in the Netherlands (1,200 adults in 36 release points) the treated orchard was also not significantly different from the control (M. Kers, pers. comm.). In southern France releases at elevated rates of 17,000-24,000 led to negligible losses irrespective of initial infestation level (Faivre-D'Arcier *et al.*, 2001). In contrast to this, spring and early summer releases of around 5,500 adults/ha were considered effective depending on proper timing – i.e. availability of prey without the infestation becoming too high – and avoidance of insecticides (Faivre-D'Arcier *et al.*, 2001). The relatively low infestation of psyllids during some trials made assessment of control effects on yield difficult. Release of *A. nemoralis* eggs in France did show effect, but was labour intensive, and predator mortality was high (Rieux *et al.*, 1994). Release of nymphs in the US was effective, but at elevated release rates of 300 nymphs per tree, releases were principally aiming at demonstrating an effect (Unruh & Higbee, 1994).

Field releases of *A. nemoralis* nymphs in experimental plots in Denmark yielded reductions of pear psyllids of up to 40%. Releases had an effect across orchards, though not significant when separately analysing the orchard where the psyllid infestation was lowest. Only in the orchard with the highest early infestation did high release (two releases of 30 nymphs/tree) give better control than low release (two releases of 10 nymphs/tree). An assessment of yield did not show any significant difference (Sigsgaard, 2005a; Sigsgaard, unpubl.).

The current practice of releasing adult anthocorids apparently has variable degrees of success. The underlying mechanisms have not been studied in detail. If variable results from published experimental releases of adults are related to dispersal of the highly mobile adult

anthocorids, releases of nymphs may present a possible alternative strategy. The generally small plots of release in experiments may also lead to 'flooding' from the surrounding untreated part of the orchard, something which appeared to be the case in a preliminary experiment in 2003 (Sigsgaard, 2005a).

Aleochara bilineata (Coleoptera: Staphylinidae) a staphylinid, which acts both as a parasitoid and a predator, is being used to control *Delia* spp. (Diptera: Anthomyiidae) in artichoke and other vegetables in northern France (Table 1). Published results from control of cabbage root fly *Delia radicum* (L.), a major pest of cabbage, were positive. The costs of rearing adults compared favourably with insecticidal control (Finch et al., 1999) though high numbers had to be released. Other difficulties include accurate timing as rove beetles attack fly maggots (the damaging stage) and too early release will lead to rove beetles migrating away.

In IPM and organic apple orchards the rosy apple aphid, *Dysaphis plantaginea* Pass. (Homoptera: Aphididae), is the second most important pest after the codling moth (*Cydia pomonella* (L.) (Lepidoptera: Tortricidae)). Damage thresholds are very low, and this has resulted in intense spraying across Europe and the appearance of resistance to pesticides. *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), *E. balteatus* (Diptera: Syrphidae), *A. bipunctata* (Coleoptera: Coccinellidae) and *C. carnea* (Neuroptera: Chrysopidae) have all been used against various aphid species under outdoor conditions. The first three were tested in Switzerland against *D. plantaginea*. In preliminary cage experiments they performed equally well, but outdoors *A. bipunctata* larvae performed best under cool spring conditions and were therefore selected for further study (Wyss *et al.*, 1999a; Wyss *et al.*, 1999b). Larvae introduced in April controlled the aphids mainly at 1:1 and 5:1 predator-prey ratios. An early release appears decisive if biological control is to be successful in apple orchards (Wyss *et al.*, 1999a). The predator-prey ratios were favourable compared with the ratios used by Hagley (1989) to control green apple aphid *Aphis pomi* De Geer (Homoptera: Aphididae) on apple trees with chrysopids (1:10 and 1:19). Larvae of *A. bipunctata* were released in rather cold weather conditions and when aphid fundatrices were scarce. Reinforcing the action of natural populations of enemies at a crucial time seemed to work well for the indigenous predators used in these trials.

Release of *A. bipunctata* for autumn control of *D. plantaginea* also showed promise in reducing the number of observed fundatrices on apple trees in spring 1999, though early pyrethroid treatment was superior (Kehrli & Wyss, 2001). Failure of releases of *Chrysoperla rufilabris* (Burmeister) and *A. aphidimyza* to control *A. pomi* (Grasswitz & Burts 1995) may have been due to too late releases or to 'flooding' of small experimental plots.

Aphidoletes aphidimyza are used in a few Danish plant nurseries to control aphids (Table 1), while in the US chrysopids are used on a much larger scale than in Europe.

In France, apterous *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) from field collected apterous individuals are now reared and sold for control of aphids in garden crops and ornamentals (Table 1). The reduced ability to disperse makes it easier to target releases and reduces the risk of spread.

Parasitoids

For field releases *Trichogramma* wasps are the most successful biocontrol agents and are used against various Lepidopteran pests (Smith, 1996).

One of the most cited and successful examples of biological control in Europe is the control of the European corn borer, *O. nubilalis*, with *T. brassicae*.

Cultural controls such as adjusting planting dates can reduce the level of *O. nubilalis* larval injury. In agroecosystems characterized by high crop diversity, crops that are less attractive than other crops to ovipositing moths during a particular corn borer generation may gain a significant degree of protection by their proximity to more attractive crops. Finally, winter survival of *O. nubilalis* is influenced by tillage practices, and sanitation by ploughing at the end of the maize-growing season is compulsory in some countries.

In the mid-nineties *T. brassicae* cost the same or up to 60% more than insecticides (Bigler, 1994). To appreciate the comparative role of biological control, information on areas treated with insecticides can be helpful. Data on areas treated with insecticides against corn borer are hard to get, but ten years ago approximately 300,000 ha were treated with insecticides in France and a couple of thousand hectares are treated in Germany. The future use of *T. brassicae* may depend on future spread of Bt maize.

After twenty years of intensive research, *T. brassica* products are now annually applied to approximately 7,000 ha in Switzerland, 11,000 ha in Germany, perhaps 150 ha in Austria, and 80,000 ha in France (F. Bigler, pers. comm.). In initial field trials in southern Switzerland larval attack was reduced by 70% when the parasitoid was released at a rate of 300,000 individuals/ha (a mixture of mature, prepupae and young larvae), released at 50 points/ha. One third were released at the beginning of the 1st generation of the pyralid, the remaining at the beginning of the 2nd generation (Bigler & Brunetti, 1986).

After initial success an apparent failure was experienced in 1980. Apparently parasitoids loose their ability to parasitise efficiently when continuously reared on a factitious host. The failure was corrected when rearing methods were changed from continuous rearing on a factitious host (*Sitotroga cerealella* (Olivier) (Lepidoptera: Gelichiidae) or *Ephestia kuehniella* Zwolfer (Lepidoptera: Pyralidae)) to a basic rearing on the true host under semi-natural conditions, while the factitious host was used to produce higher numbers for release. Currently, *T. brassicae* is released in plastic or paper packets designed to provide protection for the wasps against weather extremes and predation until emergence in the field. 'Trichocaps' packets are hollow cardboard capsules (2 cm diam.) each containing approximately 500 parasitised *E. kuehniella* eggs (Kabiri *et al.*, 1990). *Trichogramma* inside capsules are induced into an overwintering (diapause) state in the insectary and then stored in refrigerated conditions for up to nine months. This system allows for production during winter months and distribution to growers when needed in the summer with a possibility to manipulate and extend the emergence period of parasitoids and increasing the "residual" activity of a single application to approximately one week.

In commercial orchards only extremely low levels of codling moth can be tolerated, as insecticide applications have generated an economic threshold of less than 1% fruit damage

(Cross *et al.*, 1999). However, thresholds will be different for small growers and for organic growers, who do not accept the use of insecticides. Thus, in Germany the commercial use of *T. cacaeciae* Marchal and *T. dendrolimi* Matsumura against the codling moth and plum moth (*Grapholita funebrana* Treitschke (Lepidoptera: Tortricidae)) has been expanded for small-scale fruit crops. *Trichogramma* species are also being offered for the control of vine moths, *Lobesia botrana* (Schiff.) (Lepidoptera: Tortricidae) and *Eupoecilia ambiguella* Hübner (Lepidoptera: Tortricidae), on grapevine in home gardens. A strain of *T. evanescens* Westwood is available against cabbage pests, especially noctuids. Research has been conducted on the biological control of *Autographa gamma* (L.) (Lepidoptera: Noctuidae) in spinach, and of pea moth (*Cydia nigricana* Fabr. (Lepidoptera: Tortricidae)) and leek moth (*Acrolepiopsis assectella* Zeller (Lepidoptera: Acrolepiidae)) in organic farming in Germany (Zimmermann, 2004).

Trichogramma can also be used in forestry. For example 480 million females of the egg parasitoid *T. minutum* Riley was aerially released on 30 ha of boreal forest infested by the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), in Canada, providing effective short-term population suppression of spruce budworm and significant foliage protection. Releases were also thought to complement late larval parasitoids, which have been suggested to be important in initiating budworm population collapse (Bourchier & Smith, 1998).

Aphid parasitoids such as *Aphidius ervi* Haliday (Hymenoptera; Braconidae) are used in a small scale in plant nurseries. Experimental releases of *A. rhopalosiphi* De Stefani Perez in cereal fields in Belgium were not more efficient than the use of flowering strip in one year, and less efficient than flowering strips in the next, making field releases far too costly compared with the value of the crop (Levie *et al.*, 2005). In the Netherlands and elsewhere trials are currently conducted with augmentative releases of *Aphelinus mali* Hald. (Hymenoptera; Aphelinidae) against the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae) (H. Helsen, pers. com.).

Predatory mites

Predatory mites are principally used in orchards, vine orchards and strawberry. Phytoseiid mites are capable of controlling *Panonychus ulmi* Koch (Acari: Tetranychidae) and *Aculus schlechtendali* Nalepa (Acari: Eriophyidea) in apple and are used in several countries. Pesticide treated orchards devastated by spider mites in the 1970's stressed the need to replace pesticides with an alternative control. In the UK organophosphate-resistant strains of *T. pyri* were found in 1982, and resistance has also developed in other apple growing regions. A management strategy was then devised that built on preserving and/or introducing these resistant strains to orchards (Solomon *et al.*, 2000). Another important phytoseiid predatory mite in orchards is *A. andersoni*. The predatory mites are commercially available. They can be transferred on twig cuttings or on felt bands.

Typhlodromus pyri can survive and reproduce on other prey than mites and on fungi and pollen, which help maintain populations when spider mites populations are low. Thus in unsprayed orchards it will rarely be necessary to release predatory mites (Solomon *et al.*, 2000).

In strawberry *T. pyri* is also found but is not effective. In strawberry and on hops the non-native phytoseiid mite *P. persimilis* is used to control *Tetranychus urticae* Koch (Acari: Tetranychidae). It usually does not overwinter and has to be released each year. Also other predatory mites haven proven effective (Cross *et al.*, 2001).

Challenges for developing biological control

In outdoor crops released natural enemies will have to function under natural weather conditions and with plants and other arthropods in the agroecosystem. The interaction of the released biocontrol agent with natural biological control and conservation biological control will be a key issue. The risk of non-target effects also need to be clarified prior to use, though this must be considered to be of transient nature when releasing native natural enemies. Finally, the economic cost will determine to a large extent the application of biological control. Several factors may affect future cost.

Choice of biocontrol agent

Prioritising among the range of natural enemies based on their expected efficiency is quite difficult. With native natural enemies, a natural starting point will be already available ecological data on the performance of the natural enemies against a given pest, in a given crop. Criteria used in a screening to select among *A. bilineata* and *A. bipustulata* (L.) included the rate of increase, host specificity and host acceptance and a development time well synchronised with that of the host (Fournet *et al.*, 2000). Screening for natural enemies will also provide information that can be used in determining rearing and release methods. The ability to actually rear the natural enemy is an important criterion.

Interaction with natural and conservation biological control

Available ecological data on a given species are needed to prioritize between control methods. For inundative/inoculative releases such data together with field cage assays can provide information about whether the natural enemy released can function under the environmental conditions given and can provide important criteria to select the most appropriate beneficial.

Timing of release can be crucial for released beneficials' ability to keep the pests under control (Campbell & Lilley, 1999). Together with timing also performance in cool spring conditions will often be an important criterion under northern conditions with pests such as aphids and psyllids, which can build up populations at low spring temperatures. Timing was of key importance in spring release experiments to control *D. plantaginea*, and early release considered key to success (Wyss *et al.*, 1999a). Other abiotic factors of importance for performance include wind speed and solar radiation, which were used to select between two *Trichogramma* parasitoids (Ehler, 1998; Fournier & Boivin, 1999).

Dispersal of the natural enemies away from the release site may limit control. For a highly mobile predator such as *A. nemoralis* dispersal may explain variable results of release experiments. It is possible that dispersal of natural enemies is less important in larger-scale farm releases than it is in small-scale experiments. The same can be said about the experi-

ments in which massive influx of pest has overwhelmed the released natural enemies. This stresses the need for larger-scale assessments.

An integrated strategy combining conservation biological control and inoculative/inundative biological control may for example be strategies that both attract natural enemies and maintain released natural enemies (Sigsgaard, 2005b). Increasing functional biodiversity in conservation biological control may also affect the outcome of inundative/inoculative biological control. Thus survival may be improved and dispersal of released natural enemies decreased by providing flowering food plants (Nagarkatti *et al*., 2003). Food sprays (sugars, protein sources) may likewise be used to maintain released beneficials in the area of release as well as to attract beneficials and shows promise for release of coccinellids (Obrycki & Kring, 1998). Such an effect was observed in releases of *Chrysoperla* sp. against aphids in beet root (Ehler *et al*., 1997). Non-target effects from inoculative/inundative biological control include cannibalism and intra-guild predation. For example released Chrysopids may prey on coccinellid larvae (Obrycki & Kring, 1998).

When native beneficials are released marking techniques may be used to assess the spread of released individuals (Hagler & Naranjo, 2004). More complex interactions (as release of more than one species) can be assessed by immunoassay or pcr-methods.

Rearing conditions and release

Both abiotic and biotic rearing conditions are often kept stable. This may lead to a selection pressure for those individuals best fitted for these conditions. For example, the use of factitious hosts/prey, which is both practical and economic, may affect performance of the biocontrol agent, as experienced with the early failure of the *T. brassicae* releases. Incompatibility between natural enemies and the field population against which they are released has also been suggested as reason for failure in other studies (Ehler *et al*., 1997). Rearing conditions kept as near as possible to the conditions under which they are meant to be used are likely to be less problematic, but may be in conflict with needs to rationalize rearing.

Standard methods for quality control have been developed for 20 natural enemies by the International Organization for Biological Control (IOBC). Criteria include sex ratio (at least 50% females), number of females released per hectare, longevity, fecundity and capacity to parasitise/prey on the natural host. Future work will include guidelines for additional natural enemies, optimising and validating current tests, optimising sample numbers, developing less labour-intensive methods, developing simple end-user tests and last but not least developing testing methods than can relate laboratory tests to field performance including tests for flight potential (Bolckmans, 2003). For example, a central release point experiment showed that diapausing and fresh parasitoids dispersed in all directions, but that percentage parasitism by fresh parasitoids was higher (van Schelt & Ravensberg, 1990).

An assessment of the quality of *T. brassicae* produced by four companies showed that requirements were met overall. For example, the number of parasitised eggs released per hectare was, with one exception, considerably higher than the standard of 100,000 given by the companies. Survival rates of 16.7-66.7% were, however, lower than the IOBC standard of 80% and fecundity was also often below IOBC-standards (Hassan & Zhang, 2001).

The release method also affects control. For example, cannibalism in release units may reduce efficiency (Daane & Yokota, 1997), or it may be necessary to protect released predators against predation. Mechanical dispersal of beneficial is often necessary to be rational.

Risk of side effects

In cases where native natural enemies can be used this should be the first priority to reduce any risk of side effects from exotic natural enemies. Probably the risk of side effects from releases of native natural enemies will be fairly small though seasonal effects can be expected when the number of one beneficial is temporarily artificially raised. An EU project 'Evaluating environmental risks of biocontrol introductions in Europe' had *T. brassica* as a case study. The parasitoid was introduced from Moldavia to western Europe some 30 years ago. It is surviving in Switzerland in low numbers all the year round. Lynch *et al.* (2001) conclude that augmentative releases lead to population-level non-target effects quite often, but that these releases are justified by their transience and boundedness in space.

With respect to exotic natural enemies their use is regulated by national legislation. The biocontrol industry has a good record of environmental safety, and code of conducts and registration only are the main rule throughout Europe. A FAO Code of Conduct from 1996 exists for release of exotic biocontrol agents (BCA). Guidelines on safe use of biological control also exist from the European and Mediterranean Plant Protection Organization (EPPO) (1998) and CABI (2002). The Organisation for Economic Co-operation and Development (OECD) published a guidance document in 2003.

On IBMA (The International Biocontrol Manufacturers Association) request IOBC work for coordination of BCA regulation in Europe. In the future EU will harmonize BCA registration. In 2005 a dialogue between the EU Commission (Directorate General Health and Consumer protection -DG SANCO) and IBMA on revision of EU directive (91/414/EEC) regulation on plant protection products was initiated, introducing compulsory mutual recognition and a special procedure for "low risk products".

Cost

Use of predators and parasitoids in outdoor crops still builds on relatively few species. Historically, successes have required a combination of lack of control with insecticides, and/or a positive economic comparison of biological control to pesticides. This criterion was also used in an evaluation of 31 augmentative biological control experiments (mostly North American) (Collier & Van Steenwyk, 2004), concluding that typically augmentation was less cost-effective than pesticide treatment and that efficiency was often too low. The use of predatory mites in strawberries was one case, which they found cost-efficient, with costs of release making up less than 1% of total costs, and is indeed a method that is becoming more widespread. However, considering augmentative biological control as a component in integrated biological control makes criteria applied for insecticides less valid. In addition, economic models are generally unsuitable for assessing the negative externalities for ecology and human health. The benefit-cost ratio of pesticides is always overestimated. Estimates of yield loss with pesticide reductions or removal are based on incomplete data. Taking these costs into account the benefit-cost ratio of even the extreme scenarios fall from the high estimates of 40 to -2.1 in

the US and to only 1.5 in Germany (Pretty & Waibel, 2005). Hence, claims that pesticides outperform biological control may in many situations only hold true as long as external costs are considered irrelevant and ecosystem services from biological control are not considered.

Strategies for future inundative/inoculative biological control in outdoor crops

The use of inundative/inoculative biological control is growing and this trend will probably continue driven by future withdrawal of insecticides and the spread of certified IPM and organic production which require plant protection alternatives. Also consumer demands for unsprayed produce will further this development. Reduced pesticide input can help conserve natural enemies and will help to improve natural biological control.

Current evidence of pesticide reductions at country level includes policy-led pesticide reduction programmes in Sweden, Denmark and the Netherlands and there have been some considerable reductions. Thus in Denmark consumption fell by 40% from 1985 to 1995 though the frequency of applications was not reduced (2.5/ha/year). A recent national Spanish initiative regards control of vectors of tomato viruses, which cause serious losses in greenhouse production and where insecticides have largely failed. In two provinces outdoor releases in and between crops and in gardens are made to control vectors. In one province reduced pesticide use has been furthered by also releasing control agents against other pests (see Table 1). The future of the programme appears promising and a general increase in interest in biological control is seen due to a combination of failure of traditional chemical control, the decrease in permitted pesticides as well as the efficacy of biological control. Economic problems of growers and competition on the market on the other hand may lead to reduced biological control, which apparently is the case in Italy.

Probably, future development of augmentation will be depending on a move away from product models that approach biological control products as little more than chemical product replacements and better exploit their capability of being integrated with natural and conservation biological control. In an integrated biological control approach the acceptable level of control from inundative/inoculative releases will be determined from case to case, in interaction with natural and conservation biological control.

The large greenhouse production and the well-developed biocontrol industry, which provides it with natural enemies, is an advantage for developing biological control for outdoor crops. The technology and know-how for rearing natural enemies is largely in place, some native biocontrol agents for outdoor crops are already in mass production and developments in mass production, quality control, storage, shipment and release of natural enemies have lowered production costs and led to better product quality. With further development in fields like long-term storage and release methods a reduction in costs of biological control may be expected. Increasing demand may assist in this process so that it will become easier and more economic to apply in the future.

Acknowledgements

Thank you to F. Bigler, D. Beltran Morales, G. Manzaroli, J. van Schelt, K. Bolckmans, E.W. Hansen, S. Borregaard and others for helping with information about biological control practices.

References

Beninato, S. & la Morella, S. 2000: Control of *Cacopsylla pyri* with massive releases of *Anthocoris nemoralis* in pear orchards. GF-2000. Atti, Giornate Fitopatologiche, Perugia 1: 367-372.

Bigler, F. 1994: Quality control in *Trichogramma* production. In: Biological control with egg parasitoids, eds. E, Wajnberg & S.A. Hassan: 93-111.

Bigler, F. & Brunetti, R. 1986: Biological control of *Ostrinia nubilalis* Hbn. by *Trichogramma maidis* Pint. et Voeg. on corn for seed production on southern Switzerland. J. Appl. Ent. 102: 303-308.

Bigler, F., Meyer, A. & Bosshart, S. 1987: Quality assessment in *Trichogramma maidis* Pintureau et Voegele reared from eggs of the factitious hosts *Ephestia kuehniella* Zell. and *Sitotroga cerealella* (Olivier). J. Appl. Ent. 104: 340-353.

Bolckmans, K.J.F. 2003: State of affairs and future directions of product quality assurance in Europe. In: Quality control and production of biological control agents. Theory and testing procedures, ed. J.v. Lenteren: 215-224.

Bourchier, R.S. & Smith, S.M. 1998: Interactions between large-scale inundative releases of *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) and naturally occurring spruce budworm (Lepidoptera: Tortricidae) parasitoids. Env. Ent. 27: 1273-1279.

Bues, R., Boudinhon, L., Toubon, J.F. & Faivre, F.d'A. 1999: Geographic and seasonal variability of resistance to insecticides in *Cacopsylla pyri* L. (Hom., Psyllidae). J. Appl. Ent. 123: 289-297.

Campbell, C.A.M. & Lilley, R. 1999: The timing and rates of release of *Phytoseiulus persimilis* against two-spotted spider mite *Tetranychus urticae* on dwarf hops. Biocon. Sci. Techn. 9: 453-465.

Collier, T. & Van Steenwyk, R. 2004: A critical evaluation of augmentative biological control. Biol. Contr. 31: 245-256.

Cross, J.V., Easterbrook, M.A., Crook, A.M., Crook, D., Fitzgerald, J.D., Innocenzi, P.J., Jay, C.N. & Solomon, M.G. 2001: Review: natural enemies and biocontrol of pests of strawberry in northern and central Europe. Biocon. Sci. Techn. 11: 165-216.

Cross, J.V., Solomon, M.G., Babandreier, D., Blommers, L., Easterbrook, M.A., Jay, C.N., Jenser, G., Jolly, R.L., Kuhlmann, U., Lilley, R., Olivella, E., Toepfer, S. & Vidal, S. 1999: Biocontrol of pests of apples and pears in northern and central Europe: 2. Parasitoids. Biocon. Sci. Techn. 9: 277-314.

Daane, K.M. & Yokota, G.Y. 1997: Release strategies affect survival and distribution of green lacewings (Neuroptera: Chrysopidae) in augmentation programs. Environ. Ent. 26: 455-464.

Ehler, L.E. 1998: Invasion biology and biological control. Biol. Cont. 13: 127-133.

Ehler, L.E., Long, R.F., Kinsey, M.G. & Kelley, S.K. 1997: Potential for augmentative biological control of black bean aphid in California sugar beet. Entomophaga 42: 241-256.

Eilenberg, J., Hajek, A. & Lomer, C. 2001: Suggestions for unifying the terminology in biological control. BioControl 46: 387–400.

Faivre-D'Arcier, F., Millot, P. & Belzunces, L.P. 2001: Lachers inoculatifs d' *Anthocoris nemoralis* F en verger de poiriers. Phytoma 544: 76-78.

Finch, S., Elliott, M.S. & Torrance, M.T. 1999: Is the parasitoid staphylinid beetle *Aleochara bilineata* an effective predator of the egg stage of its natural host, the cabbage root fly? Bull. OILB/SROP 22: 109-112.

Fitzgerald, J.D. & Solomon, M.G. 2004. Can flowering plants enhance numbers of beneficial arthropods in UK apple and pear orchards? Biocontr. Sci. Techn. 14: 291-300.

Fournet, S., Stapel, J.O., Kacem, N., Nenon, J.P. & Brunel, E. 2000: Life history comparison between two competitive *Aleochara* species in the cabbage root fly, *Delia radicum*: implications for their use in biological control. Ent. Exp. Appl. 96: 205-211.

Fournier, F. & Boivin, G. 1999: Impact of meteorological conditions on spatial dispersion of *Trichogramma evanescens* and *T. pretiosum* (Hym.: Trichogrammatidae) in cabbage. Ann. Soc. Ent. Fr. 35: 471-475.

Grasswitz, T.R. & Burts, E.C. 1995: Effect of Native Natural Enemies and Augmentative Releases of *Chrysoperla rufilabris* Burmeister and *Aphidoletes aphidimyza* (Rondani) on the Population Dynamics of the Green Apple Aphid, *Aphis pomi* Degeer. Int. J. Pest. Mgmt. 41:176-183.

Hagler, J.R. & Naranjo, S.E. 2004: A multiple ELISA system for simultaneously monitoring intercrop movement and feeding activity of mass-released insect predators. Int. J. Pest Mgmt. 50: 199-207.

Hagley, E.A.C. 1989: Release of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) for control of the green apple aphid, *Aphis pomi* Degeer (Homoptera: Aphididae). Can. Ent. 121: 309-314.

Hassan, S.A. & Zhang, W.Q. 2001: Variability in quality of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) from commercial suppliers in Germany. Biol. Contr. 22: 115-121.

Kabiri, F., Frandon, J., Voegele, J., Hawlitzky, N. & Stengel, M. 1990: Lachers inondatifs de *trichogrammes*. Strategie evolutive contre la pyrale du mais. Phytoma 428: 23-24, 26.

Kehrli, P. & Wyss, E. 2001: Effects of augmentative releases of the coccinellid, *Adalia bipunctata*, and of insecticide treatments in autumn on the spring population of aphids of the genus *Dysaphis* in apple orchards. Ent. Exp. Appl. 99: 245-252.

Lenteren, J.C. van 2000: Success in biological control of arthropods by augmentation of natural enemies. In: Biological control: Measures of success (eds. G. Gurr & S. Wratten), pp. 77-103. Kluwer Academic Publishers, Dordrecht.

Lenteren, J.C. van 2003a: Commercial availability of biological control agents. In: Quality control and production of biological control agents, ed. J.v. Lenteren: 167-179.

Lenteren, J.C. van 2003b: Need for quality control of mass produced biological control agents. In: Quality control and Production of biological control agents, ed. J.v. Lenteren: 1-18. CABI, Wallingford.

Levie, A., Legrand, M.A., Dogot, P., Pels, C., Baret, P.V. & Hance, T. 2005: Mass releases of *Aphidius rhopalosiphi* (Hymenoptera: Aphidiinae), and strip management to control of wheat aphids. Agric. Ecosyst. Env. 105: 17-21.

Lynch, L.D., Hokkanen, H.M.T., Babendreier, D., Bigler, F., Burgio, G., Gao, Z.H., Kuske, S., Loomans, A., Menzler-Hokkanen, I., Thomas, M.B., Tommasini, G., Waage, J.K., Lenteren, J.C. van & Zeng, Q.Q. 2001: Evaluating indirect ecological effects of biological control. Key papers from the symposium "Indirect ecological effects in biological control", Montpellier, France, 17-20 October 1999. 2001: 99-125.

Nagarkatti, S., Tobin, P.C., Saunders, M.C. & Muza, A.J. 2003: Release of native *Trichogramma minutum* to control grape berry moth. Can. Ent. 135: 589-598.

Obrycki, J.J. & Kring, T.J. 1998: Predaceous coccinellidae in biological control. Ann. Rev. Ent. 43: 295-321.

Pretty, J. & Waibel, H. 2005: Paying the price: The full cost of pesticides. In: The pesticide detox, ed. Pretty: 39-54.

Rieux, R., Fauvel, G., Faivre, F.d'A., Fournage, G. & Lyoussoufi, A. 1994: Study of biological control against *Cacopsylla pyri* (L.) in pear orchards by experimental release of *Anthocoris nemoralis* F. in the egg stage. II. - results and discussion. Bull. OILB SROP 17: 120-124.

Schelt, J. van & Ravensberg, W.J. 1991: Some aspects on the storage and application of *Trichogramma maidis* in corn. Colloques de l'INRA. 1991; (56): 239-242.

Sigsgaard, L. 2005a: Perspectives for augmentative releases of *Anthocoris nemoralis* and *A. nemorum* (Heteroptera: Anthocoridae) against Pear Psyllid, *Cacopsylla pyri*. Workshop on Pest and Weed Control in Sustainable Fruit Production September 1-3, 2005 Skierniewice, Poland. p 21.

Sigsgaard, L. 2005b: Oviposition preference of *Anthocoris nemoralis* and *A. nemorum* (Heteroptera: Anthocoridae) on pear leaves affected by leaf damage, honeydew and prey. Biocont. Sci. Technol. 15: 139-151.

Smith, S.M. 1996: Biological control with *Trichogramma*: Advances, successes, and potential of their use. Ann. Rev. Ent. 41, 375-406.

Solomon, M.G., Cross, J.V., Fitzgerald, J.D., Campbell, C.A.M., Jolly, R.L., Olszak, R.W., Niemczyk, E. & Vogt, H. 2000: Biocontrol of pests of apples and pears in northern and central Europe - 3. Predators. Biocon. Sci.Tech. 10: 91-128.

Unruh, T.R. and Higbee, B.S. 1994: Releases of laboratory reared predators of pear psylla demonstrate their importance in pest suppression. Bull. IOBC/WPRS 17: 146-150.

Wyss, E., Villiger, M., Hemptinne, J.L. & Muller-Scharer, H. 1999a: Effects of augmentative releases of eggs and larvae of the ladybird beetle, *Adalia bipunctata*, on the abundance of the rosy apple aphid, *Dysaphis plantaginea*, in organic apple orchards. Ent. Exp Appl. 90: 167-173.

Wyss, E., Villiger, M. & Muller-Scharer, H. 1999b: The potential of three native insect predators to control the rosy apple aphid, *Dysaphis plantaginea*. Biocontrol 44: 171-182.

Zimmermann, O. 2004: Die Anwendung von Nützlingen im biologischen Pflanzenschutz in Deutschland: Aktuellen Anmerkungen. Gesunde Pflanzen 56: 151-156.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Practical use of biological control of pest and diseases in Danish glasshouses - bottlenecks and challenges

Lene Christensen
Lenes Laboratorium, Holtumvej 58, Holtum, 7100 Vejle, Denmark

Abstract: The use of biological control (BC) in Danish glasshouses has a long history, beginning with the use of *Phytoseiulus persimilis* in cucumbers against spider mites in the middle of 1960. Since 1985 all Danish producers of cucumbers and tomatoes have been using biological control of pests. In ornamentals about 50 per cent of the Danish producers are using some kinds of biological control, or more correctly, are using integrated pest management, IPM. In the past 15 years some growers have implemented biological control against diseases. Particularly *Trichoderma harzianum* is used for control of root diseases and gray mold, *Botrytis cinerea.* Many growers still use pesticides to control pest and diseases. These are easy to buy and use in the glasshouses, although some growers are of the opinion that they are expensive. The release of new pesticides is faster than release of new beneficials and microbiological products for diseases control in glasshouses – in other words a bottleneck for more practical use of biological and microbiological products.

Key words: Pests, diseases, biocontrol

Introduction

Biological control (BC) of pests is used by many pot plant producers in Denmark. Most of the growers use BC from time to time and only a few growers use BC solely. The growers are still keen on using BC and wish to learn more but prefer BC in combination with the use of chemicals, IPM.

Biological control of pests

The pests are spider mites (*Tetranychus spp.*), thrips (*Frankliniella occidentalis*, *Thrips tabaci*), aphids (*Myzus persicae* and others), whiteflies (*Trialeurodes vaporariorum*, *Bemisia tabaci*), sciarid flies (Sciaridae), leaf miners (*Liriomyza spp.*) and caterpillars (Lepidoptera).

New chemicals have forced back the use of BC in both vegetables and ornamentals.

BC of spider mites is used by almost every producer of cucumbers. The release of *Phytoseiulus persimilis* and *Amblyseius cucumeris* in the glasshouses takes place at the beginning of the season and continues every second week but gives some troubles in the summer. It is very difficult to control spider mites by the use of chemicals due to resistance.

Many pot plant producers are using BC and chemicals in combination – the chemical treatments in the summertime.

BC of thrips is a combination between the use of *Amblyseius spp.*, *Orius spp.* and *Hypoaspis miles*. In vegetables the use of BC of thrips is high, but a wide use of spinosad and fibronil in pot plants has lessened the use of BC. The export of pot plants is also a bottleneck, because some countries do not allow for occurrence of *F. occidentalis*. If Plant Inspection Services find *F. occidentalis* in the plants, they will stop the import of pot plants from Denmark for a period of time, as we have just seen in Russia, where they have stopped the import of Danish pot plants for one month.

Another problem in pot plants is *B. tabaci*, especially in *Poinsettia*. Some countries have a zero-tolerance level for *B. tabaci*, caused by fear of virus transmissions. The control of *B. tabaci* by means of BC is often difficult, due to the fact that the production area of *Poinsettia* cuttings is situated overseas in Kenya and the Canary Islands. There they have a high infection rate of pest and often use the chemical imidachloprid, which has a persistence as long as 16 weeks for *Encarsia formosa* and *Eretmocerus eremicus*.

The normal procedure to control *B. tabaci* in Denmark is to dip unrooted cuttings in suspensions of *Verticillium lecanii* and/or *Paecilomyces fumosoroseus*. A mix between *E. formosa* and *E. eremicus* and/or use of chemicals as pyriproxyfen, buprofezin and imidachloprid is used during the season.

A successful story

BC of sciarid flies is a combination between the use of *Bacillus thuringiensis*, nematodes and *H. miles*. When growers ask advice about control of sciarid flies, 90% of the questions refer to the use of BC and the last 10% to the use of chemicals.

New pests

New pests, i.e. pests only recently seen in Denmark, have become a major problem over the last 5 years. We have seen caterpillars and larvae from *Duponchelia foevalis* in *Begonias*, *Kalanchoës* and *Cyclamen*. *Bacillus thuringiensis* and *H. miles* are used to control these pests, sometimes in combination with light traps.

Microbiological agents

Trichoderma harzianum is used against root- and leaf diseases. In pot roses *T. harzianum* is sprayed on the plants after the first and second cut to control *Botrytis cinerea* in the flowers, buds and the leaves. Mycostop (*Streptomyces spp.*) is used against root diseases, but not so often as *T. harzianum*, because of the higher price of this product.

The most common diseases

Gray mold is a problem in many pot plant cultures and also in the production of tomatoes and cucumbers.

Powdery mildew is a large problem in pot plants and vegetables. The use of sulphur and chemicals is the only solution so far, together with the use of more resistant varieties.

Root diseases caused by *Fusarium spp.*, *Phytophthora spp.*, *Pythium spp.* and *Rhizoctonia solani* in pot plants such as *Gerbera*, *Campanula*, *Cyclamen* and *Begonia* are almost entirely controlled by chemicals.

The slow release of microbiological products for diseases control in Denmark and EU is a bottleneck for the replacement of chemicals in the production. The high cost in connection with the governmental control is a problem for the often small companies producing these microbiological agents.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biological control of seedling diseases in nursery production of *Abies nordmanniana*

Inge M.B. Knudsen[1], Kirsten A. Thomsen[2], John Hockenhull[1], Dan Funck Jensen[1]

[1]Plant Pathology Section, Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark; [2]The State Forest Tree Improvement Station, The Danish Forest and Nature Agency, Krogerupvej 21, DK-3050 Humlebaek, Denmark

Abstract: Control of damping-off in Caucasian fir (*Abies nordmanniana*) during seedling emergence and establishment through seed treatments with the antagonistic fungus *Clonostachys rosea* IK726 was investigated under laboratory and field conditions.

Seed treatment with the antagonist before the dormancy-breaking cold treatment was tested on two seed lots. The seeds, cold-treated for 3 and 6 weeks at 4°C, had a moisture content of 33%. Germination and seedling development was compared for laboratory germination in vermiculite and sowing in the nursery. Fungi from diseased seedlings were identified to at least genus level.

In the laboratory trial, *Fusarium oxysporum* was found in 50% and *Papulaspora immersa* in 28% of the isolations. In the field trial, *F. oxysporum* and *Pythium* spp. were found in the diseased seedlings. The biocontrol agent *C. rosea* IK 726 enhanced seedling emergence under both laboratory and field conditions. One seed lot (031) had an optimum germination speed when cold-treated for 3 weeks, and the other seed lot (028) needed 6 weeks to obtain fast germination. This latter seed lot showed, however, significantly enhanced seedling emergence in a field experiment, when 3 weeks' cold treatment was combined with a treatment with *C. rosea* IK 726.

Overall, the results show that seed treatment with *C. rosea* IK 726 has a promising potential for improving emergence and field survival of *A. nordmanniana* seedlings.

Key words: *Pythium ultimum* var *ultimum*, *Fusarium oxysporum*, Caucasian fir, seed vigour, stratification, time of application

Introduction

Caucasian fir (*Abies nordmanniana*) is an important forest species in Denmark, particularly for Christmas tree and greenery production. Damping-off during nursery production, which may be accentuated by use of seed of poor quality, is a major problem. This has led to intensive treatments with fungicides and soil fumigants, since at present more environmentally friendly control measures are not available.

Although Caucasian fir seeds are orthodox, i.e. they tolerate drying and low storage temperatures, they can only be stored for 3-5 years (Planteavlsstationen, 2005). To break

dormancy, seeds of this species usually benefit from cold treatment (stratification) for 3-6 weeks at 4°C and the optimal seed moisture content for controlled dormancy release (avoidance of germination during cold treatment) is 33% (Jensen, 1997).

The use of antagonistic microorganisms (BioControl Agents, BCAs) to control seedling diseases in forestry nurseries has scarcely been investigated. But seed treatment with BCAs has been shown to be beneficial against seed borne pathogens and it may also protect against soil borne diseases during germination and seedling development (Ahmad & Baker, 1987. Seed treatment with *Clonostachys rosea* (IK726) is effective against *Fusarium* and *Alternaria* diseases of cereals and carrots (Knudsen *et al.*, 1995; Jensen *et al.*, 2000; Jensen *et al.*, 2004) and also *Pythium* diseases in sugar beet (Jensen *et al.*, 1996). *C. rosea* IK726 is rhizosphere competent (Knudsen *et al.*, 1996; Lübeck *et al.*, 2002) and survives better on roots than in soil (Johansen *et al.*, 2005).

As seed-borne fungi such as *Fusarium* spp. may proliferate during stratification of forest seeds, seed treatment with a BCA when starting up the cold treatment can be beneficial (Hoefnagels & Linderman, 1999). When coated on carrot seeds in different wet seed technologies such as seed priming, *C. rosea* IK 726 has been shown to be very effective in controlling seed borne diseases and stimulating seedling emergence (Jensen *et al.*, 2004). A biocontrol effect has also been shown with this isolate at low temperatures in connection with cold humid storage of acorns (*Quercus robur*), where the acorns were hot water-treated and then coated with the BCA before storage (Knudsen *et al.*, 2004). Moreover, *C. rosea* IK726 performed very well in field studies with *Abies nordmanniana* seed coating, where the isolate was compared with several commercial materials (Knudsen *et al.*, submitted).

The objectives of the present work were to: (i) test the effect of seed treatment with a BCA before cold stratification on seedling emergence and survival in a controlled environment and (ii) to compare this with a field study in a Danish forest nursery.

Material and methods

Seed

Seed lots of *Abies nordmanniana* from Tversted (no. 031) collected in 2002 and from Ambolauri, Georgia (no. 028) collected in 2001 with, respectively, low and high seed quality and respectively low (031) and high (028) dormancy levels.

Cold treatments

Seeds were cold treated at a controlled moisture content (m.c.) of 33 ± 2% (fresh weight basis), which was obtained by soaking the seeds in water for 12 h and checking the m.c. using the ISTA low constant temperature oven method (ISTA, 1999). After adjustment of the m.c. by either adding water or drying slightly at room temperature, the seeds were cold treated at 5°C in loosely folded plastic bags. The m.c. was measured indirectly by weighing every second day, and water was added when necessary. Optimal length of cold treatment (to break dormancy) was determined by comparing germination after respectively 3 and 6 weeks´ cold treatment. When seeds were prepared for the field experiments, m.c was determined four

times: before and after soaking, after approximately seven days of cold treatment, and at the end of the treatment period.

Germination tests

After cold treatment, germination tests were made in vermiculite. Four replicates (boxes) of 50 seeds per treatment were incubated at 20°C in 12 hours' light. Germination was recorded weekly until all seeds were either germinated or dead. Germination was considered to have occurred when the radicle was the same length as the seed. After germination the test was extended until the seedlings had shed the seed coat and the cotyledons were visible. Germinated seeds were recorded in the following categories: germinated and healthy seedlings, germinated and dead seedlings, or abnormal seedlings. Non-germinated seeds were opened and recorded as fresh, dead or empty (ISTA, 1999). Germination was expressed as percentage germinated seeds of the total number of seeds, as well as the total number of full seeds (total number of seeds – number of empty seeds).

Post-emerged diseased plants were removed from the boxes and washed carefully and surface sterilized. Tissue pieces were plated on semi-selective media to obtain isolates of *Rhizoctonia solani*, *Pythium* spp, *Phytophtora* spp. and *Fusarium* and other species. A similar procedure was used for isolation from diseased plants sampled from the field.

BCA production

Inoculum of *C. rosea* IK726 was prepared as a storable clay formulation according to the procedure of Jensen *et al.* (2002). The clay formulation was applied as a suspension in water.

Field experiment

Field experiments were carried out in 2004 at Fosdal nursery approximately 10 km ENE of Fjerritslev in Thy, North-Jutland (UTM 32V NJ 253 308). Preparation of seed beds and sowing density were according to normal commercial procedures at the nursery. Seed beds were prepared by a bed forming machine, seeds were sown at a density of 2000 seeds per m^2 and after sowing seeds were covered with 1 cm of coarse sand. Watering and manual weeding were done as required. The experimental design was a complete randomized block design with five blocks. Each block consisted of a 1 m wide seed bed and each plot was 5 m long. Plots were separated by 0.5 m untreated seed bed (guard strips). Blocks were separated by 0.5 m wide wheel tracks.

C. rosea was applied before stratification as 0.005 g clay inoculum per g seed resulting in 4×10^9 cfu per g seed. Seeds were mixed with liquid formulations of the BCA in plastic bags and the coating was applied by stomaching the bag for 2 min. Evaluation of density of living propagules at the time of sowing was assessed by plate dilution of washings of seed samples of three replicates of five seeds. At sowing the cfu were measured to 2.6×10^6 per g seed.

The seeds were sowed on May 11. First seedling assessment was made after 1 month (June 9) and second assessment after 2 months (July 9) by counting seedlings in 10 x 10 cm squares. A 30 x 30 cm frame with wires 10 cm apart in each direction resulting in nine squares per frame was placed eight times in each plot in a predetermined pattern with 15 cm between each site of assessment and diagonally across the plot. At the second assessment, seedlings in the three diagonally placed squares of the frame were removed for disease as-

sessment. Plants were washed and inspected by magnifying glass for disease symptoms. The seedlings were sorted into plants with 1) post-emergence symptoms or deformations, and 2) healthy plants.

Results

The germination tests of seed lots 031 and 028 disclosed that these seed lots contained 40.3 and 23.4 empty seeds, respectively. On the basis of full seeds, germination was 80.8 ± 12.3% and 75.6 ± 12.5% for seed lots 031 and 028. The corresponding germination percentages for lots 031 and 028, when empty seeds are included, are 48 and 63%, respectively. Speed of germination after 3 weeks' cold treatment calculated as mean germination time was 15.1 ± 2.3 and 29.7 ± 4.8, and after 6 weeks 13.4 ± 1.1 and 19.8 ± 2.7 for seed lots 031 and 028, respectively.

There was a tendency (although not statistically significant) that clay and *C. rosea* had a positive effect on germination after 3 weeks of cold treatment (Figure 1). This was most pronounced for seed lot 028. Concerning disease rates, seed lot 031 had a higher rate of post-emergence diseased and dead plants with 22.9% and 20.4% after 3 and 6 weeks' cold treatment respectively. The figures for lot 028 were 9.5 and 5.7% resulting in corresponding differences in total numbers of healthy seedlings (Figure 2). Isolation from diseased plants from 031 and 028 gave *Fusarium oxyporum* (50% of isolations) and *Papulaspora immersa* (28% of isolations). In seed lot 031 there was a statistically significant lower number of diseased and dead plants in the pre-*C. rosea* treated compared with the pre-clay-treated seeds after 6 weeks of cold treatment. Thus after 3 and 6 weeks of cold treatment 10.7 and 9.3% were either diseased or dead in the pre-*C. rosea* compared with 31.5 and 54.1% in the pre-clay-treated seeds, respectively (Figure 2). However, these levels were not significantly different from those in the water control. In seed lot 028, there was a statistically significant difference between the numbers of diseased plants in the pre-*C. rosea* treatment after 3 weeks (1.7%) and the control (9.5%).

The field experiment resulted in a very low number of plants (results not shown) at the first assessment 4 weeks after sowing due to a rather cold season. After 2 months, a significantly higher number of emerged seedlings were found for the *C. rosea* treatment of seed lot 028, cold-treated for 3 weeks, compared with the untreated control (Figure 3). The numbers correspond to 26% germinated healthy plants compared with 19% in the control. Analysed by a 3-factor statistical model, and sorting data by treatment period, a general significant ($p=0.0111$) effect of treatment with *C. rosea* in combination with 3 weeks of cold treatment was obtained. Moreover, a significant effect was obtained with seed lot 028 at both cold treatment periods (<0.0001).

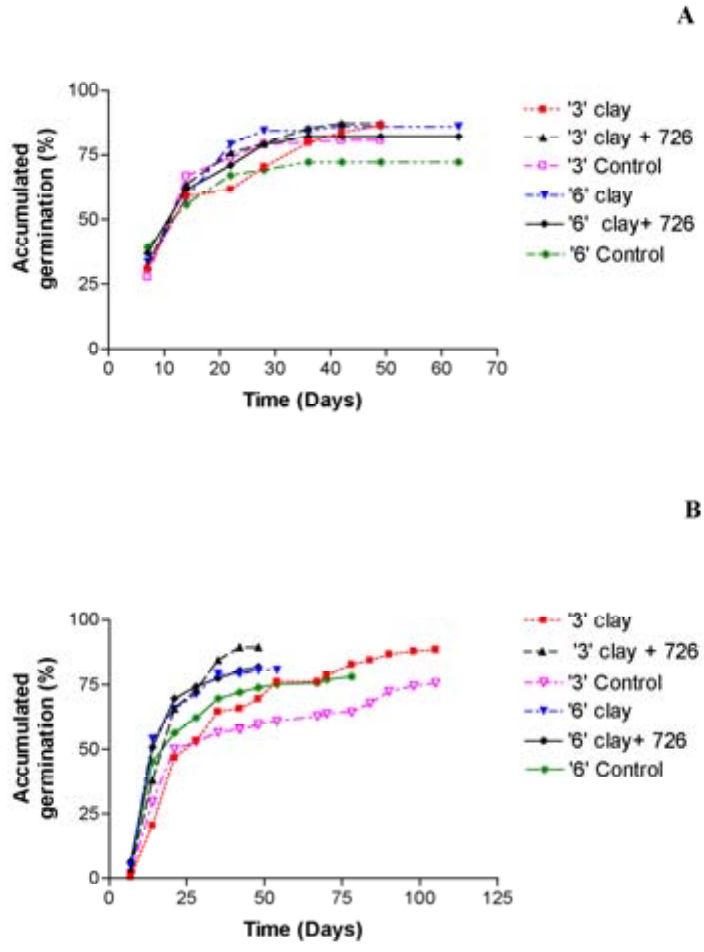

Figure 1. Accumulated laboratory germination percentages of two seed lots of *Abies nordmanniana* cold-treated for 3 ('3') or 6 weeks ('6'), respectively, and treated with clay or *C. rosea* IK726 before cold stratification compared with untreated control.

A: seed lot from Tversted, Denmark (no. 031) maturated in 2002 and

B: seed lot from Ambolauri, Georgia (no. 028) maturated in 2001.

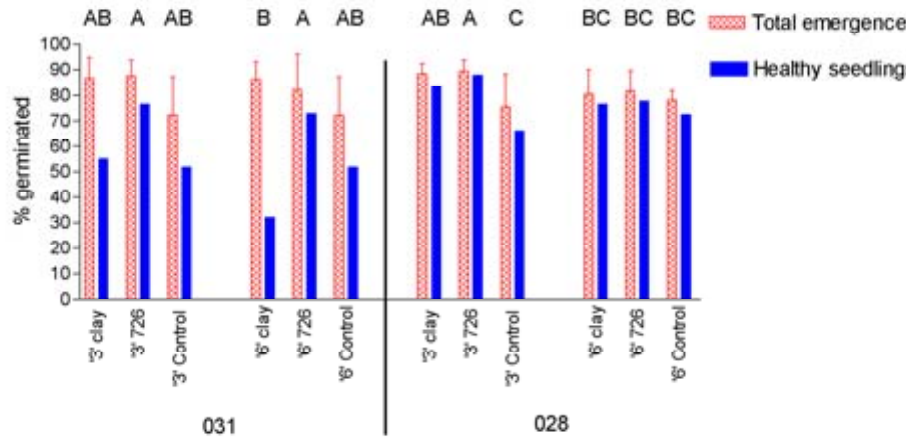

Figure 2. Total laboratory seedling emergence and healthy seedlings of *Abies nordmanniana*, Tversted 031/2002, and Ambolauri, Georgia 028/2001 cold-treated for 3 ('3') or 6 weeks ('6'), respectively and treated with clay or *C. rosea* IK726 before cold stratification compared with untreated control. Different letters above the bars indicate significant differences in numbers of healthy seedlings between treatments according to T-test for variable (Bonferonni (Dunn test)).

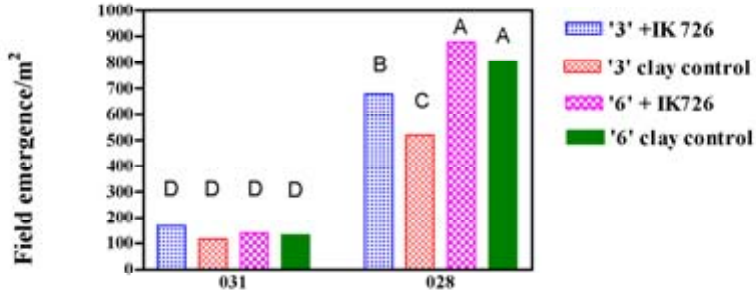

Figure 3. Field emergence assessed 2 months after sowing in a 2004 field experiment using seeds of *Abies nordmanniana* Tversted 031/2002 and Ambrolauri 028/2001, treated with the biocontrol agent *C. rosea* IK726 in a clay preparation or clay alone, applied before 3 or 6 weeks of cold treatment. Different letters above the bars indicate significant differences in numbers of seedlings between treatments according to T-test for variable (Bonferonni (Dunn test)).

If data are sorted by seed lot there is a significant effect of cold treatment period for seed lot 028 ($p<0.0001$) and for treatment with *C. rosea* for seed lot 028 ($p=0.0099$) and a small significant effect of *C. rosea* treatment of seed lot 031 ($p=0.0481$).

There was a general and significantly higher post-emergence disease level in seed lot 031 compared with seed lot 028 ($p<0.001$) and a significantly lower number of diseased seedlings in 3 week cold-treated, *C.rosea*-treated 028 seeds, compared with corresponding clay control ($p<0.5$)(results not shown). Isolations from samples of diseased plants resulted in recovery of *F. oxysporum* and *Pythium* spp.

Discussion

The importance of seed borne pathogens varied amongst the seed lots used in this study. The highest disease level was observed in seed lot 031. Seed-borne *Fusarium oxysporum* was involved, but also *Papulaspora immersa,* which was found in 28% of isolations from diseased plants in vermiculite, was also prevalent on the seeds (Knudsen *et al.*, submitted). However, *P. immersa* could not be isolated from diseased plants from the field, which is in accordance with another study from a Danish forest nursery (Larsen *et al*., 2004). It therefore seems that *P. immersa* is unable to compete with other microorganisms under field conditions. *Papulaspora* spp., along with *Penicillium, Rhizopus* and *Trichoderma,* has previously been reported on *Abies* seed in high frequencies in 111 samples in the Czech Republic (Procházková, 1991). *Papulaspora* was found in 41% of the seeds, but was not reported to be pathogenic (Procházková, 1991).

The germination test of the seed lots demonstrated the variation in optimal stratification period that is found between seed lots. Although it is usual to attempt to optimise the cold treatment period before sowing in the nursery, practical circumstances, weather, etc. may often mean that the treatment that is actually given is either shorter or longer than what was recommended for the seed lot. It has been demonstrated that *C. rosea* establishes and grows on seeds during cold stratification especially in the area of the seed coat, where the radicle protrudes at germination (Knudsen *et al.,* submitted). In the field experiment, the 3-week cold treatment of seed lot 028 was sub-optimal and resulted in lower field emergence, compared with the optimal 6 weeks' treatment. However, treatment with IK726 significantly improved the emergence of this seed lot. This shows that optimal cold treatment is of importance with regard to survival of emerging seedlings and that the BCA can have a positive effect, probably due to control of harmful fungi, during a long germination period. In the germination test, which was made in sterile vermiculite, there were no significant differences between survival of treated seeds after respectively, 3 and 6 weeks cold treatment. This indicates that the lower field emergence after 3 weeks of cold treatment was due to harmful fungi in the soil.

Forest seed are more genetically diverse and often have a lower germination capacity compared with agricultural or horticultural crop seed. In addition to this, *A. nordmanniana* seed lots often contain large fractions of empty seeds (they are difficult to remove during

processing), which may constitute a source of pathogen inoculum and food substrate for multiplication of inoculum in the soil.

In conclusion, the results show that a strategy based on application of the BCA *C. rosea* IK726 before cold treatment has a good potential for improving seedling emergence and survival of *A. nordmanniana* in the field.

Acknowledgements

This work was financed by the Danish Environmental Protection Agency (M 7041-0475). Karin Olesen, Hanne Jørgensen, Lene Tjott Müller and Sigrit Diklev are gratefully acknowledged for skilful technical assistance, and we thank the manager, Kent Nielsen, at Fosdal nursery, Thy and his staff for their help and support with establishment, maintenance and assessment of the experiments.

References

Ahmad, J.S. & Baker, R. 1987: Rhizosphere competence of *Trichoderma harzianum* Phytopathology 77: 182-189.

Hoefnagels, M.H. & Linderman, R.G. 1999: Biological suppression of seedborne *Fusarium* spp. during cold stratification of Douglas Fir seeds. Plant Disease 83: 845-852.

ISTA, 1999: International Rules for Seed Testing. Seed Science and Technology 27 (supplement). ISTA, Zürich.

Jensen, B., Knudsen, I.M.B., Jensen, D.F. & Hockenhull, J. 1996: Application of antagonistic microorganisms to seeds to control fungal plant pathogens. Combined Proceedings International Plant Propagators' Society 46: 256-262.

Jensen, B., Knudsen, I.M.B. & Jensen, D.F. 2000: Biological seed treatment of cereals with fresh and long-term stored formulations of *Clonostachys rosea*: Biocontrol efficacy against *Fusarium culmorum*. European Journal of Plant Pathology 106: 233-242.

Jensen, B., Knudsen, I.M.B. & Jensen, D.F. 2002: Survival of *Clonostachys rosea* on stored barley seeds and their biocontrol efficacy against seed-borne *Bipolaris sorokiniana*. Biocontrol Science and Technology 12: 427-441.

Jensen, B., Knudsen, I.M.B., Madsen, M. & Jensen, D.F. 2004: Biopriming of infected carrot seed with a BCA selected for control of seedborne *Alternaria* spp. Phytopathology 94: 551-560.

Jensen, M. 1997: Moisture content controls the effectiveness of dormancy breakage in *Abies nordmanniana* (Steven) Spach seeds. In: Basics and Applied Aspects of Seed Biology (Eds. Ellis, R.H., Black, M., Murdoch, A.J & Hong, T.D.) pp 181-190.

Johansen, A., Knudsen, I.M.B., Binnerup, S.J., Winding, A., Johansen, J.E., Jensen, L.E., Andersen, K.S., Svenning, M.M. & Bonde, T.A. 2005: A greenhouse study of non target effects of the microbial control agents *Pseudomonas fluorescens* DR54 and *Clonostachys rosea* IK726 in field soil grown with barley followed by sugar beets. Soil Biol. Biochem. 37: 2225-2239.

Knudsen, I.M.B., Hockenhull, J. & Jensen, D.F. 1995: Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: effects of selected fungal BCAs on growth and yield components. Plant Pathology 44: 467-477.

Knudsen, I.M.B., Jensen, B., Jensen, D.F. & Hockenhull, J. 1996: Colonization of *Gliocladium roseum* on barley roots from sand and field soil. In: *"Monitoring BCAic fungi deliberately released into the environment"*. (D. Funck Jensen, H-B. Jansson & A. Tronsmo eds.). Kluwer Academic Publishers. pp. 33-37.

Knudsen, I.M.B., Thomsen, K.A., Hockenhul, J. & Jensen, D.F. (submitted 2005) Effect of biological control agents on seedling diseases in nursery production of *Abies nordmanniana* (Steven) Spach. Forest Pathology.

Knudsen, I.M.B., Thomsen, K.A., Jensen, B. & Poulsen K.M. 2004: Effects of hot water treatment, biocontrol agents, disinfectants and a fungicide on storability and control of the pathogen *Ciboria batschiana* on English oak acorns. Forest Pathology 33: 47-64.

Larsen, J., Ravnskov, S., Møller, K. & Bødker, L. 2004: Bæredygtig produktion af småplanter i forstplanteskoler. Miljøstyrelsen. 63 pp.

Lübeck, M., Knudsen, I.M.B., Jensen, B., Thrane, U., Janvier, C. & Jensen, D.F. 2002: GUS and GFP transformation of the biocontrol strain *Clonostachys rosea* IK726 and the use of these marker genes in ecological studies. Mycol. Res 106: 815-826.

Planteavlsstationen, 2005: Planteavlsstationens Frøhåndbog. www. Planteavlsstationen.dk.

Procházková, Z. 1991: The occurrence of seed-borne fungi on forest tree seeds in the years 1986-1991. Communicationes Instituti Forestalis, Cechoslovaca 17: 107-123.

Ulocladium atrum and *Glomus mossae* control *Botrytis cinerea* grey mould and counteract darkness stress effects in pot roses

Kaare Møller[1], David Yohalem[1], Kristian Kristensen[2], John Larsen[1]

[1]Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark; [2]Department of Genetics and Biotechnology, Research Centre Foulum, Danish Institute of Agricultural Sciences, Blichers Allé, P.O. Box 50, DK-8830 Tjele, Denmark

Anstract: A three-factorial design was applied to study effects of plant darkness stress, *Ulocladium atrum* and *Glomus mossae* on *Botrytis cinerea* grey mould and plant vigour in pot roses. The interaction effect of the three treatment factors significantly influenced the disease response. Under non-stress conditions *U. atrum* reduced disease incidence from 25% to 9%. The disease incidence increased to 74% under stress, but was reduced to 14% when *U. atrum* was also applied. Hence, the plant disease status was significantly improved by *U. atrum* application in both stressed and non-stressed plants, when comparing to non-treated control plants. *G. mossae* tended to interact positively with *U. atrum* under non-stress conditions (disease reduction from 25% to 2%) and negatively in darkness (reduction from 74% to 22%). Although simultaneous application of *U. atrum* and *G. mossae* gave a significant disease reduction in stressed plants, the reduction was not significant, when comparing to untreated, non-stressed plants. *G. mossae* significantly reduced non-specific leaf wilt incidence from 42% to 23%. Darkness significantly reduced the number of leaves formed above the pruning stub by 50%. *U. atrum* efficiently counteracted this effect, but had no such effect under non-stress conditions. *G. mossae* and *U. atrum* additively increased the total number of leaves per plant (32% increase), and also increased plant top dry weight. *G. mossae* had no effect on dry weight under stress conditions, though. *G. mossae* root colonization increased significantly (from 71.2% to 81.5%) by *U. atrum* treatment. Darkness negated this effect.

Mycorrhizal fungi and crop protection: experiences from The Netherlands

Jacqueline Baar
Applied Plant Research, Wageningen University and Research Centre, P.O. Box 6042, 5960 AA Horst, The Netherlands

Abstract: Mycorrhizal fungi are soil fungi that have the ability to enhance the growth and development of the majority of plants. Mycorrhizal fungi live in symbiosis with their host plants playing a vital role in the acquisition of mineral nutrients (N, P) and water from the soils in exchange for carbon from the plants. Also, mycorrhizal fungi can suppress the development of below- and above ground pathogens. Two major groups of mycorrhizal fungi are distinguished: ectomycorrhizal fungi (EMF) that mainly associate with trees and arbuscular mycorrhizal fungi (AMF) that mostly associate with grasses, herbs, forbs and some tree species. Also, colonization of AMF can also occur on crops including onions, leeks, wheat, rye and maize.

During the last few years, the interest in mycorrhizal fungi has grown in The Netherlands. This has resulted in increased application of EMF and AMF. A selection is discussed in this presentation.

Ecological management of urban trees has increased in The Netherlands and mycorrhizal fungi recently started to become a part. At Applied Plant Research, below ground development of EMF was observed with microscopic and molecular techniques, and related to the health conditions of trees and environmental conditions. Based on these observations, treatments to improve health conditions of the trees were taken.

For agricultural systems, the near future policies in The Netherlands are reduced use of chemical fertilizers and crop protection agents including fertilizers. This enables possibilities for enhanced application of AM fungi resulting in the promotion of mineral uptake by crops and enhancement of defence mechanisms against pathogens.

Recently, a cooperative project between Plant Research International and Applied Plant Research has started with the objective to develop crops with enhanced responsiveness to AM fungi. This project focuses on crops in open arable systems. In 2004, the project has started with analysis of onion roots with molecular techniques (PCR) at organic and conventional onions farms in Zeeland and Flevopolder. Diversity of AMF was relatively high in the organically and conventionally managed fields in the Flevopolder and even in the conventionally managed fields AMF was observed. These results show that the Dutch soil conditions are suitable for the development of AMF. Recently, field experiments in the Flevopolder and Zeeland were carried out with the addition of specific inoculum resulting in improved development of the onions and reduction of fungal pathogens.

Also, for closed agricultural systems such as the growth of crops in greenhouses mycorrhizal fungi can be applied. Thus far, interest was only coming from Dutch growers of organic crops. Applied Plant Research has carried out two experiments on the addition of inoculum of AMF and cucumbers. Further research is planned for more effective application of AMF on greenhouse crops.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

The mite pathogenic fungus *Neozygites floridana* for the control of the two-spotted spider mite

Ingeborg Klingen, Karin Westrum, Nina Trandem, Inger Nordengen
The Norwegian Crop Research Institute, Plant Protection Center, Hogskoleveien 7, N-1432 Ås, Norway

Abstract: *Neozygites floridana* is a fungus in the order Entomophthorales that infects and kills the two-spotted spider mite, *Tetranychus urticae*. In a study conducted in Norwegian strawberry fields, *N. floridana* infected and killed *T. urticae* in all 12 fields studied. Infection levels up to 90% were registered, and the highest infection levels were observed late in the season. The infection levels throughout a season varied considerably. To evaluate factors that may be important for conservational biological control, the effect of pesticides used in strawberries on the *N. floridana* infection level was also studied. The pesticides tested were three fungicides; Euparen (tolylfluanid), Teldor (fenhexamid), Switch (cyprodinil + fludioxonil) and one acaricide: Mesurol (mercaptodimethur). The experiment indicates that Euparen and Switch do not affect the *N. floridana* killing capacity, but both Teldor and Mesurol do. Methods for the production and storage of *N. floridana*-infected *T. urticae* cadavers for inoculative/inundative biological control in strawberries were established in our laboratory and are presented briefly in this paper. Similar methods may be adapted for the inoculative/inundative biological control of *T. urticae* in for example greenhouse crops.

Key words: *Neozygites floridana, Tetranychus urticae*, natural enemy, biological control, strawberries

Introduction

Neozygites floridana is a fungus in the order Entomophthorales that infects and kills the two-spotted spider mite, *Tetranychus urticae*. *N. floridana* is the key regulator factor of *T. urticae* in many crops, and conservational biological control of this fungus by adaptation of pesticide spray programs are well known in maize and soybean in the USA (Cross *et al*., 1999). To our knowledge, few systematic studies have been conducted on *N. floridana* as a mortality factor of *T. urticae* in northern Europe. Some studies have, however, been conducted in Poland which indicate that *N. floridana* may be important for the regulation of *T. urticae* in several cultures (Mietkiewski *et al*., 1993, 2000). When *N. floridana* is used to control *T. urticae*, it is important both to get an infection established (inundative/inoculative control) and to conserve naturally existing infections (conservation). To conserve naturally occurring *N. floridana*, chemical pesticides, especially fungicides, need to be used carefully.

In a study conducted in Norwegian strawberry fields we aimed at establishing which *N. floridana* infection levels could be found in *T. urticae* in strawberries and to clarify the effect

of pesticides used in this crop on the *N. floridana* infection level (conservational biological control). Inoculative or inundative biological control by the use of *N. floridana* has also been suggested (Kennedy & Smitley, 1988; van der Geest *et al.*, 2000), but to our knowledge not yet used in practice. In our work we therefore also aimed at establishing methods for production, storage and release of *N. floridana* for the inoculative/inundative biological control of *T. urticae* in strawberries.

Material and methods

Conservation approach

To observe *N. floridana* infection levels in *T. urticae* populations in strawberries, leaves were collected two times during the season from 12 different fields located from the North East to the South East of Norway. The collections were conducted in 2002 and 2003. The first collection was conducted at the end of June/the beginning of July, and the second was conducted a month later. To study the variation in infection levels throughout a season, one field was also collected weekly from March to September during 2003. Leaf samples were observed for *N. floridana*-infected *T. urticae* by using a technique where *T. urticae* was washed out and observed for *N. floridana* hyphal bodies in the microscope.

To study the effect of pesticides used in strawberries on *N. floridana* and *T. urticae*, strawberry leaf disks were dipped in the fungicides Euparen (tolylfluanid), Teldor (fenhexamid) or Switch (cyprodinil + fludioxonil), in the acaricide Mesurol (mercaptodimethur) or in water (control) and placed in 25 ml vials with water agar. *T. urticae* were placed on top of treated leaf disks to feed for 48 hours. After 48 hours of feeding, half of the *T. urticae* in each treatment were inoculated with *N. floridana*, the rest were not treated with *N. floridana*. *T. urticae* was then placed individually on leaf disks in vials and observed daily for infection and death.

Inoculative/inundative approach

A method adapted from Delalibera & Hajek (2004) using Cryo tube lids, pins and fresh strawberry leaves was established in our laboratory for a small-scale production of *N. floridana*-infected *T. urticae* cadavers. A method adapted from Oduor *et al.* (1995, 1997) for storing *N. floridana* infected *T. urticae* cadavers was also established in our laboratory. We tested several methods for the inoculation of *N. floridana* into a *T. urticae* population in a strawberry field. Most of them involved inoculation of *N. floridana*-infected *T. urticae* cadavers into the field. In addition, one of the tested inoculation methods involved the use of still live but *N. floridana* infected *T. urticae*. A full description of the methods tested will be published elsewhere.

Results and discussion

Conservation approach

N. floridana infected and killed *T. urticae* in all 12 strawberry fields studied, and infection levels up to 90% were registered in August. Infection rates throughout a season varied considerably, and the highest infection rates were observed late in the season. Being this far north, *N. floridana* infections were also found surprisingly early in the spring at the first sampling date, March 18, 2003.

In our experiment on the effect of pesticides to *N. floridana* and *T. urticae*, the *T. urticae* control mortality was too high (52.5%), and new studies are being conducted. Our preliminary studies indicate, however, that tolylfluanid and cyprodinil + fludioxonil did not affect the *N. floridana* killing capacity, but that both fenhexamid and mercaptodimethur did. Tolylfluanid and fenhexamid also had a direct killing effect on *T. urticae* that is infected by *N. floridana*. Surprisingly, the acaricide mercaptodimethur did not cause mortality in *T. urticae*, but this may be due to a possible resistance in the *T. urticae* laboratory culture (Figure 1).

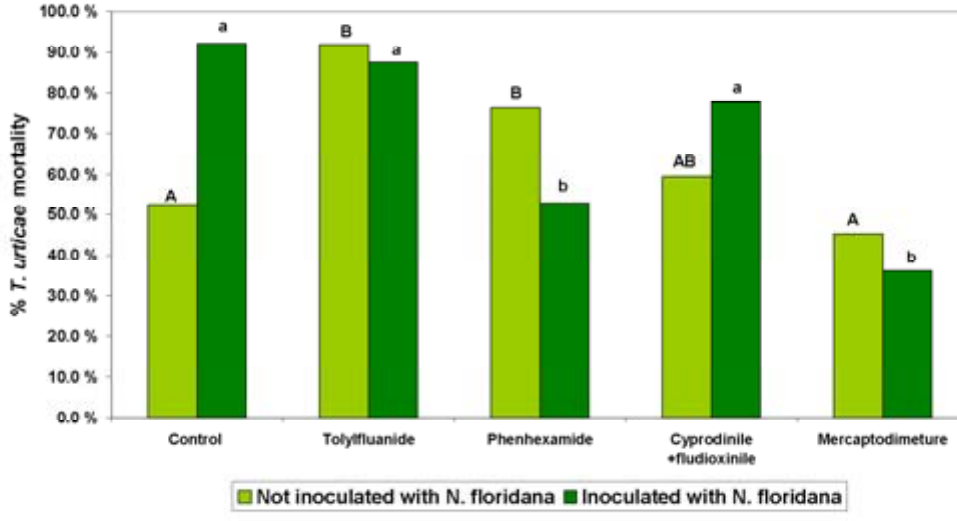

Figure 1. Effect of pesticides on *N. floridana* and *T. urticae*. Light green bars indicate % mortality of healthy (not infected) *T. urticae*. Dark green bars indicate % mortality of *N. floridana* infected *T. urticae*.

Inoculative/inundative approach

Both long- and short-term control of *T. urticae* by the use of *N. floridana* may be relevant, depending on the culture in question. For short-term control in annual field crops the most

important factor would be the establishment of a *N. floridana* epizootic as early as possible in the season. In this part of the season, however, the temperatures are quite low. Short-term control in greenhouses may also be of interest. In this system an early establishment of *N. floridana* will be less dependent on the temperature requirements of the fungus since greenhouse production probably provides optimal temperatures for the fungus. Long-term control is relevant in perennial cropping systems (e.g. strawberries). A *N. floridana* infection for short- or long-term control may be established in a *T. urticae* population by the use of *N. floridana*-infected *T. urticae* cadavers.

A small-scale production of *N. floridana*-infected *T. urticae* cadavers was successfully established at our laboratory. We are now able to have a stable production of quite a high amount of *N. floridana*-infected *T. urticae* cadavers for laboratory experiments and probably also field inoculation. Long time storage (at least 12 months) of *N. floridana*-infected *T. urticae* cadavers has also been established, and we are therefore able to start new *N. floridana in vivo* production any time of the year. Also greenhouse experiments with *N. floridana* as a biological control agent against *T. urticae* in greenhouse crops may therefore be relevant.

None of the field inoculation methods tested until now have resulted in successful establishment of *N. floridana* in our strawberry field. We hypothesise, however, that live but *N. floridana*-infected *T. urticae* is a method that can be developed for successful inoculation. Live but *N. floridana*-infected *T. urticae* will probably seek out microhabitats suitable for promoting sporulation and dispersion of the fungus before they die. Further studies are being conducted to test this hypothesis. At the time we tried to inoculate the strawberry field with live *N. floridana*-infected *T. urticae,* we were not able to produce a high amount of fungus infected *T. urticae*. Hence, only a low number (about $1/m^2$) was inoculated into the field. Since we now are able to produce higher numbers of *N. floridana* infected *T. urticae* we will, in 2006, inoculate with a higher number (at least $4/m^2$). Laboratory experiments, which may reveal other factors that are involved in a successful field inoculation, will also be conducted.

Acknowledgements

We thank Annette Folkedal, Fakjit Palintorn and Srichana Punpilas for technical assistance.

References

Cross, J.V., Solomon, M.G. Chandler, D., Jarrett, P., Richardson, P.N., Winstanley, D. Bathon, H., Huber, J., Keller, B., Lagenbruch, G.A. & Zimmermann, G. 1999: Biocontrol of pests of apple and pears in northern and central Europe: 1. Microbial agents and nematodes. Biocontrol Science and Technology 9: 125-149.

Delalibera, I. & Hajek, A.E. 2004: Pathogenicity and specificity of *Neozygites tanajoae* and *Neozygites floridana* (Zygomycetes: Entomophthorales) isolates pathogenic to the cassava green mite. Biological Control 30: 608-616.

Kennedy, G.G. & Smitley D.R. 1988: Method of controlling plant feeding mites with the fungus *Neozygites floridana*. United States Patent 4,752,468 1-12.

Mietkiewski, R., Balazy, S. & Tkaczuk, C. 2000: Mycopathogens of mites in Poland – A review. Biocontrol Science and Technology 10: 459-465.

Mietkiewski, R., Balazy, S. & van der Geest, L.P.S. 1993: Observations on a mycosis of spider mites (Acari: Tetranychidae) caused by *Neozygites floridana* in Poland. Journal of Invertebrate Pathology 61: 317-319.

Oduor, G.I., Yaniek, J.S., De Morales, G.J. & Van der Geest, L.P.S. 1997: The effect of pathogen dosage on the pathogenicity of *Neozygites floridana* (Zygomycetes: Entomophthorales) to *Mononychellus tanajoa* (Acari: Tetranychidae). Journal of Invertebrate Pathology 70: 127-130.

Oduor, G.I., Yaninek, J.S., Van der Geest, P.S. & De Morales, G.J. 1995: Survival of *Neozygites* c.f. *floridana* (Zygomycetes: Entomophthorales) in mummified cassava green mites and the viability of its primary conidia. Experimental & Applied Acarology 19: 479-488.

van der Geest, L.P.S., Elliot, S.L., Breeuwer, J.A.J. & Beerling, E.A.M. 2000: Diseases of mites. Experimental & Applied Acarology 24: 497-560.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Implementation of arbuscular mycorrhizas in greenhouse-grown vegetables

Sabine Ravnskov, John Larsen

Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark.

Abstract: The possibility of implementing arbuscular mycorrhizal fungi in commercial tomato and cucumber production to increase root health was examined. Initially, three commercial AM products were tested for colonisation in the most used cultivars of cucumber and tomato grown with different P levels in the nutrient solution. In general, the results showed that growth of cucumber was unaffected by a 50% reduction of P in the nutrient solution (to 20 ppm) and that plants were 10-15% colonised after four weeks, which is the approximate time for transplanting. On the other hand, tomato plants were only colonised at very low P levels, where the plant had unacceptable growth for commercial production. Furthermore, experiments in three commercial cucumber nurseries were conducted to evaluate the effect of AM fungi on plant growth and yield of cucumber under commercial conditions. In the first production cycle from January to July, the growers had no problems with root diseases and AM did not influence yield in two of the nurseries, but in the third nursery inoculation with mycorrhiza resulted in approximately 4% more first class cucumbers compared with plants without mycorrhiza. In conclusion, our results show that it is possible to implement mycorrhiza in commercial cucumber production, but most likely not in tomato production.

Key words: Cucumber, tomato, mycorrhiza, *Pythium*, greenhouse, Phosphorus

Introduction

Arbuscular mycorrhizal fungi are well known for their plant beneficial features such as plant growth promotion (Smith & Read, 1997) and biocontrol of root pathogens (Whipps, 2004).

Most plants form mycorrhiza under natural conditions, whereas plants in horticultural greenhouse production systems often are grown in rock wool or peat-based growth media, where AM fungi are absent. Hence, in these systems it is necessary to inoculate the plants to benefit from AM fungi.

Commercial inocula of AM fungi are available, but have in many cases not been examined for compatibility with grower practice. One of the main bottlenecks of integrating AM in horticulture is that AM is sensitive to high levels of phosphorus fertilization, which is common for horticultural crops both vegetables and ornamentals. Also compatibility with methods to control root diseases such as fungicides and biocontrol agents are important to consider (Larsen *et al*., in press).

Cucumber and tomato are among the most important greenhouse-grown vegetables in Denmark. In these systems inert growth media such as Grodan (rock wool) are used sometimes in combination with recirculation of the nutrient solution. The main disease problems in this system are caused by root pathogens (mainly *Pythium*) and foliar pathogens (mainly mildew). Cucumber colonised with mycorrhiza has shown biocontrol features against *Pythium* (Larsen *et al.*, 2003), but has no effect on mildew (Larsen & Yohalem, 2004).

The objective of the project is to examine the possibility of integrating AM fungi in production of greenhouse-grown vegetables in order to increase plant health and tolerance against plant root diseases.

Material and methods

Controlled experiments

Two similar fully factorial experiments were conducted under controlled greenhouse conditions with cucumber and tomato, respectively. Each experiment had three factors: 1) plant variety (3 levels), 2) mycorrhizal inoculum (4 levels, without, TerraVital, Biorize and Triton) and 3) phosphorus (4 levels, 0, 25%, 50% and 100% of normal practice (40 ppm P). Each treatment had four replicates with three plants each. Plants were grown for 4 weeks in 10 x 10 cm Grodan blocks. At harvest shoot dry weight and mycorrhiza root colonization were examined.

Grower experiments

Three experiments were conducted in commercial cucumber nurseries in the spring production (January to July) each using different varieties (Naomi, Euphoria, Mystica) of cucumber. Transplants were produced at low P (20 ppm P in nutrient solution) with and without mycorrhiza and after transplanting the plants followed the normal practice in each nursery. Plant development and yield were measured, and in one nursery the quality of the cucumbers (class 1 or 2) was also recorded.

Results and discussion

Overall mycorrhiza formation was reduced with increasing phosphorus levels in the nutrient solution in both cucumber (Figure 1) and tomato (Figure 2). This is a well-known phenomenon (e.g. Olsson *et al.*, 1997) and is the main bottleneck for integration of mycorrhiza in horticulture (Larsen *et al.*, in press). The different commercial mycorrhiza inocula resulted in different levels of colonisation. Inoculation with Biorize resulted in the highest level of colonisation. Cordiki *et al.* (2004) also tested several different commercial AM inocula and found that some were more efficient than others at developing mycorrhiza when mixed in a peat-based growth substrate.

However, it is important to note that no clear relationship with root colonisation intensity and plant growth and protection has been found. Also very high colonisation levels may result in plant growth depressions (Larsen & Yohalem, 2004). The examined tomato varieties over-

all developed lower mycorrhizal fungus root colonisation than found with the cucumber varieties. Such differences in compatibility between AM fungi and host plant are also well known (e.g. Ravnskov & Jakobsen, 1995).

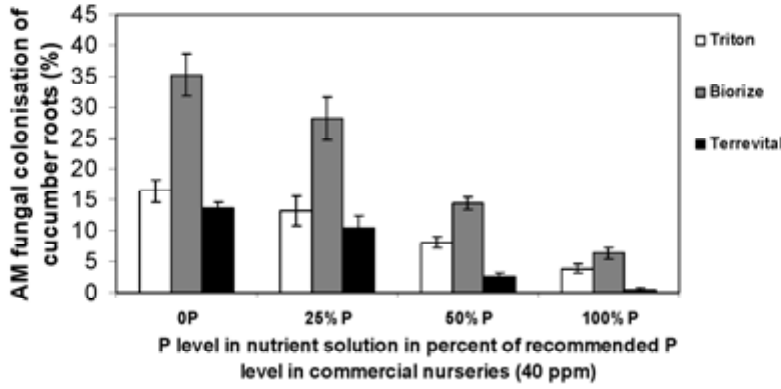

Figure 1. Average arbuscular mycorrhizal fungus root colonisation in three cucumber varieties (Naomi, Euphoria, Mystica) four weeks after sowing and inoculation with three different commercial mycorrhiza inocula (Triton, Biorize and TerraVital) as influenced by phosphorus levels in the nutrient solution (0, 25%, 50% and 100% of nursery practise). Error bars represent standard error of the mean.

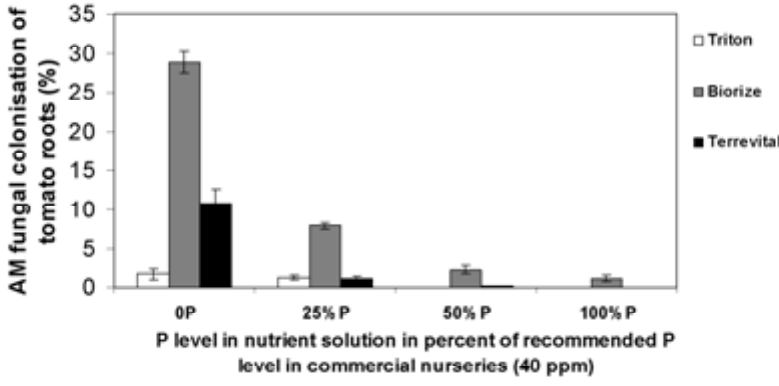

Figure 2. Average AM fungus root colonisation of three tomato varieties (Cedrico, Aromata, Axxion) four weeks after sowing and inoculation with three different commercial mycorrhiza inocula (Triton, Biorize and TerraVital) as influenced by phosphorus levels in the nutrient solution (0, 25%, 50% and 100% of nursery practice). Error bars represent standard error of the mean.

As expected, plant growth of both cucumber and tomato increased with incresing levels of P in the nutrient solution (Figures 3 and 4). In tomato the highest growth was found with 100% P, whereas in cucumber 50% P resulted in the same growth as 100% P, indicating that the P levels in the nutrient solution could be reduced to 20 ppm P, which seemed to be compatible with mycorrhiza formation.

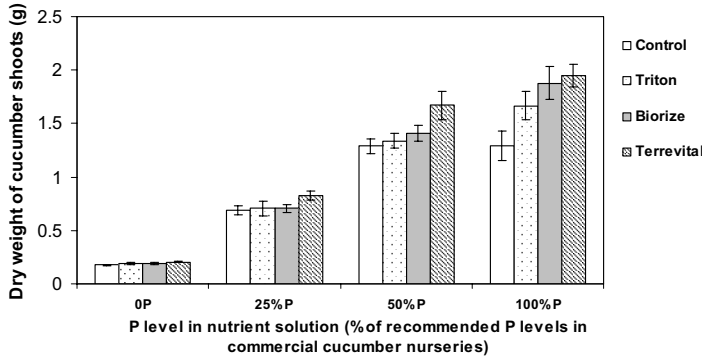

Figure 3. Average shoot dry weights of three cucumber varieties (Naomi, Euphoria, Mystica) four weeks after sowing as influenced by phosphorus levels in the nutrient solution (0, 25%, 50% and 100% of nursery practice 40 ppm P) and inoculation with three different commercial mycorrhiza inocula (Triton, Biorize and TerraVital). Error bars represent standard error of the mean.

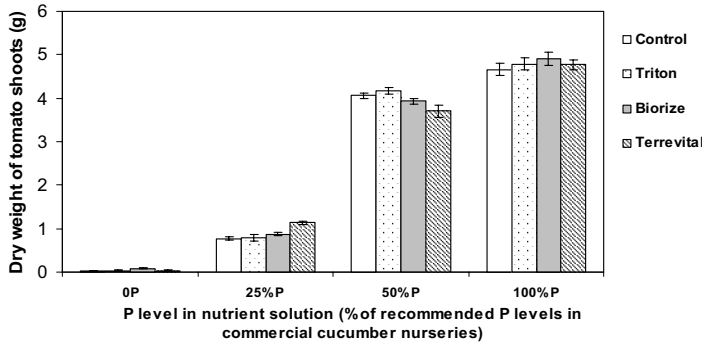

Figure 4. Average shoot dry weights of three tomato varieties (Cedrico, Aromata, Axxion) four weeks after sowing as influenced by phosphorus levels in the nutrient solution (0, 25%, 50% and 100% of nursery practice 40 ppm P) and inoculation with three different commercial mycorrhiza inocula (Triton, Biorize and TerraVital). Error bars represent standard error of the mean.

In cucumber, inoculation with mycorrhiza resulted in plant growth promotion with TerraVital at all levels of P except without P, whereas Triton and Biorize only promoted plant growth at the higest P level. The growth promotion at the 100% P coincided with almost no mycorrhiza formation, indicating that other components in the AM inocula could be responsible for the growth promotion. Inert growth systems such as rock wool-based are deficient in microbial activity, and applying mycorrhiza inocula to these systems may not only result in mycorrhiza formation, but also in introduction of mycorrhiza associated bacteria (Mansfeld-Giese *et al.*, 2002). Indeed, mycorrhiza-associated bacteria from the genus Paenibacillus have been shown to promote growth of cucumber plants (J. Larsen, unpublished). Consequently, the underlying mechanisms for the growth promoting effects of the various AM inocula need to be further investigated.

In the grower experiments with cucumber, no differences were found between mycorrhizal and non-mycorrhizal plants in terms of growth and yield. However, in the third nursery using the cultivar Naomi, plants inoculated with mycorrhiza resulted in a 3.8% increase in the yield of first class cucumber (Figure 5), which for this specific nursery would correspond to an extra profit of approximately 50,000 Euro in the spring production. None of the nurseries had root disease problems in this production, so the influence of mycorrhiza on this parameter could not be examined.

In conclusion, our results show that it is possible to implement mycorrhiza in cucumber, but most likely not in tomato. The plant health improving features of AM fungi still need to be shown in practice. Also the plant growth promoting effects of AM inocula as well as the observed increase in cucumber quality need to be confirmed.

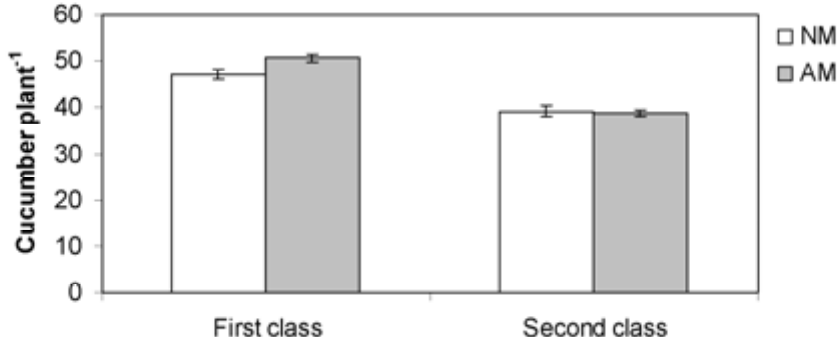

Figure 5. Numbers and quality of cucumbers of the variety Naomi harvested in the spring production January to July 2004 in a commercial nursery, inoculated (AM) or not (NM) with the AM fungus inoculum Biorize. Error bars represent standard error of the mean.

Acknowledgements

We thank the cucumber growers Søren Klarskov, Dan Bredskov and Lars Kristiansen for hosting our experiments. Also Tina Tønnersen and Steen Meier are thanked for excellent technical support. Finally, we thank The Danish Directorate for Food, Fisheries and Agro Buiness for financial support grant no. 3401-65-03-621.

References

Corkidi, L., Allen, E.B., Merhaut, D., Allen, M.F., Downer, J., Bohn, J. & Evans, M. 2004: Assessing the infectivity of commercial inoculants in plant nursery conditions. Journal of Environmental Horticulture 22: 149-154.

Larsen, J., Ravnskov, S. & Jakobsen, I. 2003: Combined effects of an AM fungus and BCA bacteria against the root pathogen *Pythium ultimum* in soil. Folia Geobot. 38: 145-154.

Larsen, J., Ravnskov, S. & Nygaard, J.N. in press: Capturing the benefits of arbuscular mycorrhiza in horticulture. In: Mycorrhizae in Crop Production (ed. Hamel, C.), The Haworth Press Inc.

Larsen, J., & Yohalem, D.S. 2004: Interactions between mycorrhiza and powdery mildew of cucumber. Mycol. Prog. 3: 123-128.

Mansfeld-Giese, K., Larsen, J. & Bødker, L. 2002: Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. FEMS Microb. Ecol 41: 133-140.

Olsson, P.A., Baath, E. & Jakobsen, I. 1997: Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. Appl. Environ. Microbiol. 63: 3531-3538.

Ravnskov, S. & Jakobsen, I. 1995: Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. New Phytol. 129: 611-618.

Smith, S.E. & Read, D.J. 1997: Mycorrhizal Symbiosis. Academic Press, London, 605 pages.

Whipps, J. 2004: Prospects and limitations of mycorrhizas in biocontrol of root pathogens. Can. Jour. Bot. 82: 1198-1227.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biological seed treatment for control of seed-borne *Alternaria* spp. in carrot seed

Birgit Jensen, Dan Funck Jensen, Inge M.B. Knudsen

Plant Pathology Section, Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: The fungal antagonists *Plectosporium tabacinum* IK1755, *Clonostachys rosea* IK726 and IK1878, *C. rosea* f. *catenulata* IK1871 and *C. solani* IK1889 were applied during priming of carrot seed, i.e. biopriming. The five BCAs have previously shown high biocontrol efficacy against the seed-borne pathogens *Alternaria radicina* and *Alternaria dauci* when applied by seed coating. Priming of carrot seeds increases speed and uniformity of seed germination and improves final stand under environmental stress conditions. However, a detrimental effect on plant health can be expected if the seeds to be primed are infected with pathogenic fungi. In the present experiments occurrences of *A. dauci* and *A. radicina* and saprophytic fungi belonging to *Ulocladium, Cladosporium,* Mucorales and *Penicillium* increased significantly during hydro-priming. However, biopriming with isolates of *Clonostachys rosea, C. solani* and *C. rosea* f. *catenulata* almost eradicated these fungi, while *P. tabacinum* was less effective. At the same time all isolates multiplied 5- to 8-fold on the seed. In establishment tests conducted in sand, water-primed seeds had a lower healthy seedling stand than unprimed seeds. In contrast, biopriming resulted in a seedling stand that was better than for both unprimed and water-primed seeds and again the *Clonostachys* spp. isolates were most efficient. The effects of IK726 coating and IK726 biopriming in combination with heat treatment of seed were tested in the field. IK726 biopriming of heat-treated seed naturally infected with *Alternaria* spp. resulted in the significantly highest plant stand of all treatments including a Thiram coating of unprimed seed. IK726 is compatible with commercial pelleting and coating technologies, and therefore the perspectives for practical use are promising.

Key words: *Gliocladium roseum*, *Fusarium tabacinum*, drum priming, heat treatment, integrated control

Introduction

Alternaria dauci (Kühn) Groves & Skolko and *Alternaria radicina* M.D. *et* E. (syn. *Stemphylium radicinum* (Meier, Drechsler et Eddy) Neerg.) are both important seed-borne pathogens, causing seed decay and damping-off in carrot (*Daucus carota* L.). Alternative methods for control of the seed-borne carrot pathogens *A. dauci* and *A. radicina* are needed especially in organic vegetable production. Potential antagonists have been isolated from carrot and barley tissue and screened in carrot seedling assays using seed naturally infected with both pathogens and with seed artificially infested with a high level of *A. radicina,* respectively. Among 141 candidates representing over 25 species, isolates of *Clonostachys* spp.

(*C. rosea* f. *rosea* (Link: Fr) Schroers *et al.* (syn. *Gliocladium roseum* Bainer, Bull), *C. solani* ((Harting) Schroers & Gams) f. *solani,* and *C. rosea* f. *catenulata* (Gilman & Abbott) Schroers, (syn. *Gliocladium catenulatum* Gilman & Abbott)) generally turned out to be the most effective antagonists against pre- and post-emergence death caused by *A. dauci* and *A. radicina* (Jensen *et al.*, 2004).

Priming of seed is a process, which helps to accelerate germination and improve seedling establishment in many crops, especially under unfavourable environmental conditions or in soils infested with plant pathogens. During priming water availability is restricted to maintain a moisture content that will allow physiological processes of germination to occur but prevent the completion of germination and radicle emergence. Priming, where the elevated but restricted moisture content is based solely upon controlled water addition, e.g. drum priming and hydro-priming (Rowse, 1996; Tylkowska & Van den Bulk, 2001; Jensen *et al.*, 2004), is used commercially in carrot both in Denmark and in other EU countries. On the other hand, priming may also have a detrimental effect on plant health if the carrot seeds to be primed are infected with pathogenic fungi (Jensen *et al.*, 2004; Tylkowska & van den Bulk, 2001). However, when the antagonistic fungus *C. rosea* was applied during a priming process based only upon water addition (biopriming), *A. dauci* and *A. radicina* were almost eradicated and seedling establishment was significantly improved (Jensen *et al.*, 2004). Priming in relation to beneficial microorganisms has mainly been studied as method to apply e.g. growth promoting bacteria and BCAs to seed with the purpose of proliferation and establishment of the microbes on seeds during priming (Harman *et al.*, 1989; Wright *et al.*, 2003a).

The main objectives of the present study were to evaluate 1) the potential of five fungal BCAs to multiply on seeds during priming and at the same time depress seed injuries caused by fungal pathogens, and 2) the feasibility of integrating such BCAs into commercial seed priming and seed coating technologies for field application.

Material and methods

Seed material

Carrot seed of the variety Royal Chantenay Rola lot 133087 was used in growth chamber and field experiments. Lot 133087 was naturally infected with *A. radicina* (7.5%) and *A. dauci* (5.1%). Lot 133002 of the same variety infected naturally with *A. radicina* (7%) and *A. dauci* (4%) was also used in the field trials.

Antagonistic fungi and their production

C. rosea, IK726 was isolated from barley roots (Knudsen *et al.*, 1995), while *C. rosea,* IK1878, *C. rosea* f. *catenulata* IK1871, *C. solani* IK1889 and *Plectosporium tabacinum* (van Beyma) M.E. Palm *et al.*, IK1755 were isolated from carrot material. Peat-bran preparations of the BCAs and a clay formulation of IK726 were prepared according to Jensen *et al.* (2002). For field trials, Danisco Seed produced IK726 on a carrier according to their internal procedures. Furthermore, the commercial product TRI003, a formulation of *Trichoderma harzianum* isolate 1295-22, was used in the field trials.

Seed coating and heat treatment

For seed coating, amounts of dry formulations of BCAs resulting in 1 x 10^7 cfu/ml were suspended in sterile water and shaken for 1 min on a vortexer. Seeds were then coated by shaking 1 g of seeds with 4 ml of the adjusted conidial suspension on a shaker (IKA-Vibrax) at 130 rpm. for 10 minutes. Subsequently, the seed were air-dried on filter paper for 1 h in a laminar flow hood before planting. Effects of antagonist treatments were compared with water treatment and with the chemical fungicide Iprodione (20 ml kg seed), while Thiram was used as fungicide control in field trials. Emergent Genetics™ conducted heat treatment of seed for fields by immersion of seed in hotwater bath at 52ºC for 15 minutes. Danisco Seed used their protected film-coating technique for application of BCAs to untreated, heat-treated, primed and bioprimed seed. Danisco Seed also evaluated the compatibility of IK726 with colour, binder and the insecticide Promet used for film-coating in plate well diffusion assays.

Seed priming and biopriming

The general steps in the priming procedure for laboratory experiments were as follows: Seeds were imbibed for 16 h in running tap water, subsequently seeds were dried to 39% moisture content (m.c.). Seeds were then immediately incubated at 15°C for 13 days. During the priming period seeds were regularly shaken. After incubation, seeds were dried to 7-8% m.c and stored at 4°C. For biopriming, a defined amount of peat-bran inoculum of the antagonists (1.5 x 10^8 cfu/20 g seed at 39% m.c.) was applied immediately after the seeds were adjusted to 39% m.c. Subsequently, seeds were treated as described for priming. Priming for field trials was performed by Emergent Genetics™ according to their internal procedures based on the principles for laboratory priming described above.

Estimation of occurrence of fungi on seed and quantification of antagonists on seed

The occurrence of *Alternaria* spp. and other dominating fungal genera after priming and biopriming was assessed with a deep freeze blotter test (ISTA 1996). Samples were taken during the priming process at 1, 8 and 14 days' priming. The presence of *Alternaria* spp. and other fungi on the seed was then recorded under a dissection microscope (Olympus SZH) after 7 and 10 days' incubation. Fungal occurrence on seed was expressed as percentage of seed where a fungal species was identified. The population density of antagonistic fungi on carrot seed (cfu/seed) was determined at 1- to 3-day intervals during the priming period by dilution plating on semi-selective medium of washing water from the seeds (Jensen *et al.*, 2002).

Assessment of seedling emergence

For growth chamber experiments treated seeds were sown in washed coarse sand moistened with tap water (3:1 v/v) in plastic boxes (50 seed/box) and incubated at 20°C ± 1°C as described by Jensen *et al.*, (2004). Each treatment was replicated four times (in total 200 seeds per treatment) and arranged in a fully randomized block design. Seedling emergence was recorded from 4 to 21 days after sowing. Dead and wilted plants were removed and post-emergence infection by *A. dauci* and *A. radicina* was confirmed by incubating the wilted plants near ultraviolet light (NUV) and examination by dissection microscopy. In field trials plots consisted of 10-metre doubled rows and each treatment had four replications in a ran-

domized block design. Seedling emergence was counted at approximately 50% emergence and at 100% emergence and expressed as plants per row.

Results and discussion

Priming of carrot seed having 7.5% and 5.1% infection with *A. radicina* and *A. dauci*, respectively, resulted in significant increase in occurence of both pathogens (Figure 1). Thus occurrence of *A. radicina* was increased 3-fold, while occurrence of *A. dauci* was increased 8-fold on primed seed. *A. alternata* occurred on almost all seed after priming. Other saprophytic fungi like *Ulocladium, Cladosporium,* Mucorales and *Penicillium* spp. occurred on < 2% of unprimed seed, but during priming occurrences of all these fungi increased to approximately 20%. Our results are in agreement with Tylkowska & Van den Bulk (2001) finding that hydropriming increased *Alternaria* spp. on carrot seed, and with Wright *et al.* (2003b) finding that sporulating fungi (no species identification) increased by 2 to 4 $\log_{10}$ cfu/seed during drum-priming. In contrast, biopriming with the *Clonostachys* isolates IK726, IK1871, IK1878 and IK1889 almost eradicated the *Alternaria* pathogens as well as the range of saprophytic fungi (Figure 1). Biopriming with *P. tabacinum* IK1755 did not suppress the naturally occurring microflora as efficiently as the *Clonostachys* spp. antagonists. While naturally occurring fungal species were significantly reduced on seed during biopriming, the applied BCAs themselves multiplied significantly during the priming period to reach a level of 4 to 8 x 10^4 cfu/seed (Figure 2a). The isolates of *C.rosea* and *C. rosea* f. *catenulata* had the highest

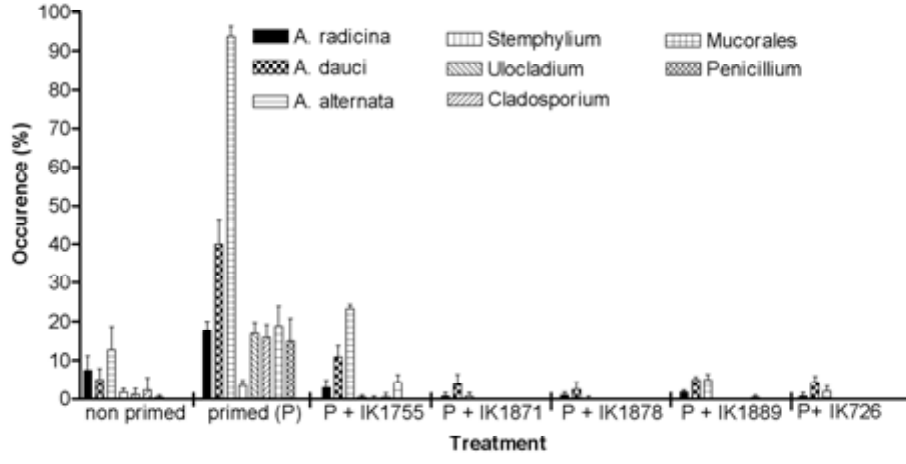

Figure 1. Effect of biopriming on occurrence of fungi on carrot seed. (IK726 = *Clonostachys rosea*, IK1878 = *C. rosea f. catenulata* and 1889 = *C. solani*).

proliferation rate. In a study with application of *Trichoderma* spp. during drum priming Wright *et al.* (2003a) found that the density of *T. harzianum* 1295-22 and *T. virens* G20 on seed either remained constant or decreased during priming of carrot seed, and they concluded that application of *Trichoderma* spp. during drum priming generally reflected survival of the inoculum rather than proliferation of these fungi. Thus, it seems that fungal isolates of *Clonostachys* spp. and *P. tabacinum* are more adapted to the high humidity during hydro-priming of carrot seed. Microscopy of seed during IK726 biopriming has revealed that the antagonist colonizes and sporulates the whole seed surface (Jensen *et al.*, 2004). Evaluation of the biocontrol efficacy in sand assays showed that biopriming with all BCAs improved plant emergence significantly compared with the priming treatment (Figure 2b). IK726 was the most effective antagonist resulting in 88% seedling emergence, which is not significantly different from the Iprodione treatment, while IK1755 was least effective with 71% seedling emergence. Moreover, all priming treatments decreased the period for maximum seedling stand from 10 to 8 days (Figure 2b).

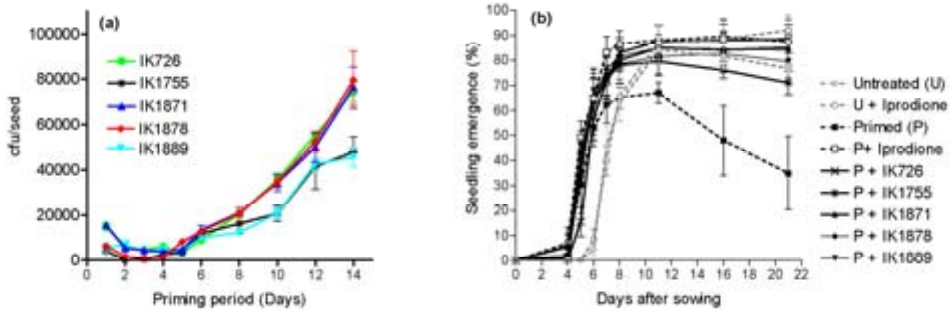

Figure 2. Biopriming of carrot seed with the fungal antagonists *Plectosporium tabacinum* 1755, *Clonostachys rosea* IK726 and IK1878, *C. rosea f. catenulata* IK 1878 and *C. solani* IK1889.
(a) Effect of biopriming on multiplication of antagonists on seed during the priming process.
(b) Effects of biopriming on establishment of carrot seedlings in a growth chamber sand test.

C. rosea IK726 was selected for further development and field trials among the initially tested and equally efficient *Clonostachys* spp. antagonists. Mainly because IK726 previously has proved effective in field control of seed-borne and soil-borne diseases (Knudsen *et al.*, 1995; Jensen *et al.*, 2000; Møller *et al.*, 2003). The present evaluation of the feasibility of integrating with commercial seed technologies showed IK726 to be compatible with colour, binder and the insecticide Promet used for film-coating at Danisco Seed, and IK726 also multiplied and survived at high number on seed following the biopriming procedure used at Emergent genetics™. The effect of biopriming or film-coating with IK726 and TRI003 on field establishment of carrot seedlings is shown in Figure 3a. Priming alone decreased the seedling

number compared with unprimed seed, which probably reflects that naturally occurring *Alternatia* pathogens have multiplied during priming. Biopriming with IK726 on the other hand increased the seedling emergence to the level of unprimed seed. The significantly highest plant stand was achieved with IK726-coated seed. The situation differed considerably when seeds were heat treated before either coating or biopriming (Figure 3b). No decrease in seedling establishment was seen after priming, probably because the heat treatment had eradicated the majority of the pathogenic *Alternaria* species. Furthermore, IK726 biopriming gave the significantly highest plant stand, which may be ascribed to control of soil-borne diseases. IK726 has previously shown biocontrol efficacy against *Pythium tracheiphilum* in field trials (Møller *et al.*, 2003). Biopriming with TRI003, with the *T. harzianum* strain 1295-22 as active ingredient, had no positive effects on seedling establishment. This is in agreement with Wright *et al.* (2003) finding that the 1295-22 was unable to proliferate during drum priming.

In conclusion fungal BCAs belonging to *Clonostachys* spp. in particular seem well adapted for application during priming of carrot seed. Since *C. rosea* IK726 is compatible with commercial pelleting and coating technologies, the perspectives for practical uses of this BCA are promising.

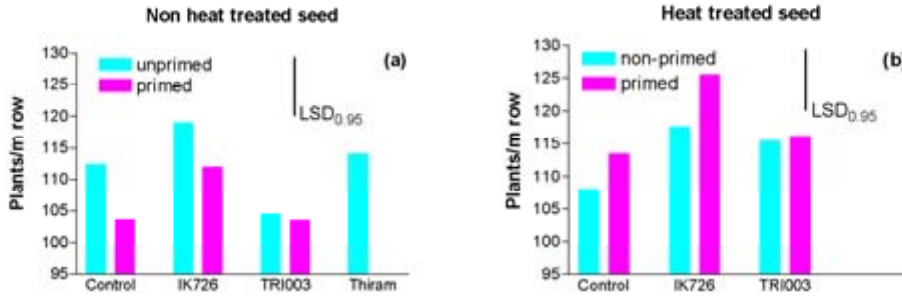

Figure 3. Coating and biopriming with IK726 and TRI003, respectively. (a) non heat treated carrot seed and (b) heat treated seed. Unprimed and primed seed were used as controls.

Acknowledgements

The research is supported by the European Commission Quality of Life and Management of Living Resources Programme (QoL), Key Action 1 on Food, Nutrition and Health QLKI-1999-0986 and the Danish Ministry of Food, Agriculture and Fisheries. Thanks to Danisco Seed a/s for coating and pelleting of carrot seed and for conducting field trials and to Emergent Genetics™ for priming, biopriming and heat treatment of seed for field trials.

References

Harman, G.E., Taylor, A.G. & Statsz, T.E. 1989: Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Disease 73: 631-637.

ISTA 1996: International Rules for Seed Testing, 1996. Seed Sci. Technol. 24 Supplement.

Jensen, B., Knudsen, I.M.B. & Jensen, D.F. 2000: Biological seed treatment of cereals with fresh and long term stored formulations of *Clonostachys rosea*: Biocontrol efficacy against *Fusarium culmorum*. Eur. J. Plant Pathol. 106: 233-242.

Jensen, B., Knudsen, I.M.B. & Jensen, D.F. 2002: Survival of conidia of *Clonostachys rosea* coated on barley seeds and their biocontrol efficacy against seed-borne *Bipolaris sorokiniana*. Biocontrol Science and Technology 12:427-441.

Jensen, B., Knudsen, I.M.B, Madsen, M. & Jensen, D.F. 2004: Biopriming of infected carrot seed with an antagonist selected for control of seed-borne *Alternaria* spp. Phytopathology 94: 551-560.

Knudsen, I.M.B., Hockenhull, J. & Jensen, D.F. 1995: Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: Effects of selected fungal antagonists on growth and yield components. Plant Pathol. 44: 467-477.

Møller, K., Jensen, B., Andersen, H.P., Stryhn, H. & Hockenhull, J. 2003: Biocontrol of *Pythium tracheiphilum* in chinese cabbage by *Clonostachys rosea*. Biocontrol Science and Technology 13: 171-182.

Rowse, H.R. 1996: Drum priming – an environmentally friendly way of improving seed performance. J. Royal Agricultural Soc. England 157: 77-83.

Tylkowska, K. & Van den Bulk, R.W. 2001: Effects of osmo- and hydropriming on fungal infestation levels and germination of carrot (*Daucus carota* L.) seeds contaminated with *Alternaria* spp. Seed Sci. Technol. 29:365-375.

Wright, B., Rowse, H.R. & Whipps. 2003a: Application of beneficial microorganisms to seeds during drum priming. Biocontrol Science and Technology 13, 599-614.

Wright, B., Rowse, H.R. & Whipps. 2003b: Microbial population dynamics on seeds during drum and steeping priming. Plant and Soil 255: 631-640.

Using simulation models as a training tool to improve biological control: Spider mites and predatory mites on greenhouse cucumbers as a case study

Gösta Nachman
Department of Population Biology, Institute of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark

Large-scale biological control experiments are both time-consuming and costly. Virtual experiments by use of computer simulation models may therefore serve as short cuts to obtain important information on complex agro-ecosystems. My presentation introduced a spatially explicit stochastic simulation model called *DynaMite*, which is part of a comprehensive system of various simulation programmes called *FIToM* (**F**rom **I**ndividuals **to** **M**etapopulations). The interactive model confronts the user with the decisions a grower has to make in order to protect his crop against an arthropod pest (in the present case spider mites attacking greenhouse cucumbers). Control options are spraying with different types of pesticides, inundative releases of predatory mites and replanting. Costs and benefits of the various control measures are evaluated, and at the end of a simulation, mimicking a growing season, the user is informed about his/her skills as a grower. The computer program may serve as a research tool to obtain better understanding of how spatial processes affect predator-prey dynamics so as to improve control strategies and as an educational tool to train students in the principles of biological control.

A preliminary version of *FIToM* is available via http://www.bi.ku.dk/staff/person.asp?ID=82. Users are welcome to contact me with comments and suggestions to improve the program.

Soil inoculation with *Metarhizium* and mycorrhiza: a method to enhance afforestation in Iceland and Faroe Islands

Edda Sigurdís Oddsdóttir[1], Charlotte Nielsen[2], Jørgen Eilenberg[2], Robin Sen[3], Guðmundur Halldórsson[1]

[1]Icelandic Forest Research, Mógilsá, IS-116 Reykjavik, Iceland; [2]Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark; [3]Environmental Sciences Group, The Macaulay Institute, Scotland, United Kingdom

Abstract: Iceland has suffered from severe ecosystem degradation since it's settlement in 874 and has lost more than 50% of the original vegetative cover. The cover of birch (*Betula pubescens* Ehrh.), the only native forest forming tree species in Iceland, has reduced greatly and now covers less than 1.2% of the land, compared to the estimated 20-25% in the year 874. Today birch is the most common species used in afforestation and is extensively used in land reclamation on degraded sites. Due to low survival rates of young tree-seedlings at these sites, it could be hypothesized that lack of beneficial soil biota is hampering afforestation. A Nordic co-operation programme aims to study the existence of beneficial soil fungi (mycorrhiza and insect pathogens) in soil from natural birch stands and degraded sites in Iceland and Faroe Islands and to inoculate birch seedlings with these beneficial soil fungi to enhance afforestation process in these countries. In our study no entomopathogenic fungi were found in soil collected from eroded land. In contrast two species, *Paecilomyces farinosus* and *Beauveria bassiana,* were documented from natural birch habitats and *Metarhizium anisopliae* was documented from grass habitats. The same pattern could be seen with mycorrhizal fungi: no fungi were found in eroded soil while several morphotypes were recorded in birch soil. The most prevalent mycorrhizal type proved to belong to the genus *Hebeloma.* Other common species were *Phialophora finlandia* and *Cenococcum geophilum*. Further, the results suggest that artificial inoculation of these beneficial fungi in areas planned for afforestation could assist in a successful re-establishment of birch in Iceland.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biocontrol of foliar diseases in horticulture: Screening and application of *Ulocladium atrum* for grey mould control

Jürgen Köhl
Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Abstract: The objective of our research was to develop a concept for biological control of *Botrytis* spp., to select antagonists and to test their use in economically important crops. Several research steps were necessary to develop such a biocontrol system: Epidemiological knowledge on diseases caused by *Botrytis* spp. in different crops was necessary to identify valid target tissues. Suitable antagonists with a high competitive ability during the colonisation of various necrotic plant tissues had to be selected. Antagonists with superior ecological competence had to be found. Knowledge on the mode of action of selected antagonists was needed to optimise targeting and timing of antagonist applications. The potential of the control strategy and applications of the selected antagonist had to be studied under field and greenhouse conditions.

Key words: *Botrytis cinerea*, *Ulocladium atrum*, antagonist screening, ecological competence

Introduction

Success of biocontrol depends on the use of antagonists with high efficacy and optimum adaptation to the niche in which they have to be active. Success of commercialisation of biocontrol agents depends on many more criteria. Consequently, many relevant criteria have to be considered in a screening programme aimed at the development of biocontrol agents besides efficacy testing.

Leaf surfaces are characterised by low soluble nutrient content and microclimatic conditions often unfavourable for microbial activity, e.g. water availability, UV radiation and temperature can fluctuate rapidly. Important criteria for applications of antagonists in the phyllosphere are cold tolerance, drought tolerance, resistance to high temperatures and UV irradiation and rainfastness of the applied inoculum (Diem, 1971; Hjeljord *et al*., 2000). Applied inoculum should be able to germinate and colonise the target tissue within the few hours during wetness periods which are often combined with low temperatures, e.g. during nights with dew formation. Important criteria for the feasibility of a commercial exploitation are, besides others, suitability for mass production and possible potential risks of certain antagonist candidates which may jeopardise registration.

Examples of testing various characteristics of antagonists under controlled conditions and field conditions are presented for the antagonist *Ulocladium atrum*. This antagonist has been selected for its ability to outcompete *Botrytis* spp. on necrotic plant tissues resulting in

suppression of sporulation of the necrotrophic pathogen. It has been demonstrated that *U. atrum* efficiently control diseases caused by *B. cinerea* in crops such as grapevine, tomatoes and ornamentals. The antagonist shows many characteristics which explain its good performance in the phyllosphere.

Efficacy testing of antagonists

The bioassay method developed for antagonist screening, based on interactions between *Botrytis* spp. and antagonistic fungi on dead leaf tissue, was an effective tool to select antagonists. Under constantly humid experimental conditions most of the antagonists suppressed sporulation of *B. aclada* efficiently in the initial screening experiments (Köhl *et al.*, 1995). Antagonists originating from necrotic leaf tissue such as *A. alternata* and *U. atrum* were highly antagonistic even after leaf wetness periods had been interrupted repeatedly on up to three consecutive days (Figure 1). On the other hand, soil-borne fungi such as *Gliocladium* spp. and *T. harzianum* showed sensitivity to interrupted leaf wetness periods especially during the early stage of colonisation at the first day after application.

Frequent and rapid fluctuations of the water potential may be highly restrictive for fungal development in the field, where periods of leaf wetness or high humidity are usually interrupted daily by dry periods. Antagonists aimed at suppression of sporulation of *Botrytis* spp. and other necrotrophic leaf pathogens on necrotic leaf tissue may only be reliable under field conditions if they are able to survive during dry periods and to start to regrowth rapidly with only short lag times after conditions become favourable for fungal growth again. Bioassays with interrupted leaf wetness periods were the key to differentiate antagonists according to their sensitivity to dry conditions during the colonisation process.

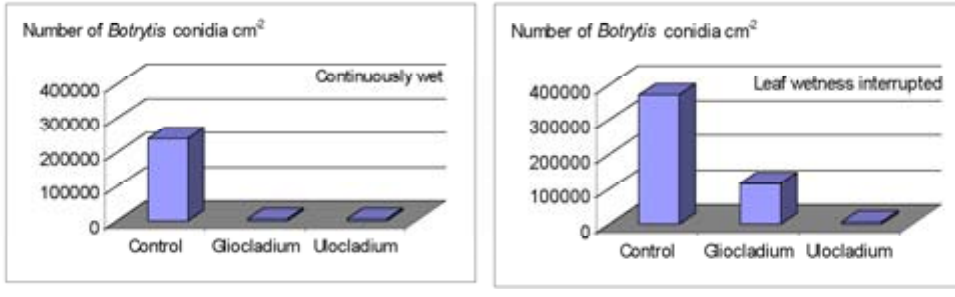

Figure 1. Effect of the antagonists *Gliocladium roseum* and *Ulocladium atrum* on sporulation of *Botrytis aclada* on dead onion leaves incubated at 18°C under continuously wet conditions (left) or with interruption of the leaf wetness period 16 h after antagonist application (From Köhl *et al.*, 1995).

Antagonist populations in the phyllosphere

After application of spore suspensions of antagonists on leaf surfaces in the open field, the biocontrol efficacy in most cases depends on the homogenous coverage of the canopy surface by the inoculum and its survival and persistence in the phyllosphere. When *U. atrum* conidia were applied in dense lily crops to control *Botrytis elliptica*, significantly less conidia were found on leaves from the bottom of the canopy compared to the middle or top levels of the canopy (Figure 2). Furthermore, the number of conidia on lily leaves declined over time. The uneven vertical distribution of antagonist inoculum may have significant epidemiological consequences since primary infections in the host canopies may commence on the older leaves at the bottom of the canopy where host and microclimate conditions are more conducive to infection and sporulation. These results indicate that the spraying system used in the study was not suitable and further evaluation of application methods may be required to improve penetration of the antagonist suspension in dense crops.

After antagonist application the number of *U. atrum* conidia declined. Rain and wind are important factors for dispersal of fungal spores. Rain splash has been shown to be an efficient mechanism for spreading large numbers of pathogen propagules from a source of inoculum but also wind dispersal may cause redistribution of fungal spores in a canopy (Vloutoglou *et al.*, 1995). The relative importance of wind or rain as dispersal mechanisms for redistributing antagonists such as *U. atrum* is poorly understood. The use of molecular markers may enable better investigation of the spatial and temporal dynamics of *U. atrum*.

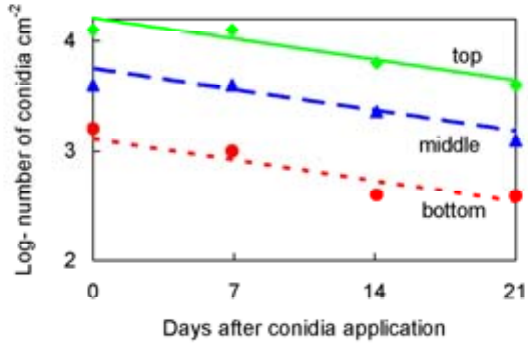

Figure 2. Number of conidia of *Ulocladium atrum* on leaves at the top, middle and bottom level of a lily canopy after spray application of suspensions containing $2x10^6$ conidia ml^{-2} (From Elmer & Köhl, 1998).

Major losses of antagonist inoculum, especially of ungerminated conidia, on leaf surfaces may be caused by rainfall. When conidia of *U. atrum* were sprayed on strawberry leaves and

incubated under dry conditions so that they remained ungerminated, it could be demonstrated that after a few hours even ungerminated conidia started to stick to the plant surface (Figure 3). The highest risk of losses caused by rainfall will thus be in situations of rain events shortly after antagonist conidia have been applied to the field. Further improvement of stickiness of conidia to leaf surfaces was possible by adding certain formulations.

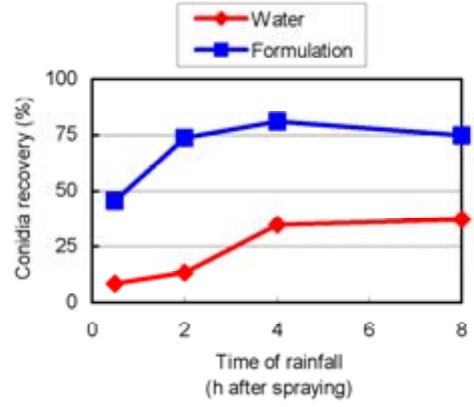

Figure 3. Effect of simulated heavy rainfalls on the recovery of conidia of *Ulocladium atrum* on leaf surfaces of strawberry. Conidial suspensions were sprayed with $1x10^6$ conidia ml^{-1} and the number of conidia cm^{-2} was assessed microscopically.

Besides fluctuations of water availability, UV irradiation is another detrimental factor for microorganisms on leaf surfaces. To study the possible effect of UV irradiation on conidia of *U. atrum*, conidia were sprayed in an onion crop on a hot and sunny day with maximum temperatures of 30°C at bright sunshine or after sunset. Leaf samples were collected during the consecutive days and conidial germination was assessed directly after sampling or after additional incubation in moist chamber. Since dry and hot weather did not support fungal development in the field, no germinated conidia were observed on leaves directly after sampling. After leaves had been incubated in the moist chamber, approximately 80% of the conidia were germinated (Figure 4). No differences were found in the germinability of conidia exposed after spraying to bright sunlight and those that had been sprayed after sunset.

Studies on the persistence of *U. atrum* conidia on cyclamen leaves under greenhouse conditions confirmed that propagules of this antagonist have the potential to survive on the surface of green leaves for at least seventy days under dry conditions. When such green leaves senesced seventy days after the antagonist application and thus became available as substrate for saprophytic colonisation by both *B. cinerea* and *U. atrum*, propagules of the antagonist still had the potential to compete successfully with the pathogen (Köhl *et al.*, 2000). Similar

results were even found in production systems with topirrigation with regular wetness periods in the cyclamen canopy.

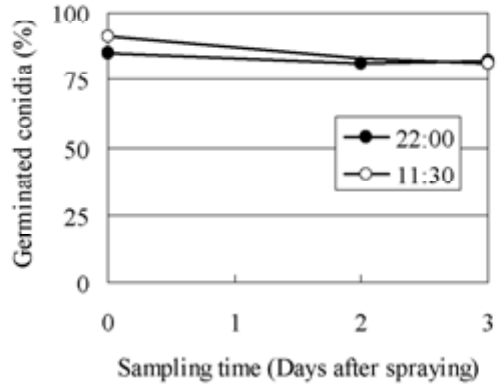

Figure 4. Percentage germinated conidia of *Ulocladium atrum* sprayed at bright sunlight (11:30) or after sunset (22:00) in an onion crop. Leaf samples were incubated in a moist chamber and conidia germination was observed microscopically.

Disease control under commercial growing conditions

Significant disease control of grey mould was achieved with spray applications of *U. atrum* in various crops, such as grapevine (Schoene & Köhl, 1999), tomato (Fruit & Nicot, 1999) and ornamentals (Köhl *et al.*, 2000). Extensive research has been carried out in greenhouse-grown cyclamen crops produced in various production systems. Regular application of conidial suspensions of *U. atrum* (1×10^6 conidia ml^{-1}) suppressed the incidence and severity of *B. cinerea* rot in cyclamen under commercial growing conditions (Table 1). Increasing the intervals between applications of the antagonist from two to four weeks resulted in similar control levels of disease incidence and disease severity. Regular applications of conidial suspensions of *Clonostachys rosea* (*G. roseum)* containing 2×10^6 conidia ml^{-1} suppressed the disease half as effectively as *U. atrum* and was similar to *U. atrum* treatments when applied with 1×10^7 conidia ml^{-1}. Such antagonist applications reached control levels similar to those of the standard fungicide programmes used by growers. The results of a series of experiments in different commercial greenhouses showed that *U. atrum* controlled *B. cinerea* when sprayed at four-week interval, except when the disease pressure of *B. cinerea* was extremely high. However, under such conditions even fungicide applications did not control the disease.

Table 1. Effect of treatments with *Ulocladium atrum* on the average number of diseased petioles of cyclamen plants of marketable age grown in a commercial greenhouse. Cyclamen were sprayed twice or three times during a growing period of approximately 230 days.

Treatment	Number of diseased petioles per plant
Water with 0.01% Tween 80	3.5 a*
Untreated	2.8 a
U. atrum, 3 applications **	0.9 b
U. atrum, 2 applications	0.7 b

* Figures with a common letter do not differ significantly (LSD, 5%).

** $1x10^6$ conidia ml^{-1}.

Conclusions

Several research steps were necessary for the development of a successful biocontrol system for foliar diseases caused by *B. cinerea* in horticultural crops based on competition between *B. cinerea* and an antagonist on necrotic plant tissues. Epidemiological knowledge on diseases caused by *Botrytis* spp. in different crops was necessary to identify valid target tissues. Only if necrotic tissues that are significant inoculum sources during *Botrytis* epidemics can be targeted with a biocontrol agent, such a strategy may result in disease control. Suitable antagonists with a high competitive ability during the colonisation of various necrotic plant tissues were selected in bioassays based on the assessment of competition on the natural substrate and avoiding any studies using *in vitro* systems, e.g. on artificial agar media. Antagonists with superior ecological competence could be found. Thorough knowledge on the mode of action of selected antagonists was needed to optimise targeting and timing of antagonist applications to different target substrates of different crops. Strong saprophytic competitive ability during colonisation of above-ground necrotic tissues depends on two parameters: (1) a high enzymatic activity for substrate utilisation (Berto *et al.*, 2001) and (2) the adaptation to the harsh microclimatic conditions in the phyllosphere. Before applying antagonists in large-scale field experiments, their ecological attributes had to be known in order to prevent failure under the prevailing environmental conditions. In our studies, we investigated the effect of different constant temperatures as well as constant and fluctuating water potential and the persistence of conidia of *U. atrum* under very different environmental conditions, e.g. in field-grown lily and greenhouse-grown cyclamen without and with top irrigation. The potential of the control strategy and applications of the selected antagonist *U. atrum* was then successfully proved in various crops under field and greenhouse conditions.

References

Berto, P., Jijakli, H.M. & Lepoivre, P. 2001: Possible role of colonization and cell wall-degrading enzymes in the differential ability of three *Ulocladium atrum* strains to control *Botrytis cinerea* on necrotic strawberry leaves. Phytopathology 91: 1030-1036.

Diem, H.G. 1971: Effect of low humidity on the survival of germinated spores commonly found in the phyllosphere. In: Ecology of leaf surface micro-organisms, eds. Preece, T.F. & C.H. Dickinson: 211-219.

Elmer, P.A.G. & Köhl, J. 1998: The survival and saprophytic competitive ability of the *Botrytis* spp. antagonist *Ulocladium atrum* in lily canopies. European Journal of Plant Pathology 104: 435-447.

Fruit, L. & Nicot, P. 1999: Biological control of *Botrytis cinerea* on tomato stem wounds with *Ulocladium atrum*. Integrated Control in Glasshouses. IOBC Bulletin 22, pp. 81-84.

Hjeljord, L.G., Stensvand, A. & Tronsmo, A. 2000: Effect of temperature and nutrient stress on the capacity of commercial *Trichoderma* products to control *Botrytis cinerea* and *Mucor piriformis* in greenhouse strawberries. Biological Control 19: 149-160.

Köhl, J., Gerlagh, M. & Grit, G. 2000: Biocontrol of *Botrytis cinerea* by *Ulocladium atrum* in different production systems of cyclamen. Plant Disease 84: 569-573.

Köhl, J., van der Plas, C.H., Molhoek, W.M.L. & Fokkema, N.J. 1995: Effect of interrupted leaf wetness periods on suppressing sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. European Journal of Plant Pathology 101: 627-637.

Schoene, P. & Köhl, J. 1999: Biologische Bekämpfung von *Botrytis cinerea* mit *Ulocladium atrum* in Reben und Cyclamen. Gesunde Pflanzen 51: 81-85.

Vloutoglou, I., Fitt, B.D.L. & Lucas, J.A. 1995: Periodicity and gradients in dispersal of *Alternaria linicola* in linseed crops. European Journal of Plant Pathology 101: 639-653.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biological control of arthropod pests in protected crops – recent developments

Annie Enkegaard
Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark

Abstract: Biological control of pests in protected vegetables has a long history and practical implementation is a routine measure for many glasshouse-grown vegetables in temperate regions. In recent years biocontrol of pests has in many countries been extended to include an increasing area of ornamental crops. In addition, other types of crops grown under protection (glass, plastic tunnels, etc.) have started to become the focus of development of biocontrol, e.g. herbs, hardy nursery stock and berries. The diversity of plants grown under protection reflects a great diversity in pest fauna, host plant characteristics and abiotic conditions and biocontrol programmes must therefore be specifically tailored for each culture. Since it can not be expected that a particular beneficial can perform equally well in all protected crops, methods attempting to remedy this fact are being sought. In the paper the recent achievements and the newest focus within the research on biocontrol of pests in protected crops will be described and exemplified.

Key words: Biocontrol, pests, protected crops, greenhouses, integrated control

Introduction

Aside from minor applications in the 1920-40s of the parasitoid *Encarsia formosa* against whiteflies in North European glasshouse tomatoes (Speyer, 1927), biocontrol in protected crops began for real in the 1960s with implementation of the predatory mite *Phytoseiulus persimilis* against spider mites (Bravenboer & Dosse, 1962) and the subsequent rediscovery and implementation of *E. formosa* in the 1970s (Hussey, 1985). The area on which these two biocontrol methods were used rapidly increased in Western Europe and Northern America (van Lenteren *et al.*, 1992). Simultanously, new beneficials were marketed for use against secondary pests and by the end of the 1980s, full biological control programmes for glasshouse vegetables were employed routinely implementing e.g. predatory mites and bugs against thrips, parasitoids and gallmidges against aphids and parasitiods against leafminers.

Early attempts to implement biocontrol in ornamentals were made in the late 1970s in a few North European countries (e.g. Wardlow, 1979; Stenseth, 1979), but the general notion was that biocontrol in ornamentals on a larger scale was unrealistic (van Lenteren & Woets, 1988), primarily because of the low damage threshold of these cultures. However, a breakthrough occurred in the late 1980s due to increasing resistance problems and reduced

availability of pesticides combined with an increasing number of commercially available beneficials (Figure 1) and a shift in release strategies from inoculative to inundative (Brødsgaard, 1995) and/or preventive releases. The uptake of biocontrol among ornamental growers has, however, been slower than among vegetable growers due to factors such as zero-tolerance for export items; complex production processes; many pest species; lack of safety periods; and recent marketing of pesticides for which resistance among pest species has not yet evolved. Despite these limitations implementation of biocontrol in ornamentals (e.g. in Chrysanthemum, roses, Gerbera and Poinsettia), especially in northern temperate regions, in some countries now amounts to up to 10-35% of the area (Enkegaard, 2003).

Despite the great success for biocontrol in protected crops, there is, of course, still much to be done. This is detailed below.

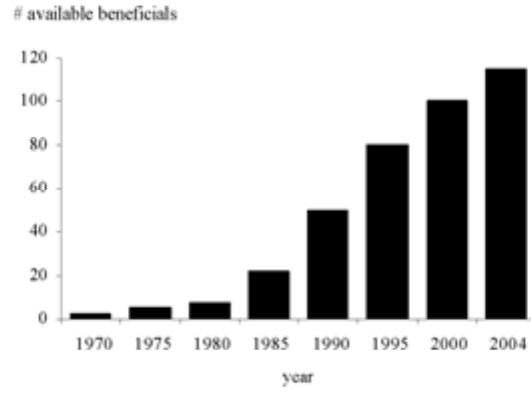

Figure 1. Development in number of commercially available beneficial arthropods. Adapted from van Lenteren & Nicoli (2004).

Implementation in additional protected crops

Protected crops are grown in a diversity of constructions offering protection to the outdoor climate, i.e. glasshouses, greenhouses, screenhouses, plastic tunnels and the like. The crops grown under protection encompass a wide variety of plant species and cultivars, including various vegetables (e.g. tomato, cucumber, sweet pepper, egg plant, melon, leafy salad, leek, radish), herbs (e.g. parsley, dill, thyme, basil), berries (e.g. strawberry, grape), medicine plants (e.g. ginseng, rose hips) and ornamentals. Ornamentals constitute the most diverse category of protected crops with more than 400 species of cutting plants, pot plants, bedding plants, green foliage, hardy nursery stocks and bulbs grown in Europe alone (van Lenteren, 2000). This great diversity in the floral composition of protected crops reflects in an equal diversity in climatic conditions, growing practices, host plant characteristics and pest fauna, and consequently also in the species of beneficials needed for biocontrol. It can therefore not

be expected that a particular beneficial can perform equally well in all protected crops and biocontrol programmes must therefore be specifically tailored for each culture.

Examples of protected cultures which recently have been targeted with such programmes are lettuce and leafy salad (e.g. Bennison *et al.*, 1999; Head *et al.*, 2002), grapes (Biobest, 2005), leek (Rat-Morris, 1999), herbs (Bennison *et al.*, 2005), hardy nursery stocks (Bennison *et al.*, 2002; van der Linden, 2002) and strawberry (Saiki & Wada, 2005). Development of biocontrol programmes involve pinpointing beneficial species useful for the specific conditions of the crop, determining release rates, frequencies and methods, integrating the use of biocontrol with other methods, e.g. against diseases, and estimating the economical feasibility of the programme. As an example, an integrated programme for strawberry with emphasis on biocontrol could be as shown in Table 1.

Improvement of efficacy of commercially available beneficials

In many cases an existing, commercially available beneficial may not always be sufficiently effective in achieving satisfactory biocontrol. This can be a result of various factors, e.g. host plant characteristics: climatic conditions or growing practices affecting the beneficial negatively; unability of the beneficial to cope with pest densitites above a certain level; poor quality; or suboptimal adaptation to pests that, at first, may be percieved as suitable hosts. An example of the latter is the aphid parasitoid *Aphidius colemani*, which is very efficient against some aphids (e.g. green peach aphids) but not others (e.g. foxglove aphids and potato aphids).

Table 1. An example of an integrated management programme for strawberry under protection.

Pest	**Control methods**	**Beneficials**
Spider mites	Biocontrol	Predatory mites (*Phytoseiulus persimilis, Amblyseius californicus*); Gall midge (*Feltiella acarisuga*)
Strawberry mites	Biocontrol	*Predatory mite (*Amblyseius cucumeris*)*
Thrips	Biocontrol	Predatory mite (*A. cucumeris*); Predatory bugs (*Orius spp.*)
Aphids	Biocontrol	Parasitoids (*Aphidius colemani, A. ervi, Aphelinus abdominalis*); Gall midge (*Aphidoletes aphidimyza*); Lady beetle (*Adalia bipunctata*); Lacewings (*Chrysoperla spp.*)
Whiteflies	Biocontrol	*Parasitoid (*Encarsia formosa*)*
Vine weevils	Biocontrol	Nematodes (*Heterorhabditis spp.*)
Caterpillars	Biocontrol	Bacteria (*Bacillus thuriengiensis*); Lacewings (*Chrysoperla spp.*)
Nematodes	Chemical/cultural	
Diseases	Chemical/cultural	

One way to overcome such problems would be just to increase the release rates and/or release frequencies of the beneficial, but this is, of course, in many case uneconomical, and other ways to compensate for suboptimal efficiency have been devised.

Reduce pest densities

Pest densities may be reduced in various ways in an attempt to help the beneficials to gain control, the most obvious being a limited use of chemicals for instance prior to beneficial release or as hot spot treatment in cultures where beneficials are already applied (e.g. van Driesche *et al*., 1999; Richter, 2005). Use of nettings to reduce influx and subsequent build-up of pests is a well-known method in warmer regions, e.g. the Mediterranean area, but may also be useful in cooler areas (Teerling & Murphy, 1999). Likewise the use of cultivars with partial resistance towards pests is a theoretical option, although marketing of such cultivars so far has not been seen in spite of research on development of methods for screening for host plant resistance against various pests (e.g. Balkema-Boomstra *et al*., 1999; Sütterlin, 1999).

Condition or select for better performance

Attempts to improve the efficacy of beneficials through selection/conditioning have been few in the history of biocontrol in protected crops. Only the selection of strains of *P. persimilis* with resistance to organophosphorous pesticides (to allow their integration in management programmes employing these) (e.g. Croft & Morse, 1979; Schulten, 1980) and with an improved adaptation to tomatoes (the original strain did poorly on tomato due to trichome exudates) (Drukker *et al.*, 1996), respectively, has resulted in commercial products. Selection of a strain of *E. formosa* better adapted to low temperatures (to facilitate its use in low-temperature (and thus energy-saving) tomatoes) (Klapwijk, 1999) has been researched but not yet implemented.

Combine several beneficial species

Other ways to circumvent insufficient effect of one beneficial is to combine the use of several species against the same pest. Thus, biocontrol of thrips is often based upon a simultaneous use of several predatory mites (some adapted to leaves, some to flowers and some to soil), predatory bugs and mirids, nematodes and fungi. Likewise biocontrol of aphids is often based upon a combined use of several parasitoids, gallmidges, lacewings, coccinellids and fungi. By using several beneficial species against the same pest it becomes easier to effectively target all life stages of the pests and to target the pest in the various phases of the production cycle.

Increase dispersal of beneficials

Suboptimal efficiency of a beneficial may result from slow dispersal. This can in some cases be remedied by increasing the number of release sites either manually or mechanically assisted by blowing beneficials out in the culture via battery-powered air blowers, such as is used in some places for distribution of the predatory mite *Amblyseius cucumeris* (van Driesche *et al*., 2002). When insect pathogenic microorganisms are used, dispersal may be increased through vectoring with bumble bees already installed in the culture for pollination (Kapongo *et al*., 2005).

Supplement food

Beneficials may be assisted in their establishment and survival if food or prey items are supplied.

This may be of assistance when target prey densities are low or even when prey is absent, as in case of preventive releases. Supplement food can be provided as "lunchboxes" directly in the product, e.g. a few spider mites are often included in products of *P. persimilis*; or the supplement food may be applied directly to crop in the form of e.g. pollen to assist *Amblyseius* mites in non-pollen crops or moth eggs for assistance to mirid bugs.

The strategy termed pest-in-first is another way to assist beneficial establishment – in this strategy, used by some growers of e.g. pepper and egg plants (e.g. Bolckmans & Tetteroo, 2002), the grower deliberately starts a small infestation of pests hereby creating a more stable foundation for establishment and build-up of the subsequently released beneficials.

Yet another method for assisting establishment of beneficials is by application of breeding units, exemplified with the slow-release system developed and implemented on a large scale for *A. cucumeris*. Here the mites are delivered in small sachets containing mould mites as a food base for reproduction. The mites reproduce in the sachets and the progeny will disperse into the crop over a 4-6-week period.

Banker plants, trap crops and push-pull strategy

Breeding units can also be in the form of plant-based rearing units, the so-called banker plants. A banker plant is a plant, e.g. a cereal, infested with a herbivore that does not attack the crop, e.g. cereal aphids. Several such plants are distributed in the crop and beneficials, e.g. aphid parasitoids, released on them. The beneficials reproduce on the herbivore and the fresh new off-spring disperse to the crop and attack the crop-harbouring pests. In this way the beneficials can be established preventively in the crop prior to any pest infestation and subsequently maintained at sufficient densities, also during periods with low levels of pests. The use of banker plants reduces the need for frequent inundative releases and are therefore generally cheaper than repeated inundative releases.

The use of banker plants for aphid parasitoids have been implemented for many years in protected crops and these systems are now commercially available. But new banker plant systems are in use or under development – examples are given in Table 2, but there are many other possibilities. Banker plants have played a very important role for implementation of biocontrol in ornamentals.

Table 2. Examples of banker plant systems in use or under development.

Plant	**Herbivore**	**Beneficial**	**Target**	**Crop**
Cereal	Cereal aphids	Gall midge (*A. aphidoletes*)	Aphids	Sweet pepper
Ranunculus	Leafminers	Parasitoids (*Diglyphus isaea, Dacnusa siberica*)	Leafminers	Lettuce
Corn	Grass mites	Predatory mite (*P. persimilis*)	Spider mites	Ornamentals
Nipplewort	Cabbage whitefly	Parasitoids (*E. formosa*)	Whiteflies	Ornamentals
Tobacco	Moth eggs	Mirids (*Macrolophus caliginosus, Hesperus* spp.)	Whiteflies	Ornamentals
Castor bean	(Pollen)	*Amblyseius*-mites	Thrips	Sweet pepper

Trap crops

Trap crops are rather similar in concept to banker plants. Trap crops are plants or cultivars especially attractive to pests and can therefore act to lure these from the crop plants. Trap plants themselves are useful for monitoring and may, in addition, assist biocontrol by acting to reduce pest densities on the crop plants. In addition trap plants may be converted into banker plants by releasing beneficials on to them. Trap plants are in use in an increasing number of biocontrol programs for protected crops – a few examples are provided in Table 3.

Table 3. Examples of systems of trap crops under development/consideration.

Crop	**Trap plant**	**Target pest**
Tomato	Tobacco	Whiteflies
Fuchsia	Tomato	Whiteflies
Alstromeria	Sweet pepper	Aphids
Tomato	Bean	Spider mites
Bedding plants	Verbena	Thrips
Cucumber	Egg plant	Whiteflies, aphids, spider mites

Push-pull strategy

The principle of trap crops is exploited in the push/pull strategy which aims at "pushing" the pests from the crop plant, thus helping to reduce pest densities, for instances by use of repellents, antifeedants or oviposition deterrents, and "pulling" them onto trap plants through the attractive action of the plant itself perhaps assisted by application of attractive substances (e.g. sex pheromones) on the trap plant and subsequently applying beneficials to the trap plants As an example, push/pull strategies for the thrips, *Frankliniella occidentalis* is under development using e.g. carvacrol as a repellent and using attractive plants and nerol or the plant volatile (*E*)-b-farnesene as attractants combined with applications of predatory bugs (*Orius spp.*) and soil-dwelling predatory mites (*Hypoaspis spp.*) on the trap plant (Bennison *et al.*, 2003; Kornherr & Blümel, 2005; Kornherr *et al.*, 2005).

Finding new beneficials

In spite of the various methods for assistance to beneficial establishment and survival described above there are cases where it is too difficult to obtain satisfactory control with the commercially available beneficial. In these cases the only remaining option if biocontrol is wished for is to find new beneficial species that are better adapted to the specific crop/climate/growing practices in which their presence is required or with better control efficacy to the particular pest in question. These particular pests may be current troublesome pests like some species of thrips and whiteflies, or they may be pests that are in the uprising

either as secondary pests or pests in the process of expanding their geographical range. Search for beneficials may also be motivated by a wish to find candidates more easily used preventively than existing beneficials or by a need to find indigenous beneficials, if use of a particular commercial beneficial is prohibited on account of being exotic.

New beneficials are marketed at regular intervals. Within the past 10 years marketed beneficials have for instances included mirid bugs against whiteflies and other pests; several species of *Amblyseius* mites against thrips, spider mites and whiteflies; a gall midge against spider mites; *Eretmocerus* parasitoids against whiteflies; and a staphylinid beetle against shore flies and sciarids. A native European lady beetle, *Adalia bipunctata*, is also a recent addition, replacing the formerly used exotic species.

Potential candidates for near-future commercialisation are the anthocorid *Anthocoris nemorum* (Meyling *et al.*, 2003), the hunter fly *Coenosia attenuata* (Sensenbach *et al.*, 2005) and perhaps shore fly parasitoids (Vänninen, this issue). But there are, of course, many more potential candidates - 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 funding needed for their discovery, evaluation and utilisation is available (Enkegaard & Brødsgaard, 2005).

Adapting biocontrol to new technologies/growing practices

Greenhouse technologies and growing practices for protected crops are not *status quo* but change over time for reasons not related to biocontrol but for energy saving purposes or in attempts to optimise plant growth and yield. These changes in technologies and practices may influence the biology of pests and beneficials in various ways and thus the outcome of biocontrol, and biocontrol pest management programmes consequently need to be adapted to these changes.

As an example, a new principle for climate control is in progress – dynamic climate, developed for energy saving purposes. The principle in dynamic climate control is to supply heat and CO_2 only when the plants can make use of it, i.e. when the light intensity is sufficiently high. As a consequence, the outdoor climate will influence the climate in the greenhouse to a larger extent than previously and larger temperature fluctuations will occur both on a daily and on a seasonal basis. The implications of this new climate control for biocontrol are presently under investigation (Jakobsen *et al.*, 2005).

Other changes in greenhouse technologies involve manipulations to increase photosynthesis and regulate plant growth and flowering through changes in light intensity, spectral composition and day length. These manipulations may be associated with changes in growing practices – e.g. pruning, choice of cultivar, changes in plant densities etc. all of which may affect biological control (Vänninen & Johansen, 2005).

Implement novel ideas

Various techniques used in pest management in outdoor crops are from time to time sought implemented in protected crops in an attempt to assist biological control. Examples of such ideas, novel to protected crops, are the use of mating disruption, sterile insect technique and plant growth promoting rhizobacteria.

Mating disruption

With the mating disruption (MD) technology the habitat of a pest is flooded with pest specific pheromones, causing confusion among males and making them unable to locate females, hereby reducing or preventing reproduction. This technique is used successfully in e.g. vineyards, orchards and forests and has recently been investigated for its potential in protected crops for instance against the tomato pinworm in tomato (Ferguson *et al.*, 1999), for the cabbage looper in pepper (McGregor *et al.*, 2002) and for tortricids in ornamentals (Quaglia, 1993). The use of MD may stand alone or be a tool to reduce pest densities to facilitate the action of released beneficials. A prerequisite, of course, is that the beneficials will work sufficiently well in the pheromone-loaded environment which for instance is the case with application of MD against the cabbage looper where the use of pheromones did not significantly interfere with the performance of *Trichogramma* wasps (McGregor *et al.*, 2002).

Sterile insect technique

Another novel technique for protected crops is the use of Sterile Insect Technique (SIT) which involves mass rearing of the pest and sterilising the males by exposing them to low doses of radiation. These sterile male flies are then released in the pest habitat where they compete with wild males for mating of the females. The result is reduced reproduction and decline in the pest population density, or perhaps even eradication.

Investigation into the use of SIT as a supplement to control *Liriomyza* leafminers is ongoing in California: here it has been possible to successfully sterilise the leafminer species in question and obtain viable and competitive males (Kaspi & Parrella, 2002). When applied this technique helps to reduce pest density making it easier to get efficient control with leafminer parasitoids. The researchers have demonstrated that a combination of SIT and biocontrol is more effective for control of leafminers than either technique alone (Weintraub & Cheek, 2005).

The SIT technique has also been investigated for the whitefly *Trialeurodes vaporariorum* where slower population developments have been obtained even though population reductions were not accomplished because of the arrhenotokous nature of the whitefly resulting in production of males from unfertilised eggs (Calvitti *et al.*, 1998). In principle, however, SIT may be used to assist released beneficials in their control of whiteflies.

Plant growth-promoting rhizobacteria

Plant growth-promoting rhizobacteria (PGPR), initially found to be interesting due to their plant growth stimulating effects, have been shown also to have resistance inducing properties both to diseases and pests. For field crops it has been shown that PGPR convey some degree of resistance to cucumber beetles and aphids on tomato and ongoing research in e.g. Poland is

investigating the possibilities of exploiting PGRB in greenhouses. It has been shown that application of these bacteria results in decreased densities of spider mites and that plants grown with these bacteria seem to be compatible with biocontrol – in fact stimulating the activity of predatory mites (Tomczyk, 1999; Tomczyk & Burda, 2005).

The future

The total area worldwide used for production of protected crops is estimated to be about 300,000 ha (of which about 50,000 ha is under glass) with about 200,000 ha used for production of vegetables (van Lenteren, 2000). Although the developments within biocontrol in protected crops in the past decades have been encouraging, the area treated with biocontrol is still rather small. 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.

References

Balkema-Boomstra, A.G., Ziljstra, S. & van der Helm, F. 1999: A rapid method to test resistance to spider mite (*Tetranychus urticae*) in cucumber. IOBC/wprs Bull. 22(10), 9-12.

Bennison, J., Maulden, K. & Wardell, G. 1999: Integrated control of the South American leafminer *Liriomyza huidobrensis* on UK glasshouse lettuce and Chinese leafy salad crops. IOBC/wprs Bull. 22(1), 9-12.

Bennison, J., Maulden, K. & Maher, H. 2002: Choice of predatory mites for biological control of ground-dwelling stages of western flower thrips within a "push-pull" strategy on pot chrysanthemum. IOBC/wprs Bull. 25(1), 9-12.

Bennison, J., Umpelby, R. & Buxton, J. 2002: IPM on protected hardy ornamental nursery stock in the UK. IOBC/wprs Bull. 25(1), 13-16.

Bennison, J., Green, K. & O'Neill, T. 2005: Best Practice Guide for integrated pest and disease management on UK protected herbs. IOBC/wprs Bull 28(1), 15-18.

Biobest. 2005: http://www.biobest.be/, accessed 31st October 2005.

Bolckmans, K.J.F. & Tetteroo, A.N.M. 2002: Biological pest control in egg plants in the Netherlands. IOBC/wprs Bull., 25(1), 25-28.

Bravenboer, L. & Dosse, G. 1962: *Phytoseiulus riegeli* Dosse als Predator einiger Schadmilben aus der *Tetranychus urticae* gruppe. Entomol. Exp. Appl. 5, 291-304.

Brødsgaard, H.F. 1995: 'Keep down' - A concept of thrips biological control in ornamental pot plants. In B. L. Parker, M. Skinner & T. Lewis (Eds.), Thrips biology and management (pp. 221-224). Plenum Publishing Corporation, New York.

Calvitti, M., Remotti, P.C., Pasquali, A. & Cirio, U. 1998: First results in the use of the sterile insect technique against *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) in greenhouses. Ann. Ent. Soc. Am. 91, 813-817.

Croft, B.A. & Morse, J.G. 1979: Research advances on pesticide resistance in natural enemies. Entomophaga, 24, 3-11.

Drukker, B., Janssen, A., Ravensberg, W. & Sabelis, M.W. 1996: Improved control capacity of the mite predator *Phytoseiulus persimilis* on tomato. Exp. Appl. Acarol. 21, 507-518.

Enkegaard, A. 2003: Sting. Newsletter on biological control in greenhouses, No. 25 July 2003. Retrieved 31st October 2005 from http://web.agrsci.dk/plb/iobc/sting/sting25.htm.

Enkegaard, A. & Brødsgaard, H.F. 2005: Biocontrol in protected crops: is lack of biodiversity a limiting factor? In Eilenberg, J. and Hokkanen, H.T.M. (eds.): An Ecological and Societal Approach to Biological Control. Springer, in press.

Ferguson, G.M., Shipp, J.L. & Hunt, D.W.A. 1999: Evaluation of Pheromone Concentrate for Control of Tomato Pinworm in Greenhouse Tomatoes. IOBC/wprs Bull. 22(1), 73-76.

Head, J., Palmer, L.F. & Walters, K.F.A 2002: Development of an integrated control strategy for leafminers in leaf salads with potential for extrapolation to other cropping systems. IOBC/wprs Bull. 25(1), 97-100.

Hussey, N.W. 1985: History of biological control in protected culture. In N. W. Hussey & N. Scopes (Eds.) Biological pest control – The glasshouse experience (pp. 11-22). Blandford Press, Poole.

Jakobsen, L., Brogaard, M. Körner, O., Enkegaard, A. & Aaslyng, J.M. 2005: The influence of a dynamic climate on pests, diseases and beneficial organisms: recent research. IOBC/wprs Bull. 28(1), 127-134.

Kapongo, J.P., Shipp, L., Kevan, P. & Broadbent, B. 2005: Optimal concentration of *Beauveria bassiana* as vectored by bumblebees for pest control on sweet pepper. IOBC/wprs Bull. 28(1), 143-146.

Kaspi, R. & Parrella, M. 2002: The potential of Sterile Insect Technique (SIT) as one of the strategies for control of *Liriomyza trifolii* (Diptera: Agromyzidae) infesting greenhouse crops. IOBC/wprs Bull. 25(1), 123-126.

Klapwijk, J.N. 1999: Biological control of tomato pests in the Netherlands. IOBC/wprs Bull., 22(1), 125-128.

Kornherr, C. & Blümel, S. 2005: Attraction of the monoterpenoids nerol and carvacrol to the predatory flower bug *Orius laevigatus* (Fieber). IOBC/wprs Bull 28(1), 159-162.

Kornherr, C., Hausdorf, H. & Blümel, S. 2005: Side effects of the monoterpenoids nerol and carvacrol on the predatory flower bug *Orius laevigatus* (Fieber) in the laboratory. IOBC/wprs Bull 28(1), 163-166.

McGregor, R.R., Gillespie, D.R., Quiring, D.M.J. & Foisy, M.R.J. 2002: Mating disruption of cabbage loopers (*Trichoplusia ni*, Lepidoptera: Noctuidae) and the response of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) to host pheromones in pepper greenhouses. IOBC/wprs Bull. 25(1), 173-176.

Meyling, N.V., Enkegaard, A. & Brødsgaard, H.F. 2003: Two *Anthocoris* bugs as predators of glasshouse aphids – voracity and prey preference. Entomologia Experimentalis et Applicata 108, 59-70.

Quaglia, F. 1993: Populations dynamics of tortricids (*Cacoecimorpha pronubana* (Hb.) and *Epichoristodes acerbella* (Walk.)) on ornamentals, with special reference to the potential use of sex-pheromones for monitoring, mass-trapping and mating disruption. Frustula Entomologica, 16, 1-7.

Rat-Morris, E. 1999: Biological control of *Thrips tabaci* on protected leek seed crops. IOBC/wprs Bull. 22(1), 201-204.

Richter, E. 2005: Can integrated pesticides improve biological control of *Bemisia tabaci* in *Euphorbia pulcherrima*? IOBC/wprs Bull. 28(1), 209-212.

Saiki, Y. & Wada, T. 2005: Biological control in strawberry in Japan. IOBC/wprs Bull. 28(1), 213-216.

Schulten. 1980: A strain of *Phytoseiulus persimilis* (Acari: Phytoseiidae) resistant to organophosphorus compounds for control of spider mites in greenhouses. In A. K. Minks & P. Gruys (Eds.), Integrated control of insect pests in the Netherlands. Wageningen: Centre for Agricultural Publishing and Documentation, 119-120.

Sensenbach, E.J., Wraight, S.P. & Sanderson, J.P. 2005: Biology and predatory feeding behaviour of larvae of the hunter fly *Coenosia attenuata*. IOBC/wprs Bull. 28(1), 229-232.

Speyer, E.R. 1927: An important parasite of the greenhouse whitefly. Bull. Ent. Res. 17, 301-308.

Stenseth, C. 1979: Biological control in Norway 1978. In J. C. van Lenteren & J. Woets (Eds.), Sting. Newsletter on biological control in greenhouses, No. 2, May 1979. Retrieved 31st October 2005 from http://web.agrsci.dk/plb/iobc/Sting2.pdf

Sûtterlin, S. 1999: Plant resistance in rose cultivars to *Frankliniella occidentalis*: a commercial test. IOBC/wprs Bull. 22(10), 53-58.

Teerling, C.R. & Murphy, G. 1999: Experiences with insect exclusion screening of greenhouse vents in Ontario, Canada. IOBC/wprs Bull. 22(1), 247-250.

Tomczyk, A. 1999: The use of plant growth promoting rhizobacteria (PGPR) to decrease the susceptibility of cucumber to spider mites. IOBC/wprs Bull. 22(1), 251-254.

Tomczyk, A. & Burda, W. 2005: Behaviour and activity of *Phytoseiulus persimilis* (A.-H.) on mite infested cucumber plants cultivated in the presence of plant growth promoting rhizobacteria (PGPR). IOBC/wprs Bull. 28(1), 267-270.

van der Linden, A. 2002: State of integrated crop protection in Dutch nursery stock and future prospects. IOBC/wprs Bull. 25(1), 269-272.

van Driesche, R.G., Hoddle, M.S., Lyon, S. & Sanderson, J.P. 1999: Use of insect growth regulators to reduce rates of *Eretmocerus eremicus* needed for biological control of whiteflies on poinsettia. IOBC/wprs Bull. 22(1), 61-64.

van Driesche, R., Lyon, S., Sanderson, J., Smith, T., Lopes, P., MacAvery, S., Rusinek, T. & Couch, G. 2002: Greenhouse trials in Massachusetts and New York with *Amblyseius cucumeris*: effects of formulation and mechanical application. IOBC/wprs Bull. 25(1), 273-276.

van Lenteren, J.C. 2000: A greenhouse without pesticides: fact or fantasy? Crop Protection, 19, 375-384.

van Lenteren, J.C. & Nicoli, G. 2004: Quality control of mass-produced beneficial insects. In K. M. Heinz, R. G. van Driesche & M. P. Parrella (Eds.), Biocontrol in Protected Culture (pp. 503-526). Ball Publishing, US.

van Lenteren, J.C. & Woets, J. 1988: Biological and integrated pest control in greenhouses. Ann. Rev. Entomol., 33, 239-269.

van Lenteren, J.C., Benuzzi, M., Nicoli, G. & Maini, S. 1992: Biological control in protected crops in Europe. In J.C. van Lenteren, A.K. Minks & O.M.B. de Ponti (Eds.) Biological control and integrated crop protection: Towards environmentally safer agriculture. (pp. 77-89). Pudoc, Wageningen.

Vänninen, I. & Johansen, N.S. 2005: Artificial lighting (AL) and IPM in greenhouses. IOBC/wprs Bull. 28(1), 295-304.

Wardlow, L. 1979: Integrated pest control on chrysanthemums. Forward, Dec. 1979.

Weintraub, P. & Cheek, S. 2005: Need for new biocontrol agents in greenhouse IPM – a European perspective. IOBC/wprs Bull. 28(1), 317-324.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Parasitoids as biocontrol options of shore flies (Diptera: Ephydridae)

Irene Vänninen, Marika Linnamäki
Agrifood Research Finland, Plant Production Research, FIN-31600 Jokioinen

Abstract: *Aphaereta debilitata* Morley (Hym.: Braconidae), *Kleidotoma psiloides* Westwood (Hym.: Figitidae), and *Hexacola neoscatellae* Beardsley (Hym.: Figitidae) are all larval parasitoids of either *Scatella stagnalis* or *S. tenuicosta*. Parasitoids have the advantage over other biocontrol agents such as predatory mites and insect parasitic nematodes of being able to function also in wet conditions such as heavily irrigated rockwool cubes or peat pots. We studied the basic biology of *A. debilitata* at constant 25ºC and conducted a cage experiment with two release rates and strategies of the parasitoid against *S. tenuicosta* in summer temperature conditions. The high parasitisation efficacy and the ability of *A. debilitata* to suppress the host population when released in the first period of the 40-day experiment showed that the parasitoid has a good potential of being an inoculative biocontrol agent of shore flies.

Key words: Greenhouse vegetables, cucumber, lettuce, herbs, inoculative biological control

Introduction

The algal feeding shore flies *Scatella* sp. (Diptera, Ephydridae) (Zack & Foote, 1978) are nuisance and cosmetic pests of lettuce, herbs and cucumber seedlings. The control of flies with chemicals has proven difficult (Lindquist & Casey, 1994). Hydrogen peroxide reduces algal growth and thus the reproduction of flies but can be phytotoxic (Vänninen & Koskula, 1998). Nematodes and predatory mites control shore flies in peat only when very high release rates are used (Vänninen & Koskula, 2003, 2004).

Three parasitoid species, *Aphaereta debilitata* Morley (Hym.: Braconidae) (Vänninen & Koskula, 1998), *Kleidotoma psiloides* Westwood (Hym.: Figitidae) (Michael de Courcy-Williams, pers. comm.) and *Hexacola neoscatellae* Beardsley (Hym.: Figitidae) (Diamond *et al*., 2001) are all larval parasitoids of either *Scatella stagnalis* or *S. tenuicosta*. The advantage of parasitoids may be their ability to exert control in wet media, such as heavily irrigated rockwool not conducive to efficacy of neither nematodes (Vänninen & Koskula, 2003) or predatory mites (Vänninen & Koskula, 2004). Furthermore, the parasitoids may be more specialised on shore flies than nematodes and predatory mites. The host ranges of the above parasitoids are currently not known. As to *A. debilitata*, however, only a few Alysiinae species inhabit more than one microhabitat and most species appear to restrict their host range to one family only (Vet & van Alphen, 1985).

We studied the development of and host utilisation by *A. debilita* to see if the parasitoid could exert control on shore flies known to reproduce very quickly, especially in summer conditions with temperatures of 25°C and above.

Material and methods

Parasitisation of host larvae, parasitoid development times and sex ratio at constant 25°C

Fifteen host larvae aged <1 and 1 day, and 20 host larvae aged 2 or 3 days were exposed to one parasitoid female for 16.5 hours. In the dishes, the larvae lived on a small circle of moist capillary matting covered with green algae. There were four replicate dishes per age class. After 16.5 hours females were removed. On day six after egg-laying of flies, 10 larvae from each dish were removed and placed individually to small plexiglass ring chambers (diam. 1.5 cm, height 1 cm) to determine the egg-to-adult development times of parasitoids. The sex ratio was calculated based on the total of parasitoids from the dishes and the chambers.

Life-time fertility and hatching dynamics of* A. debilitata *at constant 25°C and 16L:8D

Batches of 50 1-day old shore fly larvae were exposed to female parasitoid as long as the females lived. In the first experiment, the larvae lived on agar overgrown with green algae in Petri-dishes. In the second experiment, they lived on algal-covered circles of capillary matting. Both experiments were started with 12 newly hatched females, each having constant access to a male. Every 24 hours the females were offered a new batch of 50 larvae. When unparasitised larvae were known to approach hatching, daily checking of the dishes commenced to record hatching of parasitoids.

Control efficacy of* A. debilitata *against* S. tenuicosta *in insect cages

The experiment (from 6 September until 16 November 1998, i.e. for 40 days) was a completely randomised one in a 38 m² greenhouse compartment. During the experiment, shore flies bred on algae growing on the surface of peat pots. The pots (vol. 0.3 l, 36 pots per cage measuring 0.6 m^2 in area and 80 cm in height) were filled with Finnish sphagnum peat and then inoculated with suspension of green algae to induce profuse algal growth. Temperature in the greenhouse compartment was set at 25°C and was monitored with a thermohygrometer. The cages were illuminated (16L:8D) by high-pressure sodium lamps.

On day 0 (Sept. 6-7), 36 young female and 36 male shore flies were released in each of the cages. Then the cages were allotted randomly to three treatments, each with six cages: 1) untreated control without parasitoids; 2) one-time release of A. debilitata (Ad-once), rate 1.4 females per pot (with males); and 3) five releases at 4-6 days intervals, consisting of 15, 10, 50, 15 and 15 females of *A. debilitata* (Ad-repeated), making a total of 2.45 females per pot.

The control efficacy was assessed by measuring the following parameters: 1) number of adult flies emerging per pot sampled every 10 days from the cages (removed pots were replaced with new ones overgrown with algae to provide fresh breeding medium for the flies throughout the experiment); 2) direct population counts of adult flies (and parasitoids) on the surface of new, fresh pots every 4 days (n=5 pots per cage); and 3) percentage parasitisation rate determined from a fixed number of pupae sampled per treatment from pots taken to de-

termine the per pot emergence of insects. The combination of these three monitoring methods was necessary to understand the dynamics of the interaction between flies, parasitoids and their breeding habitat, which consisted of both old and fresh pots. Here we only report efficacy based on adult counts from fresh pots extremely attractive to the flies.

Results and discussion

Parasitisation of host larvae of different ages, parasitoid development times and sex ratio at constant 25°C

During the 16.5 hour exposure period, the females parasitised significantly more hosts when these were 1 or 2 days old than when these were still very small (<1 day) or large, 3-day-old, i.e. closer to pupation (F=7.15, DF=3, P=0.0062, GLM analysis of variance). The development time of female wasps was significantly longer, or 13.5-14.7 days, depending on host age, than that of males (12-14 days) (Kruskall-Wallis test at $p<0.05$). Thus at 25°C, the development time of parasitoid females is 4-5 days longer than that of the host (cf. Vänninen, 2001; Fischer & Gros, 2004). The proportion of female progeny averaged 45.7 ± 5.5% (S.E.) over host age categories and was not significantly different between host ages (F=1.64, DF=3, P=0.2364; GLM analysis of variance). Based on these results, we used 1-day-old larvae in the experiment where the fertility of parasitoids was measured.

Life-time fertility and hatching dynamics of* A. debilitata *at constant 25°C and 16L:8D

The females' progeny production peaked on day four at 43.5 daughters and sons (Figure 1). By day 7, the daily fertility dropped below 10 daughters and sons. During its entire life, one female produced an average of 227 and 202 daughters and sons in the agar and capillary mat dishes, respectively. The cumulative fertility of days 1-4 comprised 74% of the total life-time fertility.

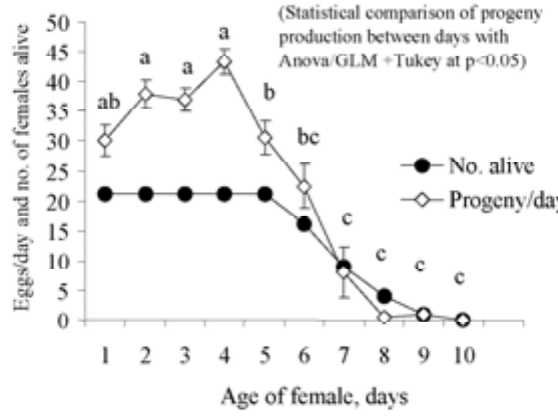

Figure 1. Fertility of *Aphaereta debilitata* during the life-time of the females (n=24).

When combining the information obtained from this experiment with the unreported results concerning mortality of immature parasitoids in the previous experiment, an intrinsic rate of increase of 0.261 d^{-1} was calculated. This is very close to the r_m-value of 0.263 d^{-1} of the host obtained by Vänninen (2001), but smaller than what the biological parameters measured for the host by Fischer & Gros (2004) suggest. In any case, the r_m-value of *A. debilitata* compared with that of its host is high enough to suggest it could control the host when released inoculatively.

Cage experiment: control efficacy of *A. debilitata* against *S. tenuicosta*

Adult shore fly counts showed clearly that both release strategies of *A. debilitata* reduced fly numbers below the level observed in the untreated control (Figure 2). In Ad-repeated cages, the number of flies remained below 5 per pot on all counting days and was always significantly lower than those in the controls. These numbers corresponded to control efficacies of 70-92%, depending on the day, except on the last sampling day, when it was 50%. A more detailed analysis of the data has revealed that the fourth and fifth releases were unnecessary, thus a good control efficacy would have been obtained with repeated releases totalling 1.8 females per pot.

We conclude that *A. debilitata* shows good potential of controlling shore flies. This preliminary conclusion should be corroborated with experiments in actual production conditions, where the removal of parasitised pupae with the crop may slow down the population development of the parasitoids, and where releases must be targeted at appropriate phases in the production cycle (about a week after seedling emergence). Furthermore, an economical and efficient massproduction method of both the flies and the parasitoids should be developed. Such studies could include the search for easily-reared surrogate hosts for the parasitoids.

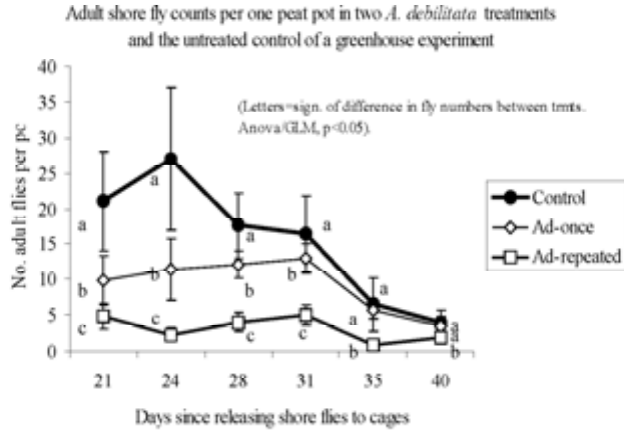

Figure 2. Number of shore flies per pot (total n=36 per cage, 5 pots counted every 4 days).

Acknowledgements

We thank Koppert B.V. for financial support.

References

Diamond, J.C., Carney, V.A., Murphy, G.D. & Allen, W.R. 2001: First Canadian record of *Hexacola neoscatellae* (Hymenoptera: Figitidae: Eucoilinae), a parasitoid of the shore fly, *Scatella stagnalis*. Great Lakes Entomol. 34: 51-53.

Fischer, S. & Gros, P. 2004: La mouche *Scatella tenuicosta* Collin, commensale des cultures sous abri. Rev. Suisse Viticult., Arboricult., Horticult. 36: 215-221.

Lindquist, R. & Casey, M. 1994: Pesticide evaluations against sweetpotato whiteflies and shore flies, Part I. Ohio Florists' Assoc. Bull. 772: 6-8.

Vänninen, I. 2001: Biology of the shore fly *Scatella stagnalis* in rockwool under greenhouse conditions. Entomol. Exp. Appl. 98: 317–328.

Vänninen, I. & Koskula, H. 1998: Effect of hydrogen peroxide on algal growth, cucumber seedlings and the reproduction of shore flies (*Scatella stagnalis*, Diptera, Ephydridae) in rockwool. Crop Prot. 17: 547–553.

Vänninen, I. & Koskula, H. 2003: Biological control of the shore fly (*Scatella tenuicosta*) with steinernematid nematodes and *Bacillus thuringiensis* var. thuringiensis in peat and rockwool. Biocontrol Sci. Technol. 15: 51–67.

Vänninen, I. & Koskula, H. 2004: Biocontrol of the shore fly *Scatella tenuicosta* with *Hypoaspis miles* and *H. aculeifer* in peat pots. BioControl 47: 137-152.

Vet, L.E.M. & van Alphen, J.J.M. 1985: A comparative functional approach to the host detection behaviour of parasitic wasps. 1. A qualitative study on Eucoilidae and Alysiinae. Oikos 44: 478-486.

Zack, R.S. & Foote, B.A. 1978: Utilization of algal monocultures by larvae of *Scatella stagnalis*. Environ. Entomol. 7: 509-511.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biological control of tarsonemid mites with predatory *Amblyseius* mites in outdoor strawberries

Erik W. Hansen
EWH BioProduction, Centervej Syd 4, DK-4733 Tappernoje, Denmark

Abstract: The strawberry mite *Phytonemus pallidus* has become an increasing problem within the last five years in Scandinavian strawberry fields. Chemical pesticides seem to have a very limited effect – in fact pesticide application seems to aggravate the problem due to its killing off natural enemies. Trials and observations in Scandinavia indicates that the predatory mite *Amblyseius cucumeris*, applied for biocontrol in glasshouses, is a good candidate for biocontrol of strawberry mites. The predatory mite is presently used on ca. 600 ha in Scandinavia.

Key words: Strawberry mite, outdoor strawberry, biocontrol, *Phytonemus pallidus, Amblyseius cucumeris*

Introduction

The pest mite

The strawberry mite *Phytonemus pallidus* (Banks) (Acarina: Tarsonemidae) (= *Tarsonemus pallidus*) (Figure 1) has become an increasing problem within the last five years in Danish, Swedish and Finnish strawberry fields. Chemical pesticides seem to have a very limited effect, and field observations indicate that the problem is getting worse when pesticides are used. This is due to the fact that natural beneficials are killed by the pesticides. The strawberry mite is 0.1 mm long and has minimum 4-6 generations per year. The development time from egg to adult is around 3 weeks at 15°C, but as fast as 7 days at 25°C (Karl, 1965). The egg laying is concentrated to young leaves and leaf buds, and the damage is seen from 10 to 20 mites per leaf. This means that the mite can build up high numbers of damaging mites and suddenly result in serious symptoms and damage to the plants. The symptoms of damage are short-haired leaf stems, plants which never become large and normal (but often brown) (Figure 2) and berries which are partly dried out. The growth of the plants stops completely. Some growers have completely lost the whole crop due to the damage from *P. pallidus*.

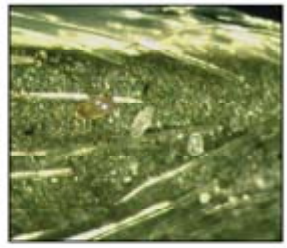

Figure 1. The strawberry mite *Phytonemus pallidus*.

Figure 2. Damaged strawberry plant.

The beneficial species

The predatory mite *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) (= *Neoseiulus cucumeris*) (Figure 3), which today is used as a routine measure for thrips control in cucumber, sweet pepper and ornamentals in glasshouses, can control a wide range of damaging mites, among them the strawberry mite. It is polyphagous, consuming a broad spectrum of prey and even eating pollen and fungi as alternative food. *Amblyseius cucumeris* is about 0.4 mm long, its colour is brown-clear. Development time from egg to adult is around 9 days at 25°C. Females lay approx. two eggs per day (van Houten *et al.*, 1995). The potential population increase in the summertime in Danish strawberry fields (20-25°C) with plenty of food mites available is very high, therefore the potential for being a good predator is in place.

Figure 3. The predatory mite *Amblyseius cucumeris*.

Trials and observations

Trials in Scandinavia

Trials in Finland, covering 90 strawberry fields (Tuomo Tuovinen, Jokioinen, 1998), and observation trials in Denmark (EWH BioProduction, 2001-2005) (Figure 4) and Sweden have shown that when the predatory mite is released in June, July or August, it will always give a

partial or complete control of the strawberry mite at the end of September at the latest. The earlier the release is made, the higher the effect. *Amblyseius cucumeris* will reproduce in the field and predate on the population of strawberry mites. *Amblyseius cucumeris* is in Denmark and Southern Sweden active until the middle or end of October, until the temperature is around 10ºC in daytime. Frost will hamper the development of *A. cucumeris* and also of the pest mite. After release *A. cucumeris* can be found on the underside of the leaves and especially in unfolded leaves where strawberry mite colonies are concentrated. In 2005 around 250 ha of strawberries in Denmark were treated with predatory mites (EWH BioProduction, Erik Hansen). The figure in Finland is around 200 ha (Jan Hulshof, Biotus OY, pers. comm.), and it is unknown for Sweden (but estimated to about 250 ha) and Norway (but estimated to about 10 ha).

Pesticides and biological control

It is obvious that some pesticides like Vertimec, Mesurol and various pyrethroids have a very toxic effect on the predatory mites, and release of predatory mites should therefore be delayed until minimum 4 weeks after spraying with these insecticides. Use of Pirimicarb for aphid control as well as weed control by various herbicides does not harm the predatory mites. The use of *Bacillus thuringiensis* products like Dipel for control of caterpillar as well as use of sulfur, Nissorun and Torque for mite control is not harmful to *A. cucumeris*. Nor do fungicides harm the predatory mites.

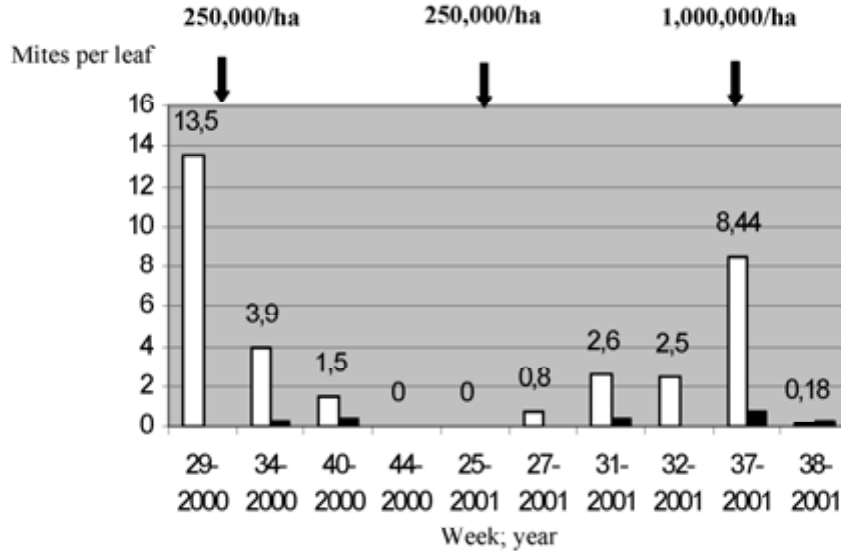

Figure 4. Danish trial with biological control of strawberry mites (white bars) with *Amblyseius cucumeris* (black bars).

The strategy based on trials and observations year 2000-2005

These following recommendations apply to the use of *A. cucumeris* in open Danish strawberry fields:

- Release *Amblyseius cucumeris* preventively in August at the latest, and as early as possible
- Release 500,000 *Amblyseius cucumeris* in new plantings (less than one-year-old plants)
- Release 1,000,000 *Amblyseius cucumeris* in plants more than one year old
- Release 2,000,000 *Amblyseius cucumeris* on serious infestations and in "hot-spots"

The predatory mites must be released on dry plants and can be distributed by hand or, more efficiently, by a modified sowing machine (Figure 5). The formulation of predatory mites is a mix of bran and vermiculite, specially designed for mechanical distribution.

Figure 5. Distribution of predatory mites in a strawberry field with a modified sowing machine.

Future aspects for mite control in strawberries

It is obvious that a good solution is available and used on approx. 600 ha in Scandinavia. Other predatory mite species must be looked upon as candidates. These are candidates which are frost tolerant, so the control period can be prolonged until early spring. This could help to reduce the early season (March-May) build-up of the pest mite population.

References

Karl, E. 1965: Untersuchungen zur Morphologie und Ökologie von Tarsonemiden gärtnerisher Kulturpflanzen II: *Hemitarsonemus latus* (Banks), *Tarsonemus confusus* (Ewing), *Tarsonemus talpae* (Schaarschmidt), *Tarsonemus smithi* (Ewing) und *Tarsonemoides belemnitoides* (Ewing). Biologischer Zentralblatt H3: 331-357.

Houten, Y.M. van, Rijn, P.C.J. van, Tanigoshi, L.K. & Stratum, P. van: 1993: Potential of phytoseiid predators to control western flower thrips in greenhouse crops, in particular during the winter period. Bulletin IOBC/WPRS 16/8: 98-101.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Clonostachys rosea controls *Pythium tracheiphilum* in Chinese cabbage under field conditions

K. Møller[1], B. Jensen[1], H. Paludan Andersen[1], H. Stryhn[2], J. Hockenhull[1]

[1]Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark; [2]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI C1A 4P3, Canada

Abstract: *Clonostachys rosea* (isolate IK726) controlled leaf and head rot of Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*), caused by *Pythium tracheiphilum*, in two field trials in 1995 and 1999 on naturally infested land in commercial crops in Denmark. Significant 2-3-fold disease reductions were obtained at a high application rate (10^8-10^9 conidia m^{-2}) of *Clonostachys rosea* (isolate IK726) in both years. Disease reduction by *Trichoderma harzianum* (Supresivit) was close to significance at a high application rate (1 g product m^{-2} corresponding to 7×10^9 c.f.u. m^{-2}) in both years. In both trials, the percentage of marketable heads increased significantly by 10% following a full application rate of *C. rosea*, while Supresivit gave a significant 13% yield improvement in 1995 (high application rate) but not in 1999. No disease control or yield improvement was found when the two agents were applied at tenfold reduced rates. A Danish *T. harzianum* isolate significantly increased yield by 13% in 1995 but gave no disease control. Yield improvements obtained by Supresivit and the Danish isolate of *T. harzianum* may have resulted from plant growth promotion effects. Products such as Binab T (*T. harzianum* + *T. polysporum*), Mycostop (*Streptomyces griseoviridis*), Polyversum (*P. oligandrum*) and Aliette (fosetyl-Al) as well as two Danish isolates of *P. oligandrum* and one of *T. virens* were also tested in 1995, but none of these gave any disease control or yield effects.

Key words: *Brassica campestris* ssp. *pekinensis*, fosetyl-Al, *Gliocladium roseum*, *Pythium oligandrum*, *Streptomyces griseoviridis*, *Trichoderma virens*, *Trichoderma polysporum*

Introduction

The presented material is based on work by Møller & Hockenhull (1997), Møller (1999) and Møller *et al.* (2003).

In the late 1980s, a new foliar disease of fieldgrown Chinese cabbage appeared in Denmark. It was the objective of this study to search for possible biological means to control this disease, based on knowledge of the pathogen and its infection biology, which is presented in outline in the below.

The causal agent

The causal agent is the soilinhabiting *Pythium tracheiphilum* Matta (Møller & Hockenhull, 1997), which has also been reported to cause a disease problem in lettuce (*Lactuca sativa* L.) (Matta 1965). While in lettuce the vascular system of the root and rarely the above ground parts are attacked (Matta, 1965), in Chinese cabbage it is exclusively the foliage, which is affected. Primary attacks show as a progressing dry rot in the basal mid-rib area of outer leaves, spreading inwards to the heart and head leaves and often resulting in a complete collapse and rot of the head. Secondary bacterial invasion often leads to a general soft rot of the plant (Møller & Hockenhull, 1997). Leaf and head rot of Chinese cabbage was proposed as common name for the disease (Møller & Hockenhull, 1997).

Importance of the disease

In the 1990s the area annually grown to Chinese cabbage in Denmark was about 6-800 ha, enough to cover both the Danish demand and a certain export. However, field losses due to *P. tracheiphilum* combined with a shift in market demand caused a number of growers to give up growing Chinese cabbage and caused severe economical problems to others. Since its first appearance, losses due to the disease soon became important and recurrent in many fields. Harvest losses of up to 50% of the normal about 35-40.000 marketable heads per ha were observed, not only in fields grown to Chinese cabbage year after year, but also in a few fields, where neither Chinese cabbage nor lettuce had previously been grown. Incidence of attacked plants sometimes exceeded 70%, also in fields in which Chinese cabbage was grown for the first time.

Infection biology and aetiology

According to field observations, plant emergence is not affected in infested fields, and plants are juvenile resistant to leaf infection until three to four weeks after sowing, when they become fully susceptible. In the field, primary attacks do not appear until the beginning of head closure, approximately 5-7 weeks after sowing (Møller & Hockenhull, 1997; Møller, 1999), depending on weather conditions and variety. Prior to this, senescing, shed or flaccid cotyledons and first true leaves may be colonized from the soil by the pathogen, which is thus vitalized by fresh nutrition, after a low-nutrition or fungistatic period in uncropped soil. Primary attacks are initiated by soil-borne inoculum, which is dispersed to the basal area of outer leaves by wind, rain and irrigation water. The above mentioned colonized cotyledons add to this inoculum. The disease spreads inwards within the plant by direct growth from infected to non-infected leaves and probably via the stem. Infected outer leaves are rapidly colonized (few days) and disintegrate on the soil surface within 7-14 days. Inoculum contained in the disintegrated leaf material may contribute to secondary spread to neighbouring plants by water and wind dispersal. Field observations indicate that the inoculum is often uniformly distributed in the field, primary attacks appearing synchronously at the time of head initiation throughout the field, but secondary dispersal may be assumed to aggravate disease development in the crop. The disease develops most severely in waterlogged field areas and under humid weather conditions (Møller & Hockenhull, 1997; Møller, 1999). Attacked plants may

still produce marketable heads, if the disease has only spread to the cover leaves and the outermost head leaves, which may be removed by trimming at harvest.

Control possibilities

Since the disease problem was new, no control measures were at hand, and with the increasing public concern over environmental effects caused by pesticides, it seemed unlikely that chemical agents capable of controlling this soil-borne pathogen were to appear on the market. The varieties commonly grown were all experienced as highly susceptible, and cultural practices suitable to reduce disease incidence were not obvious. Since, as mentioned above, severe disease outbreaks were observed also in fields with no history of lettuce or Chinese cabbage cropping, application of longer rotation schemes did not seem likely to offer a solution either.

In consequence, identifying and developing suitable biocontrol measures became interesting. In 1995 we screened antagonists, which had previously been reported able to control various *Pythium* spp. Screenings took place in a commercial Chinese cabbage crop on naturally infested land. For comparison, one chemical agent, claimed to control oomycetous organisms in general (*i.e.* also *Pythium* spp.) was also included. From the screenings, two promising antagonists were selected and tested in new field experiments.

Experimental strategy

Timing, targeting and mode of application

Since attacks take place by dispersal from the soil surface to leaf sheaths and since biocontrol must meet attacks preventively, the soil surface between plants was the target for applications in the 1995 experiment. First treatments were made at the first sign of head initiation, six weeks after sowing and second treatments were made one week later. All agents were applied in water suspension, using either a backpack or a bicycle-mounted sprayer.

Selection of antagonists and agents and their origin

Five fungal and one streptomycete antagonist species as well as a chemical agent were selected for the field screenings in 1995, based on reports of their capacity to control one or more *Pythium*-species (Table 1).

The selected antagonists were represented by four commercially available formulations and five Danish isolates in the 1995 screening. The latter were propagated on a peat-bran substrate or in V8-suspension (*Pythium* antagonists). At the end of propagation, suspensions were prepared and concentrations adjusted for field application, which was made within a few hours. However, a dry diatomaceous clay-based *wp* formulation of the Danish isolate IK726, securing a long shelf life, was developed in 1996 (Jensen *et al.*, 1996) and this formulation was used in 1999. In the 1995 screening, two agents qualified for further testing, which was carried out in 1999. Species, origin and application rates of agents tested in 1995 and 1999 are presented in Table 2.

Table 1. Reports of control capacity of antagonists and agents selected for experiments.

Antagonist species/agent	Pathogen controlled	Authors
Fosetyl-Al	Oomycetes	Fernando & Lindernan, 1994
	Phytophthora spp.	Schutte, 1994
		El-Hamalawi *et al.*, 1995
		Thinggaard, 1995
Clonostachys rosea	*P. ultimum*	Steinmetz & Schönbeck, 1994
		Birgit Jensen, pers. comm.
Trichoderma harzianum	*P. ultimum*	Wolffhechel, 1989
		Steinmetz & Schönbeck, 1994
	P. aphanidermatum	Sivan *et al.*, 1984
	Pythium spp.	Svedelius, 1988
Trichoderma polysporum	*Pythium* spp.	Svedelius, 1988
Trichoderma virens	*P. ultimum*	Wolffhechel, 1989
		Knauss 1992
		Howell *et al.*, 1993
		Wilhite *et al.*, 1994
P. oligandrum	*P. ultimum*	Veselý, 1977
		Lutchmeah & Cooke, 1985
		Walther & Gindrat, 1987
		McQuilken *et al.*, 1990
	P. splendens	Thinggaard *et al.*, 1988
	P. debaryanum	Veselý, 1977
Streptomyces griseoviridis	*Pythium* spp.	Tahvonen, 1988
		White *et al.*, 1990
		Lahdenperä, 1996

Table 2. Application rates of antagonist isolates and product formulations used in field experiments.

Isolate/product	Antagonist species/agent	Rates of application, units m^{-2}		Origin
Experiment 1995		1st treatment	2nd treatment	
Aliette	Fosetyl-Al (80% a.i.*)	0.24 g a.i.*	0.24 g a.i. *	Rhône-Poulenc
Binab T	*Trichoderma harzianum* + *Trichoderma polysporum*	10^4 c.f.u. [1]	10^4 c.f.u. [1]	Bio-Innovation
Mycostop	*Streptomyces griseoviridis*	6.7×10^7 c.f.u. [1]	6.7×10^7 c.f.u. [1]	Kemira Agro Oy
Polyversum	*Pythium oligandrum*	7×10^5 g.oosp. [2]	7×10^5 g. oosp. [2]	Remeslo
Supresivit	*Trichoderma harzianum*	7×10^9 c.f.u. [1]	7×10^9 c.f.u. [1]	Fytovita
G2	*Trichoderma virens*	7×10^9 conidia [3]	5×10^9 conidia [3]	Wolffhechel (1989)
IK726	*Clonostachys rosea*	1×10^8 conidia [3]	8×10^8 conidia [3]	Knudsen (1992)
MM1	*Pythium oligandrum*	3×10^5 propagules [4]	1×10^7 propagules [4]	Madsen (1995)
MM2	*Pythium oligandrum*	9×10^5 propagules [4]	1×10^7 propagules [4]	Madsen (1995)
T3	*Trichoderma harzianum*	5×10^9 conidia [3]	10×10^9 conidia [3]	Wolffhechel (1989)
Experiment 1999	*Trichoderma harzianum*			
Supresivit				Fytovita
- low rate:		7×10^8 c.f.u. [1]	7×10^8 c.f.u. [1]	
- high rate:	*Clonostachys rosea*	7×10^9 c.f.u. [1]	7×10^9 c.f.u. [1]	
IK726				Knudsen (1992)
- low rate:		1×10^8 c.f.u. [5]	6×10^7 c.f.u. [5]	
- high rate:		1×10^9 c.f.u. [5]	6×10^8 c.f.u. [5]	

[1] Colony forming units according to product specifications. [2] Germinable oospores according to product specifications. [3] Haemocytometer counts. [4] Haemocytometer counts of mixture of oospores and sporangia. [5] Plate dilution counts. [*] active ingredient.

Experimental design and statistical treatment of results

A complete randomized block design was used in both years. Plots were integral segments of the commercial crop, sharing also field cultivation methods with it. Minimum 1-m guard belts separated plots, and 15-m guard belts secured the experimental area from any routine fungicide treatment practiced in the remainder of the field. All plants were scored individually, using 4 and 8 level indices to evaluate disease and harvest results, respectively. A double set of control plots was included in each replication. Scoring took place at harvest time.

Disease and harvest score data were analysed by a generalized linear mixed model for ordinal data (Breslow & Clayton, 1993; Goldstein, 1995) in a two-way design with treatments and blocks. This model extends a usual logistic regression model for a binary response (e.g. healthy vs. diseased) in two ways. First, it allows for outcomes, which fall into several ordered categories, as do the disease and harvest scores in this study. Second, it takes into account the (spatial) field variation by including random plot effects for the plots in the design. Recent advances in algorithms and software have made analysis of such models possible in specialised statistical software (MLwiN program; Yang *et al.*, 1998).

In order to control the overall error level when performing multiple comparisons, the standard significance level of P=0.05 was Bonferroni-corrected by division of P with the number of treatments compared with control treatments in each experiment. Hence levels were P=0.005 in 1995 and P=0.0125 in 1999.

Results and discussion

Of the ten agents screened in 1995, one gave a statistically reliable control effect; another was close to doing so. The other eight had no control effect (Table 3).

Hence, *C. rosea* IK726 significantly reduced the disease level and increased the harvest result in both years of testing. In 1995, application of *C. rosea* IK726 at full rate (1995: 10^8-10^9 conidia m^{-2}; 1999: 10^8-10^9 c.f.u. m^{-2}) significantly reduced the estimated percentage of attacked plants. Hence, in 1995 the disease level was reduced from 16.6% (untreated) to 7.0% (Table 3, odds ratio 2.67) and in 1999 from 47.6% to 19.8% (odds ratio 3.76). At the same time, fullrate *C. rosea* IK726 treatment was associated with a significant yield increase of about 10% in both 1995 and 1999 (Table 3). Lowrate application of *C. rosea* (10^7-10^8 c.f.u. m^{-2}) had no effect on disease and harvest yield in either of the two years, however.

Considering that a cautious significance threshold was applied, in 1995 disease control effects of treatments with the Supresivit formulation of *T. harzianum* at an application rate of 7×10^9 c.f.u. m^{-1} were close enough to significance to make it a candidate for the 1999 tests (Table 3). Its candidateship was further supported by a significant 13% yield improvement associated with this treatment. However, although the trends were similar in 1999, none were significant (Table 3).

In the 1995 screening, the Danish *T. harzianum* isolate T3 clearly had no significant control effect. Still, a significant 13% yield increase resulted in treatments with this agent (Table 3), comparable with that found in the 1995 Supresivit-*T. harzianum* treatments. In 1999, how-

ever, neither high nor low application rates of Supresivit had any significant control or yield effects.

Table 3. Disease control and yield results in 1995 and 1999 trials.

Treatment	Effects on disease			Effects on harvest results		
	% Diseased plants[a]	Odds ratios[b]	P-values[c]	% Marketable plants[a]	Odds Ratios[b]	P-values[c]
1995 trial						
Fosetyl-Al (Aliette)	17.1	0.96	0.89	57.3	1.00	0.99
C. rosea IK726	7.0	2.67	0.001[d]	68.2	1.59	0.0056[d]
T. virens G2	12.3	1.42	0.21	63.8	1.31	0.11
P. oligandrum (Polyversum)	13.5	1.28	0.39	54.6	0.89	0.48
P. oligandrum MM1	13.1	1.32	0.32	67.1	1.51	0.014
P. oligandrum MM2	17.6	0.93	0.80	60.0	1.11	0.51
S. griseoviridis (Mycostop)	14.8	1.15	0.61	60.4	1.13	0.45
T. harzianum + *T. polysporum* (Binab T)	20.3	0.78	0.37	52.9	0.83	0.26
T. harzianum T3	12.7	1.37	0.26	70.0	1.73	0.0013[d]
T. harzianum (Supresivit)	9.2	1.97	0.019	70.2	1.75	0.0010[d]
Untreated	16.6	1	•	57.4	1	•
1999 trial						
IK726, low[e]	48.2	0.97	0.94	85.0	1.02	0.95
IK726, high[f]	19.9	3.76	0.0012[d]	93.8	2.72	0.0007[d]
Supresivit, low[e]	36.0	1.64	0.21	88.3	1.36	0.29
Supresivit, high[f]	29.4	2.22	0.045	89.3	1.51	0.16
Untreated	47.6	1	•	84.8	1	•

[a]Estimated percentages of attacked and marketable plants are calculated from the probabilities of plant disease score > 0 and harvest score ≤ 4, respectively.
[b]Odds ratios are calculated as: [p1/(1-p1)]/[p2/(1-p2)], where p1 and p2 are the probabilities of observing healthy/ marketable plants in response to the two treatments (p1: control agent; p2: untreated).
[c]*P*-values relate to the comparisons of treated plots to untreated.
[d]Indicates significance **after** Bonferroni correction of the significance level from P=0.05 to P=0.005 in 1995 and P=0.0125 in 1999.
[e]0.1 x full application rate.
[f]Full application rate.

The present study demonstrated that reproducible biocontrol of leaf and head rot of Chinese cabbage could be obtained under field conditions by *C. rosea* IK726 application. Disease levels were reduced to about 40% relative to the controls in both years in these treatments. By comparison, strobilurine-based fungicides, which have recently been released for use in Chinese cabbage in Denmark, may reduce leaf and head rot by 50-75% (Klaus Paaske, pers. com.).

The 1995 strategy of targeting the top soil layer at application and avoiding direct treatment of plants aimed at blocking the disease cycle at soil level, avoiding deposition of the agent on consumable plant parts. Further, the phylloplane was not considered suitable for sustaining long term survival of antagonists. However, the accumulation of soil at the bases of outer leaves, which are also sites of primary attacks, is considerable, and these sites may offer niches for antagonist survival and pathogen control, in which the accumulated soil volume

may buffer the diurnal variations in moisture and temperature. Hence, the application strategy applied in 1999 also sought to utilize this potential benefit. While the disease reduction obtained by *C. rosea* IK726 treatments was quite comparable in the two experimental years, despite the differences in application strategy, it should be noted that the general disease level was considerably higher (47.6%) in 1999 than in 1995 (16.6%) (Table 3). Hence, the capacity of the antagonist to reduce disease under an increased disease pressure may possibly in part be due to the change in application strategy.

Yield improvements were recorded for treatments with *C. rosea* IK726, Supresivit and the T3 isolate of *T. harzianum*. While the yield improvements obtained in *C. rosea* IK726 treatments seem to be the likely results of disease control, in the case of Supresivit and the T3 isolate of *T. harzianum* their non-significant control results indicate that plant growth promotion effects may have been involved. Such effects have previously been recorded in cultures other than Chinese cabbage by e.g. Chang *et al.* (1986), Windham *et al.* (1986), Besnard and Davet (1993) and Inbar *et al.* (1996). The yield improvement obtained by Supresivit application in 1995 could not be repeated in 1999, and the higher disease pressure may have played a role in this. The *T. harzianum*-containing product BinabT was applied at a considerably lower rate than the two other agents, which may in part explain why no measurable effects on disease or yield were found for BinabT. The low BinabT application rate was due to the low c.f.u. content of the product (Table 1). While for Supresivit 1 g of product per m^2 sufficed to obtain an application rate of 7×10^9 c.f.u. m^{-2}, 100 kg of BinabT would have been required to obtain a comparable rate, which would be inpracticable.

The handling of all tested agents was found easy and suitable for field application purposes at the rates given. Hence, also the clay-based dry formulation of *C. rosea* IK726 proved easy to handle in spray applications in 1999.

At present commercial formulations of biocontrol agents are not likely to hold c.f.u. levels higher than 10^9 to 10^{10} per g, as in e.g. Supresivit. Hence, the rates of application required for *C. rosea* to efficiently exert control and improve yield or for Supresivit to improve yield were very high in view of the market price of such products in general. As indicated by the results of this study, biocontrol of field diseases with these products seems to be technically realistic, but a considerable reduction of the general product price level would be required to obtain a cost-efficient production. Large-scale production associated with increased demands for products with a documented effect could help reduce prices. Other possibilities of reducing required application rates may be found in optimizing timing and targeting of application as well as in adopting cultural practices, which may enhance the effect of biocontrol. In the case of leaf and head rot of Chinese cabbage, straw mulching might add to the biocontrol effect of top soil inoculum by physically hampering inoculum dispersal to the primary sites of attack.

References

Besnard, O. & Davet, P. 1993: Mise en évidence de souches de *Trichoderma* spp. à la fois antagonistes de *Pythium ultimum* et stimulatrices de la croissance des plantes. Agronomie 13, 413-421.

Breslow, N.E. & Clayton, D.G. 1993: Approximate inference in generalized linear mixed models, Journal of the American Statistical Association 88, 9-25.

Chang, Y., Chang, Y., Baker, R., Kleifeld, O. & Chet, I. 1986: Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Disease 70, 145-148.

El-Hamalawi, Z.A., Menge, J.A. & Adams, C.J. 1995: Methods of fosetyl-Al application and phosphonate levels in avocado tissue needed to control stem canker caused by *Phytophthora citricola*. Plant Disease 79, 770-778.

Fernando, W.G.D. & Linderman, R.G. 1994: Chemical control of stem and root rot of cowpea caused by *Phytophthora vignae*. Plant Disease 78, 967-971.

Goldstein, H. 1995: Multilevel statistical models, 2nd ed. Edward Arnold, London.

Howell, C.R., Stipanovic, R.D. & Lumsden, R.D. 1993: Antibiotic production by strains of *Gliocladium virens* and its relation to biocontrol of cotton seedling diseases. Biocontrol Science and Technology 3, 435-441.

Inbar, J., Abramsky, M., Cohen, D. & Chet, I. 1996: Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. European Journal of Plant Pathology 100, 337-346.

Jensen, B., Knudsen, I.M.B., Jensen, D.F. & Hockenhull, J. 1996: Development of a formulation of *Gliocladium roseum* for biological seed treatment. IOBC wprs Bulletin 19, 164-169.

Knauss, J.F. 1992: *Gliocladium virens*, a new microbial for control of *Pythium* and *Rhizoctonia*. Florida Foliage 18, 6-7.

Knudsen, I.M.B. 1992: Isolation of potential antagonists against seed borne diseases from Danish agricultural soils. Minor Ph.D. Thesis, Plant Pathology Section, The Royal Veterinary and Agricultural University, Denmark, pp 131.

Lahdenperä, M.-L. 1996: Activity of Mycostop biofungicide in rockwool. Biological and integrated control of root diseases in soilless cultures. IOBC wprs Bulletin 19, 174-178.

Lutchmeah, R.S. & Cooke, R.C. 1985: Pelleting of seed with the antagonist *Pythium oligandrum* for biological control of damping-off. Plant Pathology 34, 528-532.

Madsen, A.M., Robinson, H.L. & Deacon, J.W. 1995: Behaviour of zoospore cysts of the mycoparasite *Pythium oligandrum* in relation to their potential for biocontrol of plant pathogens. Mycological Research 99, 1417-1424.

Matta, A. 1965: Una malattia della lattuga prodotta da una nuova specie di *Pythium*. Phytopathologia Mediterranea 4, 48-53.

McQuilken, M.P., Whipps, J.M & Cooke, R.C. 1990: Control of damping-off in cress and sugar-beet by commercial seed-coating with *Pythium oligandrum*. Plant Pathology 39, 452-462.

Møller, K. 1999: Studies of the infection biology and biocontrol of leaf and head rot of Chinese cabbage (causal agent: *Pythium tracheiphilum)* and of some taxonomical aspects of the genus Pythium. Ph.D. thesis. Section for Plant Pathology, the Royal Veterinary and Agricultural University of Copenhagen.

Møller, K. & Hockenhull, J. 1997: Leaf and head rot of Chinese cabbage, - a new field disease caused by *Pythium tracheiphilum* Matta. European Journal of Plant Pathology 3, 245-249.

Møller, K., Jensen, B., Andersen, H. Paludan, Stryhn, H. & Hockenhull, J. 2003: Biocontrol of *Pythium tracheiphilum* in Chinese cabbage by *Clonostachys rosea* under field conditions. Biocontrol Science and Technology 13, 171-182.

Schutte, G.C. 1994: The timing of fosetyl-Al (Aliette) treatments for *Phytophthora* root rot control in the summer rainfall region. Citrus Journal 4, 26-28.

Sivan, A., Elad, Y. & Chet, I. 1984: Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. Phytopathology 74, 498-501.

Steinmetz, J. & Schönbeck, F. 1994: Conifer bark as growth medium and carrier for *Trichoderma harzianum* and *Gliocladium roseum* to control *Pythium ultimum* on pea. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 101, 200-211.

Svedelius, G. 1988: Erfarenheter av de biologiska preparaten Binab T och Mycostop vid bekämpning av svampsjukdomar på gurka och tomat. Växtskyddsrapporter, Jordbruk 52, 59-63.

Tahvonen, R.T. 1988: Microbial control of plant diseases with *Streptomyces* ssp. Eppo Bulletin 18, 55-59.

Thinggaard, K. 1995: Root rot in *Campanula carpatica* caused by *Phytophthora cryptogea*. European Journal of Plant Pathology 101, 111-114.

Thinggaard, K., Larsen, H. & Hockenhull, J. 1988: Antagonistic *Pythium* against pathogenic *Pythium* on cucumber roots. EPPO Bulletin 18, 91-94.

Veselý, D. 1977: Potential biological control of damping-off pathogens in emerging sugar beet by *Pythium oligandrum* Drechsler. Phytopatologische Zeitschrift 90, 113-115.

Walther, D. & Gindrat, D. 1987: Biological control of *Phoma* and *Pythium* damping-off of sugar-beet with *Pythium oligandrum*. Journal of Phytopathology 119, 167-174.

White, J.G., Linfield, C.A., Lahdenperä, M.-L. & Voti, J. 1990: Mycostop - a novel biofungicide based on *Streptomyces griseoviridis*. Brighton Crop Protection Conference on Pests and Diseases. 221-226.

Wilhite, S.E., Lumsden, R.D. & Straney, D.C. 1994: Mutational analysis of the biocontrol fungus *Gliocladium virens* in relation to suppression of *Pythium* damping-off. Phytopathology 84, 816-821.

Windham, M.T., Elad, Y. & Baker, R. 1986: A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76, 518-521.

Wolffhechel, H. 1989: Fungal antagonists of *Pythium ultimum* isolated from a disease suppressive *Sphagnum* peat. Växtskyddsnotiser 53. 7 – 11.

Yang, M., Rasbach, J. & Goldstein, H. 1998: MLwiN macros for advanced multilevel modelling. Institute of Education, University of London.

Interactions among entomophagous insects in IPM programmes

Henrik F. Brødsgaard, Annie Enkegaard

Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark

Abstract: Interspecific interactions among arthropods in protected crops has become increasingly important as the use of still more complex biological control programs have been implemented into glasshouse crops. In many areas of Northern Europe, ornamental growers are trying to use biological control of pests by means of beneficial arthropods. This is mainly because the number of registered effective pesticides is decreasing. One of the many features in which ornamental crops differ from vegetables is the wide range of arthropod pest species that may attack an ornamental crop species. Many crops are attacked by six to ten different pest species that have to be controlled simultaneously by a set of beneficials. Due to the facts that many pest species are difficult to control, the damage thresholds are very low, and the cropping cycles often are very complex, several beneficial species have to be introduced not only against the pest complex in general but against each pest species. Many of the presently used beneficial arthropods are not monophagous. Hence, intraguild predation among introduced predators or predators switching away from the target pests are likely to occur. Several examples from laboratory experiments document intraguild predation among biological control candidates. Fewer studies suggest intraguild predation in glasshouse crops. However, both control failures and control delays due to intraguild predation are reported and in such cases the outcome of the control programs may differ from what can be expected based on the experience that has been generated from the much simpler greenhouse vegetable systems.

The positive and negative aspects of using polyphagous predators have been discussed for years and the use of monophagous beneficials in biological control programs have been emphasised by many authors. In the present presentation, we will discuss the role of intraguild predation with a system approach and with focus on system stability and end control results.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biocontrol of *Botrytis cinerea* in strawberry: factors influencing interactions between the pathogen and its fungal antagonists

Gunn Mari Strømeng[1,2], Linda Gordon Hjeljord[1], Andrew Dobson[2], Arne Stensvand[2], Arne Tronsmo[1]

[1]Department of Chemistry, Biotechnology and Food Science, The Norwegian University of Life Sciences, 1432 Ås, Norway; [2]Department of Plant Pathology, Plant Protection Centre, The Norwegian Crop Research Institute, 1432 Ås, Norway

Abstract: Many fungi show antagonistic activity against *Botrytis cinerea* in the laboratory, and biocontrol may be an alternative to fungicide sprayings to control grey mould caused by *B. cinerea* in strawberry. *Trichoderma* has long been known to be antagonistic to *B. cinerea*, but field trials have shown that it has not been consistent in the control of grey mould in strawberry. This could be caused by lack of adaptation to the phyllosphere of this soil fungus. We have found that interactions between *B. cinerea* and its antagonists are largely dependent on temperature, nutrient availability, and relative spore concentrations. In an attempt to find new antagonists that are better suited to establish in the phyllosphere, we have collected fungal isolates from strawberry plants grown outdoors in Norway. The isolates were screened for antagonistic activity against *B. cinerea* by using a detached flower assay, and based on the results of this assay, isolates were selected for field testing. Field trials were carried out in 2004 and 2005. In 2004, *Epicoccum nigrum*, *Trichoderma hamatum*, *Aureobasidium pullulans*, and *Acremonium* sp. were tested. There was a high disease pressure in the field, with 54% fruit rot in the untreated control plots. *E. nigrum* and *A. pullulans* both significantly reduced the disease incidence, to 44 and 37% rot, respectively. The other agents did not reduce disease incidence. In 2005, *Acremonium* sp. and *T. hamatum* were replaced by *Clonostachys roseae* and *Ulocladium* sp., and *T. atroviride* strain P1 was also included in the trial. Heavy rainfalls during flowering caused a considerable number of flowers to die from aggressive infection by *B. cinerea*. Disease incidence in ripe fruits was low, with only 3% fruit rot in the control plots. There were no significant differences between treatments in 2005.

Key words: Bioassay, *Botrytis* fruit rot, grey mould, *Fragaria* × *ananassa*, latent infection, indigenous fungi

Introduction

Grey mould, caused by the fungus *Botrytis cinerea* Pers ex Fr., is the most important disease in strawberry (*Fragaria* × *ananassa*) in Norway. *B. cinerea* infects strawberry through open flowers (Powelson, 1960), and fungicides are applied during flowering to protect flowers from infection. Because of an extended flowering season, fruits and flowers are both present

in the field during most of the spraying period, leading to the possibility of fungicide residues in ripe fruits. Biological control using fungal antagonists may have potential as control strategy (Tronsmo & Dennis, 1977). *Trichoderma* species have been extensively tested as biocontrol agents in strawberry, but control has been inconsistent in Norway (Tronsmo, 1986; Stensvand, 1997; Stensvand, 1998). To improve biocontrol efficacy, we need to identify factors that are important for the outcome of the interactions between *B. cinerea* and its antagonists under field conditions. Because *Trichoderma* species are soil fungi, they may not be the best suited for control of foliar pathogens. It has been shown that spores of *Trichoderma* spp. germinate much slower than spores of *B. cinerea* at temperatures that commonly occur during flowering in Norway (Hjeljord *et al.*, 2000), and it seems reasonable to assume that antagonist spores need to germinate at least as fast as *B. cinerea* spores. Furthermore, it is evident that nutrient availability in the substrate the fungi grow on has great influence on the interactions between antagonist and pathogen. At low nutrient availability, many antagonists suppress *B. cinerea*. However, with increasing nutrient availability *B. cinerea* becomes more difficult to suppress (Hjeljord *et al.*, 2006). Strawberry flowers, which are very rich in nutrients due to the presence of pollen and nectar, seem to favour the pathogen (Chou & Preece, 1968). Screening potential antagonists against *B. cinerea* under nutrient-rich conditions therefore seems to be very important to ensure that the most suitable isolates are selected for field testing.

In an attempt to improve biocontrol of *B. cinerea* in strawberry under field conditions, we collected indigenous fungi from aboveground parts of field-grown strawberry plants. Isolates were screened for antagonistic activity against *B. cinerea* using a detached flower assay, and selected isolates were tested in field trials in 2004 and 2005.

Material and methods

The fungal isolates that had been collected were identified to genus, or sometimes to species by morphological characteristics. The fungi were grown on agar media (PDA, malt agar or V8). For the bioassay, known concentrations of spores of the potential antagonist and spores of *B. cinerea* were mixed in a water suspension. The spore suspension was applied as three 10 µl droplets in each flower. Flowers were then incubated at high humidity, at either 15 or 20°C, and examined daily for development of sepal necrosis. Isolates selected for field testing were grown on agar medium (PDA or V8) to produce spores. Spores were scraped off the agar, and the suspensions were filtered if needed, to avoid clogging of the sprayer by fungal hyphae. The concentrations of spores in the suspensions were quantified using a haemacytometer. Concentrations of the yeasts *Aureobasidium pullulans* Au16 and *Acremonium* sp. Acr603 were adjusted to 10^8 cells per ml suspension, while the concentrations of the fungi *Trichoderma hamatum* T20, *T. atroviride* P1, and *Clonostachys rosea* (*Gliocladium roseum*) Gr313 were 10^7 conidia per ml suspension, and the concentrations of the fungi *Epicoccum nigrum* Ep1 and *Ulocladium* sp. Ulo17 were 10^5 conidia per ml suspension. Field trials were carried out in 2004 and 2005 at the Norwegian Crop Research Institute at Ås, Norway. The

experimental field was established in August 2003 with cv. Korona in double row beds with drip irrigation. Herbicides and insecticides were applied prior to flowering, but no fungicides were applied at any time. The trial was set up as a randomised block design with three blocks and approximately 40 plants in each plot. The field received overhead irrigation during the day (1 minute per hour from 0800 to 1900 hours) to maintain moist conditions to enhance establishment of the fungi. Application of antagonists was conducted using a knapsack sprayer (1.5 bar, 130 l/1000 m double row). In 2004, pieces of agar with sporulating *B. cinerea* Bc101 were placed in the field just before flowering to ensure flower infections. The following year, it was assumed that the build-up of natural inoculum from the previous year was sufficient for disease development. In 2004, five sprays with the antagonists were carried out over a two-week period, and in 2005 eight sprays were carried out over a three-week period. In both years the field was harvested three times per week for a total of 12 and 9 times in 2004 and 2005, respectively. Berries infected through contact with diseased fruits were defined as healthy in these experiments, since the investigation was aimed at preventing infection during flowering. Flowers and fruits showing symptoms of grey mould were harvested as soon as they were detected, and healthy berries were harvested when ripe. At all harvest dates in 2004 and at two harvest dates in 2005, samples of healthy berries from each treatment were incubated at room temperature to test for latent infections of *B. cinerea*. Statistical analysis was performed using the analysis of variance (GLM-procedure of Minitab 14.20), and treatments were compared using Tukey's test (P = 0.05). Data on accumulated daily precipitation and mean daily temperatures for both seasons were obtained from a local weather station at Ås.

Results and discussion

The bioassay showed that the fungal isolates differed in their ability to prevent *B. cinerea* from causing sepal necrosis. In flowers inoculated with *B. cinerea* only, symptoms started to appear after three days, and after 5-6 days sepal necroses had developed from 100% of the inoculum droplets. The incidence of sepal necrosis was reduced when *B. cinerea* was coinoculated with potential antagonists. *E. nigrum* gave the best result and was able to reduce sepal necrosis to 0–35%. Coinoculation with *T. atroviride* P1 reduced sepal necrosis to 63-83%. All the fungi selected for field trials were able to reduce sepal necrosis incidence, but the efficacy was largely dependent on the relative spore concentrations of the pathogen and the antagonist. In general, the antagonists were more effective when concentrations were higher than the concentrations of the pathogen than if they were inoculated at the same concentration.

In 2004, four isolates were tested in the field; *T. hamatum*, *E. nigrum*, *A. pullulans*, and *Acremonium* sp. There was a very high disease pressure in the field, with 54% fruit rot in the control plots (Figure 1). Treatment with *E. nigrum* and *A. pullulans* both significantly reduced fruit rot incidence compared with the control plots (by 19 and 31 per cent, respectively, P = 0.004). *T. hamatum* and *Acremonium* sp. did not reduce fruit rot incidence compared with the

control, but treatment with *Acremonium* sp. resulted in significantly less fruit rot than treatment with *T. hamatum*. In 2004, the flowering season was characterised by dry weather, and only 11.2 mm accumulated precipitation was recorded during the period of spraying. The harvest season had much more rainfall, with 122.8 mm accumulated precipitation. The wet weather coincided with berry ripening and probably triggered development of stem-end rot of *B. cinerea*, which we assume originated from latent flower infections. As water films are necessary for spore germination of *B. cinerea* (Jarvis, 1962), flower infection was probably favoured by overhead irrigation, but it is also possible that dewfall during night may have provided sufficient free water for the fungus to infect the flowers. Symptoms developed in incubated berries 2-3 days after picking, but there were no significant differences in postharvest rot between the treatments. The combination of high disease pressure and wet weather during harvest favoured disease development, and therefore the results of this field trial indicate that indigenous antagonists have a potential as biocontrol agents of *B. cinerea* in strawberry.

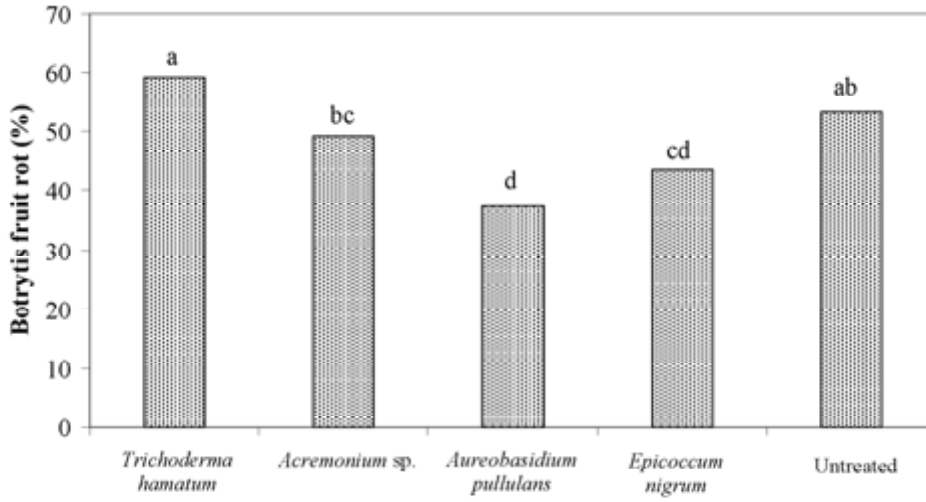

Figure 1. Control of grey mould (*Botrytis* cinerea) in strawberry cv. Korona, by spraying spore suspensions of indigenous, antagonistic fungi during flowering, Ås, Norway 2004. Mean of three replicates, significant differences indicated by different letters on top of the bars (Tukey's test, P = 0.05).

In 2005, we tested the same isolates of *E. nigrum* and *A. pullulans* as in 2004, but we replaced *T. hamatum* and *Acremonium* sp. with *C. rosea* and *Ulocladium* sp. In addition, we included *T. atroviride* P1, which has been tested in several earlier field trials (data not shown). Weather conditions in the 2005 season differed greatly from those of the previous year. During flowering 85.4 mm rain was recorded, while the harvest season was dry with only 32.2 mm rain. A

considerable number of flowers were killed by *B. cinerea* during flowering because of the wet weather combined with a high disease pressure. Approximately half of the potential yield was lost in the field due to flower blight prior to fruit development, and there were no significant differences between treatments (Figure 2). The yield loss due to Botrytis fruit rot was very low, with only 3% in the control plots. There were no significant differences between the treatments at harvest or in the postharvest tests.

The results of these trials indicate that the precipitation pattern during the season had a great influence on when symptoms of *B. cinerea* flower infections appeared. Heavy rains during flowering promoted disease in earlier stages of development. Sprays with antagonists in the wet flowering season did not reduce flower blight compared with the control plots. The reason for this could be that the antagonists were unable to inhibit aggressive infection of the pathogen promoted by the climatic conditions, or that rainfall after spraying washed away the antagonist spores. The preliminary conclusions from this work are that indigenous antagonists show potential in reducing fruit rot caused by latent flower infection with *B. cinerea*, but aggressive flower infections may be more difficult to prevent. Future work includes testing of antagonists under different controlled humidity conditions and studies of antagonistic mechanisms. In future field trials we will increase the spore concentrations of the antagonists to see if this gives improved biocontrol of flower infection by *B. cinerea*.

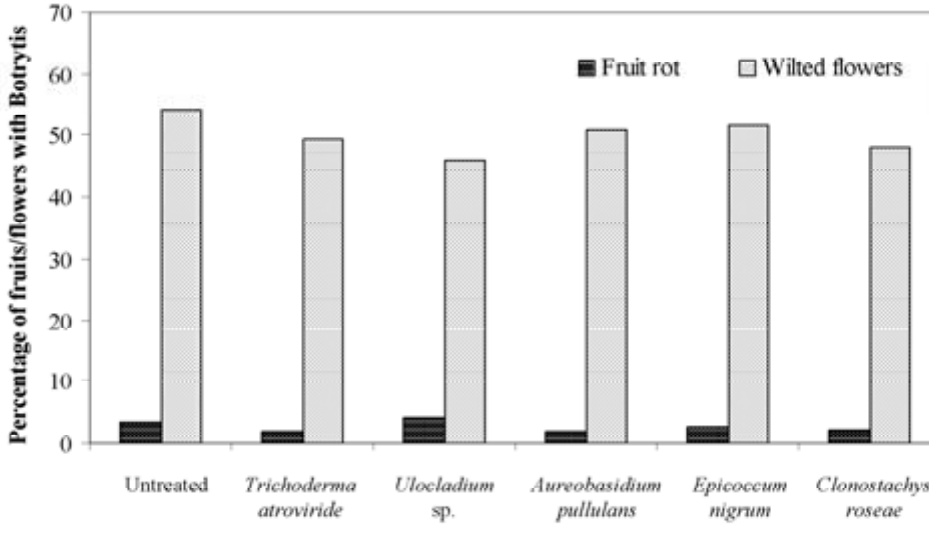

Figure 2. Control of grey mould (*Botrytis cinerea*) in strawberry cv. Korona, by spraying spore suspensions of indigenous, antagonistic fungi during flowering, Ås, Norway 2005. Mean of three replicates.

References

Chou, M.C. & Preece, T.F. 1968: The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. Ann. Appl. Biol. 62: 11-22.

Hjeljord, L.G., Strømeng, G.M., Stensvand, A. & Tronsmo, A. 2006: Biological control of grey mold in strawberry: what we know and what we need. IOBC wprs Bull. 29: *In press.*

Jarvis, W.R. 1962: The infection of strawberry and raspberry fruits by *Botrytis cinerea* Fr. Ann. Appl. Biol. 50: 569-575.

Powelson, R.L. 1960: Initiation of strawberry fruit rot caused by *Botrytis cinerea*. Phytopathology 50: 491-494.

Tronsmo, A. & Dennis, C. 1977: The use of *Trichoderma* species to control strawberry fruit rots. Neth. J. Plant Path. 83:449-455.

Tronsmo, A. 1986: *Trichoderma* used as a biocontrol agent against *Botrytis cinerea* rots on strawberry and apple. Scientific Reports of The Agricultural University of Norway 65 (17), 22 p.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Entomopathogenic nematodes against vine weevil (*Otiorhynchus sulcatus*) in field grown strawberries

Solveig Haukeland
The Norwegian Crop Research Institute, Plant Protection Centre, Hogskoleveien 7, 1432 Aas, Norway

Abstract: The use of entomopathogenic nematodes (EPN) against the vine weevil *Otiorynchus sulcatus* was studied under field conditions. A number of field trials were conducted using two commercial products of EPN, Nemasys H (*Heterorhabditis megidis*) and Nemasys L (*Steinernema kraussei*). The results from these trials indicate that low temperature is still a limiting factor for the successful use of EPN against *O. sulcatus* in northern Europe. Furthermore, it was observed that application methods of EPN in field grown strawberries requires improvement.

Key words: Strawberry, biological control, root weevils, *Otiorhynchus sulcatus*, entomopathogenic nematodes, *Heterorhabditis megidis*, *Steinernema kraussei*

Introduction

In Norway, strawberries are produced mainly outdoors using direct drills or under black polythene mulch. The production area is approximately 1725 ha, with an estimated annual value of 345 m. NOK (43 m. Euro). Most strawberry production occurs in the southern part of the country, and it is in the southernmost and western part that root weevils are major problem. There are several species of root weevils that are associated with strawberries in Norway, but it has been shown that the most damaging species is the vine weevil *Otiorhynchus sulcatus* (Stenseth, 1979; Hesjedal, 1982; Moorehouse *et al.*, 1992). Post harvest application of the pesticide azinphosmethyl against the adult weevils is the most common control method today. Azinphosmethyl will no longer be available from 2006, and there are currently few alternative pesticides on the market that are effective against vine weevil adults.

Entomopathogenic nematodes (EPN) are an option for controlling the soil dwelling larvae. In Norway there are three products on the market comprising two species (*Heterorhabditis megidis* and *Steinernema kraussei*). In 2004-2005 a number of field trials were conducted to examine the efficacy of these commercial EPN in field grown strawberries. Some of the results from this work are presented here.

Materials and methods

A total of 5 field trials were conducted, 4 in the southern part of the country and one in the

north-western part. Each trial was set up as randomised blocks along strawberry rows, with four plots per treatment comprising 16 plants. EPN treatments were applied manually as a drench per plant in a 100 ml volume with doses of 30,000, 25,000 or 15,000 per plant. Treatments were conducted in early spring or late autumn to target the overwintering larvae. The spring treatments were assessed after about one month and the autumn treatments after 6-7 months (overwintering). At each assessment 8 plants per plot were dug up and the number of live *O. sulcatus* larvae or pupae per plant were counted. Comparisons were made between untreated control plants and EPN-treated plants using the mean number of larvae per plant (in blocks).

Results and discussion

In the first spring trial, temperatures were unusually mild for the time of year and never dropped below 12°C. In this trial Nemasys H and Nemasys L worked quite well at the highest dose per plant (30,000/plant) applied two times with a week's interval. The mean number of larvae were reduced by 89% and 77% for Nemasys H and Nemasys L, respectively. For all the following trials, two spring trials and two autumn trials, temperatures were below 12°C on average, ranging from 6°C to 9°C in early spring and late autumn to below 0°C in winter. In these trials Nemasys H did not work well at all (always less than 30% reduction) and the results confirm that this nematode product does not work at low temperatures. Nemasys L, which is considered a cold-active product, did not perform as well as expected. Nemasys L treatments reduced the number of larvae at best by 50% and 64% at 25,000 and 30,000 per plant. In strawberry fields infested with root weevils, plants are usually attacked by at least two or more larvae, which is sufficient to reduce yields or kill the plant. Therefore a 50% to 60% effect of a nematode treatment is not acceptable.

Nemasys L (*Steinernema kraussei*) is reported to be effective at temperatures down to at least 5°C according to trials in England (Willmott *et al.*, 2002). Similar effectiveness could not be demonstrated for the field trials conducted in Norway. The field trial in England was conducted in a controlled manner, whereby strawberry plants in pots containing a known number of weevil eggs were buried in the field outdoors, after which nematodes were applied as a drench in different doses per pot. In the Norwegian trials naturally infested strawberry fields were used and nematodes were applied in a similar manner to the English trial but on plants that were growing under black plastic mulch.

It appears that the practical application or use of EPNs in open fields may result in rather inconsistent or poor results. Application of EPNs has to be improved in such a way that they are able to reach and infect the pest target in the soil and root system.

Acknowledgements

Many thanks to Ana Solberg at the Agricultural Extension Service (Agder) for much of the field work. Also thanks to technical staff and students at the Plant Protection Centre for work

with the soil samples and weevil larvae. This work was funded by the Research Council of Norway.

References

Hesjedal, K. 1982: Life cycle and fecundity of weevil species in strawberry fields. Forskning og forsøk i landbruket. 33: 143-149.

Moorhouse, E.R., Charnley, A.K. & Gillespie, A.T. 1992: A review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Annals of Applied Biology. 121: 431-454.

Stenseth, C. 1979a: Rotsnutebiller på jordbær. Reprinted from Gartneryrket. 69: 231-233.

Willmott, D.M., Hart, A.J., Long, S.J., Edmondson, R.N. & Richardson, P.N. 2002: Use of a cold-active entomopathogenic nematode *Steinernema kraussei* to control overwintering larvae of the black vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) in outdoor strawberry plants. Nematology. 4: 925-932.

The future of biological control: gaps, challenges and options

Jørgen Eilenberg[1], Annie Enkegaard[2], Niels B. Hendriksen[3], Dan Funck Jensen[4], Jørgen B. Jespersen[2], John Larsen[2], Anne Mette Madsen[5], Hans Peter Ravn[6], Sabine Ravnskov[2]

[1]Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark; [2]Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark; [3]Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, Frederiksborgvej 399, DK-4000, Roskilde, Denmark; [4]Department of Plant Biology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871, Frederiksberg C, Denmark; [5]National Institute of Occupational Health, Lerso Parkallé 105, DK-2100, Kobenhavn Ø, Denmark; [6]Forest & Landscape, The Royal Veterinary and Agricultural University, Horsholm Kongevej 11, DK-2970 Horsholm, Denmark

Abstract: The Danish Centre for Biological Control was established in 2003. During the period 2003-2005 four workshops were arranged, covering various aspects of biological control: potential occupational health problems, environmental effects, teaching, and implementation into practice. The paper briefly outlines the results of the workshops and the future prospects for biological control.

Key words: Biological control, Danish Centre for Biological Control

The objectives of the Danish Centre for Biological Control

The Danish Centre for Biological Control was established in 2003. The overall objectives were to support the following activities:

- Research and development of biological control products for application
- Biological research to support natural regulation of pests, diseases and weeds
- Risk assesment
- Teaching and other dissemination of results and possibilities

The centre consists of the main institutions in Denmark working with different aspects of biological control: The Royal Veterinary and Agricultural University, the Danish Institute of Agricultural Sciences, the National Environmental Research Institute and the National Institute of Occupational Health.

During the period 2003-2005 the centre obtained financial support for specific network activities. A webpage was etablished (www.centre-biological-control.dk) and four well-attended workshops were arranged.

The workshops 2003-2005

The Centre organised four workshops/conferences in the period 2003-2005, of which three were international and one was national:

2003: Occupational health risks of producing and handling organisms for biological control of pests in agriculture
2004: Health and environmental risks by the use of organisms for biological control of pests and diseases in agriculture
2005: Teaching biological control (national, in Danish)
2005: International workshop on implementation of biocontrol in practice in temperate regions - present and near future

These activities allowed us to focus on problems and challenges concerning the future development of biological control. At the three international workshops, invited guest speakers from abroad ensured a broad perspective. Much new knowledge was presented and discussed. Abstracts from all workshops are available on our web-page and can be consulted for specific information about the presentations. Further, concerning the last workshop the proceedings provide the information given in the presentations.

Future gaps, challenges and options

At the last workshop we tried to summarize the outcome in a few bullet points, which we found significant for developing biological control to meet future demands:

Question 1: Is biological control adequately utilized and implemented in Europe?
Biological control is now implemented and is an existing possibility for many growers, for example in greenhouse production of vegetables. There was, however, consensus that this method holds more potential in Europe. There are many unexploited possibilities and society will get value for money by allocating funding for studies to enhance biological control as one option among several alternatives to chemical control.

Question 2: What are the major existing constraints?
It was agreed that there is still a mostly unexplored global or regional diversity of species with attractive properties for biological control. We are not in doubt that fundamental studies of host-antagonist relationships can still provide us with new options for biological control.

More attention should be given to identifying limitations and bottlenecks for implementation of biocontrol with focus on compatibility with prevailing farming practice. From the initiation of any biological control programme, the implementation into practice should be an integral element.

Technological exploitation, for example in terms of application methods, is far from being solved. At our meeting, like at many other meetings, scientists and other people with a strong background in especially application methods were underrepresented. Knowledge gaps concerning application methods include studies that can improve the survival and activity *in situ* of microorganisms as well as methods to improve the survival and dispersal of beneficial insects and mites.

The economical constraints are also obvious. In our opinion collaborative studies involving biologists, economists and sociologists are strongly needed to elucidate the economic benefits of biological control. The immediate response from consumers to biological control is mostly very positive. The rather long and expensive registration process in the EU for microbial control agents is, however, a serious restriction for placing products on the market.

Question 3: Can biological control meet future demands?

We believe that biological control holds great potential for meeting future demands for sustainable pest control in our region. The number of challenges is, however, high and the nature of some of these challenges is partly unknown. We have identified the following factors that can promote further implementation of biological control:

- More communication with consumers to ensure that perception of biological control as an effective and yet environmentally friendly strategy is maintained
- A critical attitude to avoid types of biological control that may have a negative impact on human health and/or environment
- A rapid uptake of new methodologies and technologies allowing us to study biological interactions with more sophisticated methods
- An effort to integrate biological control with new cropping methods, for example by ensuring that biological control methods are in agreement with principles of organic farming
- Adaptation of biological control to future changes in European land use, such as the recent shift to new crops (e.g. plants for biomedicine) or non-productive use (golf courses etc.).
- Preparation for the situation created by the increased influx of exotic organisms resulting from increasing trade and transportation. These organisms possess the potential of developing into invasive organisms that represent a threat to native biodiversity. Especially classical biological control may offer good opportunities for environmentally friendly control methods.

Acknowledgements

We thank the programme FØTEK III (administered first by FELFO, later by The Strategic Research Programme) for financial support to The Danish Centre for Biological Control.

Posters

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biological control of house flies and stable flies by inundative release of the parasitiod wasp *Spalangia cameroni* on two Norwegian pig farms

Tone Birkemoe, Arnulf Soleng, Karen Riddervold, Anders Aak
Norwegian Institute of Public Health, Department of Pest Control, P.O. Box 4404 Nydalen, NO-0403 Oslo, Norway

Abstract: *Spalangia cameroni* is a common, naturally occurring parasitoid wasp on pupae of house and stable flies in Norway. This parasitoid has proved efficient in controlling houseflies on Danish farms where pigs were kept on deep bedding. In the present study we tested the ability of *S. cameroni* to suppress flies on farms with another manure system; pens with slits (in one end) above a gutter in which a scraper remove manure approximately every second day. Bedding (wood shavings or straw) is added to the pens daily. Fly-breeding occur in the gutter at points not covered by the scraper. The rooms were washed with water approximately every eleven-twelve week. Biweekly releases of wasps (150-190 wasps per square metre) were performed from April to October at two farms during 2004 and 2005. Each farm had two almost identical rooms with sows and piglets. Wasps were released in one room while the other room acted as a control. During the second year of the study the two rooms were exchanged. Fly numbers was monitored by sticky traps and a visual index based on number of flies per animal. Parasitism was estimated weekly by use of house fly sentinel pupae in 2004. The per cent parasitism of sentinel pupae in 2004 averaged 5% in the release rooms on both farms with a maximum of 16% in July and August. This low parasitism is likely to be caused by a sub-optimal positioning of the sentinel pupa. A survey of natural occurring pupae on both farms in August showed a parasitism of 20-40% for stable and house flies. There was an overall significant effect of treatment (wasp release) on house fly number on the farms. Furthermore, both stable fly and house fly numbers were kept below nuisance level at one farm in 2004. However, the effect varied between farms and years and more information is needed in order to recommend wasp release to farms with this particular manure system.

Key words: Biological control, house flies, hymenoptera, parasitoid, stable flies, wasps

Introduction

Spalangia cameroni is a common, naturally occurring parasitoid wasp on pupae of house and stable flies in Norway (Birkemoe *et al.*, 2004). It has proved successful in controlling house flies on Danish farms, when pigs and cattle are kept on deep bedding (Skovgård, 2004; Skovgård & Nachman, 2004). In the present study we tested the ability of *S. cameroni* to suppress flies on farms with a different manure system.

Materials and methods

Manure system

The manure is removed with a mechanical scraper through a gutter down to a cellar approximately every second day. Bedding (wood shavings or straw) is added to the pens daily after manure has been manually scraped into the gutter. The facilities are washed with water approximately every eleven-twelve week. Fly-breeding mainly occur in the gutter at points not covered by the scraper and in the cellar below the pig facilities.

Experiments

Biweekly releases of wasps (150-190 wasps per square metre) were performed from April to October at two farms in close proximity to Oslo during 2004 and 2005. Each farm had two almost identical rooms (each of 160 m^2 at Farm A and 272 m^2 at Farm B) with sows and piglets. Wasps were released in one room while the other room acted as a control. The control and treatment rooms were reversed during the second year of the study. House fly and stable fly numbers were monitored by a visual index based on number of flies per animal estimated at each visit and by sticky traps (6 per room, 0.18 m^2 surface per trap) hanging for 24 hours. Parasitism was estimated weekly by use of house fly sentinel pupae in 2004. Data on parasitism for 2005 is not yet available. Air temperatures were recorded by a logger at a central position in all rooms. No chemical treatment was carried out at Farm A during the experiment. At Farm B, however, both rooms were sprayed with insecticides in July 2005.

Results and discussion

Parasitism

The parasitism of sentinel pupa in 2004 was higher in the release room as compared with the control at both farms. The per cent parasitism of sentinel pupae averaged 5% in the release rooms on both farms with a maximum of 16% in July and August. This low parasitism is likely to be caused by a sub-optimal positioning of the sentinel pupa. A survey of natural occurring pupae on both farms in August showed a parasitism of 20-40% for stable flies and house flies in the release rooms.

Fly densities

An overall ANOVA test based on average number of flies per trap for each treatment with year and farm as categorical variables showed a significant effect of treatment (wasp release) on house flies ($F_{1,7}$=48.7, p=0.03). The overall effect on stable flies was not significant ($F_{1,7}$=17.9, p=0.15).

The results from Farm A were much more promising than the results from Farm B. In 2004, the treatment room on Farm A had a very low number of house flies and stable flies as compared with the control, which indicates an effect of the wasp release. In 2005, when the rooms were reversed, the number of stable flies was low whereas the house fly numbers reached higher values than the control at two dates in July-August. This coincided with a

breakdown of the air ventilation fan and scraper in the release room, leading to very high indoor temperatures and a build-up of manure. In order to lower the temperature inside all doors were kept wide open letting flies from the rest of the farm enter.

The nuisance level of 13-25 house flies per animal set by the Danish Pest Infestation Laboratory is difficult to compare with our numbers as we did not distinguish between house flies and stable flies. However, based on all flies the total number of flies per animal only exceeded 25 once in the release room on Farm A in 2004. In 2005, however, the number of flies was higher, and it exceeded 50 flies per animal when the scraper was damaged.

One possible difference between the farms is the apparent lack of permanent spots with manure in Farm B. In Farm A, fly pupae was found at the same locations throughout the experimental period. This might have sustained a lager population of wasps and ensured a build-up in wasp number throughout the summer. Another plausible explanation for the difference in success is a larger fly production in the manure cellar on farm B compared with farm A.

Conclusions

Release of *S. cameroni* may suppress stable flies and house flies below nuisance levels on farms with the manure system tested. However, the effect was not consistent on the two investigated farms, and more information is needed in order to recommend wasp release to farms with this particular manure system.

Acknowledgements

Torhild Tveito Compaore, Nina Huynh, Reidar Mehl, Preben Ottesen and Håvard Øyrehagen kindly helped with field work. Nina Huynh has given most valuable help in the laboratory. A special thanks to Bjørn Gevelt and Ole Tom Bøe allowing us to use their farms for the experiments.

References

Birkemoe, T., Soleng, A. & Riddervold, K. 2004: Abundance of parasitic Hymenoptera on pupas of *Musca domestica* and *Stomoxis calcitrans* (Diptera: Muscidae) on pig farms in Vestfold, Norway. Norw. J. Entomol. 51: 159-164.

Skovgård, H. 2004: Sustained releases of the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) for control of house flies, *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) on dairy farms in Denmark. Biol. Control 30: 288-297.

Skovgård, H. & Nachman, G. 2004: Biological control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) by means of inundative releases of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 94: 555-567.

Entomopathogenic fungi for control of stable flies *Stomoxys calcitrans*

M.V. Boese[1,2], T. Steenberg[1], S.A. Nielsen[2]

[1]Department of Integrated Pest Management, The Danish Pest Infestation Laboratory, Danish Institute of Agricultural Sciences, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark; [2]Department of Life Science and Chemistry, Roskilde University, Building 18.1, Universitetsvej 1, P.O.BOX 260, DK-4000 Roskilde, Denmark

Abstract: The stable fly *Stomoxys calcitrans* is a major pest in confined cattle facilities and in pig farms. It is a considerable nuisance to the animals when present in high numbers, and may cause a reduction in milk yield and weight gain. Therefore, the economic importance of this pest raises a demand for improved management/control methods. Entomopathogenic fungi may have potential as a microbial control component of an integrated fly management program. To date, several laboratory and a few field studies have evaluated entomopathogenic fungi for control of the house fly *Musca domestica*, and for some strains of hyphomycetes success have been achieved. In contrast, very little focus has been put on the susceptibility of the stable fly towards entomopathogenic fungi, and these studies suffer from problems with high control mortality. Furthermore, until presently, biological control of the stable fly has almost exclusively been directed against the larval and pupal stages.

The aim of this project therefore is to select a hyphomyceteous fungus with high virulence against adult stable flies. Twelve isolates, representing four species (*Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *Lecanicillium muscarium*), were chosen for the study. Several of the isolates originate from flies from Danish livestock farms. The initial selection criterion was that the fungus must be able to grow at abiotic conditions found in stables during summers (in Denmark). Current bioassays with four isolates are focusing on the selection of the most virulent isolate. This will be followed by transmission studies in cages to determine the practical control potential of this group of fungi. Preliminary results will be presented at the workshop.

Predators as biological control agents in winter oilseed rape fields - Results on predators of the EU-project MASTER

W. Büchs[1], D. Felsmann[1], Z. Klukowski[2], A. Luik[3], C. Nilsson[4], O. Schlein[1], I.H. Williams[5]

[1]BBA, Institute for Plant Protection in Field Crops & Grassland, Messeweg 11/12, D-38108, Braunschweig, Germany; [2]Department of Crop Protection, Agricultural University Wroclaw, 50-205 Wroclaw, Cybulskiego str. 32, Poland; [3]Estonian Agricultural University, Kreutzwaldi 64, Tartu EE 51017, Estonia; [4]Swedish University of Agricultural Sciences, P.O. Box 44, S-230 53 Alnarp, Sweden; [5]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom

Abstract: The role of predators (mainly Carabidae, Staphylinidae and Araneae) as biological control agents of insect pests has been investigated in two different winter oilseed rape management systems in 2003 and 2004. An ICM-system (ICM = Integrated Crop Management) with reduced soil cultivation and no insecticide applications has been compared with a Standard oilseed rape management system. The poster presents a short overview of results achieved during 4 years within the EU-project MASTER (Integrated pest management strategies incorporating bio-control for European oilseed rape pests – QLK5-CT-2001-01447). The following tasks are highlighted:

- Identification of key predator species in European oilseed rape field and assessment their abundance on the basis of a literature review and a Europe wide joint field trial in 5 countries
- Determination of the spatio-temporal within-field distribution of pest larvae & key predators by means of a SADIE-analyis
- Consumption rates and feeding preferences of key species of carabids by conducting choice and non-choice laboratory feeding trials, detection of pest remains by microscopical gut dissection and PCR (Polymerase Chain Reaction)-technique
- Predation rates and management related density of web spiders within the vegetation layer
- Assessment of Staphylinidae-larvae from oilseed rape flower stands and their role in regulation of Meligethes-larvae

Further general information on the EU-project can be found on the project website www.iacr.bbsrc.ac.uk/pie/master/master.htm

Earwig in pome fruit production - a beneficial?

Maja Rohr Hansen[1], Lene Sigsgaard[1], Peter Braun[2]

[1]Department of Ecology, Zoology Group, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark; [2]Department of Agricultural Sciences. Højbakkegård Allé 21, DK-2630 Taastrup, Denmark

Abstract: In the search for new solutions to the pest problems in orchards a possible solution could be to enhance the conditions for the naturally occurring predators. One of these is the common earwig *Forficula auricularia*, which is very common in orchards. This paper will present aspects of its biology and potential use in conservation biological control.

Key words: Common earwig, biological control

Introduction

During the last 20 years the use of many pesticides for pest control in orchards have been forbidden in Denmark (Lindhard *et al.*, 2003). This has contributed to an increased interest in alternative control measures against insect pests. A very common orchard predator is the common earwig *Forficula auricularia* Linneaus. The common earwig is an omnivorous insect and can contribute positively to pest control in apple and pear orchards (Solomon *et al.*, 2000). Earwigs also feed on plant tissue and can feed on fruits as well; however, in pome fruit this is considered of minor importance compared with the beneficial effect. Rearing is difficult, but conservation biological control strategies can be used to enhance earwig numbers. One such strategy is the use of artificial houses. This paper is based on a BSc thesis (Hansen, 2005).

Aim

A good knowledge of the biology of the common earwig, its dietary requirements and habitat needs, is a necessary prerequisite to design orchards in which the earwig will thrive and contribute to the control of insect pests – conservation biological control (Solomon *et al.*, 1999).

Biology

The common earwig belongs to the order Dermaptera. The order consist of 1.800 species divided into families (Gullan & Cranston, 1996). The common earwig belongs to the family

Forficulidae and is today spread all over the world (Behura,1956).

The common earwig has a one-year life cycle. The adults mate in late summer or early fall. After mating the male and female overwinter in the nest, which the female has excavated. In late winter or early spring the female lays its eggs and then drives the male out to protect the eggs. In the following months she tends the eggs until they hatch; without this maternal care the eggs will not hatch. After hatching, the female will continue to nurse the nymphs until they leave the nest (Lamb, 1976).

It is debated whether the common earwig lays one or two clutches of eggs (Behura, 1956; Lamb & Wellington, 1975; Stap *et al.*, 1987), but researchers have recently shown the existence of two sibling species. Crosses between individuals from these two sibling species are sterile (Wirth *et al.*, 1998). It is not known to which sibling species the common earwig in Denmark belongs, i.e. whether it lays one or two clutches of eggs.

The nymphs pass through four nymphal instars before becoming adults. From the third instar onwards nymphs do not return to the nest chamber but can be found in orchard trees day and night (Helsen *et al.*, 1998). The earwig is active at night and needs natural or artificial refugees for protection through the day (Solomon *et al.*, 1999).

The common earwig has a lower temperature threshold for development of 6°C (Helsen *et al.*, 1998). Development from egg to 4th instar takes about 617 day degrees (°DD) and from egg to adult about 880°DD. By calculating day degree temperature sum above 6°C from 1 January the date when the earwig becomes adult can be found (Helsen *et al.*, 1998). In Denmark temperature measurement from Roskilde 2004 has been gathered. Figure 1 shows that according to data from Helsen (1998) applied to temperature data from Roskilde earwigs would enter the adult stage in August 2004.

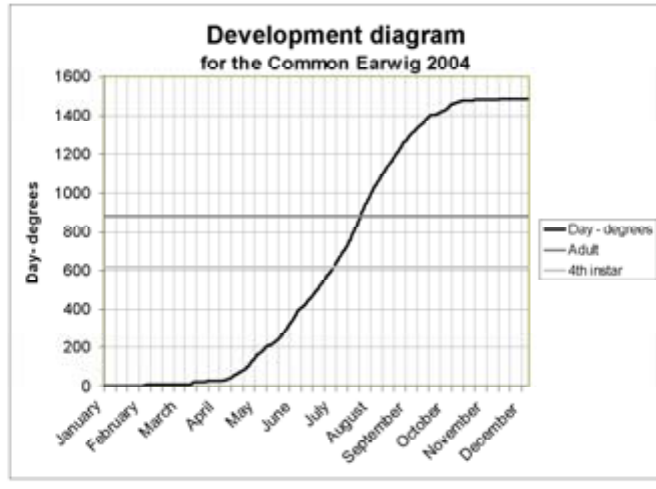

Figure 1. Development of the common earwig from egg to adult based on temperature dependent development data from Helsen *et al.* (1998) and temperature data from Roskilde, 2004, provided by DMI. The light grey line shows the day degrees needed for the common earwig to reach 4th instar nymphs (617°DD), the dark grey line the day degree requirements for development into adult (880°DD) (Helsen *et al.*, 1998). The black line shows the cumulated day degrees with a T_o of 6°C.

Food

The common earwig preys upon many species of insects, in addition to fungi, pollen and plant tissue. Several experimental trials have shown that earwigs are able to significantly suppress populations of aphids and psyllids. Significant pest suppression has for example been documented for *Aphis pomi* DeGeer (Carroll & Hoyt, 1984) and *Eriosoma lanigerum* Hausmann (Stap *et al.*, 1987; Mueller *et al.*, 1988). The earwig can cause secondary damage on already injured fruit and may cause primary damage to soft-skinned varieties of apple (Discovery). Damage to apples and pears are normally considered insignificant. In other crops the common earwig is considered a pest (Solomon *et al.*, 2000).

Habitat

The common earwig requires a protected place in the trees during the day. Young trees do not provide sufficient natural refuges for earwigs (Solomon *et al.*, 1999). It is possible to set up artificial earwig refuges and thereby increase the population of earwigs in the trees. The simplest design is a container with straws or corrugated cardboard attached directly to the tree or placed in a bottle. The artificial refuges are desirable for the earwigs, but it can also become a great help for the growers. The use of artificial refuges allows the grower to move earwigs between trees or remove them completely if they should have a negative impact on the trees.

Conclusion

Biological control in orchards can be improved by providing good conditions for the common earwig. In young trees there are few natural refuges and artificial refuges can help improve biological control. It should be kept in mind that the earwig can eat plant material and therefore may damage fruits or leaves. But this can be controlled by physically moving the refugees at daytime.

It is important to know whether the common earwig produces one or two clutches of eggs in Denmark, as this may influence the ability of earwigs to control pests. Temperature is also an important factor. Data suggest that in Denmark the main contribution from earwigs to biological control will be in late summer. However, information on temperature dependent development for Danish populations of earwigs would be useful.

References

Behura, B.K. 1956: The biology of the European earwig, *Forficula auricularia* L. Annals of Zoology 1, 117-142.

Carroll, D.P. & Hoyt, S.C. 1984: Augmentation of European earwigs (Dermaptera: Forficulidae) for biological control of apple aphid (Homoptera: Aphididae) in an apple orchard. Journal of Economic Entomology, 77, 738-740.

Gullan, P.J. & Cranston, P.S. 1996: The Insects. An Outline of Entomology. Chapman & Hall, London, UK.

Hansen, M.R. 2005: Ørentvisten i kernefrugtavlen - et nyttedyr. BSc Thesis. Department of Ecology, Zoology Group, KVL.

Helsen, H., Vaal, F. & Blommers, L. 1998: Phenology of the common earwig *Forficula auricularia* L. (Dermaptera: Forficulidae) in an apple orchard. International Journal of Pest Management, 44, 75-79.

Lamb, R.L. 1976: Parental behaviour in the Dermaptera with special reference to *Forficula auricularia* (Dermaptera: Forficulidae). Canadian Entomologist, 108, 609-619.

Lamb, R.L. & Wellington, W.G. 1975: Life history and population characteristics of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae), at Vancouver, British Columbia. Canadian Entomologist, 107, 819-824.

Lindhard, H. *et al.* 2003: Vurdering af muligheder for forebyggelse og alternativ bekæmpelse i frugt og bær. Danmarks JordbrugsForskning. Bilag 4 til rapporten "Muligheder for forebyggelse og alternativ bekæmpelse inden for gartneri og frugtavl". Arbejdsrapport fra Miljøstyrelsen nr. 38.

Mueller, T.F., Blommers, L.H.M. & Mols, P.J.M. 1988: Earwig (*Forficula auricularia*) predation on the woolly apple aphid *Eriosoma lanigerum*. Entomologia Experimentalis et Applicata, 47, 145-152.

Solomon, M., Fitzgerald, J. & Jolly, R. 1999: Artificial refuges and flowering plants to enhance predator populations in orchards. IOBC/ wprs Bulletin, 22(7), 31-37.

Solomon M., Cross, J.V., Fitzgerald, J., Campbell, C.A.M., Jolly, R. & Olszak, R.W. 2000: Biocontrol of pest of apples and pears in Northern and Central Europe. 3. Predators. Biocontrol Science and Technology, 10, 91- 128.

Stap J.S., Mueller, T.F., Drukker, B., van der Blom, J., Mols, P.J.M. & Blommers. L.H.M. 1987: Field studies on the European earwig (*Forficula auricularia* L.) as predator of the woolly apple aphid (*Eriosoma lanigerum*). Mededelingen van de Faculteit Landbouwwetenschappen, Universiteit Gent, 52 (2a), 423-431.

Wirth, T., Le Guellec, R., Vancassel, M. & Veuille, M. 1998: Molecular and reproductive characterization of sibling species in the European earwig (*Forficula auricularia* L.). Evolution, 52, 260-265.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Field application of entomopathogenic nematodes to control the cherry fruit fly, *Rhagoletis cerasi* L. (Diptera, Tephritidae): the "how and when" as key to success?

Annette Herz[1], Kirsten Köppler[1,4], Heidrun Vogt[1], Ellen Elias[2], Peter Katz[2], Arne Peters[3]

[1]Institute for Plant Protection in Fruit Crops, Federal Biological Research Centre for Agriculture and Forestry, Schwabenheimer Str. 101, D-69221 Dossenheim, Germany; [2]Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany; [3]e-nema GmbH, Klausdorfer Str. 28-36, D-24223 Raisdorf, Germany; [4]Department of Zoology, University of Heidelberg, Neuenheimer Feld 230, 69120 Heidelberg

Abstract: In recent studies we demonstrated the high potential of entomopathogenic nematodes (*Steinernema feltiae*, *S. carpocapsae*) to infect larvae of the cherry fruit fly, *Rhagoletis cerasi* L. (Diptera, Tephritidae) after leaving the cherry for pupation in the soil in laboratory, semi-field and field trials. These results pointed to a new approach for biological control of this serious pest on cherries in Europe. Here we explore the requirements for feasible and efficient introduction of this method into practice. One challenge is the development of an application technique which maintains quality and persistence of nematodes and which is feasible for the grower. We tested the suitability of a tractor mounted spray boom for treatment under the canopy area to apply the entomopathogenic nematodes (EPN) in several cherry plantations. The achieved application rate was evaluated by petri-dish samples placed on the ground during spraying. Soil samples were taken at regular intervals to assess infectivity of EPN in laboratory bioassays using the host *Galleria mellonella*. The applied rate of EPN met the expectations. Activity of EPN was satisfying directly after application but dropped during the following weeks. The results are discussed regarding suitable application schedules and prospects for implementation under different conditions of cherry growing.

Key words: Fruit flies, entomopathogenic nematodes, application technology, *Rhagoletis cerasi*

Introduction

The cherry fruit fly, *Rhagoletis cerasi* L. (Diptera, Tephritidae) is the main insect pest on cherries in Europe. The fly causes regular infestations, especially in sweet cherry. Growers are urged by the market to produce almost completely undamaged cherries. This leads to regular application of insecticides. Dimethoate is the current product of choice, but the registration for this purpose is banned in an increasing number of European countries. Treatments with insecticides have to be stopped in due time before harvest in order to avoid

residues on the crop, thus leading to unreliable control in many situations. Organic cherry growers are left without any option to combat *R. cerasi* efficiently.

In previous studies, we demonstrated the potential of several species of entomopathogenic nematodes (EPN) to infect and kill larvae of the cherry fruit fly when they crawl into the soil to find a place for pupation (Koeppler *et al.*, 2004). Entomopathogenic nematodes are known to be efficient biological control agents for a number of soil-dwelling insect stages and are nowadays commercially available for control of various pests in outdoor and protected crops as well as turfgrass (Grewal *et al.*, 2005). Beside the pathogenicity for the specific target pest and the quality management during the production process, proper application techniques and timing are crucial for their efficacy under field conditions.

For control of the cherry fruit fly, EPN need to be applied to the soil. As the dropping of *R. cerasi* larvae usually occurs from the whole canopy area, the total soil surface under the canopy must be treated with nematodes. Our aim was to develop an appropriate technique to apply EPN in cherry plantations. In home gardens and for treatment of single cherry trees, the use of a watering can may be suitable. But plantations with many trees require a more time-efficient technique. We tested a tractor-mounted spray boom to apply the EPN in high water volume in several cherry plantations and we evaluated nematode activity and persistence afterwards. A suitable application technique is necessary to assess the potential of EPN for control of the cherry fruit fly under field conditions. It is also an important prerequisite to developing this method towards practical implementation, especially concerning the easy adoption by farmers.

Material and methods

Nematodes

Trials were performed using the product nemaplus® (e-nema GmbH, Raisdorf, Germany) containing the nematode *Steinernema feltiae*. The product was dissolved in water according to producer's instruction before filling it into the tank. Depending on the experiment, nematodes were applied at a rate of 125,000, 250,000 or 500,000 EPN/m² with water. Before spraying, several open petri-dishes were put on the ground within the spraying area and were closed directly after the spraying boom had passed the petri-dish. In the laboratory, the volume inside the petri-dishes and the number of nematodes in 50 µl droplets were determined to calculate the applied rate of nematodes. Furthermore, percentage of motile nematodes was determined as an indicator for nematode fitness.

Spraying equipment

We used a tractor-mounted spray boom, made for applying herbicides, but with only one pole (Figure 1). The length of the pole was 2 m. Pairs of nozzles (Type Albuz® 117APG 80°, flat fan nozzle) were fixed on four positions along the pole. The nozzles are recommended for hop culture and have a flow rate of 10.48 l/min at 3.5 bar at 20°C according to the manufacturer. The tank volume was 350 l and the tank content was recirculated. Filters and sieves in the system were removed before application. We tested the effect of spraying at different pres-

sures on applied nematode rate and quality. Petri-dishes were distributed along the spraying line to evaluate the applied volume per m² and to obtain tank mix samples for determination of nematode concentration and motility as described above.

Application of EPN in the field

Field application of EPN using the equipment described above was performed at several cherry plantations during June and July 2005. The spraying was done at a pressure of 3.3 bar and tractor velocity of 1.5 km/h to achieve the application of 1 l spray liquid per m². Pre-application and post-application irrigation was also done at a rate of 1 l water per m² in order to obtain optimum soil moisture and to rinse any nematodes sticking on plant surfaces into the soil. No further irrigation was performed afterwards.

Estimation of EPN persistence in the field

On each experimental site, four soil samples (10x10x10 cm) were taken under the canopy area of different trees at particular intervals after the application. In the laboratory, activity of nematodes in the soil samples was evaluated by placing 10 last instar *Galleria mellonella* larvae into the soil samples and evaluating larval mortality after 7 days' incubation at 25°C.

Figure 1. Spray equipment using a tractor-mounted spray boom for soil application of the nematode *Steinernema feltiae* against the cherry fruit fly in cherry plantations.

Results and discussion

Suitability of the spraying equipment

In Germany, sweet cherry is a high value crop and consumers demand an insect-free as well

as insecticide-free product. This urges the grower to apply plant protection measures but also to observe regulations for avoiding environmental side-effects and pesticide residues on the crop. For applying pesticides, the use of axial fan sprayers and nozzles which cause low drift-age is state of the art, and most cherry growers possess such equipment. In a previous experiment we used an axial fan sprayer and the nozzles described above at a pressure of 3.5 bar. But a reduction in nematode motility by more than 40% was observed. Probably, this had been caused by a short increase to 10 bar within the spraying system before nozzles opened (data not shown). Furthermore, no more than 0.4 l per m² spray liquid could be created.

With the tractor-mounted spray boom we achieved the desired volume of 1 l spray liquid per m² at a tractor velocity of 1.5 km/h. In addition, the applied rate and the quality of nematodes were maintained at acceptable levels in comparison with the control (stock solution before spraying, Figure 2). Motility of nematodes was not significantly reduced at different spraying pressures.

Whether such a system is adopted will depend on the availability to cherry growers. In conventional farming practice, this equipment is used for herbicide treatments and can be used after careful cleaning to apply nematodes. However, organic farmers do not use herbicides and therefore may not possess the right equipment. Hence, further exploration of other tools to apply the EPN properly is needed to reach also the requirements of this target group.

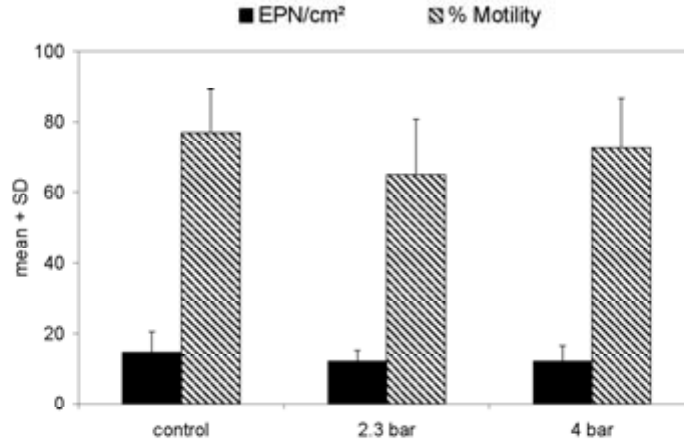

Figure 2. Application rate (EPN/cm²) and motility (% of total number) of *Steinernema feltiae* in spray liquid before spraying (control) and after spraying at different pressures with a tractor mounted spray boom in petri-dish samples. Applied rate was 125,000 EPN/m² by releasing 1 l spray liquid per m².

Persistence of nematodes in the soil

Application regimes and soil conditions varied between the experimental sites where field

trials were performed (Table 1). The treatment was done shortly before harvest time, depending on local weather conditions end of June or beginning of July. Soil samples were taken on the day of the treatment after post-application irrigation as well as after one week, two weeks and four weeks.

Table 1. Experimental sites where field application of entomopathogenic nematodes against larvae of the cherry fruit fly were performed in 2005.

Experimental site	**EPN/m²**	**Area [m²], date of application**	**Soil type, pH-value**
Dossenheim/Baden-Württemberg	500,000	640, one row, 23rd June	29% clay, 54% silt, 17% sand, pH = 6.92
Mittelehrenbach/Bavaria	250,000	1100, six rows, 5th July	34% clay, 41% silt, 25% sand, pH = 7.15
Wendershausen/Hesse	500,000	1200, five rows, 12th July	No data available
Stecklenberg/Saxony-Anhalt	500,000	2400, four rows, 14th July	24% clay, 67% silt, 9% sand, pH = 6.75

Infection rates of exposed wax moth larvae in soil samples taken at the day of application varied between 55% (Dossenheim), 97% (Mittelehrenbach), 100% (Wendershausen) and 73% (Stecklenberg). In Figure 3, activity of EPN in subsequent soil samples is shown in relation to the initial infection rate.

One week after application, the relative activity of EPN in soil samples dropped on average to 60% of the initial value. It is not possible yet to determine if this activity is sufficient to suppress the cherry fruit fly population. This will be done in the following spring by comparing densities of emerged adults in treated and untreated areas in the experimental cherry plantations. But a longer persistence of nematodes in the soil is desirable because drop of cherry fruit fly larvae may last several weeks, especially if trees are not completely harvested. In turf, irrigation frequency after the application proved to be a major factor to maintain field efficacy of applied nematodes (Georgis & Gaugler, 1991), and several irrigation treatments are usually recommended for 2 to 4 weeks' post-application to maintain humid conditions which favour the homogenous distribution and survival of nematodes in the soil (Wright *et al.*, 2005). In Germany, cherry plantations are usually rainfed or drip-irrigated. As an alternative, the use of sprinkler irrigation systems which treat the whole surface under the tree canopy may help to

retain soil moisture also in cherry plantations after application of nematodes without interfering too much with other activities of the grower during the busy harvest time.

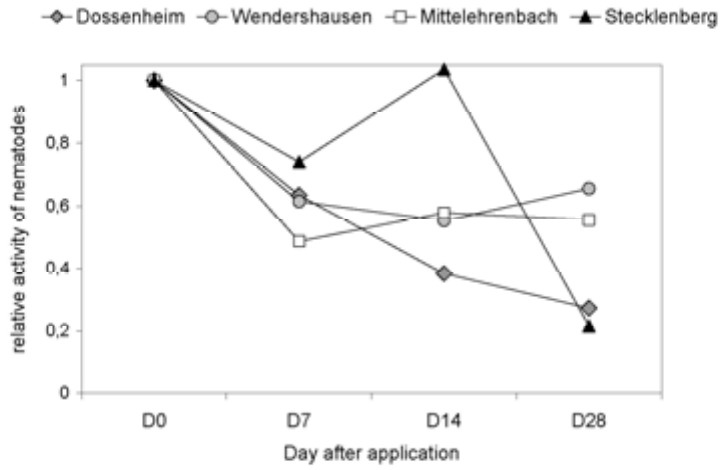

Figure 3. Relative activity of *Steinernema feltiae* in soil samples taken after application in four different cherry plantations (infection rate in soil samples taken on the day of application (D0) is set to 1).

Acknowledgements

This project is funded by the Deutsche Bundesstiftung Umwelt. We thank the gardener team of the Institute for Plant Protection in Fruit Crops, Dossenheim, for development of the spraying technique. Particular thanks to Mrs. Simone Bogun for her skillful technical assistance during field trials.

References

Georgis, R. & Gaugler, R. 1991: Predictability in biological control using entomopathogenic nematodes. Journal of Economic Entomology 84: 713-720.

Grewal, P.S., Ehlers, R. & Shapiro-Ilan D.I. 2005: Nematodes as Biological Control Agents. CABI Publishing, Wallingford, UK, 528 pp.

Koeppler, K., Peters, A. & Vogt, H. 2004: Basic results in biological control of the European cherry fruit fly *Rhagoletis cerasi* L. (Diptera: Tephritidae) with entomopathogenic nematodes. 11th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing. Proceedings of the conference, Weinsberg, Germany, 3-5 February 2004: 48-54.

Wright, D.J., Peters, A., Schroer, S. & Patterson Fife, J. 2005: Application Technology. In: Nematodes as Biological Control Agents, eds. Grewal, Ehlers and Shapiro-Ilan: 91-103.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Competition between insect pathogenic fungi and other fungi, with emphasis on plant disease antagonists

Linda Gordon Hjeljord, Richard Meadow[1], Annette Folkedal[1]

Norwegian University of Life Sciences, Dept. of Chemistry, Biotechology and Food Science, Ås, Norway; [1]Norwegian Crop Research Institute, Plant Protection Centre, Ås, Norway

Abstract: Experiments were performed to determine the ability of the insect pathogenic hyphomycete, *Metarhizium anisopliae*, to germinate, grow and sporulate in the presence of *Trichoderma atroviride*. *Trichoderma* spp. includes mycoparasites commonly found in soil and used in biocontrol of plant diseases. When conidia of *M. anisopliae* and *T. atroviride* were coinoculated on agar media, *T. atroviride* overgrew *M. anisopliae* and prevented its establishment at temperatures from 6-30°C. This effect was lessened when *M. anisopliae* and *T. atroviride* were inoculated at different points on the medium, giving the insect pathogen time to establish a colony and secrete antifungal metabolites. Although both fungi had growth optima at 25-30°C, separately inoculated *M. anisopliae* was best able to compete with *T. atroviride* at temperatures ≥ 18°C, and the antibiosis zone was widest at 30°C. When *M. anisopliae* and *T. atroviride* were coinoculated onto black vine weevil larvae (*Otiorhynchus sulcatus*) at 20°C, the ability of the insect pathogen to infect and kill the larvae and sporulate on the cadavers was not affected by the presence of *T. atroviride* conidia. These results indicate that while the virulence of *M. anisopliae* to the larvae was not reduced by *T. atroviride*, the ability of the insect pathogen to grow saprophytically and increase its inoculum could be reduced on substrates containing *T. atroviride* conidia.

Key words: Competition, secondary metabolite, biological control, fungal interactions

Introduction

In order to infect target insects after application to soil or plant surfaces, conidia of insect pathogenic fungi must remain germinable when exposed to abiotic and biotic stress factors. Certain applications assume an increase in inoculum of the insect pathogens by saprophytic growth and/or sporulation on or near the plant to be protected. As part of an investigation to develop *Metarhizium* and *Beauveria* strains for biocontrol of the black vine weevil (*Otiorhynchus sulcatus*) and other pests, we are investigating the ability of the insect pathogens to compete with other fungi commonly isolated from soil and above-ground plant surfaces, with emphasis on isolates with potential for biocontrol of plant diseases. Here we report on experiments designed to test the effect of *T. atroviride* on the virulence and saprophytic growth of *M. anisopliae* isolates.

Material and methods

Fungal isolates

Stock cultures of *M. anisopliae* and *T. atroviride* were stored in 20% glycerol at –80°C. For inoculum production, *M. anisopliae* isolates NCRI 5/96, NCRI 9/96, NCRI 1/01, NCRI 6/96 and NCRI 250/02 were cultured on SDA at 20°C for 14d. Conidia were washed from the agar in sterile distilled water containing 0.01% Tween 80 and adjusted to the desired concentration. *T. atroviride* P1 (ATCC 70458) was cultured on PDA for 14 d at 20°C, and conidia were washed from the agar in sterile distilled water and adjusted to the desired concentration. Suspensions were prepared on the day of use and stored in the meantime at 4°C.

Competition on agar media

Three inoculation techniques were used: 1) One 10µl drop of a conidial suspension of a *M. anisopliae* isolate and one 10 µl drop of *T. atroviride*, both at a concentration of 8×10^5 ml^{-1}, were placed 50 mm apart on plates of PDA and SDA. 2) Conidial suspensions of *M. anisopliae* and *T. atroviride* were mixed in equal amounts to a final concentration of 8×10^5 ml^{-1} and a 10 µl drop of the mixture was placed on SDA and PDA plates. 3) *M. anisopliae* conidia were used at a concentration of 5×10^6 ml^{-1}, while *T. atroviride* conidia were at a concentration of 5×10^5 ml^{-1}. PDA and SDA plates were inoculated with a 10 µl drop of the mixed suspension 10 mm from the edge of the plate, and then four 10 µl drops of the *M. anisopliae* suspension were placed 15, 30, 45 and 60 mm from the mixed drop. Control plates were inoculated with the *M. anisopliae* isolate alone. Two replicate plates of each medium and each *M. anisopliae* isolate were inoculated and incubated at 20°C until the *T. atroviride* mycelium extended across the plate. In another experiment, 10 SDA plates for each *M. anisopliae* isolate and *T. atroviride* were inoculated using technique 3 and incubated at 6, 12, 18, 24, 30 and 35°C. Diameters of the *M. anisopliae* and *T. atroviride* colonies were measured daily, until the *T. atroviride* mycelium extended across the entire plate. Inhibition % was calculated as 100 x (1-Mt/Mc), where Mt is the area of the *M. anisopliae* colony exposed to *T. atroviride* and Mc is the area of the *M. anisopliae* colony growing alone. All experiments were performed at least twice.

Bioassay

Adult black vine weevils (*Otiorhynchus sulcatus)* were collected from farmers' fields in western Norway in the autumn of 2004. The adult vine weevils were kept at 17°C in large plastic containers with lids with a gauze-covered opening and were fed strawberry leaves. Eggs were collected daily and were put on carrot pieces in autoclaved peat potting mix moistened with distilled water and the larvae were reared at 17°C. For the bioassay, final instar larvae were submerged for 10 sec. in sterile distilled water or in conidial suspensions of *T. atroviride*, *T. atroviride* + *M. anisopliae* NCRI 9/96, or *M. anisopliae* NCRI 9/96 alone, all at a final concentration of 1×10^7 conidia ml^{-1}. The larvae were then placed in 100 ml cups containing sterile soil with carrot pieces at 20°C and after 18 d were examined for mortality and sporulation of *M. anisopliae* or *T. atroviride*. There were 8 repetitions of each treatment, with 5 larvae per repetition.

Results and discussion

Competition on agar media

Two common laboratory media were used for competition experiments, SDA and PDA. SDA is the richer of the two, containing 4% glucose compared with 2% in PDA. Growth and sporulation of *T. atroviride* was better on PDA, while *M. anisopliae* grew and sporulated better on SDA. As results of competition experiments were similar on both media, only experiments on SDA are reported.

When equal amounts of *M. anisopliae* and *T. atroviride* conidia were mixed and placed on SDA, conidia of both fungi germinated at about the same time, e.g. 50% germination time at 21°C was 20 and 21 h, respectively. The radial extension of both fungi was most rapid at 30°C. However, the faster-growing *T. atroviride* hyphae covered those of *M. anisopliae*, which was unable to grow beyond the inoculation drop. No sporulation of the insect pathogen was seen in any of the mixtures, regardless of temperature.

When the fungi were placed on SDA, the degree of inhibition of *M. anisopliae* depended on the distance separating it from *T. atroviride* (Table 1). As the distance increased, the *M. anisopliae* colony was increasingly able to establish a mature colony. By 120 h after inoculation, the appearance of an antibiosis zone around the *M. anisopliae* colonies indicated production of metabolites inhibitory to *T. atroviride*; this was most obvious at 30°C. The results indicate that *M. anisopliae* was most sensitive to competition shortly after germination and that within four to five days after germination the colonies were able to defend a food base.

Table 1. Average area (mm^2) and inhibition (%) of *Metarhizium anisopliae* colonies on SDA at 21°C when inoculated together with, or separated from, *T. atroviride*. A drop of the mixture was placed on the agar, and drops of *M. anisopliae* alone were placed at increasing distances from the mixture at 15 mm intervals.

Distance from the mixture (mm)	**0**	**15**	**30**	**45**	**60**
M. anisopliae colony area (mm^2)	0	78	133	177	254
Inhibition (% of control)	100	70	48	30	0

When the average inhibition of colonies at increasing distance from the mixture was compared at different temperatures, it was seen that *M. anisopliae* isolates were least inhibited by *T. atroviride* at temperatures ≥ 18°C (Figure 1). The standard deviation between isolates indicates that certain strains may be more resistant to inhibition at low temperatures, although *M. anisopliae* in general grew slowly at temperatures below 15°C. All strains were most competitive at higher temperatures and, since *T. atroviride* did not grow at 35°C, the three of the five *M. anisopliae* strains that did grow at this temperature were uninhibited.

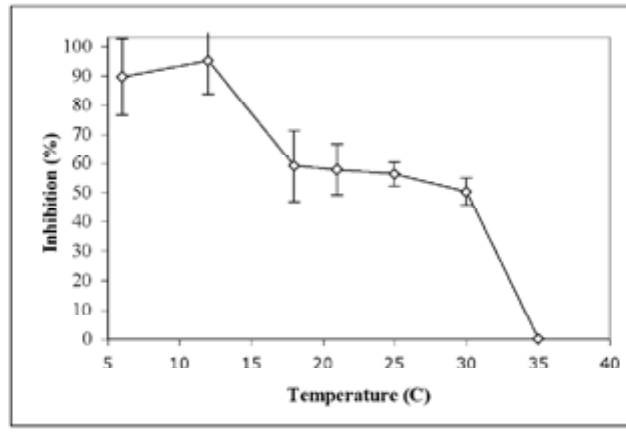

Figure 1. Effect of temperature on inhibition of *Metarhizium anisopliae* by coinoculated *Trichoderma atroviride*. Bars indicate standard deviation between average inhibition of colony area seen in different *M. anisopliae* isolates. Inhibition is shown as % of *M. anisopliae* colony growing alone.

Bioassay

There was little difference in mortality between *Otiorhynchus sulcatus* larvae treated with *M. anisopliae* alone or mixed with *T. atroviride* (Table 2). The mortality in larvae treated with *T.*

Table 2. Mortality of *Otiorhynchus sulcatus* larvae inoculated with conidial suspensions of *Metarhizium anisopliae* alone or mixed with *Trichoderma atroviride*, or *T. atroviride* alone, and incubated on sterile soil for 18 d at 20°C. The control larvae were dipped in water but not inoculated.

	Control	***M. anisopliae***	***M. anisopliae* + *T. atroviride***	***T. atroviride***
Mortality (%)	12.5	90	87.5	52.5
M. anisopliae sporulation on dead larvae (%)	0	72.2	85.7	61.9
T. atroviride sporulation on dead larvae (%)	0	0	0	0

atroviride alone was higher than in the uninoculated control, but when the dead larvae were incubated, only *M. anisopliae* sporulation was seen. The mortality in the *T. atroviride* treatment was thus probably a result of cross-contamination between treatments. No sporulation of *T. atroviride* was seen on the dead larvae, although small colonies observed on soil and larval sheddings indicated that the inoculum was germinable. Living larvae are not a food source for this soil saprophyte, and thus there was no competition between *M. anisopliae* and *T. atroviride* inoculated directly onto the larvae. Further studies are needed to determine the effect of *T. atroviride* on *M. anisopliae* inoculated into soil.

Conclusions

Taken together, the results presented indicate that *M. anisopliae* is most sensitive to *T. atroviride* and other common saprophytes from soil and plant surfaces (data not shown) when attempting to colonize a food base for saprophytic growth. Application of conidia of the insect pathogen to non-sterile soil or plant surfaces may not only result in inhibition of its ability to increase its inoculum potential through saprophytic growth, but may also result in a loss of germinability on insect cuticle due to conidial debilitation by the natural microflora. A key factor is the extent to which the substrate is colonized by other microorganisms at the time of *M. anisopliae* application, as a natural or introduced food base would be subject to competition from more rapidly growing saprophytes. However, if the conidia of the insect pathogen have little competition for a few days following application, the colonies might be able to defend the substrate through production of inhibitory metabolites, and thus be able to increase the inoculum through sporulation.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Mortality factors of Diamondback moth *Plutella xylostella* in the field, Kenya

Christine Kastrup, Lene Sigsgaard
Department of Ecology, Zoology Group, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: The aim of the study was to investigate mortality factors of Diamondback moth in kale (*Brassica oleracea* L.var. *acephala*) fields in the lowland of Kenya. Two field experiments were done during February and April 2005 at two different field sites. The first experiment was a pre-release mortality study, designed to quantify the natural contribution of parasitoids to Diamondback moth larval and pupal mortality. The second experiment was designed to assess the presence of predators on the soil surface, using pitfall traps. The natural contribution of parasitoids to Diamondback moth larvae and pupal mortality was below 20 per cent in both sites. Parasitoids observed were *Oomyzus sokolowskii, Diadegma mollipla* and *Apanteles sp*. Pitfall traps revealed high spider activity, in particular species belonging to Lycosidae. Other common predatory species include a carabid beetle and an ant (*Pheidole megacephala*).

Key words: Diamondback moth, *Plutella xylostella*, biological control, parasitoids, predators

Introduction

Plutella xylostella (L) (Lepidoptera: Plutellidae), the Diamondback moth (DBM) is a destructive pest on cruciferous plants worldwide. Its pest status has risen rapidly since the 1960s when large scale application of insecticides began in vegetable crops (Talekar & Shelton, 1993). The DBM has developed resistance against most of the insecticides available, including toxins of the microbial agent *Bacillus thuringensis* (Sun *et al*., 1978; Tanaka, 1992; Baxter *et al*., 2005; Sarfraz & Keddie, 2005). More sustainable integrated pest management (IPM) is therefore needed (Talekar & Shelton, 1993). Natural mortality factors can play a role in restricting population densities. The aim of the study was to investigate natural enemies of Diamondback moth in kale (*Brassica oleracea* L.var. *acephala*) fields in the lowland of Kenya.

Materials and methods

In order to investigate natural enemies' contribution to DBM mortality, two field experiments were done. The first experiment was a pre-release mortality study, designed to quantify the natural contribution of parasitoids to DBM larval and pupal mortality. The second

experiment was designed to assess the presence of predators on the soil surface, using pitfall traps.

Field experiments were conducted during 2005 at two different sites in Kenya: Yatta and Athi River. Yatta is situated 60 km North East of Nairobi at an altitude of approximately 1252 m above sea level. Athi River is situated 40 km South East of Nairobi along the Mombassa road at an altitude of approximately 1491 m above sea level.

Both fields were divided into sixteen approximately 10x10 metre long plots. Four plots, one in each row, were used for the experiment.

Pre-release mortality study

To asses the natural enemies' contribution to DBM mortality, six treatments were applied: a) Cage closed with glue at the plant base, b) Cage closed without glue, c) Cage open with glue d), cage open without glue, e) No cage with glue and f) No cage without glue. Each treatment consisted of four plants (with six fully extended leaves each), and one plant from each treatment was placed in each plot. At the beginning of the experiment, leaves were infested with ten neonate larvae each. One leaf per plant was collected at each sampling date. Larvae or pupae found at the collected leaves were counted, recorded and incubated separately until emergence or death upon which the cause was recorded.

The experiment in Athi River was performed in the dry season from 3 February until 13 February and repeated in the rainy season from 7 April until 17 April. In Yatta the experiment were performed from 17 February until 27 February and repeated from 21 April 2005 until 1 May.

Presence of predators in the field

Predator activity was monitored using five randomly placed pitfall traps in each of the four plots. Every other day the traps were collected and the invertebrates were preserved in 70% alcohol. Monitoring commenced 3 February 2005 and continued until 13 February 2005 in Athi River and in Yatta lasted from 17 February 2005 until 27 February 2005.

Results and discussion

Pre-release mortality study

The parasitism of DBM larvae and pupae varied a lot (Figure 1). Since data from treatments with and without glue were not significantly different, they have been pooled and are in Figure 1 seen as three different treatments (cage closed (CC), cage open (CO) and no cage (NC)). In general, parasitism was below 5% except for the experiment in Yatta during February where parasitism reached 20% for the CO treatment. The parasitoid found in highest numbers was *Oomyzus sokolowskii*. Other parasitoids found were *Diadegma mollipla* and *Apanteles sp*. No parasitoids were found in closed cages.

Significantly more larvae were refound in the closed cage (CC) compared with the two other treatments (CO and NC). While closed cages protected DBM larvae against natural enemies, open cages and no cages did not. The difference in mortality between closed cages and the other two treatments (CO and NC) provides a measure of mortality caused by natural

enemies. In the closed cage 33-48% of the DMB larvae infested on the plants were refound. In both the open cage and no cage treatments 14-26% of the larvae were refound. Based on these data it can be concluded that larvae exposed to natural enemies suffered up to twice as high mortality, showing that the contribution to DBM mortality can be substantial.

However, there was an unexplained mortality in the closed cage, which could not have been caused by natural enemies. This unexplained mortality could have been caused by abiotic factors such as water-stressed plants and high temperatures. However, mortality caused by abiotic factors appears to be equivalent for all three treatments. This is based on the fact that mortality in the open cage was not different from the no cage treatment, meaning that the cage did not provide any protection against mortality caused by abiotic factors.

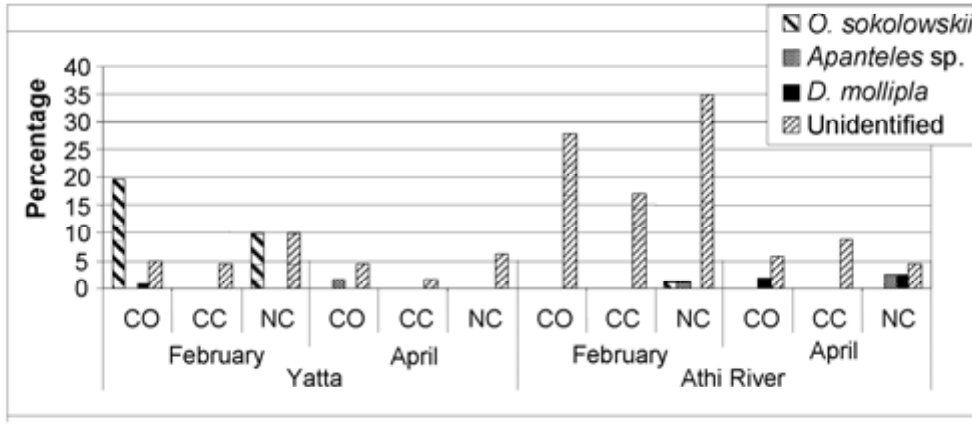

Figure 2. The percentage of DBM parasitized by; *Oomyzus sokolowski*, *Apanteles sp.* and *Diadegma mollipla*; and dead DMB where the cause of death was unidentified. The results are from the experimental sites in Yatta and Athi River, Kenya. (Treatments: Cage open (CO); cage closed (CC); no cage (NC)).

Presence of predators in the field

A number of potential predators were present in the field. Pitfall traps revealed high spider activity. Especially two spiders, not yet identified, belonging to Lycosidae were present in high numbers. Up to 6% of invertebrate trapped, belonged to this family. Other common and potential DBM predators found were a carabid beetle (15% of invertebrates trapped) and an ant (*Pheidole megacephala*) (65% of invertebrates trapped). *P. megacephala* is a predator on termites. Further studies on the predator prey range are needed to conclude if they are predators of DBM.

Possibilities for biological control of DBM in the temperate vs. tropical areas

DBM can also be a serious pest in temperate areas. Research suggests that its origin is in Af-

rica, which is why natural enemies, suitable for classical biological control, possibly could be found in these areas (Kfir, 1998).

Acknowledgements

We thank Dr. Bernhard Löhr from the International Centre of Insect Physiology and Ecology (ICIPE) for valuable discussion and support during Christine Kastrups stay in his laboratory. We also would like to thank Ana Milena Varela (ICIPE) for identification of the ants.

References

Baxter, S.W., Zhao, J.Z., Gahan, L.J., Shelton, A.M., Tabashnik, B.E. & Heckel, D.G. 2005: Novel genetic basis of field-evolved resistance to Bt toxins in *Plutella xylostella*. Insect Molecular Biology. 14(3): 327-334.

Kfir, R. 1998: Origin of the diamondback moth (Lepidoptera: Plutellidae). Annals of the Entomological Society of America, 91(2): 164-167.

Sarfraz, M. & Keddie, B.A. 2005: Conserving the efficacy of insecticides against *Plutella xylostella* (L.) (Lep., Plutellidae). Journal of Applied Entomology, 129(3): 149-157.

Sun, C.N., Chi, H. & Feng, H.T. 1978: Diamondback moth resistance to diazinon and methonyl in Taiwan. J. Econ. Entomol. 71: 551-554.

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

Tanaka, H. 1992: Occurrence of resistance to *Bacillus thuringensis* formulation Toarro Ct in diamondback moth and virius trials for integrated control in greenhouse watercress. In: Proceedings of the Second International workshop. Shanhua, Taiwan, Asian vegetable Development Center: 165-173.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Fungal bands as a method for biocontrol of *Strophosoma* weevils

Christina Krabbe, Charlotte Nielsen, Susanne Harding, Jørgen Eilenberg
Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: In Danish greenery production *Strophosoma capitatum* and *S. melanogrammum* frequently cause damage due to the adult stage feeding on the needles. Chemical control is not permitted against these weevils in Denmark. Therefore alternative control is needed; this could for example be biocontrol using entomopathogenic fungi. The adults of *Strophosoma* spp. are flightless (with fused elytra) and as a consequence adults only disperse by walking. In spring and autumn the adult weevils have long activity periods where they move back and forth between the soil and the canopy. The weevils' life cycle and behaviour suggest that adults could be targeted during these activity periods.

The objective of our study was, under laboratory conditions, to test the effect of fungal bands placed around the tree stem by observing the behaviour and fungal infection of individual weevils, when placed 8–10 cm below a fungal band impregnated with either *Beauveria bassiana* or *Metarhizium anisopliae*.

The study documented that the majority of the weevils moved upward and got in contact with the fungal band and thus were at high risk of getting infected. The bands impregnated with *B. bassiana* caused the highest infection level (63%) compared with *M. anisopliae* impregnated bands (27%), but further testing in the laboratory and in the field is needed before any recommendation can be given. In addition, the fungal bands acted as a physical barrier to the weevils, because the weevils do not like footing to the structure covered with fungus.

Key words: *Strophosoma*, weevils, fungal bands, *B. bassiana*, *M. anisopliae*, behaviour, biocontrol, laboratory studies

Introduction

In Danish greenery plantations the two weevil species *Strophosoma melanogrammum* and *S. capitatum* (Coleoptera: Curculionidae) cause serious damage due to the adult stage feeding on the needles in spring and autumn resulting in economic losses (Harding, 1993; Eilenberg *et al.*, 2003; Anonymous, 1999). Both species are flightless (with fused elytra) and as a consequence adults only disperse by walking (Sedlag & Kulicke, 1979; Palm, 1996). During their activity period they move back and forth between the soil and the canopy several times (Nielsen *et al.*, 2004). This behaviour gives the potential for using a control strategy that targets the adult weevils on the tree stem.

To control the weevils in the plantations, growers have earlier used synthetic chemicals. However, since 2003 no chemical pest control is allowed in state forestry in Denmark (Anonymous, 1998) due to environmental and health factors. For privately owned forests, there is a recommendation from the Danish state authorities to phase out chemical pesticides (Ravn, 2000). Therefore alternative control methods are highly needed in order to control the weevils in greenery plantations. One possibility is biocontrol using entomopathogenic fungi. In field tests the fungus *Metarhizium anisopliae* (Metch.) Sorok. (Ascomycota: Hypocreales) showed promising results as a potential biocontrol agent of *Strophosoma* spp. when applied to the soil (Eilenberg *et al.*, 2003; Nielsen *et al.*, 2004). Although most studies have concentrated on *M. anispliae,* the entomopathogen *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) may also have a potential since it cause natural occurring infections in *Stophosoma* populations and has shown infectivity in laboratory bioassays (Nielsen *et al.*, 2004).

Fungal bands placed around tree trunks have successfully been used in other host–pathogen systems. For example, fibre bands impregnated with *Beauveria brongniartii* effectively control the Asian longhorn beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae) on *Salix* sp. (Dubois *et al.*, 2004; Dubois *et al.*, 2004).

The objective of the present study was to test the effect of fungal impregnated bands on behaviour and mortality of *Strophosoma* spp. in the laboratory. Thereby, the potential for fungal bands as biocontrol will be evaluated.

Materials and methods

Fungal bands

M. anisopliae isolate KVL 03-143 (Bipesco 5) and *B. bassiana* isolate KVL 98-20 were used for impregnating bands. The bands were made of gauze compress covered with 6.5% Sabouraud dextrose agar (SDA) mixed with 3% water agar inoculated with a conidial suspension adjusted to 1×10^9 conidia per ml for *M. anisopliae* and 1×10^8 conidia per ml for *B. bassiana*. The conidial germination was >98% for both *M. anisopliae* and *B. bassiana*. Bands were incubated for approximately one to two weeks at 20°C and stored in the fridge at 5°C until used in assays. As control a clean gauze compress was used.

Test weevils

The weevils were collected in spring 2005 from Bidstrup, Zealand, Denmark. Cohorts of 25-50 weevils were kept in a climate room at 20°C until use.

Experimental set-up

For each treatment a small tree was constructed by using a branch of approximately 40 cm with small twigs eclipsed to the top end symbolising a tree stem. Each stem was placed in autoclaved sand in a pot. Approximately 10 cm above the sand a fungal band was eclipsed tightly to the stem. Each experiment consisted of a control treatment, a *B. bassiana* treatment and a *M. anisopliae* treatment. For each experiment 15 weevils were placed successively on the lower part of the stem approximately 8–10 cm below the band. Altogether ten experiments were carried out over a three-months period. Each experiment was carried out with either *S.*

melanogrammum or *S. capitatum* only, thus in six of the experiments *S. melanogrammum* was used as test insect and in four of the experiments *S. capitatum* was used.

The behaviour of each weevil was recorded by scoring the behaviour into one of the following four categories: 1) the weevil had no contact with the band, 2) the weevil had peripheral contact with the band, 3) the weevil had full contact with band but never crossed the band and 4) the weevil crossed the band. Each individual weevil only got one chance to either cross the band or avoid it. After end session, each weevil was placed in a 30 ml bio-assay cup. Each bioassay cup contained a small twig of Nordman fir, *A. nordmanniana*, placed in 3% water agar in order to keep the plant material fresh and ensure high humidity. The cups were covered with a semi permeable PVC plastic lid and incubated in a climate room with L:D = 16:8 hours at a temperature of 20°C. Mortality and sporulation were recorded for up to five weeks. For practical reasons the weevils were checked twice a week.

Data analysis

Behavioural data were statistically analysed by logistic regression using the GENMOD procedure in SAS (SAS Institute 1999).

Results and discussion

Fungal infection

Preliminary laboratory experiments documented infection in *Strophosoma* spp. after exposure to fungal-impregnated bands placed around tree stems. No significant differences were found in the level of fungal infection between the two *Strophosoma* species tested (data not shown), therefore data for the two weevil species were merged in further analysis. Death due to infection occurred one to five weeks after exposure, with the majority of weevils dying four to five weeks after exposure (Table 1). Unfortunately, a relatively high mortality was registered among control weevils; however no infection with entomopathogenic fungi was observed, except for one experiment where one weevil in the control treatment was infected with *B. bassiana*. This was probably due to a natural occurring infection prior to use as test insect.

Table 1. Total per cent mortality and per cent sporulation in *Strophosoma* spp. two–three and four–five weeks after exposure to fungal impregnated bands.

Treatment	N	2–3 weeks		4–5 weeks	
		Dead (%)	Sporulation (%)	Dead (%)	Sporulation (%)
Control	150	42	-	63	0.7
M. anisopliae	150	51	14	78	27
B. bassiana	150	67	45	85	63

In the majority of the experiments (seven out of ten) higher infection levels of *Strophosoma* spp. were obtained when exposed to *B. bassiana* impregnated bands compared with *M. anisopliae* impregnated bands (Figure 1).

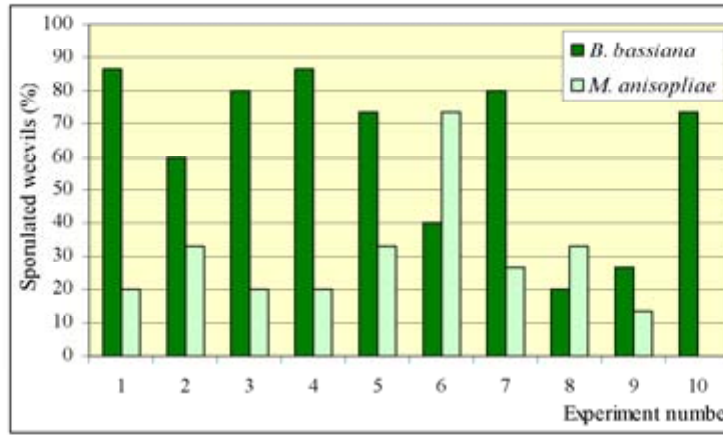

Figure 1. Diagram of per cent fungal-sporulated *Strophosoma* weevils within five weeks. Experiment no. 1–5 and no. 10 were tested on only *S. melanogrammum* and no. 6–9 on *S. capitatum*. Each experiment was conducted with 15 replicates. Data also included those weevils that did not get in contact with the fungal bands.

Behaviour

No significant difference in behaviour was found between the two *Strophosoma* species collected in spring. However, the behaviour of *Strophosoma* spp. differed significantly when presented to fungal bands compared with control bands ($P < 0.05$). Thus, weevils were more likely to cross control bands than fungal bands. This suggests that weevils were able to distinguish whether bands were impregnated with fungus or not.

Table 2. Total percentage for each behavioural category of *Strophosoma* spp. when presented for bands around the stem.

Treatment / Behavioural category	Control (%) N = 150	*M. anisopliae* (%) N = 150	*B. bassina* (%) N = 150
No contact	11	10	15
Peripheral contact	7	23	21
Full contact	7	43	46
Crossing	75	25	17

The behaviour of *Strophosoma* spp. was not significantly affected by fungal species used for impregnation ($P = 0.1693$). However, the percentage of weevils crossing the fungal band was slightly higher for *M. anisopliae* than for *B. bassiana* impregnated bands (Table 2). Several explanations for the differences in behaviour are possible. One hypothesis could be that the weevils do not like footing the fungal band structure. Another hypothesis could be that the weevils are able to recognise the fungus and thereby avoid it. Nevertheless, this hypothesis needs to be tested in further experiments.

In conclusion, fungal bands may act both as biocontrol agent as well as a physical barrier for the weevils.

Acknowledgements

We thank Karen Marie Kjeldsen for skilled assistance in the laboratory, Jan Martin for collecting weevils and Ib Skovgaard for statistical advice.

References

Anonymous 1998: Evaluering af Pesticid Handlingsplan II og aftalen om afvikling af pesticidanvendelse på offentlige arealer, Miljøministeriet 2003. (Online) Available from: http://www.mst.dk/ Last consulted on 08-11-2004 (*In Danish).*

Anonymous 1999: Skov og Landskab, FSL. (Online) Available from http://www.sl.kvl/Videntjeneste/Pyntegroent/5/05--d--05-03.aspx Last consulted on 08-11-2004 (*In Danish).*

Dubois, T., Hajak, A.E., Jiafu, H. & Li, Z. 2004: Evaluating the Efficiency of Entomopathogenic Fungi Against the Asian Longhorned Beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae), by Using Cages in the Field. Environ. Entomol. 33 (1): 62–74.

Dubois, T., Li, Z., Jiafu, H. & Hajak A.E. 2004: Efficacy of fiber bands impregnated with *Beauveria brongniartii* cultures against the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Biol. Contr. 31: 320–328.

Eilenberg, J., Nielsen, C., Vestergaard, S., Harding, S. & Augustynuik, A. 2003: Biological control of weevils (*Strophosoma* spp.) in Danish greenery plantations. IOBC/WPRS Bull. 26: 55–58.

Harding, S. 1993: Gråsnuder – et aktuelt skadedyr [*Strophosoma* – a current pest]. Fakta om skovinsekter. Skoven 8: 330–331. (*In Danish).*

Nielsen, C., Eilenberg, J., Harding, S. & Vestergaard, S. 2004: Biological Control of Weevils (*Strophosoma melanogrammum* and *S. capitatum*) in Greenery Plantations in Denmark. Pesticides Research No. 91. 84 p.

Palm, E. 1996: Nordeuropas Snudebiller. De kortsnudede arter (Coleoptera: Curculionidae) – med særligt henblik på den danske fauna [Weevils (Coleoptera: Curculionidae) from the Northern part of Europe]. Danmarks Dyreliv Bind 7. Steenstrup, Denmark: Apollo Books. 356 p. (*In Danish.)*

Ravn, H.P. 2000: Status for de vigtigste Skadevoldere – ind i det ny årtusind med eller uden pesticider [Status for the most important pests]. Beretning, Skov- og landskabskonferencen 2000: 98–104. (*In Danish).*

SAS Institute Inc. 1999: SAS/STAT User's Guide, Version 8. 1st ed. SAS Institute Inc. Cary, NC, USA.

Sedlag, U. & Kulicke, H. 1979: Zur Biologie, zum Schadauftreten und Bekämpfung des Dichtscuppingen Gtaurüsslers (*Strophosomus capitatus;* Coleoptera: Curculionidae) in Kiefernkulturen. Beiträge für die Forstwirtscharf: Heft 2. (*In German).*

A general simulation model based on the interaction between the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) and its host *Stomoxys calcitrans* (Diptera: Muscidae) in animal stables

Daniel Larsen[1,2], Henrik Skovgård[1], Gösta Nachman[2]

[1]Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Danish Institute of Agricultural Sciences, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark; [2]Department of Population Biology, Institute of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark

Abstract: A general stage structured model with temperature as the main driving force was developed for simulation of the interaction between *Spalangia cameroni* Perkins and its host, *Stomoxys calcitrans* (L.) in animal stables. *S. cameroni* is already being used for controlling *S. calcitrans* and the house fly *Musca domestica* in stables on a commercial basis.

The model is based on a deterministic approach with the equations founded on theoretical considerations. This allows the model to run on data of any pair of parasitoid and their hosts. The model is applied to real systems by choosing the equation parameters to make them fit actual data. Apart from temperature the environment is considered constant. This includes unlimited food source of the host *S. calcitrans*, which seems appropriate for systems such as manure in animal stables.

Since *S. cameroni* is a solitary pupal parasitoid the host population could be divided into four stages, an egg stage, larval stage, pupal stage and an adult stage. Using the same equations, each of these stages could easily be split into different stages.

Calibration of the parameters and sensitivity analyses is still to be completed, but the preliminary results from model runs so far shows an increase in the stable fly population with increasing daily average temperatures. This increase is seen as the optimum temperature of approximately 30°C is approached, above which fly numbers decline. When parasitism is applied to the model, stable fly levels are suppressed by up to 95% as the parasitoid population peaks. The time delay between fly and parasitoid population peaks corresponds to approximately the development time of immature parasitoids, which is about 30 days.

For validation of the model, seasonal data from stables in Denmark will be used. These studies give estimates of fly numbers in stables where *S. cameroni* have been released regularly throughout the season and some without application of parasitoids.

Population genetic studies of *Entomophthora muscae*

Malene Lihme[1], Annette Bruun Jensen[2]

[1]Institute of Microbiology, University of Copenhagen, Øster Farimagsgade 2D, DK-1353 Copenhagen K. Denmark; [2]Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: In the current projects we aim to examine the population genetic structure of *Entomophthora muscae*, an insect pathogen that commonly infects house flies (*Musca domestica*).

Primers were designed to amplify variable regions in protein coding genes of *E. muscae*, currently promising loci have been found. This will make it possible to amplify several *Entomophthora* specific sequences directly from infected flies. Single stranded conformation polymorphism (SSCP) will be used to screen the PCR products for multiple sequence types and each type will subsequently be sequenced.

Flies infected with *Entomophthora* will be collected from six farms on Zealand and we hope that this multilocus genotyping can tell if there is any genetic structure in and between the six geographic *Entomophthora* populations. In addition it might also be possible to show if any recombination is taking place.

Birch distillate efficiently repels Arionidae slugs from Chinese cabbages

Bengt Lindqvist, Irene Vänninen, Kari Tiilikkala
Agrifood Research Finland MTT, Plant Protection, 31600 Jokioinen, Finland

Abstract: Birch distillate is a side-product of burning birch wood in a patented owen system to make charcoal (Charcoal Finland Oy, Alavieska). The distillate contains about 10 000 different bioactive substances, many acting as natural defence against pests and decomposers of birch trees. Preliminary tests suggested the distillate has potential as plant protectant against thirty or so different pest organisms. In laboratory tests, the distillate was highly repellent to slugs in the family Arionidae. In 2005, two semi-field experiments were conducted to determine the repellent efficacy of the distillate against *Arion lusitanicus* and *A. fasciatus* in outdoor conditions.

Chinese cabbages were grown in plastic pots that received six treatments: 1) control (no collar around the pot rim, no distillate); 2) no collar+distillate applied at two weeks intervals to the outward rim of pots in a mixture of carrier substance (30% distillate, 70% carrier); 3) like 2, but treatment intervals of one week; 4) collar only (cut from a plastic plate and attached around the pot rim to protect the distillate from rain and sun); 5) collar + distillate applied biweekly; and 6) collar+distillate applied at one weeks intervals. The experiment was a completely randomized one with four replicates per treatment. Percentage damaged leaf area was estimated visually weekly. In treatments 2, 3, 5 and 6, damage remained below 3% throughout the four-week experiment, while it reached 85% and 59% in treatments 1 and 4, respectively, within the first week and was 100% after two weeks.

The experiment was repeated over a 6,5-week period with the same six treatments and daily damage observations. Now weeds were eliminated from around the pots and care was taken to prevent cabbage leaves from extending to soil surface to ensure slugs could not enter the pots from soil surface along plants. Distillate reapplications were stopped after three weeks when all control plants (treatments 1 and 4) died of damage. Observations in other treatments continued for 2,5 weeks after that. In all distillate treatments, leaf damage was less than 5% for 4-5 weeks, and treatment 6 was free of damage except the last observation day. In control treatments 1 and 4, damage reached 100% in less than three weeks. Thus birch distillate efficiently repels Arionidae slugs when applied as a protective barrier around plants, and the efficacy lasting as long as 3 weeks without reapplication when the distillate is protected from rain and sun.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

The impact of transgenic plants on natural enemies: a critical review of laboratory studies

G.L. Lövei[1], S. Arpaia[2]

[1]Department of Integrated Pest Management, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, DK-4200 Slagelse, Denmark; [2]ENEA – National Agency for New Technology, Energy and Environment, Research Centre Trisaia, S.S. 106 Jonica Km 419.5, I-75012 Rotondella (MT), Italy

Abstract: We reviewed laboratory tests to study the impact of genetically modified plants on arthropod natural enemies. A total of 18 species of predators and 14 species of parasitoids have been tested, most in a few experiments only. Certain groups (braconid wasps) or species (the green lacewing, *Chrysoperla carnea*) have attracted much effort, while representatives of others, including whole orders (e.g., Diptera) have no species ever tested. We conclude that laboratory tests are not the "worst case" scenarios intended by the experimental designs, and often are ecologically not realistic: they typically provided *ad libitum* feeding, no prey choice, single prey type, no combination of stress factors and usually uniform temperatures. None of these are representative of field conditions and most could be easily mimicked in more complex laboratory tests. In most cases (4.5%), studies could not indicate the level of power required to detect any impact. Small sample size and large variability are factors that mask all but very large differences in potential effects. For predators, 126 parameters were quantified, most commonly including survival/mortality (37 cases), development time (22), and body mass/size (20). For parasitoids, 128 parameters were quantified, the majority involving lectins or proteinase inhibitors. Most frequently fecundity (23 experiments), adult longevity, extent of parasitism (17 each), body size, mortality, and larval development time were measured. An aggregative scoring (summarising all quantified parameters) indicated that laboratory tests quantified a remarkable number of cases (30% for predators, 39,8% for parasitoids) where the impacts of the GM plant were significantly negative. These involve different parameters, organisms, test methods and significance levels, but collectively they indicate that the use of genetically modified crops may result in negative effects on natural enemies of crop pests.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Biological control: socioeconomic considerations

Ingeborg Menzler-Hokkanen
Ruralia Institute, University of Helsinki, Lönnrotinkatu 3-5, 50100 Mikkeli, Finland

Abstract: Biological control cannot as yet seriously 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 in Europe is the rule rather than exception. Short-term private benefits usually dictate at farm level which method of pest control will be used. Pest management decisions, however, do not only provide private benefits and costs to the farmer, but also affect the society at large. Negative impacts on the society mainly relate to pesticide usage, which involves at least two major categories of externalities. Firstly, human health can be affected by pesticide use. 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. Valuing these externalities is a difficult and complicated task. Research on the environmental and socio-economic costs of pesticides has shown that the replacement of chemical pesticide treatments by biological controls would bring immense socio-economic benefits to the society: the benefits from controlling the pests would still accrue, but the negative externalities would disappear. The benefits that could be accrued by the society from a higher degree of adoption of biological control methods should therefore be incorporated into the decision making and support structures, which determine the farmer's choice of pest management methods. 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, while currently this price support is in favour of the chemical pesticides. In addition to this bias in the pricing structure, a major obstacle in the development of economically competitive biological control methods has been the requirement for lengthy, costly, and for the most part, useless registration procedure for microbial control agents, following the rules originally intended for chemical pesticides.

Key words: Pest management decisions, economic evaluations, externalities, societal costs, environmental costs, biological control, greenhouse industry, protected crops, contract farming

Introduction

Biological control has many highly interesting economic and social dimensions, many of which are hardly touched in the scientific literature. The different approaches to biological control (Eilenberg *et al*., 2001) and their applications offer many opportunities for economic and societal analysis, but these have largely been neglected. While some biological control applications are attractive 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). In some other cases 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 (Menzler-Hokkanen, 2006). A holistic socio-economic analysis of biological control would be desperately needed and would fit for example as an EU research project; here my aim is just to initiate discussion and to illustrate the field.

Socio-economic aspects of pest management

Farm-level issues

A farmer's choice of the pest management method is influenced by many factors. These include:

- 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 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

Sometimes the farmer does not even have 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. Otherwise, at the farm level, the overriding 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 a negative balance is obtained (Kurppa, 1990).

Biological control cannot as yet seriously 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.

Society-wide issues

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 mainly relate to pesticide use, 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 some of these in a series of papers analysing the economic benefits of alternative pesticide use 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 are £6 worth of benefits to the 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 in the US these costs amount 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 are more than double of that those paid by farmers and could be viewed as society subsidies to support this form of pest management.

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).

How to promote biological control?

In order for the society to accrue the benefits from a higher degree of adoption of biological control methods, the decision making and support structures determining the farmers' choice of pest management methods should be influenced. Firstly, the development of new biological control methods for situations where satisfactory solutions do not currently exist should be strongly supported by governments, including 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% account 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 that 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.

Less costly regulation procedures would enhance commercialization of biological control agents, as can be exemplified by the commercial success of invertebrate agents. Unlike microbials, these have so far 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 to the production of insects or mites (allergies) can be managed and avoided without major costs (Ehlers, 2005). Existing and threatening overregulation of the biological control market in the EU also contradicts the objectives of developing sustainable, ecologically and economically sound agriculture and forestry management systems.

Economics of biological control

Different approaches to 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 programmes 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).

European greenhouse industry: where biological control makes a difference

European greenhouse industry has been able to keep and increase its competitiveness in the domestic markets, and it has recently strongly entered also the fresh products markets in North America. For example, in 1998 and in 1999 annually about 35,000 metric tons of fresh tomatoes were imported into the USA from the Netherlands – about ten times more than just five years earlier (Cantliffe & Vansickle, 2003). Productivity in European greenhouses is nearly three-fold, and in some cases ten-fold, compared with field production in Florida. Product quality is also generally much higher from greenhouse versus field-produced vegetables. Competitive cost structures for greenhouse production and greater product quality have allowed producers from Holland and Spain to increase their presence in the USA markets, creating greater demand by consumers for their produce (Cantliffe & Vansickle, 2003).

How did that happen? In my view, much of that success is to be credited to probably the best example of well-functioning biological control, anywhere. Only a few decades ago, greenhouse production in Europe was suffering from severe pest problems, difficult to solve by the available chemical means. The rapid multiplication of greenhouse pests necessitated routine spray programmes, and commonly more than 20 sprays had to be applied to a single cucumber crop in the UK. Under severe selection pressure, the pests quickly developed resistance to one pesticide after the other. The red spider mite *Tetranychus urticae*, one of the main pests in greenhouses, was the first to develop resistance: strains resistant to azobenzene appeared already in 1949 (Hussey & Scopes, 1985). Besides the increasing efficacy problems, many practical problems had to be faced. It is often necessary to make many spray treatments in the evenings, in order to reduce phytotoxic damage to the plants by the pesticides due to sunlight. Furthermore, many chemicals are poisonous, making it essential to operators to wear cumbersome, protective clothing for their safety.

Biological control in the greenhouses had a modest start in the early and mid 1930s with *Encarsia formosa* for whitefly control, and *Phytoseiulus persimilis* for spider mites towards the end of 1960s (Hussey, 1985a). Commercial biological control in greenhouses started in Europe in 1968, with two companies supplying the natural enemies. Currently there are 26 natural enemy producers in Europe, including the three largest in the world. Two natural enemy species were available in 1970, but now approximately 100 species are mass-produced and sold in Europe (van Lenteren, 2000). Progress in the uptake of the biological control agents by the growers was extraordinarily rapid: by the end of the 1970s up to 74% of the UK greenhouse cucumbers were produced using *P. persimilis* and 46% *E. formosa*; for tomatoes the figures were 26% and 40% (Gould, 1985). By 1982 already 83% of cucumber growers in Finland applied *P. persimilis*. Currently biocontrol-based IPM is used on a large scale in all main vegetable crops in Northern Europe, for example in the Netherlands over 90% of all tomatoes, cucumbers and sweet peppers are produced under IPM. Worldwide, however, only 5% of greenhouse production is using biocontrol-based IPM (van Lenteren, 2000).

Cost of control is not a limiting factor in greenhouse production. For example in tomatoes and ornamentals pest control costs are only about 1-2% of the total production costs (van

Lenteren, 2000); therefore, whatever method works best, it will be used by the growers. While many arguments can be presented in favour of biological pest controls, the growers will choose their pest management usually according to the expected short-term private benefits (see section one of this chapter). So why have greenhouse producers in Northern Europe increasingly chosen to rely on biological control?

Partially the answer must lie on the overall economics of pest control. The first integrated programmes for the greenhouse – using biocontrol – were designed to cost only half the sums incurred in the full chemical programmes, which often involved more than 20 sprays per crop. It turned out that this target was far exceeded, being undoubtedly a major factor in the initially rapid uptake of the technology. For example, the largest cucumber producer in the UK used to pay £20,000 per year for pesticides (without considering the application costs); this approached zero with the use of in-house produced *Phytoseiulus* and *Encarsia*. Perhaps the most important result from using biological control is the increased crop output, which follows the cessation of pesticide applications. Yield increases up to 25%, but usually around 10-15% have been documented (Hussey, 1985b). Abandoning pesticides brings along numerous other benefits. Perhaps the most important, but unquantifiable, advantage to come from biological control was the extra time, which growers found at their disposal following its use. During the summer months, pesticides had to be applied at the end of the normal working day to avoid phytotoxicity. These applications were both costly and inconvenient as the operators involved, while paid at overtime rates, were kept away from their normal family life and other social activities (Hussey, 1985b). The application of toxic chemicals within greenhouses also creates serious hazards to operators who apply them, and their substitution by completely safe natural methods has been welcomed by both governments and growers. Furthermore, lack of pesticide residues allows products to be sold at any (optimum) time, without regard to safety periods between pesticide application and marketing. Production of 'natural food' has additionally provided growers with the possibility to increase the value of their produce by selling pesticide-free commodities at a premium.

It appears that reliance on biological control in combination with superb scientific, technical and professional skills of all actors involved, coupled with modern infrastructure, has created a superior greenhouse production system in Northern Europe, unmatched anywhere in the world. For example, in the Netherlands, only 0.5% of the agricultural land is covered with glasshouses, but they produce about 20% of the total value of agricultural production in that country (van Lenteren, 2000). In my view, such a success would not have been possible without biological control. In addition, while the greenhouse environment makes only a minute contribution to the national environment, the success of the biological approach in that system has provided an important stimulus to its use in other systems outdoors.

Acknowledgements

Support from the EU project MASTER: MAnagement STrategies for European Rape pests (QLK5-CT-2001-01447) is gratefully acknowledged. A travel grant from the University of

Helsinki to attend the Flakkebjerg workshop is deeply appreciated.

References

Bowles, R.G. & Webster, J.P.G. 1995: Some problems associated with the analysis of the costs and benefits of pesticides. Crop Protection 14: 593-600.

Cantliffe, D.J. & Vansickle, J.J. 2003: Competitiveness of the Spanish and Dutch greenhouse industries with the Florida fresh vegetable industry. Florida Cooperative Extension Service, IFAS, University of Florida, publication HS918: 1-8. Available at < http://edis.ifas.ufl.edu/CV284 >, accessed on 29.12.2005.

Cullen, J.M. & Whitten, M.J. 1995: Economics of classical biological control: a research perspective. In: Hokkanen, H.M.T. and Lynch, J.M. (eds), Biological Control: Benefits and Risks. Cambridge Univ. Press, Cambridge, U.K., pp. 270-276.

Ehlers, R.-U. 2005: Risk and reason - socio-economic aspects of IBCA regulation. In: Bigler, F., Babendreier, D. and Kuhlmann, U. (eds), Environmental Impact of Invertebrates in Biological Control of Arthropods: Methods and Risk Assessment. CABI Publishing (in the press).

Eilenberg, J., Hajek, A. & Lomer, C. 2001: Suggestions for unifying the terminology in biological control. BioControl 46:387-400.

Federici, B.A. 1999: A perspective on pathogens as biological control agents for insect pests. In Handbook of Biological Control, ed. T.S. Bellows and T.W. Fischer, pp. 517-548. Academic Press, San Diego, CA, USA.

Frost & Sullivan. 2001: European biopesticides market. Available at <http://www.frost.com>, accessed on 15.04.2005.

Gould, H.J. 1985: The advisory problem. In: N.W. Hussey & N. Scopes (eds), Biological Pest Control: the Glasshouse Experience. Blandford Press, Dorset, UK, pp. 219-223.

Greathead, D.J. 1995: Benefits and risks of classical biological control. In: Hokkanen, H.M.T. and Lynch, J.M. (eds), Biological Control: Benefits and Risks. Cambridge Univ. Press, Cambridge, U.K.

Gutierrez, A.P., Caltagirone, L.E. & Meikle, W. 1999: Evaluation of results. Economics of biological control. In Handbook of Biological Control, ed. T.S. Bellows and T.W. Fischer, pp. 243-252. Academic Press, San Diego, CA, USA.

Hokkanen, H.M.T. & Hajek, A.E. (eds) 2003: Environmental Impact of Microbial Insecticides: Need and Methods for Risk Assessment. Kluwer Academic Publsihers, Dordrecht, the Netherlands.

Hussey, N.W. 1985a: History of biological control in protected culture. Western Europe. In: N.W. Hussey & N. Scopes (eds), Biological Pest Control: the Glasshouse Experience. Blandford Press, Dorset, UK, pp. 11-22.

Hussey, N.W. 1985b: The economic equation. In: N.W. Hussey & N. Scopes (eds), Biological Pest Control: the Glasshouse Experience. Blandford Press, Dorset, UK, pp. 224-228.

Hussey, N.W. & Scopes, N.E.A. 1985: Introduction. In: N.W. Hussey & N. Scopes (eds), Biological Pest Control: the Glasshouse Experience. Blandford Press, Dorset, UK, pp. 8-10.

Kennett, C.E., McMurtry, J.A. & Beardsley, J.W. 1999: Biological control in subtropical and tropical crops. In Handbook of Biological Control, ed. T.S. Bellows and T.W. Fischer, pp. 713-742. Academic Press, San Diego, CA, USA.

Kurppa, S. 1990: Inset pest damage, predicting and control in Finnish cereal cultivation during the 1980s. PhD-dissertaton, Faculty of Agriculture and Forestry, University of Helsinki, Finland, ISBN 951-729-37-6. p. 1-53.

Langewald, J. & Neuenschwander, P. 2002: Challenges in coordinating regional biological control projects in Africa: classical biological control versus augmentative biological control. Biocont. News Inform. 23: 101N-108N.

Lisansky, S.G. & Coombs, J. (1994): Development in the market for biopesticides. Proceedings of the Brighton Crop Protection Conference – Pest and Diseases, 1049-1054.

Lubulwa, G. & McMeniman, S. 1998: ACIAR-supported biological control projects in the South Pacific (1983-1996): an economic assessment. Biocont. News Inform. 19: 91N-97N.

Menzler-Hokkanen, I. 2006: Socioeconomic significance of biological control. In: J. Eilenberg & H.M.T. Hokkanen (eds), An Ecological and Societal Approach to Biological Control, Springer, Dordrecht, The Netherlands, pp. 13–25.

Mumford, J.D. & Norton, G.A. 1984. Economics of decision making in pest management. Annu. Rev. Entomol. 29: 157-174.

Perkins, J.H. & Garcia, R. 1999: Social and economic factors affecting research and implementation of biological control. In Bellows, T.S. and Fischer, T.W. (eds), Handbook of biological control. Academic Press, San Diego, CA, USA, pp. 993-1009.

Törmälä, T. 1995: Economics of biocontrol agents: an industrial view. In: Hokkanen, H.M.T. and Lynch, J.M. (eds), Biological Control: Benefits and Risks. Cambridge Univ. Press, Cambridge, U.K., pp. 277-282.

van Lenteren, J.C. 2000: A greenhouse without pesticides: fact or fantasy? *Crop Protection* 19: 375-384.

van Lenteren, J.C., Roskam, M.M. & Timmer, R. 1997: Commercial mass production and pricing of organisms for biological control of pests in Europe. Biological Control 10: 143-149.

van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G. & Hokkanen H.M.T. *et al.*, 2003: Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl 48:3-38.

van Lenteren, J.C., Bale, J., Bigler, F., Hokkanen, H.M.T. & Loomans, A.J.M. 2006: Assessing risks of releasing exotic biological control agents. Annu. Rev. Entomol. (in press).

Waibel, H., Fleischer, G., Becker, H. & Runge-Metzger, A. 1998: Kosten und Nutzen des cemischen Pflanzenschutzes in der deutschen Landwirtschaft aus gesamtwirtschaftlicher Sicht. Agrarökonomische Monographien und Sammelwerke. Wissenschaftsverlag Vauk Kiel KG, Kiel, Germany. 254 p.

Webber, H.J. 1967: History and development of the citrus industry. In The Citrus Industry, Vol. 1, W. Reuther, H.J. Webber and L.D. Batchelor (eds), University of California Press, pp. 1-39.

Webster, J.P.G. & Bowles, R.G. 1996: Estimating the economic costs and benefits of pesticide use in apples. Brighton Crop Protection Conference, Pests & Diseases, 1996, 4B1: 325-330.

Webster, J.P.G., Bowles, R.G. & Williams, N.T. 1999: Estimating the economic benefits of alternative pesticide usage scenarios: wheat production in the United Kingdom. Crop Protection 18: 83-89.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Conservation biological control with insect pathogenic fungi

Nicolai V. Meyling, Annette B. Jensen, Charlotte Nielsen, Anette J. Lauritzen, Jørgen Eilenberg

Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract: Conservation biological control employs the naturally occurring beneficial organisms in the regulation of crop pests. This is achieved by modification of the environment or existing practices to protect and enhance specific natural enemies. In our group, several current research projects are focusing on this biocontrol strategy involving insect pathogenic fungi. Knowledge of the ecology of the natural enemies in question is necessary if conservation biological control strategies are to succeed. One group of fungi in focus is aphid pathogens from Entomophthorales that can cause natural epizootics in aphid populations. One project focuses on these fungi at the molecular level for their association with specific aphid hosts. Another project focuses on performance of entomophthoralean fungi during the winter and the following early initiation of infections in spring. Successful infection processes of insect pathogenic fungi are crucial for conservation biological control. A Ph.D. project focuses on the infection process of the fungus *Pandora neoaphidis* in comparison with *Beauveria bassiana* using different microscopic and biochemical techniques. A recently completed Ph.D. project investigated natural occurrence of the fungus *B. bassiana* in an organically grown field and adjacent hedgerow. It was found that the field margin habitat provided an important reservoir of *B. bassiana* genotypes compared with the agricultural field.

Key words: Natural occurrence, *Pandora neoaphidis*, host specificity, winter survival, infection process, *Beauveria bassiana*, genetic diversity, Entomophthorales, Hyphomycetes

Introduction

The strategy of conservation biological control was redefined by Eilenberg *et al.* (2001) as: '*Modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests*'. This implies that under the strategy no natural enemies are released, but it involves the enemies that are already present in the cropping system and/or its surroundings. One group of natural enemies that can be included in conservation biological control of pests in agricultural fields are the insect pathogenic fungi.

In contrast to the great body of research devoted to the populations of predators and parasitoids, limited knowledge is available about the basic ecology of these fungi in agrosystems. Such knowledge is necessary to exploit this biocontrol strategy. We conduct different research projects in order to increase our understanding of specific insect pathogenic fungi that have potential for the regulation of pests in agricultural fields in Denmark. These

studies include natural occurrence, molecular investigations of the diversity of populations and associations with insect hosts as well as details of the infection process.

Genotyping of aphids and their fungal pathogens

Under natural conditions fungal pathogens from Entomophthorales can cause complete suppression of outbreaks in aphid populations (Nielsen *et al.*, 2001). Understanding natural host ranges in the environment is important for selecting the most suitable conservation strategy. Aphids in cereals reproduce by parthenogenesis during the summer, thus the aphid populations in the crop are composed of a mosaic of aphid clones at the time the aphid pathogenic fungi establish in their hosts. The objective of this project is to study the association between genotypes of the aphid hosts and the naturally occurring fungal pathogens.

Sampling of aphid populations

Aphids have been sampled in two constitutive years (2004 and 2005) in a single winter wheat field from the arrival of the aphids (at the beginning of June) until the crash in the aphid population (late July). The field was divided into 60 plots each of 20 x 20 metre. Each week two aphids from each plot were sampled and incubated individually in small plastic cups supplied with a wheat straw. 100 aphids were in addition sampled from a single plot in 2005. The aphids were observed daily for fungal infection and after dispersal of conidia from fungal cadavers; the cadavers were stored in the freezer until the DNA extraction. After a week the surviving aphids and their progeny were also stored in the freezer until the DNA extraction.

In 2004 complete suppression of the aphid population was observed and it was primarily caused by the fungus species *Pandora neoaphidis*. In 2005 the entomopathogens did not cause a similar dramatically crash in the aphid population, the predominant fungus was *Entomophthora planchoniana* and parasitoids were far more common than the previous year.

The host aphids, in particular the cereal aphid *Sitobion avenae*, were genotyped using 7 microsatellite markers (S4.Σ, S16b, S17b, S3.R, S3.43, S5.L, S10 (Wilson *et al.*, 2004)) to investigate the association with fungus genotypes down to host clones. Preliminary results of 2004 showed a high clonal diversity of the aphids in the field, but we were not able to detect a specific pattern of the *P. neoaphidis* infected aphid clones.

Selected Entomophthoralean species (*P. neoaphidis* and *E. planchoniana*) are investigated at the molecular level using sequence data. The ITS region of *P. neoaphidis* from *S. avenae* and other host aphid species were sequenced and the sequences were almost identical. Similar results were found in a recent study of *P. neoaphids* (Tymon *et al.*, 2005). The lack of sequence differences in the ITS region indicates that the isolates are closely related; however, they can still be composed of genetically different lineages (Tymon and Pell, 2004). *E. planchoniana* from different aphid hosts displayed some variation in the sequences of the ITS 1 and the first part of the LSU rDNA, an indication that this fungus has high host specificity.

Winter survival of aphid pathogenic Entomophthorales

Knowledge of performance of aphid pathogenic entomophthoralean fungi during the winter and the following early initiation of infections in spring is a prerequisite for the development of long-term management strategies for aphid control. A new project initiated in September 2005 aims to investigate the winter survival and the mechanisms of the early initiations of infections in spring of insect pathogenic fungi from the order Entomophthorales The project will focus on three common aphid pathogens: *P. neoaphidis, E. planchoniana* and *Conidiobolus obscurus*. As test insects, two pest aphids in cereals, the English grain aphid, *S. avenae* and the bird cherry-oat aphid, *Rhopalosiphum padi* will be used.

In temperate regions the majority of aphid pest species are holocyclic (Dixon, 1998). This implies that the survival of aphid pathogenic Entomophthorales is critical during the winter when most of their host insects are in the egg stage. Thus Entomophthorales that attack aphids must survive in the environment without the original host stages for approximately six months of the year.

Most entomophthoralean fungi produce thick-walled zygospores or azygospores, generally referred to as resting spores, to resist periods of unsuitable weather or lack of appropriate host insects (Hajek, 1997). In contrast to most other Entomophthorales, no *in vivo*-produced zygo- or azygospores have ever been observed for *P. neoaphidis* (Uziel & Kenneth, 1986).

It has generally been assumed that survival structures, although formed in cadavers above the soil, typically fall to the ground and accumulate in the soil. Soil has consequently been considered a reservoir for several entomophthoralean fungi (Hajek & Wheeler, 1994; Hajek *et al.*, 1998; Nielsen *et al*., 2003). Entomophthoralean fungi have further been documented on plant material such as the bark of trees and damp wood (Keller, 1987; Steinkraus & Kramer, 1989; Feng *et al*., 1992; Hajek *et al.*, 1998; C. Nielsen, unpublished.). Nevertheless, the environment for survival is still unknown for the aphid pathogenic entomopthoralean fungi. This is mainly due to the fact that the morphology of the survival structures is not always known and, if known, the survival structures are difficult to recognise in a complex soil substrate and on plant material. Furthermore, the exact requirements for initiation of germination and conidiation have not been completely elucidated making bioassays (measuring the mortality of insects) of environmental samples incomplete. Development of DNA isolation techniques followed by specific PCR amplification of target DNA will thus provide a unique method for detection of entomophthoralean fungi without prior knowledge of the requirements for initiation of germination or recognition of the survival structures in complex media.

Specifically, the following hypotheses will be tested in the project:

- Soil and/or bark are important sources of inoculum for aphid pathogenic entomophthoralean fungi.
- Factors activating the germination of overwintering entomophthoralean inoculum are specific to fungal species.
- Specialized thick walled conidia constitute one possible survival stage for *P. neoaphidis*.

Infection processes of insect pathogenic fungi

Microscopy studies

Successful infection processes of insect pathogenic fungi are crucial for the development of epizootics in insect pest populations and are thus important to understand for implementation of conservation biological control strategies. The infection processes of insect pathogenic fungi in the Hypocreales, Ascomycota (for example *Metarhizium anisopliae* and *Beauveria bassiana*) are well studied (e.g. Butt *et al*., 1995; Schreiter *et al*., 1994), whereas the entomophthoralean fungi have received comparatively little attention (e.g. Magalhaes *et al*., 1989). A Ph.D. project focuses on the infection process of the fungus *P. neoaphidis* in comparison with *B. bassiana*. The infection process for the fungi are studied both visually, using different microscopic techniques, and biochemically by investigating cuticle degrading enzymes and their properties.

The objective of the microscopy study is to visualize and elucidate the infection process, especially penetration and colonization, of aphids by *B. bassiana* and *P. neoaphidis*, using primarily Confocal Laser Scanning Microscopy (CLSM). The material making up the arthropod exoskeleton has autofluorescent properties and can easily be excited by ultraviolet or visible light (Klaus *et al*., 2003). In our preliminary investigations, using CLSM, we found that the exoskeleton of the English grain aphid *S. avenae* is indeed autofluorescent and is excited by ultraviolet light. Stacks of optical slices can be reconstructed into 3D objects over time, visualizing the infection process of aphids by selected isolates of *B. bassiana* and *P. neoaphidis*. To make this possible it is crucial that the entomopathogens are also visible in CLSM at the same time. Staining of the fungi is an option and was investigated using cfda (carboxy fluoresin di-acetate), Nile Red, Calcofluor white and Syto-13. So far, good images have been obtained using Nile Red and Calcofluor white M2R, which stained the lipids and β-polysaccharides, respectively. Calcofluor white: excitation at 351 and 364 nm, emission recorded at 400-500 nm; Nile Red: excitation at 543 nm, emission recorded at 580-620 nm.

There are often difficulties with staining fungi properly, such as fading of the probe over time when exposed to excitation light. Furthermore, there can be problems in getting the stain deep into the tissue of the specimen. Transformation of the fungus with a fluorescent protein, such as the green fluorescent protein (*gfp*) could be a solution to the above-mentioned problems regarding fungal staining (Lorang *et al*., 2001). We will continue our work with CLSM, staining and *gfp*-tagging of fungi to hopefully obtain 3D registration of the infection processes by insect pathogenic fungi in aphids *in situ*.

Enzyme assays

The key steps in the infection process are adhesion, germination, differentiation and penetration. The latter requires the use of cuticle-degrading enzymes and mechanical force. Of the cuticle degrading enzymes in the hypocrealean fungi, the subtilisin Pr1 has been shown to be an important virulence determinant (Butt *et al*., 1998). Pr1 like enzymes are widely distributed and are important pathogenicity-related enzymes of other invertebrate pathogens (Butt *et al*., 1998). We aim to study the infection processes of *P. neoaphidis* biochemically by identi-

fying and characterising the enzymes involved. Examples of questions that will be addressed in the future research are:

- What is the virulence of different *P. neoaphidis* strains?
- Does *P. neoaphidis* possess the same cuticle-degrading enzymes as hypocrealean fungi?
- Is the regulation of cuticle-degrading enzymes produced by *P. neoaphidis* under induction, derepression or repression control?

Hypocrealean entomopathogenic fungi in agricultural fields and hedgerows

Entomopathogenic fungi belonging to the order Hypocreales in the Ascomycota include species that have long been investigated for their biological control potential using inoculation and inundation biocontrol strategies. Many of these species occur naturally in the soil environment and as natural infections in most groups of insects in Denmark. They are thus potential candidates as conservation biological control agents. However, limited knowledge is currently available of their ecology in agricultural system, which is a prerequisite for future exploitation using the conservation strategy.

A recent PhD project focused on the natural occurrence of this group of fungi in a single organically farmed field (Bakkegården) and its adjacent hedgerow in Denmark (Meyling, 2005). The study documented that the fungus species *B. bassiana* was common in the soil environment of both agricultural field and hedgerow. However, the fungus *Paecilomyces fumosoroseus* was dominating in the hedgerow soil and was almost absent from field soil. The distribution of *B. bassiana* in the agricultural field soil was clumped and it appeared to be correlated with the cropping system (Meyling, 2005). This observation establishes some interesting hypotheses about how crops affect the occurrence of entmopathogenic fungi in the soil. Furthermore, *B. bassiana* was isolated for the first time from leaf surfaces of plants in the hedgerow (Meyling & Eilenberg, in press). Isolates obtained from phylloplanes were of different genotypes and in general the hedgerow habitat harboured many different genetic groups of *B. bassiana*. In contrast, isolates from the agricultural field soil all belonged to a single genetic group. This suggests that specific factors in agricultural fields select for certain genotypes of *B. bassiana*. Hedgerows constitute thus a valuable reservoir of genetic diversity of *B. bassiana* in agricultural landscapes (Meyling, 2005).

The project showed that there is a potential for the inclusion of hypocrealean entomopathogenic fungi, particularly *B. bassiana*, in conservation biological control strategies. However, the knowledge of factors that determine the observed occurrences needs to be established further in the future. We continue to study these systems in a new project funded by FØJO III.

Acknowledgements

Christina Wolsted, Martin Davidsen and Haldis Egholm are thanked for skilled technical assistance. The presented studies are funded by The Villum Kann Rasmussen Foundation (ABJ), The Royal Veterinary and Agricultural University (NVM and AJL) and The Danish Research Council for Technology and Production Sciences (CN).

References

Butt, T.M., Ibrahim, L., Clark, S.J. & Beckett, A. 1995: The germination behaviour of *Metarhizium anisopliae* on the surface of aphid and flea beetle cuticles. Mycological Research. 99: 945-950.

Butt, T.M., Segers, R.J., Leal, S.C.M. & Kerry, B.R. 1998: Variation in the Subtilisins of Fungal Pathogens of Insects and Nematodes. In: Molecular Variability of Fungal Pathogens (eds. Bridge, P. Couteaudier, Y. & Clarkson, J.). Chapter 11 pp 149-169. CAB International, Wallingford, Oxon, UK.

Dixon, A.F.G. 1998: Aphid Ecology. An Optimization Approach. Second edition. Chapman & Hall, London, UK. 300 pp.

Eilenberg, J., Hajek, A. & Lomer, C. 2001: Suggestions for unifying the terminology in biological control. Biocontrol 46: 387-400.

Feng, M.G., Nowierski, R.M., Klein, R.E., Scharen, A.L. & Sands, D.C. 1992: Spherical hyphal bodies of *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Zygomycetes: Entomophthorales) on *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae): A potential overwintering form. Pan-Pacific Entomologist 68: 100-104.

Hajek, A.E., Tatman, K.M., Wanner, P.H. & Wheeler, M.M. 1998: Location and persistence of cadavers of gypsy moth, *Lymantria dispar*, containing *Entomophaga maimaiga* azygospores. Mycologia 90: 754-760.

Hajek, A.E. 1997: Ecology of terrestrial fungal entomopathogens. Advances in Microbial Ecology 15: 193 – 249.

Hajek, A.E. & Wheeler, M.M. 1994: Application of techniques for quantification of soil-borne entomophthoralean resting spores. Journal of Invertebrate Pathology 64: 71-73.

Keller, S. 1987: Observations on the overwintering *of Entomophthora planchoniana*. Journal of Invertebrate Pathology 50: 333-335.

Klaus, A.V. Kulasekera, V.L. & Schawaroch V. 2003: Three-dimensional visualization of insect morphology using confocal laser scanning microscopy. Journal of Microscopy 212: 107-121.

Lorang, J.M., Tuori, R.P., Martinez, J.P., Sawyer, T.L., Redman, R.S., Rollins, J.A., Wolpert, T.J., Johnson, K.V., Rodriguez, R.J., Dickman, M.B. & Ciuffetti, L.M. 2001: Green fluorescent protein is lighting up fungal biology. Appl. Environ. Microb. 67:1987–1994.

Magalhaes, B.P., Butt, T.M., Humber, R.A., Shields, E.J. & Roberts, D.W. 1989: Formation of appressoria *in vitro* by the entomopathogenic fungus *Zoophthora radicans* (Zygomycetes: Entomophthorales). J. Invert. Pathol. 55: 284-288.

Meyling, N.V. 2005: Population ecology and genetic diversity of entomopathogenic fungi (Ascomycota: Hypocreales) in agroecosystems and field margins. Ph.D. thesis, Department of Ecology, The Royal Veterinary and Agricultural University, Denmark.

Meyling, N.V. & Eilenberg, J. in press: Isolation and characterisation of *Beauveria bassiana* isolates from phylloplanes of hedgerow vegetation. Mycological Research.

Nielsen, C., Hajek, A.E., Humber, R.A., Bresciani, J. & Eilenberg, J. 2003: Soil as an environment for winter survival of aphid-pathogenic Entomophthorales. Biological Control 28: 92-100.

Nielsen, C., Eilenberg, J. & Dromph, K. 2001: Entomophthorales on ceral aphids: Characterisation, growth, virulence, epizootiology and potential for microbial control. Pesticide Research from the Danish Environmental Protection Agency 53:1-77.

Schreiter, G., Butt, T.M., Beckett, A., Moritz, G. & Vestergaard, S. 1994: Invasion and development of *Verticillium lecanii* in the Western Flower Thrips, *Frankliniella occidentalis*. Mycological Research 98: 1025-1034.

Steinkraus, D.C. & Kramer, J.P. 1989: Development of resting spores of *Erynia aquatica* (Zygomycetes: Entomophthoraceae) in *Aedes aegypti* (Diptera: Culicidae). Environmental Entomology 18: 1147-1152.

Tymon, A.M. & Pell, J.K. 2005: ISSR, ERIC and RAPD techniques to detect genetic diversity in the aphid pathogen *Pandora neoaphidis*. Mycological Research 109: 285–293.

Tymon, A.M., Shah, P.A. & Pell, J.K. 2004: PCR-based molecular discrimination of *Pandora neoaphidis* isolates from related entomopathogenic fungi and development of species-specific diagnostic primers. Mycological Research 108: 419–443.

Uziel, A. & Kenneth, R.G. 1986: *In vitro* resting spore formation in *Erynia neoaphidis*. In: Samson, R.A., Vlak J.M., Peters, D. (eds.), Fundamental and applied aspects of invertebrate pathology. Proc. Intern. Colloq Insect Pathol.,Veldhoven, The Netherlands 1986, p. 230.

Wilson, A.C.C., Massonnet, B., Simon, J.C., Prunier-Leterme, N., Dolatti, L., Llewellyn, K.S., Figueroa, C.C., Ramirez, C.C., Blackman, R.L., Estoup, A. & Sunnucks, P. 2004 : Cross-species amplification of microsatellite loci in aphids: assessment and application. Molecular Ecology Notes, 4, 104-109.

Phytophagous insects associated with Giant Hogweed: Potential for biological control in Europe?

C. Nielsen, H.P. Ravn

The Royal Veterinary and Agricultural University, Forest and Landscape Denmark, Horsholm Kongevej 11, DK-2970 Horsholm, Denmark

Abstract: Giant Hogweed, *Heracleum mantegazzianum* Sommier & Levier (Apiaceae), is an invasive weed in Europe and has spread rapidly during the last decades. The plant is native to the Caucasus, South-West Asia, where it occurs in forest edges and meadows, or at stream sides in montane areas. Strikingly impressive in size and height, *H. mantegazzianum* was brought to European botanical gardens as an ornamental in the late 19th century. Due to a high competitive ability and a large seed production the plant has established in many countries of Europe, especially in Central Europe, where river banks, damp places and waste grounds represent typically habitats.

Once established, *H. mantegazzianum* can become the dominant vegetation forming monospecific stands, which may reduce biodiversity and degrade habitat quality. Another main reason to control the plant is the health hazard to humans. The reaction of human skin to contact with plant sap and subsequent sun exposure causes severe blistering followed by postinflammatory hyperpigmentation.

The need for sustainable solutions to stop further spread and prevent future invasions led to the initiation of the EU funded "Giant Alien Project". The overall objective was to develop an integrated management strategy that comprises effective, practicable and sustainable means of controlling Giant Hogweed. Biological control may act as component of an integrated approach to prevent the spread of the plant, and herbivorous insect species associated with *H. mantegazzianum* have been sought in the invaded area of Europe as well as in the area of origin in the North Western Caucasus.

The results of field surveys revealed more than 160 insect herbivores on *H. mantegazzianum*, however, the majority of these insects fed polyphagously on the plant. A total of 40 insect herbivores were oligophagous or monophagous insect herbivores. Especially sap suckers such as aphids (Aphididae: *Cavariella* sp.) and plant bugs (Miridae: *Orthops* sp.) were found in high abundances but without sufficient damage to reduce plant vigour. Due to the high fecundity and large roots that allow early and rapid growth of overwintering plants, the best candidates for biological control are flower or root feeding species that may reduce seed production or deplete energy resources stored in the root.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Screening for antagonistic microorganisms for *Venturia inaequalis* control by means of DGGE community analysis

Arjen Speksnijder, Carin Lombaers-van der Plas, Jürgen Köhl
Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Abstract: *Venturia inaequalis* causes apple scab. The control of the disease in organic apple production depends on the use of copper, which will be restricted in future for environmental reasons. Survival of *Venturia* on fallen leaves during winter results in carryover of the disease to the next growing season.

In a field experiment different treatments were applied to the leaves in autumn with the goal to reduce apple scab incidence in the spring. The hypothesis is to stimulate microorganisms in the leaves, which interfere with the pathogen mycelium during saprophytic expansion of the stroma in the autumn after leave fall. This may result in a reduction of initials of overwintering pseudothecia and consequently of sexually produced ascospores in spring.

In the search for environmental friendly microbial biocontrol agents and stimulation of antagonistic populations *in situ*, conventional screenings techniques are based on isolation and antagonistic testing. But molecular techniques like DGGE fingerprinting allows to bypass culturing techniques and even can identify not yet culturable organisms.

DGGE fingerprinting is applied to leaf samples in autumn to characterise microbial communities. Multivariant analysis of data on microbial fingerprints and on pathogen development in the leaf samples during winter and spring is applied to identify populations with antagonistic potential. Such potential antagonistic organisms are further identified by their specific DNA sequence.

Preliminary results identify possible antagonists. The identification based on the sequence can help to focus on specific isolation or stimulation of a possible antagonist.

The influence of natal host and learning on later host preference of *Spalangia cameroni* (Hymenoptera: Pteromalidae)

A.M. Torp[1], H. Skovgård[1], H. Phillipsen[2]

[1]Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Danish Institute of Agricultural Sciences, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark; [2]Department of Ecology, Section of Zoology, The Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark

Abstract: The pupal parasitoid *Spalangia cameroni* is used in Denmark as a biocontrol agent against the stable fly, *Stomoxys calcitrans* (L.) and the house fly, *Musca domestica* L. in confined dairy cattle and swine installations with straw beddings. Because of low production costs the parasitoid is reared on pupae of the 'black dump fly' *Hydrotaea aenescens* instead of house fly pupae. This has led to speculations whether field released *S. cameroni* reared on Black dump fly pupae have similar control effect had they been cultured on house fly or stable fly pupae instead.

Present study aims to examine whether the natal host influences on the female parasitoid later host preference/choice. This includes newly emerged female parasitoids, which is assumed to have gain little experience on natal host and females that have parasitized for several days on natal host and therefore are regarded as experienced. Furthermore, the female parasitoids were given a choice between one or more host species.

In both the 'choice' (more hosts) and the 'no choice' (one host) trials *S. cameroni* did not distinguish between the pupal hosts whatever the parasitoid had gain experience on natal host or not. However, in the 'no choice' experiment parasitism by the un-experienced parasitoids was higher than the experienced ones whereas in the 'choice' experiment this picture could only be observed when *S. cameroni* had black dump fly as natal host. There was no difference in the sex ratio whether *S. cameroni* was reared on house fly or black dump fly pupae or the parasitoid was experienced or un-experienced.

Overall mortality (parasitism and host feeding combined) of the un-experienced *S. cameroni* in the 'choice' and the 'no-choice' trials was significantly higher (74% and 62%) than for the experienced ones (58% and 47%), respectively. Finally, the mortality of house fly pupae attained the highest level (70% and 64%) irrespective the experience level of *S. cameroni* or if the parasitoid were un-experienced or had a 'choice' or 'no choice'. In contrast, black dump fly had the lowest parasitism level (60% and 48%), which again was independent of experience or if the female parasitoid had a choice or not.

Present study has shown that rearing *S. cameroni* on the black dump fly or house fly pupae seems to have little influence on the later performance of this parasitoid in field populations of house fly or stable fly.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

Microbial activity for a sound environment - Results from bacterial inoculations in potatoes and vegetables

M. Wikström[1], M. Hökeberg[2], J. Fatehi[2], B. Gerhardson[2], C. Welch[2]

[1]Findus R&D AB, P.O. Box 530, S-267 25 Bjuv, Sweden; [2]The MASE laboratories, P.O. Box 148, S-751 04 Uppsala, Sweden

Abstract: The aim of the research carried out within the MASE-programme - Microbial Activities for a Sound Environment - is to supply background knowledge that will facilitate development and use of microbial based products in food and feed production. MASE, like the sister programme DOM - Domestication of Microorganisms – aiming at accumulating knowledge and support to the biotechnology industry regarding the fermentation, formulation and safety assessment of "non-conventional" microorganisms, both have the Swedish Foundation for Strategic Environmental Research – MISTRA - as a main funder. The MASE research activities are carried out in close collaboration with industrial partners and are focusing on six main areas: environmentally sound production of vegetables, potatoes, golf courses, cereals, sugar beets, and on biopreservation of food and feed with the use of microorganisms.

Field experimental research within the MASE vegetable and potato projects, were performed mainly in the south of Sweden and in Spain. Between two and eight bacterial isolates were fermented and inoculated to seeds, tubers or roots in 42 full scale field experiments during 2004 and 2005. Seed inoculations were performed in spinach, carrots, dill and peas. Root inoculations were performed in iceberg lettuce, broccoli, cabbage, kale, swedes, peppers and tomato. The treated plants in most of these tested crops showed more enhanced emergence and a more rapid growth than uninoculated controls. Since a rapid emergence often meant an advantage over diseases and weeds, this also often led to significant yield increases. The obtained yield increases could be explained either by direct plant growth promotion and/or by biological control of diseases. Based on the results obtained we see a good potential for utilising the tested bacteria in practical plant production. The fact that some of the bacterial isolates regularly induced a positive effect in several of the tested crops, also point to good economic possibilities and commercial potential for developing these as product ingredients. However, unpredictable factors here are costs for registration and for large scale commercial production, factors that are presently researched within the sister DOM-programme in collaboration with industrial partners.

Proceedings of the International Workshop "Implementation of Biocontrol in Practice in Temperate Regions – Present and Near Future". Research Centre Flakkebjerg, Denmark, November 1-3, 2005

The effect of two release methods on parasitism by *Spalangia cameroni* on house flies on Norwegian pig farms

Håvard Øyrehagen, Tone Birkemoe
Norwegian Institute of Public Health, Department of Pest Control, P.O. Box 4404 Nydalen, NO-0403 Oslo, Norway

Abstract: The present study tested whether the method of release of wasps had any effect on the rate of house fly pupae parasitism. We used two methods of release: 1) sprinkling of wasps in the pens as the producer recommended, and 2) release of wasps from protected plastic tubes above the pens. Parasitism was monitored by use of sentinel pupae one week prior to and four weeks after wasp release. The highest rate of parasitism on house flies was found after 2 weeks, reaching almost 25% of the total number. The parasitism decreased during week 3 but remained at values around 15% in week 4. There was no significant difference in parasitism between the two treatments. However, the data indicate that sprinkling may increase parasitism the first week after release relative to release from tubes, whereas release from the tubes may contribute to wasps being active for a longer time period.

Key words: Biological control, house flies, hymenoptera, parasitoid, release method, wasp

Introduction

An ongoing project in Norway is testing the parasitoid wasp *Spalangia cameroni* for control of houseflies *Musca domestica* on pig farms by use of imported wasps from Denmark. The wasps arrive from the producer in small boxes. Some of the wasps are already hatched from the pupae, while others are still inside the fly pupae in the boxes. The Danish producer recommends sprinkling of wasps in the pens. However, we experienced that the pigs (especially piglets) ate the wasps that were sprinkled in this manner. Furthermore, with a daily cleaning of the pens many wasps would be lost, especially those not yet hatched from the fly pupae.

The aim of the present study was, therefore, to test whether the method of release had any effect on the survival of wasps and the rate of parasitism.

Materials and methods

The test was carried out in two separate periods in 2005, one from May to June and the other from August to September (spring and autumn). In the present study a group of 5-6 pens with sows and piglets were used as a test unit (repetition) for each release method. On two of the farms we had only one repetition, on the two others we had two and three repetitions. We used two methods of release: 1) sprinkling of wasps in the pens as the producer

recommended, and 2) release of wasps from protected plastic tubes above the pens. Parasitism was monitored by use of sentinel pupae one week prior to and four weeks after wasp release. The sentinel pupae were also placed in plastic tubes, perforated and tied to the interior of the gutters and on the outside of the pens where the farmers scraped the manure out. We had 15 test units, wasps were sprinkled in 8 and released from tubes in 7. These were localized on six different farms where pigs were kept on a shallow layer of bedding. Sentinel pupae were changed once a week and, after collection, kept in a climate chamber for wasps to hatch. Those still not hatched were dissected and checked for parasitism. Only preliminary data from the spring period (May to June) are presented.

Results and discussion

No parasitic wasp species were found prior to the release of *S. cameroni* on any of the farms (Figure 1). The highest rate of parasitism on house flies was found after 2 weeks, reaching almost 25% of the total number. The parasitism decreased during week 3 but remained at values around 15% in week 4.

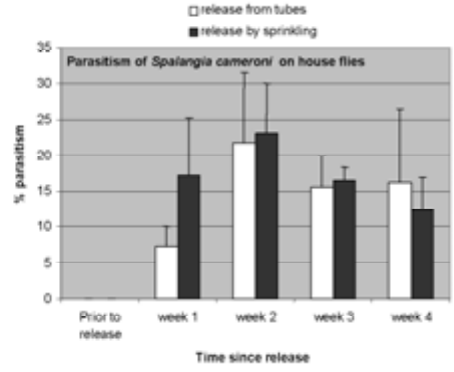

Figure 1. Parasitism (mean ± SE) of *S. cameroni* on sentinel pupae of house flies following wasp release by sprinkling and use of tubes hanging from the roof.

There was no significant difference in parasitism between the two treatments (Anova test between treatments $F_{1,33} = 0.31$, $P = 0.58$) (Figure 2).

However, the mean difference in parasitism after one week indicates a higher initial parasitism when the wasps were sprinkled out compared with the release from tubes. Also, it may look like the wasps were active longer when they were released from tubes, as this treatment showed a slightly higher parasitism after four weeks compared with the sprinkling method. This can reflect the distance the wasps had to fly from the release point and that they are using longer time to localize the fly pupae. The method of releasing wasps from tubes could have

resulted in wasps being released from a too concentrated release point as it is reported by Skovgård (2002) that these wasps have only a limited ability of dispersal.

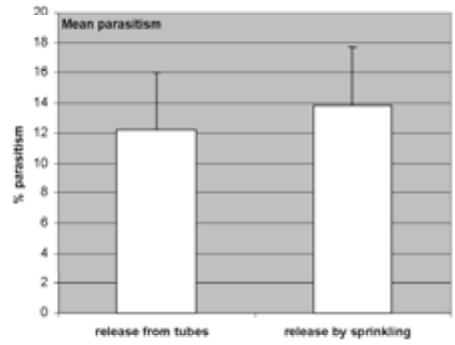

Figure 2. Mean parasitism in the two treatments following release of *S. cameroni*.

There was no overall significant difference in parasitism between the two release methods; release of wasps from tubes or sprinkling out in pens. However, the data indicate that sprinkling may increase parasitism the first week after release relative to release from tubes, whereas release from the tubes may contribute to wasps being active for a longer time period. When the results from the autumn are analysed and included, we hopefully will get better and clearer results.

Acknowledgements

We would like to thank all the farmers for letting us perform this present study on their farms. Thanks also to Arnulf Soleng, Anders Aak and Torjus Lundevall for field assistance. Furthermore, Arnulf Soleng kindly commented on this manuscript.

References

Skovgård, H. 2002: Dispersal of the filth fly parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) in a swine facility using flourescent dust marking and sentinel pupal bags. Environ. Entomol. 31: 425-431.

List of participants

Jorgen Aagesen
J.A. Consult, Espevej 6
DK-8462 Harlev, Denmark
j.a.consult@mail.dk

Cornel Adler
Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Vorratsschutz, Königin-Luise-Straße 19, D-14195 Berlin, Germany
c.adler@bba.de

Tannie Andersen
Miljøfluen I/S, Vesterhedensvej 31, DK-9362 Gandrup, Denmark
andersen@miljofluen.dk

Jacqueline Baar
Applied Plant Research, P.O. Box 6042, NL-5960 AA Horst, The Netherlands
jacqueline.baar@wur.nl

Michael Villy Boese
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
michaelvilly.boese@agrsci.dk

Aase Borges
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
aase.borges@agrsci.dk

Wolfgang Büchs
Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11/12, DE-38108 Braunschweig, Germany
w.buechs@bba.de

Henrik Frolich Brodsgaard
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
henrik.brodsgaard@agrsci.dk

Lars Bodker
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
lars.bodker@agrsci.dk

Camilla Beck Christensen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
camillabeck.christensen@agrsci.dk

Lene Christensen
Lenes Laboratorium, Holtumvej 58, DK-7100 Vejle, Denmark
lenelab@ofir.dk

Hanne-Birgitte Christiansen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
gitteb.christiansen@agrsci.dk

Claus Dahl
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
claus.dahl@agrsci.dk

Lars Damberg
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
lars.damberg@agrsci.dk

Jorgen Eilenberg
The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
jei@kvl.dk

Barbara Ekbom
Swedish University of Agricultural Sciences, P.O. Box 7044, SE-750 07 Uppsala, Sweden
barbara.ekbom@entom.slu.se

Annie Enkegaard
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
annie.enkegaard@agrsci.dk

Christopher J. Geden
USDA-ARS, Mosquito and Fly Research Unit, Center for Medical, Agricultural and Veterinary Entomology, P.O. Box 14565, Gainesville, FL 32604, Florida, USA
cgeden@gainesville.usda.ufl.edu

Jonas Geldmann
University of Copenhagen, Institute of Population Biology, Department of Population Biology, Gl. Kongevej 25, 4.th., DK-1610 København V, Denmark
jgeldmann@stud.ku.dk

Henrik Grondal
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
henrik.grondal@agrsci.dk

Erik Hansen
EWH BioProduction, Centervej Syd 4, DK-4733 Tappernøje, Denmark
bio@bioproduction.dk

Lise Stengård Hansen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
lises.hansen@agrsci.dk

Nicolai Hansen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
nicolai.hansen@agrsci.dk

Siv Beate Harbo
Norwegian University of Life Science, Department of Chemistry, Biotechnology and Food Science, P.O. Box 5003, N-1432 Ås, Norway
sivharbo@frisurf.no

Solveig Haukeland
Norwegian Crop Research Institute, Høgskoleveien 7, N-1432 Ås, Norway
solveig.haukeland@planteforsk.no

Niels Bohse Hendriksen
National Environmental Research Institute, Department of Environmental Chemistry and Microbiology, P.O. Box 358, Frederiksborgvej 399, DK-4000 Roskilde, Denmark
nbh@dmu.dk

Annette Herz
Federal Biological Centre for Agriculture and Forestry, Institute for Plant Protection in Fruit Crops, Schwabenheimer Str. 101, DE-69221 Dossenheim, Germany
a.herz@bba.de

Linda Hjeljord
Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, P.O. Box 5003, N-1432 Ås, Norway
linda.hjeljord@umb.no

Heikki Hokkanen
University of Helsinki, Department of Applied Biology, P.O. Box 27, FIN-00014 Helsinki, Finland
heikki.hokkanen@helsinki.fi

Margareta Hökeberg
BioAgri AB, P.O. Box 914, SE-751 09 Uppsala, Sweden
margareta.hokeberg@bioagri.se

Annette Bruun Jensen
The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
abj@kvl.dk

Birgit Jensen
The Royal Veterinary and Agricultural University, Department of Plant Biology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
bje@kvl.dk

Dan Funck Jensen
The Royal Veterinary and Agricultural University, Department of Plant Biology, Plant Pathology Section, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
dfj@kvl.dk

Jorgen Brochner Jespersen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
jorgenb.jespersen@agrsci.dk

Christine Kastrup
The Royal Veterinary and Agricultural University, Department of Ecology, Zoology Group, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
vakuole@dsr.kvl.dk

Ingeborg Klingen
The Norwegian Crop Research Institute, Plant Protection Centre, Høgskoleveien 7, N-1432 Ås, Norway
ingeborg.klingen@plantforsk.no

Inge M.B. Knudsen
The Royal Veterinary and Agricultural University, Department of Plant Biology, Thorvaldsensvej 40, DK-1871 Frederiksberg, Denmark
ik@kvl.dk

Christina Krabbe
The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
ckrabbe@dsr.kvl.dk

Jürgen Köhl
Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands
jurgen.kohl@wur.nl

Anne-Pia Larsen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
annepia.larsen@agrsci.dk

Daniel Larsen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
daniel.larsen@agrsci.dk

Michael Larsen
The Royal Veterinary and Agricultural University, Veterinary Pathobiology, Section for Parasitology, Dyrlægevej 10, DK-1870 Frederiksberg, Denmark
aph@post8.tele.dk

Anette Jansons Lauritzen
The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, 3. sal, DK-1871 Frederiksberg C, Denmark
ajl@kvl.dk

Malene Lihme
University of Copenhagen, Institute of Microbiology, Øster Farimagsgade 2D, DK-1353 Copenhagen K, Denmark
malenelihme@hotmail.com

Robert Linderman
USDA-ARS, Horticulture Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, Oregon 97 330, USA
lindermr@science.oregonstate.edu

Jan Lukáš
Research Institute of Crop Production, Department of Stored Product Pest Control, Drnovska 507, 161 02 Prague, Czech Republic
lukas@vurv.cz

Gabor Lövei
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
gabor.lovei@agrsci.dk

Henning Bang Madsen
University of Copenhagen, Institute of Biology, Department of Population Biology, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark
hbmadsen@bi.ku.dk

Richard Meadow
Norwegian Crop Research Institute, Plant Protection Centre, Department of Entomology and Nematology, Høgskoleveien 7, N-1432 Ås, Norway
richard.meadow@planteforsk.no

Steen Meier
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
steen.meier@agrsci.dk

Ingeborg Menzler-Hokkanen
University of Helsinki, Ruralia Institute, Lönnrotinkatu 3-5, FIN-50100 Mikkeli, Finland
ingeborg.menzler-hokkanen@helsinki.fi

Nicolai Vitt Meyling
The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
nvm@kvl.dk

Kaare Moller
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
kaare.moller@agrsci.dk

Gösta Nachman
University of Copenhagen, Institute of Biology, Department of Population Biology, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark
gnachman@bi.ku.dk

Bent Jorgen Nielsen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
bent.nielsen@agrsci.dk

Charlotte Nielsen
The Royal Veterinary and Agricultural University, Forest and Landscape Denmark, Hørsholm Kongevej 11, DK-2970 Hørsholm, Denmark
chn@kvl.dk

Magnus Gammelgaard Nielsen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
magnusg.nielsen@agrsci.dk

Steen Lykke Nielsen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
steenl.nielsen@agrsci.dk

Edda Oddsdottir
Icelandic Forest Research, Mógilsá, IS-116 Reykjavik, Iceland
edda@skogur.is

Alphonse Owuor
Dudutech Kenya Limited, Natural Enemies Division, Moi South Lake Road, P.O. Box 1927, Naivasha, Kenya
ne_dudutech@kenyaweb.com

Klaus Paaske
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
klaus.paaske@agrsci.dk

Hans-Henrik Rasmussen
Cillus A/S, Kobbervej 8, DK-2730 Herlev, Denmark
hhr@cillus.dk

Sabine Ravnskov
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
sabine.ravnskov@agrsci.dk

Ejvind Rubæk
Cillus A/S, Kobbervej 8, DK-2730 Herlev, Denmark
cillus@cillus.dk

Matthias Schöller
Biological Consultance Ltd., Hosemannstraße 8, D-10409 Berlin, Germany
bip@biologische-beratung.de

Lene Sigsgaard
The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, 3. sal, DK-1871 Frederiksberg C, Denmark
les@kvl.dk

Henrik Skovgård
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
henrik.skovgaard@agrsci.dk

Arnulf Soleng
Norwegian Institute of Public Health, Division of Infectious Disease Control, P.O. Box 4404, Nydalen, NO-0403 Oslo, Norway
arnulf.soleng@fhi.no

Tove Steenberg
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
tove.steenberg@agrsci.dk

Olaf Strauch
Christian-Albrechts-University, Institute for Phytopathology, Department of Biotechnology and Biology Control, Klausdorfer Str. 28-36, D-24223 Raisdorf, Germany
o.strauch@e-nema.de

Gunn Mari Stromeng
The Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Høgskoleveien 7, N-1432 Ås, Norway
gunn-mari.stromeng@planteforsk.no

Karin Thygesen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
karin.thygesen@agrsci.dk

Annemette Torp
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
annemette.larsen@agrsci.dk

Tina Tonnersen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark
tina.tonnersen@agrsci.dk

Solveig Vibe-Petersen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
solveig.v-petersen@agrsci.dk

Irene Vänninen
Agrifood Research Finland, Plant Protection, 31600 Jokioinen, Finland
irene.vanninen@mtt.fi

Linnea Wang
Norwegian Crop Research Institute, Plant Protection Centre, Høgskoleveien 7, N-1342 Ås, Norway
linnea_wang@hotmail.com

Minna Wernegreen
Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark
minna.wernegreen@agrsci.dk

Mariann Wikström
Findus R&D AB, P.O. Box 530, S-267 25
Bjuv, Sweden
mariann.wikstrom@se.findus.com

Håvard Øyrehagen
Norwegian Institute of Public Health, Division of Infectious Disease Control, P.O. Box 4404 Nydalen, N-0403 Oslo, Norway
havard.oyrehagen@fhi.no

Summary

This publication contains proceedings of the International Workshop on "Implementation of Biocontrol in Temperate Regions in Practice – Present and Near Future" held at Research Centre Flakkebjerg, Denmark on November 1 to 3, 2005, initiated by the Danish Centre for Biological Control, and arranged by the Department of Integrated Pest Management, Danish Institute of Agricultural Sciences. The workshop dealt with practical application of biological control of pests (i.e. plant diseases, weeds, arthropods, mammals and endoparasites) in a wide range of areas: field crops, greenhouses, forestry, animal husbandry and stored products, and was attended by 80 participants from 10 countries, mostly from Northern Europe.

Plant production

Horticulture

Livestock

Grøn Viden is issued in separate horticulture, plant production and livestock farming series. For more information on our publications please visit our website www.agrsci.dk