IOBC / WPRS

Working Group "Integrated Control in Glasshouses"

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Groupe de Travail "Lutte Intégrée en Cultures sous Verre "

Precedings of the meeting

at

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Edited by Joop C. van Lenteren

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Preface

This bulletin contains the precedings of the 10th full meeting of the IOBC / WPRS working group "Integrated Control in Glasshouses". The bulletin contains more contributions than ever, showing how fast developments are in the field of integrated pest and disease management in greenhouses. Also, we see many new names, indicating that the group of researchers is steadily increasing and that IPM has definitely settled in greenhouses.

The structure of the meeting in Brest will be slightly different from the previous ones. As a result of the final discussion in Vienna (1996) we have included more speakers to introduce the topics for the discussions in the sections. Also, several speakers have been invited to present new scientific challenges and a number of policy issues that might either frustrate or stimulate implementation of biological control, such as the registration of microbial agents, guidelines for importation of macrobial agents, risk assessment and quality control of biocontrol agents.

Two members of our working group have passed away since our last working group meeting. Prof. Danny Degheele (Belgium), responsible for many IOBC activities, died in May 1997 after a long illness. A tragic car accident in March 1999 abruptly finished the life of Dr. Giorgio Nicoli (Italy), the always positive stimulus of greenhouse IPM in Italy. We thank them both for their valuable contributions to our working group.

The "Brest 1999" meeting is organized by J.C. Maisonneuve and C. Marrec. The enthusiastic attitude of Jean Charles Maisonneuve will certainly make it a pleasant meeting. On behalf of the 100 participants, I like to thank our French hosts for all the work done to make the meeting a scientific and social success. Also, I like to thank Ineke Kok, who spent a lot of time in contacting authors and getting papers in the right format.

This will be my final year as convenor or the "northern European glasshouse IPM group". It was a pleasure to cooperate with so many colleagues world wide during more than 15 years. Our group has not limited its activities to Europe. We had two meetings outside the WPRS region, one in Hungary (1997) and another in the USA (1993). The fruitful collaboration with the EPRS and NRS sections was much appreciated and has led to many new contacts. Also cooperation with the "southern European glasshouse IPM group" has developed well, for which I am particularly thankful to Ramon Albajes. I wish the new convenor and the working group members a productive future, with many new successes in greenhouse IPM.

Joop C. van Lenteren
Convenor IOBC / WPRS Working Group
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Integrated pest management in *Dendranthema indicum*

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Abstract: Many pest species attack chrysanthemums. The most important pests in Germany are *Frankliniella occidentalis, Thrips tabaci, Tetranychus urticae, Liriomyza huidobrensis, Phytomyza* sp., *Macrosiphoniella sanborni, Myzus persicae* and *Aphis gossypii*. Sometimes caterpillars can be found on the plants. This attack often requires regular weekly applications of pesticides in chrysanthemum. Experiments with the repeated introduction of beneficials, the integration of pesticides and of a botanical (NeemAzal-T/S) have been conducted to control these pests.

Excepting *F. occidentalis* and *M. sanborni* all pest species were controlled well with beneficials. Due to an increase of the thrips population in the summer time the thrips had to be controlled with insecticides in that time. Nevertheless the application of beneficials and insecticides reduced the yearly consumption of insecticides by about 80%.

Looking for an integratable insecticide, a new neem formulation (NeemAzal-T/S) was tested. Both the combination of pesticides and NeemAzal-T/S or beneficials and NeemAzal-T/S had very good results in thrips control. When NeemAzal-T/S is applied at weekly intervals, none of the other pest species can be found on the plants. When the neem formulation is applied at longer intervals some pest species like *Aphis gossypii* infect the plants. Then the integration of insecticides or beneficials is necessary.

Key words: biological control, *Frankliniella occidentalis, Liriomyza huidobrensis, Phytomyza* sp., *Macrosiphoniella sanborni, Aphis gossypii, Amblyseius cucumeris, A. barkeri, Aphidius colemani, Aphidoletes aphidimyza,* Neem-extract, NeemAzal-T/S, Azadirachtin.

Introduction

The attempts to control pest species in chrysanthemum with integrated control methods reach back to the early 70s (Scopes and Biggerstaff 1973). Later on, Wardlow (1985) and others (Buxton and Wardlow 1991, Buxton and Finlay 1993, Sher and Parella 1996) worked on this problem. In Germany integrated control in cutflowers is not very common. This may be due to the number of pest species attacking the chrysanthemums. It is much higher than for example in balcony plants or potplants. The reason for this is that chrysanthemums are often continually reared for a long time in the same greenhouse, all plant stages are often grown together and the plants are produced all year round in many cases.Thus pests like thrips, spider mites or leafminers can overwinter in the soil and move from on bed to the other. The main pest is *Frankliniella occidentalis.* In addition other thrips species, the spidermite *Tetranychus urticae*, aphids like *Macrosiphoniella sanborni, Aphis gossypii* and others, leafminers like *Liriomyza huidobrensis* or *Phytomyza* sp. but also whitefly, broad mites and symphyla can damage the plants all year round. In the summer bugs fly into the greenhouse and suck on flowers and flowerbuds. In addition some air- and soilborne fungi attack the plants. Under those conditions the regular use of pesticides is the normal procedure in chrysanthemum cultivation. To change this situation, experiments with integrated pest management methods (use of beneficials and integration of pesticides and use of botanicals) have been conducted.

Methods

The experiments started 1995 in a horticultural enterprise, where chrysanthemum cutflowers are produced all year round on 5500 m² under glass. The temperature is kept at 20°C in winter time. Darkening in summer time, additional light in winter time and adding of carbon dioxide guarantee a good quality of the flowers. The steam sterilization of the soil, which normally was done once a year took place the last time in 1995. At the beginning, beneficials were released in two glasshouses on an area of 1250 m². The number of beneficials and the frequency of introduction from week 8 to week 32 in 1995 and from week 7 to week 18 in 1996 are shown in table 1 and 2. The plants were regularly monitored for pest species and beneficials. In addition thrips and whiteflies were monitored with blue and yellow sticky traps. Later on the trials where done on the whole production area of the horticultural enterprise. But as the results are very near to each other the results of one of the above mentioned glasshouses are presented in this paper only.

Tab. 1: Introduction of beneficials during the period 14th of February to the 1st of August 1995

Beneficial	No. of introductions	Sum of beneficials/m²
Amblyseius barkeri a. *A. cucumeris*	10	704.0
Orius sp.	2	0.9
Chrysoperla carnea-larvae	13	78.4
Aphidius sp.	6	1.8
Dacnusa sibirica a. *Diglyphus isaea*	3	3.2
Aphidoletes aphidimyza	4	1.8
Hypoaspis sp.	3	50.0

Tab. 2: : Introduction of beneficials during the period 8th of February to 23rd of April 1996

Beneficial	No. of introductions	Sum of beneficials/m²
Amblyseius barkeri a. *A. cucumeris*	11	420.0
Orius sp.	4	3.0
Chrysoperla carnea-larvae	5	13.6
Aphidius sp.	2	0.4
Dacnusa sibirica a. *Diglyphus isaea*	1	0.2
Hypoaspis sp.	2	40.0

Results

The control of the leafminer *L. huidobrensis* with *D. sibirica* and *D. isaea* worked well. One month after the introduction, no new mines could be found and after a short-while the infection was finished. The symphyla, which attack the roots of the young plants were controlled by *Hypoaspis miles*, so no loss of plants occured. In 1995 larvae of *A. aphidimyza* could never been found on the plants, although the adults were introduced 6 times under favorable conditions for the gallmidge. Control of the black-brown chysanthemum aphid *Macrosiphoniella sanborni* did not occur. Spidermites and broadmites were never found on the plants, whereas they damaged them in earlier years. This may be due to the regular introduction of the predatory mites *A. cucumeris* and *A. barkeri*. These two mite species were

able to keep the Californian flowerthrips for 4 to 9 month (depending on the variety and the bed they were grown on) at an innocuous level; then chemical control of the thrips was necessary. No integratable insecticides were on the market at that time, so that the chemical control stopped the biocontrol for a while. The varieties reacted differently against the thrips infestation. Damage of the flowers is normally visible with white and yellow varieties only when more than 40 *F. occidentalis* are caught per blue sticky trap (5cm x 12 cm) and week, whereas darker varieties show damage at much lower numbers. When the catches indicated that damage was to be expected, chemical control had to be applied. The activity of the thrips was very similar in both greenhouses, but the catches were often twice as much in house 8 than in house 7. The activity of *F. occidentalis* in greenhouse 7 is shown in figure 1. Those pest species that fly into the glasshouses as adults (like the bug *Lygus pratensis* and other *Lygus* species) could not be controlled with beneficials; thus chemical control was necessary. But nevertheless, the use of pesticides could be reduced by 80% in this part of the experiment. The quality of the plants was better, especially the colours of the flowers were improved. But the costs for the biocontrol was with about 0,042 DM/ plant more than double the price of the normal pest control with chemicals.

After this partial success in 1995 and 1996 the grower decided to finish the expensive steam sterilisation of the soil, which was normally done between week 22 and 28. As a result of this, the flower thrips reproduced quickly in the glasshouses. From that point onwards registered pesticides were used until summer 1997. During that time the blue sticky traps detected a higher infestation of the thrips than in the time of biocontrol. In weeks 31 to 45 especially the sensitive varieties showed thrips damage. Under these circumstances pesticides were effective, when the buds were sprayed.

In another horticultural enterprise a new biological insecticide had been experimentally tested against the chrysanthemum aphid *M. sanborni*. This is a preparation with the name NeemAzal-T/S. It contains 4% Azadirachtin (1% Azadirachtin-A), 51% sesame oil and 45% detergent. It has been registrated in Germany since October 1998 for the control of whitefly, aphids and leafminers. After two applications of NeemAzal-T/S (0,5%) the population of the chrysanthemum aphid was extinguished and the flower thrips showed a population decrease. As most of the compositae tolerate NeemAzal-T/S applications well, the biological insecticide was than applied in the two above mentioned glasshouses regularily. This was done at the beginning weekly, later on fortnightly and at longer intervals. When insecticides were also used at the beginning, the thrips catches on the blue sticky traps were nil (fig. 1). When NeemAzal-T/S was applied at the wider intervals, aphids could be found again on the plants. Thus beneficials like *Aphidius colemani* were released against *Aphis gossypii*. The predatory mites *A. cucumeris* and *A. barkeri* were released for thrips control; the Californian flower thrips was in this way kept at a harmless level.

The costs for the integrated control with NeemAzal-T/S and beneficials were lowered to about 0,018 DM/plant. The costs for postage of the beneficials and for the application have to be added.

Discussion

This longterm experiment has shown that biocontrol in chrysanthemums is possible. In summertime pesticides had to be added for the control of *F. occidentalis*, but the overall use of chemicals could be recuced by 80%. The costs of this procedure were relatively high. It seems that this way of pest control works only together with the steam sterilisation of the soil, which is expensive and from a biological point of view undesirable, because this sterilisation

destroys not only noxious soilborne fungi and soil living stages of *F. occidentalis* but also the natural antagonists of these pests. But without soil sterilisation the chemical control failed to avoid damage on some varieties. This is partly due to the fact, that it is not easy to apply pesticides in the dense chrysanthemum stands and because of the hidden mode of life of *F. occidentalis*. The thrips could be well reduced chemically in the bud stage of the chrysanthemums because in that stage many *F. occidentalis* can be found outside the bud or in the just opening buds, where it can be hit by the pesticide spray.

The new pesticide NeemAzal-T/S reduces the population of the Californian flower thrips slowly but effectively, when it is applied at weely or fortnightly intervals. Since aphids reproduce when the intervals are longer between the applications, aphids have to be controlled with chemicals or with beneficials. Parasitoids and predatory mites are not harmed by the botanical insecticide.

Acknowledgements

I thank Les Wardlow for correcting the English.

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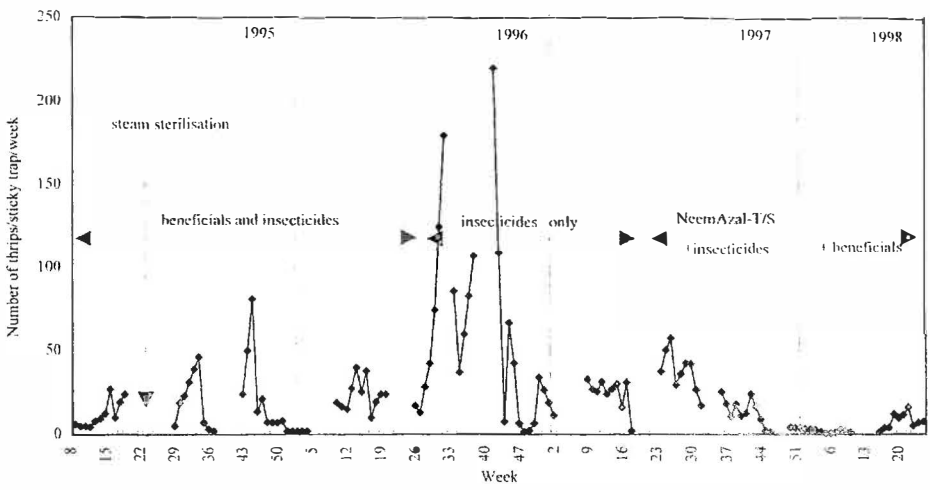

Figure 1: Activitiy of F. occidentalis in year round chrysanthemums

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Integrated biological control of tomato crown and root rot by combination of chitosan with endophytic bacteria

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Abstract: The potential of chitosan either alone or in combination with *Bacillus pumilus* (PGPR strain SE34), for inducing defense reactions in tomato plants inoculated with the vascular fungus, *Fusarium oxysporum* f.sp.*radicis-lycopersici*, was studied. Ultrastructural investigations revealed a substantial increase in the extent and magnitude of the cellular changes induced by *B. pumilus* when chitosan was supplied to bacterized tomato plants. These changes concerned a marked enlargement of the callose-enriched wall appositions deposited onto the inner cell wall surface in the epidermis and the outer cortex and restricting fungal development towards the vascular stele. Results of this work provide the first evidence that combination of biocontrol approaches is a promising step towards elaborating integrated pest management programmes.

Key words: cytochemistry, elicitor, induced resistance, integrated control, ultrastructure

Introduction

Plant-pathogen interactions are mediated by a complex network of molecular and cytological events that determine the choice between susceptibility and resistance. Recent advances in molecular biology have given rise to the notion that exogenous and/or endogenous factors could substantially affect the host physiology, leading to coordinated defense gene activation in plants normally expressing susceptibility to pathogen infection (Ward et al., 1991). Activation of the natural plant defense system has been shown to occur upon exogenous applications of either chitosan (Lafontaine and Benhamou, 1996), salicylic acid or chemicals. In all cases, characterization of the biochemical changes associated with elicitor-induced resistance revealed a correlation between establishment of resistance and accumulation of defense molecules (Benhamou, 1996). The stage is now set for this investment in knowledge to generate novel biocontrol approaches for protecting plants against microbial diseases. However, induced resistance alone is generally less effective than pesticides in controlling pathogen attack. Therefore, an integrated system combining complementary biological control approaches may hold the promise of attaining the efficacy of chemicals while reducing environmental pollution.

One specific aspect of integrated biological control concerns the possibility of stimulating the natural plant disease resistance process by combining the effects of biotic inducers. Recently, chitosan, a polymer of N-glucosamine, was reported to induce resistance to *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) in susceptible tomato plants. (Benhamou et al. 1994; Lafontaine and Benhamou 1996). Although valuable results could be obtained in terms of reduction of yield losses associated with tomato crown and root rot in greenhouse trials, the level of induced plant protection could never reach that of fungicides. The next step was to determine whether or not a combination of chitosan with another compatible biocontrol strategy such as endophytic bacteria could lead to enhanced synthesis

and accumulation of plant defense molecules and to better control of tomato crown and root rot

The objectives of the present work were to investigate the potential benefits of a chitosan-endophytic bacteria combination on the cytologically visible consequences of the induced response in tomato plants challenged with FORL.

Materials and methods

Plant material and microbial cultures

Seeds of tomato (*Lycopersicon esculentum* Mill., cv. Bonny Best) were surface-sterilized and allowed to germinate in Petri dishes.The isolate of *Bacillus pumilus* (strain SE34) was stored in nutrient broth containing 10% glycerol at -80 °C. To produce bacterial cells for root inoculation, the bacterial strain was retrieved from storage and streaked on nutrient broth agar.The isolate of FORL, known to be highly virulent on tomato, was grown on potato-dextrose agar (PDA) at 25 °C.

Preparation of chitosan

Purified chitosan (Sigma Chemical Co., St Louis, MO) was prepared according to Benhamou et al. (1994). For experimental use, purified chitosan was dissolved in 0.05 N HCl and the pH adjusted to 5.5 with 2 N NaOH to obtain a concentration of 10 $mg \cdot ml^{-1}$ (stock solution). The resulting solution was diluted in the plant nutrient solution to obtain a pH of 5.8.

Plant inoculation with B. pumilus, strain SE 34, and treatment with chitosan

Germinated tomato seeds were placed along an actively growing colony of *B. pumilus*. Controls included germinated seeds transferred to bacterial-free nutrient broth agar medium. Plates were incubated at 25 °C in the dark for 48 h. Tomato seedlings (5 days postgermination) were transplanted to 6-cm pots containing a mixture of peat-perlite-vermiculite (2:1:1) amended or not with chitosan (final concentration, 1 mg ml) and fertilized every other day with a nutrient solution

Root inoculation with Fusarium oxysporum f.sp. radicis-lycopersici

Tomato seedlings at the three-leaf stage were inoculated by introducing 3 discs of actively growing *Fusarium* mycelium as close as possible to the root system. Control plants were treated similarly but with fungus-free PDA discs. Root samples were collected four days after pathogen inoculation and processed for electron microscopy.

Tissue processing for electron microscope investigations

Root samples, collected at the sites of fungal entry, were fixed in 0.1 M sodium cacodylate-buffered 3% (v/v) glutaraldehyde and post-fixed with 1% (w/v) osmium in the same buffer prior to be dehydrated in a graded ethanol series and embedded in Epon 812. Thick sections, were stained with 1% (w/v) aqueous toluidine blue while ultrathin sections were contrasted with uranyl acetate and lead citrate for direct examination in a JEOL 1200 EX transmission electron microscope or processed for cytochemical labeling.

Cytochemistry

Pectin was localized using an *Aplysia* gonad lectin conjugated to gold (Benhamou et al. 1988). Cellulose was localized using a gold-complexed exoglucanase (Benhamou et al. 1987). Callose, a polymer of ß-1,3-glucans, was localized using a purified ß-1,3-glucanase (Benhamou 1992b). Chitin was localized using wheat germ agglutinin (WGA), in a two step procedure (Benhamou 1989).

Results and Discussion

Ultrastructural features of root tissues from non-treated tomato plants (controls)
In FORL-inoculated control plants, the pathogen multiplied abundantly in all tissues, causing severe cell damage including cytoplasm disorganization and organelle disintegration. Primary cell were often reduced to strands of disorganized fibrils and were nearly free of pectin while cellulose was randomly distributed. In such invaded tissues, host defense reactions were never observed.

Ultrastructural features of root tissues from chitosan-treated tomato plants
Examination of root samples from chitosan-treated plants revealed that fungal colonization was mainly limited to the outermost root tissues. Restriction of pathogen ingress towards the vascular stele was accompanied by the formation of structural barriers at sites of potential fungal penetration and by the accumulation of electron-dense deposits in both cells and intercellular spaces.

Ultrastructural features of root tissues from B. pumilus-treated tomato plants
In bacterized tomato plants grown in the absence of chitosan, striking differences in the rate and extent of fungal colonization were observed as compared to controls. Pathogen growth was restricted to the outer root tissues including the epidermis and the cortex, and occasionally the paratracheal parenchyma. Bacterial cells also were restricted to the outer root tissues where they colonized a few intercellular spaces. Discrete host structural changes, including increase in host cell wall density, local accumulation of polymorphic deposits at sites of potential fungal penetration and occlusion of epidermal cells and intercellular spaces with an amorphous material, were observed.

Ultrastructural features of root tissues from tomato plants treated with a combination of chitosan and B. pumilus
In bacterized tomato plants grown in the presence of chitosan, pathogen growth was mainly restricted to the epidermis. The extent and magnitude of the host response increased as compared to that observed in root tissues from plants grown in the absence of chitosan.The highly restricted fungal invasion was associated with strong host defense-related structural modifications, including the deposition of enlarged wall appositions. Another striking host reaction was the formation of an opaque matrix which usually lined the wall junctions in most intercellular spaces. A qualitative evaluation of labeling with the ß-1,3-glucanase showed that higher amounts of callose accumulated in root tissues from tomato plants treated with *B. pumilus* and chitosan. In these tissues, callose occurred not only over the appositions but also over the host cell walls as well as over the plugs formed in the intercellular spaces. Chitin occurred over the walls of invading hyphae at a time when the fungal cells were markedly altered. This was taken as an indication that chitinase synthesis in bacterized tomato roots was not an early process in the cascade of events leading to the expression of plant resistance.

Although earlier observations suggested the potential of endophytic bacteria in triggering the expression of plant defense mechanisms (Tuzun and Kloepper 1985; Benhamou et al. 1996), the data reported here provide evidence that bacterial treatment, in combination with exogenous chitosan application, induces enhanced physiological and biochemical changes at sites of attempted pathogen penetration.The substantial increase in the extent and magnitude of the cellular changes induced by *B. pumilus* in the presence of chitosan suggests that more than one type of elicitors is required for maximizing stimulation of defense mechanisms involved in induced resistance. While it seems obvious that *B. pumilus* and chitosan act synergistically in the induction of an amplified defense response, our knowledge of the actual elicitor signal transduction pathways remains rudimentary. Several lines of

evidence have shown that callose formation is modulated by the intracellular concentration of free Ca^{++} which is known to control the activity of ß-1,3-glucan synthase (Köhle et al. 1985). Considering the polycationic properties of chitosan, chitosan-elicited membrane alterations in the epidermal cells may have promoted internal osmotic imbalances, resulting in electrolyte leakage and in increased Ca^{++} entrance in the cytoplasm. Such an enhanced inflow of Ca^{++} may have contributed to trigger enhanced callose synthesis by an increased activation of the ß-1,3-glucan synthase.

The incorporation of a biocontrol preparation into the arsenal of strategies currently developed for controlling diseases caused by soilborne fungi is a promising step towards elaborating integrated pest management programmes which would be safe for the environment.

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Integrated control of the South American leaf miner *Liriomyza huidobrensis* on UK glasshouse lettuce and Chinese leafy salad crops

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Abstract: The quarantine pest *Liriomyza huidobrensis* is proving difficult to eradicate from leafy salad crops with pesticides. A biological control programme was evaluated on commercial crops of lettuce and Chinese leaves. The leaf miner parasitoids *Dacnusa sibirica* and *Diglyphus isaea* gave up to 92% parasitism on lettuce and 61% parasitism on Choi Sum. *Myzus persicae* were controlled by the aphid parasitoid *Aphidius colemani* but caterpillars were not well controlled by *Bacillus thuringiensis*.

Keywords: South American leaf miner, *Liriomyza huidobrensis*, lettuce, Chinese leafy salads, Choi Sum, Pak Choi, *Dacnusa sibirica*, *Diglyphus isaea*, biological control, integrated control

Introduction

The South American leaf miner, *Liriomyza huidobrensis* was first confirmed in the UK in 1989 and is still a notifiable quarantine pest, subject to MAFF Plant Health and Seeds Inspectorate (PHSI) eradication or containment measures on propagation and production nurseries respectively. Eradication has not proved possible on several nurseries growing lettuce and Chinese leafy salads, due to the lack of effective approved pesticides. Research in the Netherlands has shown that leaf miner parasitoids, particularly *Dacnusa sibirica*, with some contribution by *Diglyphus isaea*, can give effective control of *L. huidobrensis* on protected lettuce beween March and September (van der Linden, 1993). Research on biological control of *L. huidobrensis* in the UK has been limited to the use of entomopathogenic nematodes on tomatoes (Williams & Macdonald, 1995) and on lettuce (Williams, personal communication). The objective of the work described here was to investigate the establishment and efficacy of *D. sibirica* and *D. isaea* against *L. huidobrensis* on various leafy salad crops. A secondary objective was to investigate the biological control of aphids and caterpillars on these crops, to avoid the need for pesticides which might disrupt the biological control programme.

Materials and methods

Sites and Treatments

The experiment was carried out on two commercial nurseries, Site 1 (lettuce) and Site 2 (Chinese leafy salads), both of which had persistent infestations of *L. huidobrensis*. At each site, one glasshouse block approximately 550 m^2 was treated with a biological control programme. (The other glasshouses were treated with pesticides as used in normal commercial practice and including those in the PHSI containment schedule for *L. huidobrensis*). Two consecutive crops were used in the experiment at each site. The lettuce crops were planted on 1 July and 11 September and the Chinese leaves crops were planted on 21 August (Choi Sum) and 21 October (Pak Choi). Each glasshouse was planted with a single

crop of the same age i.e. there were no overlapping planting dates. The glasshouses at both sites were left empty for five to six weeks between the two crops for commercial reasons.

Biological control programme

Leaf miner parasitoids were introduced from planting at two per m^2 per week. In the first lettuce crop, 90% *D. sibirica* and 10% *D. isaea* were used. In the second lettuce crop and both Chinese leaves crops, 50% of each species were used. This was principally because *D. isaea* had significantly contributed to control in the first lettuce crop, despite having been introduced in relatively low numbers (0.2 per m^2, compared with *D. sibirica* at 1.8 per m^2). The aphid parasitoids *Aphidius colemani* and *Aphidius ervi* were each introduced weekly at 0.5 per m^2 to both the lettuce and Chinese leaves crops. To minimise introductions, commercial 'banker plants' were used for each aphid parasitoid species. *Bacillus thuringiensis* was applied by the growers at first caterpillar damage and repeated as necessary.

Assessments

Soil and crop canopy temperatures were monitored continuously. Weekly plant assessments were made from planting to harvest. Numbers of plants with leaf mines were recorded on 100 randomly selected plants. Numbers of mines, mined leaves per plant, numbers of plants with caterpillar damage, and numbers of aphids and parasitised aphids were recorded on 20 infested plants. Twenty leaves containing leaf miner larvae from each house were assessed by dissection for parasitism by *D. sibirica* or *D. isaea*. Any leaf miner pupae in the samples were incubated until emergence, to check for parasitism by *D. sibirica*.

Results and Discussion

On some sampling occasions very few leaf miner larvae could be found for dissection. A mean of 10 and 12 larvae per sampling date were dissected from both lettuce crops and the Choi Sum crop respectively. Only trace levels of larvae were detectable in the Pak Choi crop.

Biological control of L. huidobrensis on lettuce, Site 1

In the first lettuce crop, levels of *L. huidobrensis* remained low, peaking at 23% mined plants at harvest on 5 August (Figure 1). Within one week of the first parasitoid releases, *D. sibirica* had parasitised the first detected leaf miner larva. Four weeks later at harvest, 92% of the 24 leaf miner larvae examined were parasitised; 42% by *D. sibirica* and 63% parasitised or host-fed by *D. isaea (*some of the larvae were parasitised by both species). On this date, the mean leaf miner density was only 0.7 mines per plant.

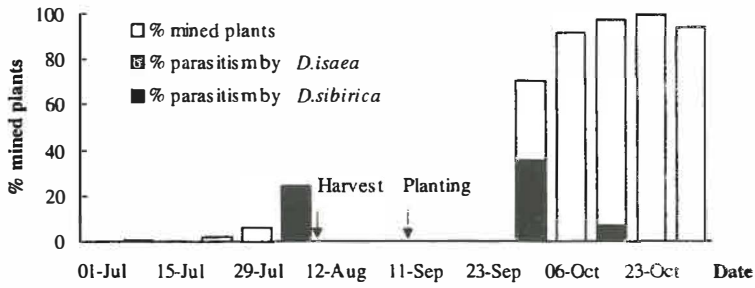

Figure 1. Percentage plants mined by *L. huidobrensis* and percentage parasitism by *D. sibirica* and *D. isaea* on lettuce, Site 1.

Unlike experience in the Netherlands (van der Linden, 1988, 1993), these results indicate that *D. isaea* can establish on lettuce at low host densities and can search as effectively as *D. sibirica* for hosts in a crop at ground level. At harvest, there was a mean of 0.4 mined leaves per plant (1.5 mined leaves per infested plant) which were easily removed before packing.

In the second lettuce crop, levels of *L. huidobrensis* were higher than in the first crop, reaching 99% mined plants on 23 October (Figure 1). Parasitism of larvae was detected only on 30 September and that of pupae on 14 October. From 6 October, less larvae were available for parasitism as a higher proportion of leaf miner pupae were present. Temperatures remained above 10°C, which should have allowed oviposition and development of both parasitoids (Minkenberg, 1989, Nicoli, in press). At the final assessment, the mean number of mined leaves was 2.4 per plant (2.5 per infested plant).

Biological control of L. huidobrensis on Chinese leaves, Site 2

The first crop (Choi Sum) was heavily infested with *L. huidobrensis* adults at planting on 21 August. A nicotine spray was applied for control of adults on 26 August and parasitoid introductions were delayed until 28 August. One week after starting parasitoid introductions, leaf mines were found on 98% plants, the mean host density was 5.8 mines per plant and 38% of the young larvae were already parasitised by *D. sibirica* (Figure 2). During the second week a maximum of 61% leaf miner parasitism/hostfeeding was recorded; 50% parasitism by *D. sibirica* and 11% host-feeding by *D. isaea*. Parasitism by *D. isaea* occurred during the third and fourth weeks and exceeded that by *D. sibirica* on 17 September. The delayed parasitism by *D. isaea* was probably due to its preference for third instar larvae for oviposition (Minkenberg & van Lenteren, 1986). Improved parasitism by *D. isaea* at increased host densities has been recorded on both lettuce and tomato (van der Linden, 1993, Sampson & Walker, 1998), but in this experiment the host density had already declinined to 4.6 mines per plant when parasitism by *D. isaea* was first detected. On 24 September, just prior to harvest, there was a mean of 2.2 mined leaves per plant and 3.4 mines per plant, although many of the mines were now empty. Temperatures remained above 10°C.

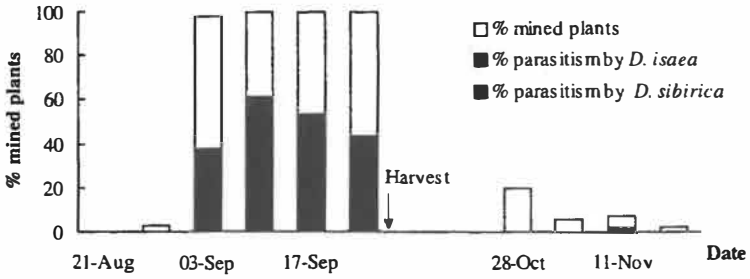

Figure 2. Percentage plants mined by *L. huidobrensis* and percentage parasitism by *D. sibirica* and *D. isaea* on Chinese leaves, Site 2.

In the second crop (Pak Choi), very few *L. huidobrensis* larvae developed and hence mining levels were low (Figure 2), despite high levels of adult puncturing shortly after planting. This was probably due to minimum crop canopy temperatures sometimes approaching the minimum development threshold for *L. huidobrensis* (Head, personal communication). Only three *L.huidobrensis* larvae were detected on 11 November and one of these was parasitised

by *D. sibirica*. On 27 November, there was a mean of only 0.002 mines per plant (0.1 mine per infested plant).

Biological control of aphids and caterpillars

No aphids were detected on the lettuce. Low numbers of *Myzus persicae* were found on the Choi Sum, but most were parasitised by *A. colemani*. The 'banker plants' were unreliable in quality; numbers of cereal aphids on them were often low or undetectable, so weekly releases of parasitoids were necessary. Slight caterpillar damage occurred on both lettuce crops but was more severe on the Choi Sum. *Bacillus thuringiensis* did not prevent further damage.

Future work

This work indicates that both *D. sibirica* and *D. isaea* have a role to play in biological control strategies for *L. huidobrensis* on lettuce and Chinese leafy salads. However, the programme used here, designed to evaluate the potential of each parasitoid, was too expensive for commercial use. If overlapping crops had been present in the glasshouses, the parasitoids could have moved from harvested crops to younger crops (van der Linden, 1993), and this should have allowed reduced releases. Banker plants have allowed cost-effective parasitism on lettuce by *D. sibirica* although *D. isaea* preferred to stay on the banker plants (van der Linden, 1993). Further work is justified on refining strategies for reliable, cost-effective integrated control and containment of *L. huidobrensis* on leafy salad crops which need to be free of leaf mines at sale. Research is also necessary on improved biological control of caterpillars and cost-effective strategies for aphid control, within a sustainable IPM programme.

Acknowledgements

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Percent parasitism and adult emergence of *E. formosa* from greenhouse whitefly pupae with multiple oviposition wounds

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Abstract
Greenhouse whitefly pupae exposed to *Encarsia formosa* adult females often exhibit one to several oviposition wounds. Reports of adult whitefly emergence from wounded pupae have raised concern regarding the possibility of resistance of greenhouse whitefly to *E. formosa*. Whitefly pupae exposed to *E. formosa* adult females were obtained from a commercial insectary and white pupae with one or more oviposition wounds were dissected to determine percent parasitism, measured as the number of pupae that contained a live *E. formosa* larva. Percent emergence of *E. formosa* was assessed from both black pupae and white pupae with one to several oviposition wounds.

Key Words: greenhouse whitefly, *Encarsia formosa*, emergence

Introduction

More than 70 years ago, the parasitic wasp *Encarsia formosa* Gahan was first described from greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) from the United States. Shortly thereafter, the life history of this parasitoid was described by Speyer (1927). Since its initial discovery, *E. formosa* has become a popular choice for biological control of greenhouse whitefly in commercial greenhouses throughout the world (Hussey, 1985; van Lenteren, 1997).

During the last IOBC meeting for the IPM in Greenhouses Working Group (Vienna, 1996), some IPM specialists reported adult emergence of greenhouse whitefly (GWF) from white pupae with multiple oviposition wounds made by *E. formosa*. These observations raised concern regarding the possibility that GWF may be developing resistance to *E. formosa*. This study was undertaken to determine percent parasitism and percent adult emergence of *E. formosa* from GWF pupae with one to several black oviposition wounds. Percent parasitism was measured as the number of pupae that contained a live *E. formosa* larva. Percent emergence of adult parasitoids from white pupae with black oviposition wounds and black pupae was also assessed.

Materials and Methods

Parasitized GWF pupae used in this study were obtained from Applied Bio-nomics Ltd. (Sidney, B.C.). Greenhouse whitefly were reared on tobacco (*Nicotiana tabacum* L.); tobacco foliage and pupae from 5 production dates were shipped to the University of Alberta (Edmonton, Alberta) for examination. All pupae had been exposed to *E. formosa* adult females and each shipment consisted of both white and black pupae. Upon close examination, almost all of the white pupae exhibited

multiple oviposition wounds, observed as black spots on the dorsal, and occasionally, on the lateral sides of each pupa.

Percent parasitism Ten white pupae with oviposition wounds were examined from each of the five samples on the day they were received (total sample size, n=50). Pupae were removed from tobacco foliage using a fine artists brush. The number of oviposition wounds present on the dorsal and lateral surfaces of each pupa was recorded. Pupae were then dissected with fine dissection needles in Yaeger's solution. The contents of each pupa was examined with both stereo- and compound microscopes for *E. formosa* larvae.

Percent emergence Sixteen pupae were examined from each sample (10 white pupae with black oviposition wounds and 6 black pupae; total sample size, n=50 and n=30, respectively). Pupae exposed to *E. formosa* that lacked oviposition wounds were not available for examination. Pupae were removed from the lower surface of tobacco foliage using a fine artists brush. All pupae were examined with a stereomicroscope after removal to ensure that they had not been damaged and the number of oviposition wounds on each white pupa was recorded. Individual pupae were placed within clear vials and examined daily for parasitoid emergence (room temperature; approx. 24 °C). Pupae were examined through the side of the vial to reduce handling of the specimens. All pupae were examined with a stereomicroscope at the end of the observation period to confirm parasitoid emergence.

Results and Discussion

Percent parasitism Percent parasitism data are reported in Table 1. Forty-five of the 50 hosts examined (90%) contained one or more *E. formosa* larvae. Of these, 9 were superparasitized (18%). These contained two *E. formosa* larvae; a large, late-instar larva and a small, dead, early-instar larva.

Table 1. Percent parasitization of white GWF pupae with oviposition wounds made by *E. formosa*

Sample No.	No. examined	Mean no. of wounds (range)	No. parasitized	No. with a live larva[1]	No. with a dead larva	No. super-parasitized
1	10	6.3 (3-13)	10	9 (4;5)	1	4
2	10	6.2 (1-11)	10	9 (8;1)	1	1
3	10	4.4 (1-7)	10	10 (7;3)	0	2
4	10	18.0 (5-43)	8	8 (6;2)	0	2
5	10	7.8 (4-14)	7	7 (6;1)	0	0
Total	**50**		**45**	**43 (31;12)**	**2**	**9**
% Total			**90.0**	**95.6 (68.9;26.7)**	**4.4**	**18.0**

[1]total number of pupae that contained a live larva (no. with a moving larva; no. with a sessile, late-instar larva)

Thirty-one pupae (68.9%) contained a live (moving), early-instar *E. formosa* larva and an additional 12 pupae (26.7%) contained a sessile, late-instar larva. Late-instar larvae were normally less active than early-instar larvae, and although reduced movement was observed for some late-instar larvae after these were gently prodded with a blunt probe, most late-instar larvae did not move during examination. There was no evidence, however, to suggest that these larvae were damaged or dead. Forty-three of the 45 parasitized pupae contained either a sessile, late-instar larva or a live (moving) larva (95.6%).

Of the 50 pupae examined, 5 did not contain an *E. formosa* larva(e). Each of these pupae contained a fully-developed, adult whitefly when dissected. There was no evidence of parasitization within these hosts; dead *E. formosa* larvae and/or melanized eggs/larvae were not observed. It is possible that these hosts were deemed unsuitable by *E. formosa* during oviposition.

Percent emergence Parasitoid emergence data from white pupae with black oviposition wounds are reported in Table 2. One pupa was lost during the observation period (total sample size, n=49). Thirty-six of the 49 pupae turned black after being placed within the vials; *E. formosa* adults emerged from all 36 pupae (73.5% emergence). Parasitoids failed to emerge from 13 pupae. Three of these pupae had turned light grey and each contained a dead adult parasitoid. Each of the remaining 10 pupae had desiccated but retained their white colouration.

Table 2. Parasitoid emergence from white GWF pupae with one or more oviposition wounds

Sample No.	No. examined	Mean no. of wounds	*E. formosa* adult emergence	*E. formosa* dead[1]	Mean days to emergence (range)[2]
1	10	6.3 (1-10)	6	1,3	11.0 (9-12)
2	10	4.5 (2-9)	8	1,1	9.1 (8-11)
3	10	4.0 (1-8)	9	0,1	9.2 (9-11)
4	9	25.2 (6-63)	5	0,4	10.6 (9-12)
5	10	4.8 (2-11)	8	1,1	10.3 (9-13)
Total	**49**		**36**	**13**	
% Total			**73.5**	**26.5**	

[1]damaged, desiccated; [2]days following receipt of shipment

Parasitoid emergence from black pupae are reported in Table 3. Adult *E. formosa* emerged from 27 of the 30 black pupae (90%); the remaining 3 pupae each contained a dead *E. formosa* adult that failed to emerge.

Table 3. Parasitoid emergence from black GWF pupae

Sample No.	No. examined	*E. formosa* adult emergence	*E. formosa* dead	Mean days to emergence (range)[1]
1	6	6	0	6.2 (5-8)
2	6	6	0	6.8 (6-8)
3	6	5	1	7.0 (6-8)
4	6	6	0	5.8 (5-7)
5	6	4	2	6.5 (5-6)
Total	**30**	**27**	**3**	
% Total		**90.0**	**10.0**	

[1]days following receipt of shipment

Percent emergence of *E. formosa* from white pupae with oviposition wounds was lower than for black pupae. White pupae are more prone to damage than black pupae and removal of white pupae from foliage may cause unnoticeable damage which could increase the likelihood of pupal desiccation. Therefore, percent emergence from white pupae in this study was probably underestimated. Desiccated pupae were dissected but the contents had shrivelled and were difficult to identify. Only *E. formosa* adults emerged from white pupae, and because percent parasitism of white pupae was high (95.6% contained a live larva), there was no evidence to suggest that greenhouse whitefly had begun to develop resistance to *E. formosa*.

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Life table characteristics of the predatory gall midge *Feltiella acarisuga*

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Abstract: Life table characteristics of the predatory gall midge *Feltiella acarisuga* were investigated at 22±2°C, 80-90% r.h., L:D 16:8 with cucumber as host plant, spider mites as prey and honeydew either present or absent as food source for adults. Juvenile development time was approx. 20 days and the juveniles suffered a mortality of 22.1%. *F. acarisuga*'s fecundity and innate capacity of increase was significantly increased with a continuous availability of fresh honeydew as compared to water: the fecundity increased to 24.4 eggs per female (2.4 times) and r_m to 0.1128 day^{-1} (1.6 times).

Key words: *Feltiella acarisuga*, fecundity, juvenile development, juvenile mortality, sex ratio, spider mites, *Tetranychus urticae*, biological control, cucumber, honeydew, glasshouse crops

Introduction

The gall midge *Feltiella acarisuga* Vallot (Diptera: Cecidomyiidae) the larvae of which prey upon spider mites (*Tetranychus* spp., Acari: Tetranychidae) (Roberti, 1954; Vacante, 1985; Gillespie *et al.*, 1994; Gillespie & Quiring, 1995) is a recent addition to the range of beneficials marketed to control glasshouse pests. Reports on promising trials with biocontrol of spider mites on roses (Vacante, 1985), sweet pepper (Jude Bennison, unpubl.), tomatoes (Wardlow & Tobin, 1990) and aubergine (Bennison *et al.*, 1996) have been made and several producers and suppliers of biological control agents claim that *F. acarisuga* is a useful supplement to the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) for control of *Tetranychus urticae* Koch and *T. cinnabarinus* Boisduval.

Even though *F. acarisuga* is commercially available, few detailed studies on its biology and impact on prey populations have been published (Gillespie *et al.*, 1994; Gillespie & Quiring, 1995; Opit *et al.*, 1997; Svendsen, 1999). Knowledge of the biology of a natural enemy and its interactions with its intended prey, as well as with other organisms in the glasshouse environment, is a prerequisite realising its full potential as a biocontrol agent and for understanding variable outcome of biological control.

To add to the knowledge on *F. acarisuga*'s biology the following experiments were undertaken.

Materials & Methods

Rearing

F. acarisuga was reared in plexi glass cages (45×45×58 cm) with forced ventilation in a climate room at 22±2°C, 80-90% r.h., L:D 16:8 with cucumber (*Cucumis sativa* L. cv. Danora F.1) as host plant, spider mites (*T. urticae*) as prey and peach-potato aphids (*Myzus persicae*

Sulzer (Homoptera: Aphididae)) present as source of honeydew for adult gall midges. For details, see Enkegaard *et al.*, 1999.

Egg and larval development and mortality
Twenty to sixty 1-3 days old adult *F. acarisuga* (sex ratio (♀/(♀+♂)) ~ 0.6) were introduced for oviposition in each of 4 plexi glass cages (45×45×58 cm) containing a small spider mite-infested cucumber plant with 1-3 leaves and a sweet pepper plant with aphids. The adults were removed after 5-24 h. The cages were placed in a climate room at 22±2°C, 80-90% r.h., L:D 16:8.

Parts of the leaves were removed at certain dates, kept in the climate room at moist conditions (90-98% r.h.) and observed daily for 3-5 days. Thus, leaves removed on day 1 and 3 after adult introduction were counted daily for eggs and larvae on days 1-3 and 3-7, respectively; leaves removed on day 7 were counted daily for larvae and pupae between days 7-10; and the remaining leaves were counted *in situ* for presence of larvae and pupae between days 10-14. Mortality was calculated for each period and subsequently added to give the overall mortality in the egg and larval stages. In addition, daily observations were made to determine the overall development time from egg to pupae.

Pupal development, pupal mortality and sex ratio
Approximately 60 1-3 days old adult *F. acarisuga* (sex ratio ~ 0.6) were introduced into a plexi glass cage as described above for oviposition. The cage was placed in the climate room for 5 h after which the adults were removed. Between days 7-13 after adult introduction, the leaves were inspected daily *in situ* and the larvae and pupae present were counted. On day 14 the leaves were cut into small pieces each holding 1-5 pupae. These leaf pieces were kept in Petri dishes (5 cm ∅) under moist conditions (90-98% r.h.) in the climate room. The number of emerging gall midges were counted and sexed daily until emergence ceased when the unhatched pupae were counted.

Fecundity and the influence of honeydew
Since *F. acarisuga* eggs are difficult to see without damaging the host plant and thus very time consuming to count, female fecundity was calculated as the number of juveniles produced 9 days after the onset of egg laying. Eight or twelve male and 12 female *F. acarisuga* (max. 24 h old) that had emerged with access to water only, were introduced into each of 6 plexi glass cages containing a small spider mite-infested cucumber plant with 2 leaves. All adults had access to water. In addition, three of the cages contained a sweet pepper plant with aphids. The cages were placed in the climate room at conditions as described above. On the 9th day after the introduction of adults, the leaves were removed and the larvae and pupae present were counted.

Results

Juvenile development and mortality
The egg and larval stages of *F. acarisuga* lasted a little over a week, the average development time (±*s.e.*) being 8.6±0.05 days (*n*=846). The average pupal development time (±*s.e.*) was 11.7±0.1 days (*n*=282). The mortality rates in the sequential periods of egg and larval development were: 4.3% (day 1-3), 4.4% (day 3-7), 3.4% (day 7-10), and 4% (day 10-14). Thus, eggs and larvae suffered an overall mortality (±*s.e.*) of 16.1±0.94% (*n*=1,525). The pupal mortality (±*s.e.*) was 6.0±1.5% (*n*=250).

Sex ratio
The sex ratio among the offspring of *F. acarisuga* was female biased with 220 out of 282 adults being female, giving a ratio (♀/(♀+♂)) (±*s.e.*) of 0.78±0.03.

Fecundity and the influence of honeydew
A continuous supply of honeydew for ovipositing female had a significant positive effect (χ^2 analysis, $P<0.001$) on the number of juveniles produced. When honeydew was available an average (±*s.e.*) of 21.7±5.4 juveniles per female were produced as opposed to 8.9±5.2 juveniles per female in the absence of honeydew. From the mortality experiment, a mortality rate of 11% for juveniles developing during the 9-day experimental period can be assumed. Hence, the estimated fecundity (±*s.e.*) of *F. acarisuga* females is 24.4±6.1 and 10.0±5.8 eggs per female with and without access to honeydew, respectively.

Life table parameters
Assuming an adult lifespan of 3½ days under our experimental conditions (Stig Jacobsen, pers. obs.; Gillespie *et al.*, 1994; Gillespie & Quiring, 1995) and an evenly distributed fecundity throughout the female's adult life, life tables for *F. acarisuga* with cucumber as host plant, spider mites as prey and honeydew either present or absent for adults were constructed (Birch, 1948). *F. acarisuga* had a mean generation time (G) of 21.5 days. In the presence of honeydew the net reproductive rate (R_0) and the innate capacity for increase (r_m) was 11.3 and 0.1128 day^{-1}, respectively, as opposed to 4.6 and 0.0711 day^{-1}, respectively, in the absence of honeydew. Thus, access to honeydew increased the innate capacity for increase 1.6 times.

Discussion

Wardlow and Tobin (1990) observed that the development of *F. acarisuga* on tomato at 21°C lasted about one week for larvae and 7-10 days for pupae. These values are similar with the present results but higher than reported by Gillespie *et al.* (1994). These authors found an overall development time of about 13.6 days (interpolated from their data). The host plant and further details in methodology were, however, not specified and an interpretation of the faster development is therefore not possible.

No reports on the juvenile mortality of *F. acarisuga* are available in the literature.

The sex ratio (♀/(♀+♂)) of *F. acarisuga* reported here was 0.78. This is higher than sex ratios of field-collected *F. acarisuga* which varied between 0.63-0.71 with an overall sex ratio of 0.69 (Roberti, 1954). Likewise, Enkegaard *et al.* (1999) found sex ratios varying from 0.55 to 0.8 with an overall ratio of 0.64 in rearings of *F. acarisuga*.

Gillespie and Quiring (1995) found a fecundity of *F. acarisuga* on tomato of about 14.9 4-day old juveniles per female (interpolated from their data), equivalent to 15.8 eggs per female (assuming, from the present mortality experiment, a juvenile mortality of 5.4% over the 4-day period). The environmental conditions were similar to ours and females oviposited on leaves sprayed once with a sugar solution as energy source. The fecundity registered is about 35% lower than in our experiments where female *F. acarisuga* had continuous access to fresh honeydew but about 60% higher than the fecundity we observed when females had access only to water. Thus, even though sugar seems superior to water in terms of increasing both fecundity and female life span (Gillespie *et al.*, 1994), honeydew appears to be superior in its effect on fecundity and thus also on net reproductive rate and innate capacity for increase. Presumably female gall midges benefit not only from the sugars but also from the

other substances, e.g. amino acids (Maltais & Auclair, 1952; Hussain *et al.*, 1974), present in the honeydew.

We suggest that biocontrol with *F. acarisuga* may improve if adults have access to honeydew and water and are given space and time to mate before being released in a large culture.

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Protected cultivation and research on biological control of pests in greenhouses in Brazil

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Abstract. Production under protected cultivation is a relatively recent development in Brazil. Initially, mainly ornamentals were produced. Nowadays also vegetables are grown in greenhouses. The total area under protected cultivation is about 1000 hectares. Various insect and mite pests occur, some are similar as those in other greenhouse countries, others are typical Brazilian pests. Pest control is still mainly by chemicals, which have to be applied at frequencies up to three times per week. Recently, several groups have started research on biological control of pests, a development stimulated mainly by ornamental growers.

Key words: protected cultivation, Brazil, crops, pests, chemical control, biological control

Introduction

Biological control of pests in Brazil has a long history and is applied on a large scale (e.g. Siconbiol V, 1996 and Siconbiol VI, 1998). The best known cases of large scale inundative biological control are in sugar cane, where sugar cane borer *Diatraea saccharalis* is controlled by *Cotesia flavipes;* in soybean, where the caterpilar *Anticarsia gemmatalis* is controlled by *Baculovirus anticarsia* and several soybean bugs (*Nezara viridula, Euchistus herus* and *Piezodorus guildini*) are controlled by *Trissolcus basalis*; in tomato, where the leafmining lepidopteran *Tuta absoluta* is controlled by *Trichogramma pretiosum*; and in wheat where several cereal aphids are controlled by parasitoids (mainly *Aphidius* species)(Vilela et al., 1998). About 1000 scientists are currently working on various aspects of biological control in Brazil.

Protected cultivation is a relatively new production method in Brazil, starting around 1970, principally in the states Rio Grande do Sul, Parana, Sao Paulo, Rio de Janeiro, Santa Catarina and Minas Gerais. The greenhouses are made of glass or plastic, and can be opened or closed at the sides, depending on the climate. Climate control is done with horizontal and vertical screens. It is estimated that there is a total area of 1000 to 1200 hectares under protected cultivation in Brazil (Oliveira, 1995). Flower production started in the 1970s in Holambra, which is still the location with the largest production of ornamentals. In the 1970s and the 1980s mainly flowers were produced in greenhouses, followed in the 1990s by production of vegetables. Currently, more than 400 species of plants are grown in greenhouses, the main ones being roses, chrysanthemums, gerbera, gladiola, violets, azalea, kalanchoe. The main vegetables grown in greenhouses are tomato, cucumber, melon, sweet pepper and lettuce (Vilela et al., 1998).

Pest control in protected cultivation is still mainly by chemical pesticides, and a lack of research on natural enemies for biological control of these greenhouse pests is a main advantage for greenhouse growers (Oliveira, 1995).

Greenhouse pests in Brazil

The main pests occurring in protected cultivation are summarized in table 1 (Bueno, unpublished). The pest situation in ornamentals and vegetables is very serious in Brazil and leads to frequent pesticide applications. In table 2 an example is given of daily sampling during 4 weeks (November-December 1997) with yellow traps for pest insects in tomato crops in Rio Grande do Sul (L.A.B. de Salles, personal communication, 1998). The main pests were leafminers and aphids. Surprisingly, no whiteflies and thrips were found. In the northern part of Sao Paulo State, mainly leafminers and whiteflies are occurring in eggplants (F. do Amaral Mesquita, personal communication, 1999). The high densities of these pests lead to 2-3 pesticide applications per week with alternation of chemicals.

Table 1. Main pest species occurring in protected cultivation in Brazil

Pest species	Crops
aphids	
Myzus persicae	ornamentals and vegetables
Aphis gossypii	ornamentals and vegetables
Aphis sp.	ornamentals
Dysaphis sp.	ornamentals
Uroleucon sp.	lettuce
thrips	
Frankiniella sp.	ornamentals
Thrips palmi	ornamentals
leafminers	
Liriomyza sp.	egg plant
whiteflies	
Bemisia sp.	egg plant and ornamentals
mites	
Tetranichus urticae	ornamentals

Table 2. Occurrence of pest organisms in tomatoes under protected cultivation in Pelotas, Rio Grande do Sul

Pest species	Number
Chrysomelidae	15
Cicadellidae	5
Aphididae	42
Agromyzidae	343

Research on biological control of greenhouse pests in Brazil

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The main project concerns the evaluation of parasitoids and predators as biological control agents of pests in protected cultivation, which is coordinated by V.H.P. Bueno. Within this project there are the following subprojects:

1. Development of a banker plant systems based on sorghum as banker plant, *with Schizaphis graminum* as host insect and the parasitoid *Lysiphlebus tetaceipes*, for control *Aphis gossypii* in sweet pepper (Rodrigues, 1999). Experiments in the laboratory showed that *L. testaceipes* is attacking both *S. graminum* and *A. gossypii*, and shows a higher rate of parasitism of *S. graminum* in choice and no-choice tests. In an experimental greenhouse, *L. testaceipes* migrated from the sorghum banker plant and easily found *A. gossypii* on sweet pepper plants that were furthest away from the release plant, i.e. 3.35 meter, during the first day of release.
2. Foraging behaviour of *Aphidius colemani* on *Aphis gossypii* and *Myzus persicae* at different host densities, and host preference for these two aphid species under choice and no-choice situations (Sampaio, M. V., personal communication, 1999).
3. Development and rearing of *Orius insidiosus* to control thrips in greenhouses (Mendes, S.M., personal communication, 1999).
4. Foraging behaviour of *Orius insidiosus* for thrips on cucumber (Argolo, V., personal communication, 1999).
5. The importance of pre- and post-imaginal learning for host finding by the aphid parasitoids *Lysiphlebus testaceipes*, *Aphidius colemani* and *Aphidius ervi* (R. Perez-Maluf, personal communication, 1999).
6. Comparison of pest development on chrysanthemum in commercial greenhouses of Fazenda Terra Viva, Schoenmaker group, Holambra, Sao Paulo (Bueno, Mendes & Domingos Ferreira Junior, unpublished). In 1998, the development of several aphid species (*Myzus persicae, Aphis* sp., *Aphis gossypii* and *Dysaphis* sp.) was studied in a commercial chrysanthemum crop that was sprayed according to normal practices and a chrysanthemum crop in which no chemical control was applied. In the chemically controlled crop, the spray frequency was on average 2-3 times per week. In the non-sprayed crop the following parasitoids were found: *Aphidius colemani, Diaeretiella rapae and Lisyphlebus testaceipes*. The numbers of aphids increased during the first six weeks, after which the parasitoids reduced the aphids to very low numbers. A very positive result of this observation was that the number of flowers produced in the non-sprayed chrysanthemums was the same as in the chemically controlled flowers.

A second topic concerns the development of whitefly on tomato lines with a different degree of resistance (Ciociola, A.I., personal communication, 1999).

Another research project, which was started recently, concerns the evaluation of entomopathogens for control of greenhouse pests (A. Moino Jr., personal communication, 1999).

Department of Agronomy, State University of Londrina, Parana (P. J. Neves, A. Menezes Jr)

Research on entomopathogens for pest control, mainly fungi. Several isolates of fungi are evaluated for control of thrips species.

Future developments of biological pest control in Brazil

The area under protected cultivation is still growing in Brazil. Producers are looking for new locations to find the optimal climate for production, for example cooler areas in higher locations. Producers in the main areas for greenhouse production are increasingly interested in biological pest control, because of resistance problems and environmental pollution. It must be realized that development of biological control in ornamentals will not be an easy task, because of the high cosmetic demands of the product and the many pest species that occur in the more than 400 species of plants. Development of biological control in vegetables might be easier, as lot of experience from other countries is available, the number of pest species is lower and cosmetic damage is not an important issue here.

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Biological control of the Two-spotted spider mite, *Tetranychus urticae*, on hardy nursery stock

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Abstract: Two-spotted spider mite, *Tetranychus urticae* is one of the most damaging pests of hardy nursery stock. Biological control with the predatory mite *Phytoseiulus persimilis* is not always satisfactory in low temperatures outdoors, or hot, dry environments. In this trial, the predatory mite *Amblyseius californicus* displaced *Phytoseiulus*, and became the dominant, most effective predator of spider mite on *Ceanothus* plants grown outdoors.

Introduction

Two-spotted spider mite is a major pest of a wide range of hardy ornamental nursery stock, including the important plant genera *Choisya*, *Ceanothus*, *Weigelia* and *Viburnum*. In the UK, many growers have converted to IPM programmes using a range of beneficial insects and mites to control the spectrum of pests infesting the crop. The most common beneficial used to control *Tetranychus urticae* is the predatory mite *Phytoseiulus persimilis*. In some situations, this works well, but in the summer, high temperatures and low humidities can adversely affect the predators' activity, leading to poor control. Equally, although spider mite can multiply outdoors under lower temperatures, *Phytoseiulus* may not give good control (Hussey and Scopes, 1985). Research on biological control of *T. urticae* with *Amblyseius californicus* has shown that *A. californicus* is active under both low and high temperatures (Pickett & Gilstrop, 1986, Rott and Ponsonby, 1998).
The objective of the work described was to further investigate the control of *T. urticae* by *P. persimilis* outdoors on *Ceanothus*. In the event, *Amblyseius californicus* was naturally present on the test plants, and the relationship between the two predators was studied.

Materials and methods

The trial was conducted at Laylocks Nurseries, Worcester, UK, using Ceanothus "Blue Mound" as the test plant. A natural infestation of all stages of *T. urticae* (approximately 10-20 per leaf) was present on the plants at the start of the trial on 1 August. The plants were grown in 2 litre pots and maintained outside in a container standing out area, irrigated by overhead sprinklers.

Treatments and Experimental design

The trial used a randomised block design with 4 replicates per treatment and 4 pots per replicate.
The following treatments were used:
1. Untreated
2. *Phytoseiulus persimilis* low rate (10 per plant)
3. *P. persimilis* high rate (20 per plant).

The low rate was applied to plants on 9 and 23 August, while the high rate plants received predators on 9, 23 and 29 August.
The foliage of plants within each replicate was touching, so that predators could move from plant to plant, but individual replicates and treatments were separated by at least 0.5 m, to reduce movement between treatments.

Assessments

80 leaves per treatment (20 per replicate) were removed at random every week, and the number of spider mite eggs, nymphs and adults, and the number of predatory mites per leaf were counted using a binocular microscope.

Results and Discussion

The results of all assessments are shown in Tables 1-3. During the second assessment (19/8) the predatory mite *Amblyseius californicus* was detected in the leaf samples. The *Ceanothus* plants had previously been grown under protection in a polythene tunnel before being placed outdoors in late July, and the *A. californicus* must have colonised the plants at this time. Therefore, both *A. californicus* and *P. persimilis* were present on the test plants.

Table 1
Counts of Two-spotted spider mite on plants not treated with *P. persimilis*

Date	Mean mites per 10 leaves			
	Nymphs	Adults	Eggs	*A. californicus*
1/8	41	10	142	0
19/8	17	22	271	7
26/8	89	10	166	12
3/9	11	9	46	9
9/9	7	0	0.7	7

Amblyseius californicus spread rapidly on the "untreated" plants and within 3 weeks had achieved good control. Temperatures during the period of the experiment were variable, with several cool, wet days, but this appeared to have no adverse effects on *A. californicus*.

Table 2
Control of Two-spotted spider mite on plants treated with low rate *P. persimilis*

Date	Mean mites per 10 leaves				
	Nymphs	Adults	Eggs	*A. californicus*	*P. persimilis*
1/8	43	11	99	0	0
19/8	34	8	134	4	0
26/8	18	4	53	3	1.3
3/9	4	3	9	5.3	0.7
9/9	0.7	0	0	3	0

P. persimilis was detected in leaf samples after two introductions on 9 and 23 August, but *A. californicus* became the dominant predator, and number of spider mites were reduced to almost zero by 9 September. Observation of *Amblyseius californicus* under the binocular microscope showed a high level of searching ability; these predators moved rapidly over the leaf and appeared to be more active than *P. persimilis* in the samples observed.

Table 3
Counts of Two-spotted spider mite in plants treated with high rate *P. persimilis*

Date	Mean mites per 10 leaves				
	Nymphs	Adults	Eggs	*A. californicus*	*P. persimilis*
1/8	54	6	38	0	0
19/8	34	8	86	13	2.7
26/8	12	3	29	5.3	2
3/9	3	0	0	2	1.3
9/9	0	0	0.7	3	0.7

Phytoseiulus persimilis counts were higher in the samples from the high rate treatment, but *Amblyseius californicus* still became the dominant predator.

As before, excellent control of *T. urticae* was achieved by early September.

The original source of *A. californicus* on this nursery site is unknown, but the grower had not used biological controls previously so it is unlikely that it was from one of the bio-control suppliers. Specimen plants had been imported from Italy: this may have been the original source. Samples taken the following spring showed that *A. californicus* had survived the winter period in an unheated polythene tunnel at this nursery.

There may be variation between strains of *Amblyseius californicus*: further research is needed in this area. The UK government at present is only allowing *A. californicus* to be used on small areas under licence, but samples from outdoor crops such as strawberries have shown that it is well established in areas such as Kent (Labuscchagne, pers-comm).

Acknowledgments

Dr Anne Baker of the British Museum (Natural History) kindly confirmed the identification of *A. californicus*. Thanks to Laylocks Nurseries, Worcester, UK, for provision of the trial site. This work was funded by the Ministry of Agriculture, Fisheries and Food.

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Development of an integrated pest management program for fresh cut roses in US greenhouses

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Abstract: Fresh cut roses have one of the highest aesthetic standards among greenhouse floral crops. This has traditionally resulted in frequent prophylactic pesticide applications and has left growers reluctant to consider integrated pest management methods. Increasingly stringent US pesticide regulations and high pesticide costs are now causing rose growers to reexamine their pest management methods. The objective of this project is to develop and implement IPM methods for the three key pests of fresh cut roses (western flower thrips, twospotted spider mite, and powdery mildew). Work has been completed for western flower thrips and is underway for twospotted spider mites and powdery mildew.

Key words: roses, western flower thrips, twospotted spider mite, powdery mildew, integrated control

Introduction

The high aesthetic standards of the cut rose market has traditionally led to frequent prophylactic pesticide applications. Growers have been reluctant to adopt integrated control methods because of a lack of information about thresholds for rose pests and an uncertainty about the effectiveness of IPM methods. Recent changes in US laws resulting from the Food Quality Protection Act of 1996 have led to mandatory safety reviews of many pesticides commonly used by rose growers. Manufacturers may elect to remove these products from the market rather than perform these expensive tests. Faced with the possible loss of familiar control options, many rose growers are expressing renewed interest in IPM.

Fresh cut roses have three key pests: western flower thrips (*Frankliniella occidentalis*); twospotted spider mite (*Tetranychus urticae*); and powdery mildew (*Sphaerotheca pannosa*). The objective of this project is to develop an IPM program for greenhouse grown fresh cut roses that includes sampling methods, thresholds, and reduced risk control strategies for these three key pests.

Materials and methods

Western flower thrips

Yellow sticky traps were counted weekly for the past three years to monitor thrips populations. Flower samples were also taken weekly. Thrips injury was assessed and then the flowers were dissected and all thrips counted. This permitted the calculation of the relationship between the number of thrips per flower, thrips injury, and trap catch.

Twospotted spider mite

Mite distribution is being studied in a commercial greenhouse on rose plants ('Kardinal') grown using the arching (bent cane) method. Observations began on October 8, 1998. Five leaves extending up from the bend and five leaves extending down from the bend are sampled weekly for spider mites. This will enable us to determine where on the plants mites are located and how that distribution shifts in response to changes in mite density. Studies of interplant movement have just been initiated. Clean plants that surround those with mite infestations are examined weekly for mites. Mite movement onto uninfested plants will be related to mite densities on infested plants. This study will take two years to complete.

Powdery mildew

Monitoring equipment that measures temperature, humidity, and leaf wetness was placed at the same greenhouse operation were the mite studies are being conducted on January 13, 1999. A model used to predict mildew outbreaks in grapes based on these environmental parameters will be adapted for rose powdery mildew.

Results and discussion

Western flower thrips

We have determined that 25 to 50 thrips per yellow sticky card per week means that there is an average of one thrips per flower. Thrips populations higher than this generally result in visible flower injury. We have demonstrated the efficacy of the insect pathogen *Beauveria bassiana* (BotaniGard™) for thrips control (Murphy et al., 1998). We have also shown that pesticide applications targeted at the rose flower can reduce pesticide volume with no effects on thrips mortality (Parrella et al., 1999). This part of the project is ready for grower implementation.

Twospotted spider mite

Preliminary observations of twospotted spider mite distribution show that most mites are found immediately above and below the crown area where the bend is made (Table 1). This

Table 1. Twospotted spider mite distribution on bent cane rose plants.

Leaf location	Avg. no. mites
9 upper	0.00
7 upper	0.56
5 upper	0.68
3 upper	0.82
1 upper	1.01
CROWN	
1 lower	1.46
3 lower	1.05
5 lower	0.59
7 lower	0.04
9 lower	0.16

is acceptable to rose growers, as these parts of the plant are not harvested. Mites have been observed to exhibit positive phototaxis at high densities (Helle and Sabelis, 1985), so we expect that this distribution will shift upward during the summer when mite levels become quite high. This may result in mite movement onto the harvested portion of the plant. Determination of the density that triggers this movement is an important objective of these studies because it will be used to formulate a threshold for twospotted spider mite.

Knowledge of mite distribution also permits the use of pesticide sprays directed at the crown. This provides the possibility of a refuge for predatory mites in the lower portion of the canopy. We will initiate studies with *Phytoseiulus persimilis* in the fall of 1999.

Powdery mildew

No mildew infections have been observed to date, so there is no data to report on this aspect of the study.

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Dicyphus hyalinipennis Burm. (Heteroptera: Miridae): A potential biological control agent for glasshouse pests in Hungary

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Abstract: Since 1995 a mirid bug unknown for the Hungarian fauna has been observed to migrate regularly in the summer into greenhouses with tomato grown under conditions of low insecticide pressure, multiply there in high rates and effectively control the most noxious pest *Trialeurodes vaporariorum*. In order to evaluate the usefulness of the species (identified as *Dicyphus hyalinipennis* Burm.) for biological control, its main biological features, prey range and predation capacity were studied. Female daily egg-production, developmental time of nymphal stages, and life-longevity of adults were determined on tomato, tobacco and cucumber and glasshouse whitefly as prey. Potential daily prey-consumption served as parameter for evaluating the predation capacity of *D. hyalinipennis*. Nymphs and adults of the predator were offered different developmental stages of *T. vaporariorum*, *Bemisia tabaci* and *Tetranychus urticae* on tomato, and *Aphis gossypii* alatae and apterous forms on cucumber, in no-choice tests. The reproduction ability differed in the case of number of eggs laid and number of emerged nymphs: the highest averages were obtained on tobacco, the lowest on cucumber. No significant differences were observed between host plants regarding either the nymphal development period or adult longevity. High average daily prey consumption rates indicate that *Dicyphus hyalinipennis* Burm. may be a promising biological control agent.

Key words: pests, predators, Heteroptera, Miridae, Dicyphus hyalinipennis, biological control, protected cultivation

Introduction

Production of protected crops in Hungary is an intensively developing, profitable branch of horticulture. Demand for healthy, pesticide-free vegetables as well as use of bumble-bees for natural pollination provide motivation for application of biological control of pests. During the last three decades a range of effective biological control agents has been introduced into greenhouse crop protection systems. The most important one, *Encarsia formosa*, however, is not able to keep the whitefly populations under the economic threshold level during high temperature periods in summer in Hungary.

Several mirid bugs indigenous in the Mediterranean basin have been reported as effective polyphagous predators: *Cyrtopeltis tenuis* Reuter (Benuzzi & Mosti, 1994; Hernandez-Garcia et al., 1996), *Dicyphus errans* (Benuzzi & Mosti, 1994; Ronco & Faure, 1993), *Dicyphus tamaninii* Wagner (Albajes et al., 1996; Castane et al., 1997), *Macrolophus caliginosus* Wagner (Benuzzi & Mosti, 1994; Malausa, 1989), *Macrolophus pygmaeus* Rambur (Perdikis & Lykuoressis, 1997) One of them, *Macrolophus caliginosus* has been produced and is widely applied in tomato crops (Malais & Ravensberg, 1992), also in combination with *Encarsia formosa* (Forray et al, 1996). Others, such as *Dicyphus tamaninii*, can also be conserved in open-fields (Alomar et al., 1994).

In Hungary a spontaneous introduction of a predatory bug into greenhouses has been regularly observed every year since 1995. The predator was able to reproduce and reduce the whitefly populations in protected tomatoes without any additional chemical treatments. The bug, unknown until then in Hungary, is now identified as *Dicyphus hyalinipennis* Burm., and occurred and migrated into greenhouses in many places of South Hungary. This beneficial occurs in open fields on areas rich in weed flora. Adults of its first generation enter greenhouses and under favourable conditions they reproduce at a high rate. Nymphs as well as adults were found to prey on greenhouse whiteflies (*Trialeurodes vaporariorum, Bemisia tabaci*) and aphids (*Myzus persicae, Aphis gossypii*), but feeding on spider mites, eggs of *Helicoverpa armigera* and thrips was also reported (Cegiarska et al., 1998).

This paper presents results on study performed on biology and predation capacity of *Dicyphus hyalinipennis*.

Materials and methods

Insect rearing

The predatory bug rearing was maintained under glasshouse conditions (temp. 25 $\pm$2 ^{0}C, 60 % RH, 16/8 D/L regime) on tomato and tobacco plants infested with *Trialeurodes vaporariorum*.

Main biological features

The tests, replicated six times, were conducted under laboratory conditions at temp. 25 ^{0}C, 60 % RH, and 16/8 D/L regime. The daily production of viable eggs was determined on tomato, cucumber and tobacco. Fifteen couples of two week old adults were placed on host plants for 48 hours. After two weeks incubation, the plants were checked for the presence of nymphs. For defining of nymphal development time just hatched individuals were maintained on leaf discs infested with whitefly premature stages, until eclosion. Adult longevity was determined using just hatched individuals, which were kept on tomato and tobacco plants with whitefly as a prey, until death. To avoid the interference of a new born insects, the host plants were changed forthrightly.

Potential daily consumption rates

In order to obtain an uniform level of hunger before the establishment of feeding tests, 4th-5th instar nymphs and adult females of *D. hyalinipennis* were isolated for 24 hours and supplied only with water. The tests were carried out in plastic containers containing fresh host plants leaves, on which the prey was offered for 24 hours (no-choice test): on tomato - various stages of *T. vaporariorum, Bemisia tabaci, Tetranychus urticae,* on cucumber - winged and wingless forms of *Aphis gossypii.*

Evaluation of the data

All the data were statistically evaluated using one-way ANOVA test, with the separation of appropriate means by Tuke's HSD (SPSS 6.1).

Results and discussion

Main biological features

The results are presented in Table 1. The daily production of viable eggs differs significantly between host plants: the highest rates were obtained on tobacco, the lowest ones on cucumber. Concerning nymphal development time, the 1st instar nymphs have a longer development time on tomato, while 2nd and 3rd instar show a longer developmental time on cucumber. The period of 4th instar did not differ significantly between host plants. In the case

of 5th instar nymphs, significant differences were experienced between all three host plants. According to the data, the longevity of adults does not depend on examined plants.

Table 1. Main biological parameters of the predatory bug *Dicyphus hyalinipennis* Burm.

Host plant	Daily production of viable eggs	Nymphal development time, days					Adult longevity days
		1st instar	2nd instar	3rd instar	4th instar	5th instar	
Tomato	1.46 ± 0.36	3.3 ± 0.51	2.7 ± 0.51a	3.3 ± 0.51a	4.7 ± 0.51a	5.5 ± 0.55	60.0 ± 6.7
Cucumber	0.33 ± 0.14	2.8 ± 0.41a	3.3 ± 0.51	4.3 ± 0.51	4.5 ± 0.55a	6.2 ± 0.75	-
Tobacco	4.75 ± 0.47	2.5 ± 0.55a	2.7 ± 0.51a	3.5 ± 0.54a	4.5 ± 0.55a	4.5 ± 0.55	55.3 ± 2.7

Means indicated with the letter 'a' show no significant differences between treatments

Potential daily consumption rates

Table 2 contains the potential daily predation rates of *D. hyalinipennis*. All offered kinds of prey were accepted by the predator, some of them - like spider mites and cotton aphid - were consumed in a high rates. Comparing the data obtained from the related species *D. tamaninii* (Alvarado et al., 1997), *D. h.* nymphs consumed much more wingless aphids on cucumber, while females consumed less. In the case of tobacco whitefly on tomato the predator exhibited higher predation than that of *D. t.* reported by Bamadas et al. (1998). According to the summary of *D. t.* data given by Albajes et al. (1996), feeding on glasshouse whitefly larvae occurs to be similar.

Amounts of consumed prey do not differ significantly between examined stages, except for those of aphids and mites: *D. h.* nymphs ate more than females.

Table 2. Potential daily prey consumption by the predatory bug *Dicyphus hyalinipennis* Burm.

Dicyphus hyalinipennis stage	Number of consumed individuals per 24 hours							
	Trialeurides vaporariorum			*Bemisia tabaci*		*Tetranychus urticae*	*Aphis gossypii*	
	Eggs	Larvae	Adults	Larvae	Adults	Adults	wingless form	winged form
Nymphs	18.7	13.3±1.9	9.5±1.0	9.8±0.6	4.1±0.3	48.7±3.2	41.2±2.9	35.1±1.4
Females	-	12.1±1.4	12.6±0.8	10.3±0.6	3.6±0.4	36.3±1.8	31.8±1.9	28.5±1.4

The results show that the predatory bug *Dicyphus hyalinipennis* Burm possesses features qualifying it as potential biological control agent. Positive Hungarian experiences in tomato crops where the predatory bug 'spontaneoously' invaded the greenhouses, indicate that it is worth to evaluate its biological parameters important for biological control. All the European experience coming from study and practice on predatory mirids, as well as the incerasing complexity of IPM, creates motivation for further work on the whole group of *Dicyphinae* in order to clear their role in control of noxious pests.

Acknowledgements

I thank J. C. van Lenteren for his valuable support, E. Kondorosy, M. Goula and O. Alomar for identification of the predator, Cs. Budai and Z. Ilovai for their kind help.

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Biological pest control in greenhouses in France during 1998

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Abstract

Following the launch of biological pest control in France in 1980, the area using the technique reached 821 ha in 1990 but decreased to 580 ha in 1994. The area increased to 917 ha in 1998. The techmique is used mainly in protected tomatoes.

Thie expansion of biological control has been made possible by large commercial scale mass rearing of beneficials and by the development of new strategies of release.

It seems from the data that the new pest *Frankliniella occidentalis* is now well controlled and this now allows development of biological control on new crops.

Key words : biological control, greenhouse, areas, France.

Introduction

Each year, since 1986, the Service Regional de la Protection des Végétaux (S.R.P.V. = Plant Protection Service – Ministry of Agriculture) conducted an investigation of all the areas using biological pest control in greenhouses in France.
Following major effort by French scientists and growers, biological pest control is now an acceptable way to protect crops against pests and pathogens in greenhouses and outside. The survey shown in this paper indicates the results of all that effort. Any expansion in the area employing the technique indicates its stage of development and success.

Methods

A questionnaire was sent to each horticultural organisation, especially to technicians dealing with biological control. Companies marketing insects were also consulted.
The areas given are the actual areas (ha) of greenhouses without taking into account the number of crops grown per season. It is common to grow two tomato crops per season, in that case, the area under biological control is only included once in this survey, although both tomato crops were protected with beneficials.

Results for 1998

Data are shown in Figure 1. Areas under biological control increased to 917 ha in 1998 following a significant decrease to 580 ha in 1995 due to *Frankliniella occidentalis, Liriomyza huidobrensis and Bemisia tabac.*, The sustained increase are probably due to:-

- new strategies of releases allow better control
- improved service from the rearing companies

- biological control training courses for growers and their workers

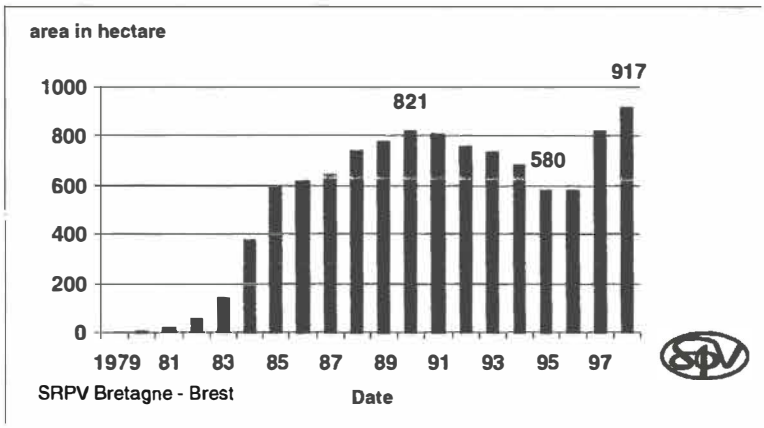

Figure N°1 : Evolution of Biological control in France under greenhouse

The crops

In salad crops, tomato is the major user of biological control at 727 ha (645 ha in 1997). The efficiency of bumblebees and the use of the polyphagous *Macrolophus caliginosus* has helped this development.

The second major crop is Strawberry at 67 ha where *Steinernema feltiae* is used against *Otiorrhynchus sulcatus*.

Subsequently, in order of extent, cucumber at 50 ha, Sweet Pepper with 21 ha and Egg-plant at 16 ha.

Thereafter there are miscellaneaous crops such as melon, beans, and some herb crops.

On ornementals, natural enemies are being used in cut flowers (Rose, Gerbera and Alstroemeria) on 6 ha and pot plants (Cyclamen, Pélargonium, Begonia, etc .) on 12 ha

Beneficials used

Against *Trialeurodes vaporariorum* : *Encarsia formosa* and *Macrolophus caliginosus*
Aphids : *Aphidius colemani, Aphidius ervi, Aphelinus abdominalis* and *Aphidoletes aphidimyza*,
Thrips : *Amblyseius cucumeris, Amblyseius degenerans*, and *Orius* sp.
Leafminers : *Dacnusa sibirica* and *Diglyphus isaea*
Mites : *Phytoseiulus persimilis*, some *Ampblyseius californicus* and *Feltiella acarisuga*
Caterpillars : *Bacillus thuringiensis* and *Trichogramma evanescens*
Leafhoppers (*Hauptidia marocana*) : *Anagrus atomus*

In pollination *Bombus terrestris* is widely used on 1,510 ha, mainly on Tomato (1330 ha). This is really the best marketing agent for biological control. Bees are also used in egg-plant, strawberry, and melon.

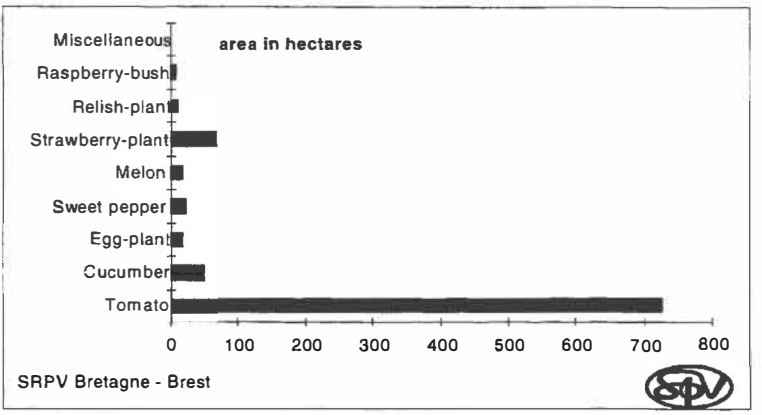

Figure N°2 : Vegetables using Biological control in France 1998

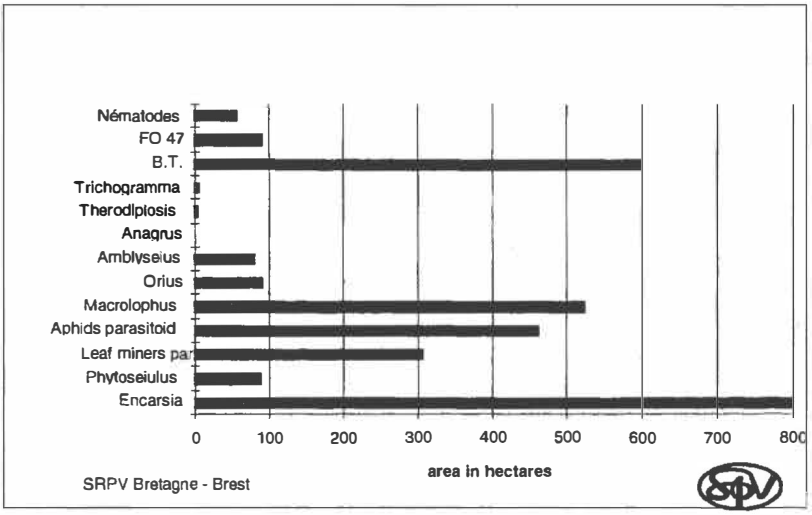

Figure N°3 : Beneficial used in Biological control in France 1998

Future : The use of banker-plants seems to develop actually in different region. Actually, various plant are tested and used to increase the efficiency of introduced beneficial. It is possible to find :

- on different vegetables, barley to control Aphids
- on Alstroemeria : Tobacco and Ricinus to control whitefly and thrips
- on Tomato : Tobacco to rear *Macrolophus*
- on melon : Eleusine to control Aphids

It is probable that the arrival of banker-plants on different crop will help to the development of a real integrated control of some pests, against which, it is difficult to be eficient actualy.

Conclusion

In 1998, the french growers have proved that biological control will be a sustainable system to protect the crops. The important increasing of the areas shows it; and promote the efforts of all the scientists and technicians.

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Parasitoid-induced mortality in the biological control of *Bemisia tabaci* on poinsettia

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Abstract: A comparison of mortality due to parasitism and host feeding on the B-biotype of the whitefly *Bemisia tabaci* was made on five cultivars of poinsettia. Two parasitoids, *Encarsia formosa* and *Eretmocerus eremicus,* were compared over a range of whitefly densities. A binomial analysis of the combined cultivar data for *E. formosa* was found to require a quadratic term (on the logit scale) to best describe the observed whitefly mortality data. Total mortality of 39% at the highest whitefly density (150 scales per plant) was predicted by the fitted response curve for *E. formosa*. Approximately 75% of the observed mortality was due to direct parasitisation. For *E. formosa*, no difference was observed between cultivars. Performance of *E. eremicus* was poor and no parasitism or host feeding was commonly observed. A low natural mortality (2%) of *B. tabaci* was observed in the absence of parasitoids.

Key words: *Bemisia tabaci, Encarsia formosa, Eretmocerus eremicus,* biological control, whitefly

Introduction

The increasing pest status of the whitefly *Bemisia tabaci* in Europe and elsewhere has created considerable research effort in investigating possible control methods for this invasive and adaptable pest. Several biotypes of *B. tabaci* have been distinguished worldwide on biological, behavioural and genomic characters and one, the B-biotype, has been given specific status as *B. argentifolii* Bellows & Perring (Bellows *et al.,* 1994). In addition to causing direct damage, *B. tabaci* is a vector of two distinct tomato yellow leaf curl geminviruses in Europe (Secker *et al.* 1998). There is also widespread pesticide resistance in both the recently introduced B-biotype and the native European Q-biotype (Cahill *et al.,* 1996). Both these considerations have prompted considerable interest in researching biological based control methods for *B. tabaci* (Gerling, 1996).

Amongst the natural enemies of *B. tabaci*, parasitoids of the genera *Encarsia* and *Eretmocerus* have received the most attention. Mass production techniques for *E. formosa* are well established and it has been used worldwide in the control of glasshouse whitefly, *Triaeurodes vaporariorum.* In addition, performance on *B. tabaci* has been shown to differ between populations of *E. formosa* (Henter & van Lenteren, 1996). Another parasitoid species from North America, *Eretmocerus eremicus* Rose & Zolnerowich, has been investigated recently using inundative releases against *B. tabaci* (Hoddle *et al.* 1998). As this species can be reared on *T. vaporariorum* it has been commercialised rapidly using the techniques developed for *E. formosa*. However, there are conflicting reports on the effectiveness of *E. eremicus* against *B. tabaci* (Heinz & Parrella, 1994). Furthermore, a second species, *E. mundus* Mercet, which was collected in Spain, has given superior results in America (Goolsby *et al.,* 1996). However, *E. mundus* does not parasitise *T. vaporariorum* and can only be mass-produced on *B. tabaci.*

The aim of this study was to quantify levels of direct parasitism and host feeding for two parasitoid species at different densities of *B. tabaci* on poinsettia and to determine a response curve to describe the observed data.

Material and methods

Levels of parasitism and host feeding were determined for *E. formosa* and *E. eremicus* on *B. tabaci* using five cultivars of poinsettia. Number of replicates for *E. formosa* were as follows: Sonora Red (n = 48), Sonora Pink (n = 14), Freedom Pink (n = 8), Freedom White (n= 8) and Marble Star (n = 15). Number of replicates for *E. eremicus* were as follows: Sonora Red (n = 48), Sonora Pink (n = 6), Freedom Pink (n = 3), Freedom White (n= 3) and Marble Star (n = 5).

Cultures of *B. tabaci* (B-biotype) were maintained on cotton (*Gossipium hirsutum* var. 'Delta Pine') in controlled environment rooms at 25°C (16:10, L:D) in a quarantine facility. Small poinsettia (*Euphorbia pulcherrima*) plants were infested by clip caging adult female *B. tabaci* on the underside of leaves for 24 hours to produce different whitefly densities on a range of plants. After 24 hours the adult whitefly were removed and the eggs were allowed to hatch into crawlers, which were free to disperse and settle on the leaf. The scales were allowed to develop until they reached the fourth instar. A single 24-hour-old wasp, either *E. formosa* or a mated female *E. eremicus* was enclosed in a cylinder cage with one poinsettia plant for 4 days at 18°C(16:10, L:D). After 4 days the wasp was recovered by introducing CO_2 until it was narcotised and fell to the floor of the cage. This provided confirmation that the parasitoid had survived the duration of the experiment. The plant was removed from the cage and kept in a greenhouse at 18°C until all the developing whiteflies and parasitoids had emerged. The scales were counted and scored for whitefly emergence, parasitoid emergence and dead scales. This experimental design was repeated five times with the plants split evenly between *E. formosa* and *E. eremicus* each time. A separate batch of plants (n = 83) were infested with whiteflies without exposure to parasitoids as controls for natural whitefly mortality.

A binomial analysis of mortality against density provided a test for overdispersion and significant differences between cultivars. A simple binomial generalised linear model was used to account for variation in the data for the control plants.

Results and discussion

Control plants

The mortality for *B. tabaci* on the control plants was 2%. The binomial analysis showed a significant overdispersion that was attributed to 6 plants with higher than average mortality (up to 15%). This mortality was small compared to that of the parasitoids and it was concluded that the base level of mortality could be ignored in the analysis of parasitoid-induced mortality.

Test plants

Figure 1 shows plots of the level of parasitism (a) and host feeding mortality (b) by *E. formosa* at different densities of *B. tabaci* (range = 3-147 scales/plant) and (c) level of parasitism by *E. eremicus* at different densities of *B. tabaci* (range = 5-157 scales/plant for all five cultivars. Observed levels of parasitism by *E. eremicus* were low and no whitefly mortality was common (Figure 1(c)). One likely explanation for this result (other than handling problems) has recently been indicated by Gerling & Fried (1997) who recorded high levels of female sterility associated with crowding of parental females in the related species, *E. mundus*. Further research into the impact of crowding on sterility for this species is needed.

The binomial analysis of the *E. formosa* data was found to require a quadratic term (on the logit scale) to describe the data best. The residual variability is greater than would normally be

described by the binomial distribution and even with the quadratic term there is still a large overdispersion. An informal check of the adequacy of the underlying model was made by sub-dividing the density range and testing to see if the heterogeneity varied according to density. As this was found not to be the case it is suggested that an over-dispersed binomial model is reasonable. The fitted equation is given by the following logit equation with a quadratic response (where n = whitefly density and r = number killed):

$$\log_e (r/n\text{-}r) = 2.108 - 0.0393.n + 0.0001477.n^2$$

The shape of the fitted response for parasitisation and host feeding by *E. formosa* on *B. tabaci* are given in Figures 1(a) and (b) respectively. Examination of the different forms of mortality shows that approximately 75% of the total mortality is through direct parasitisation and approximately 25% through host feeding.

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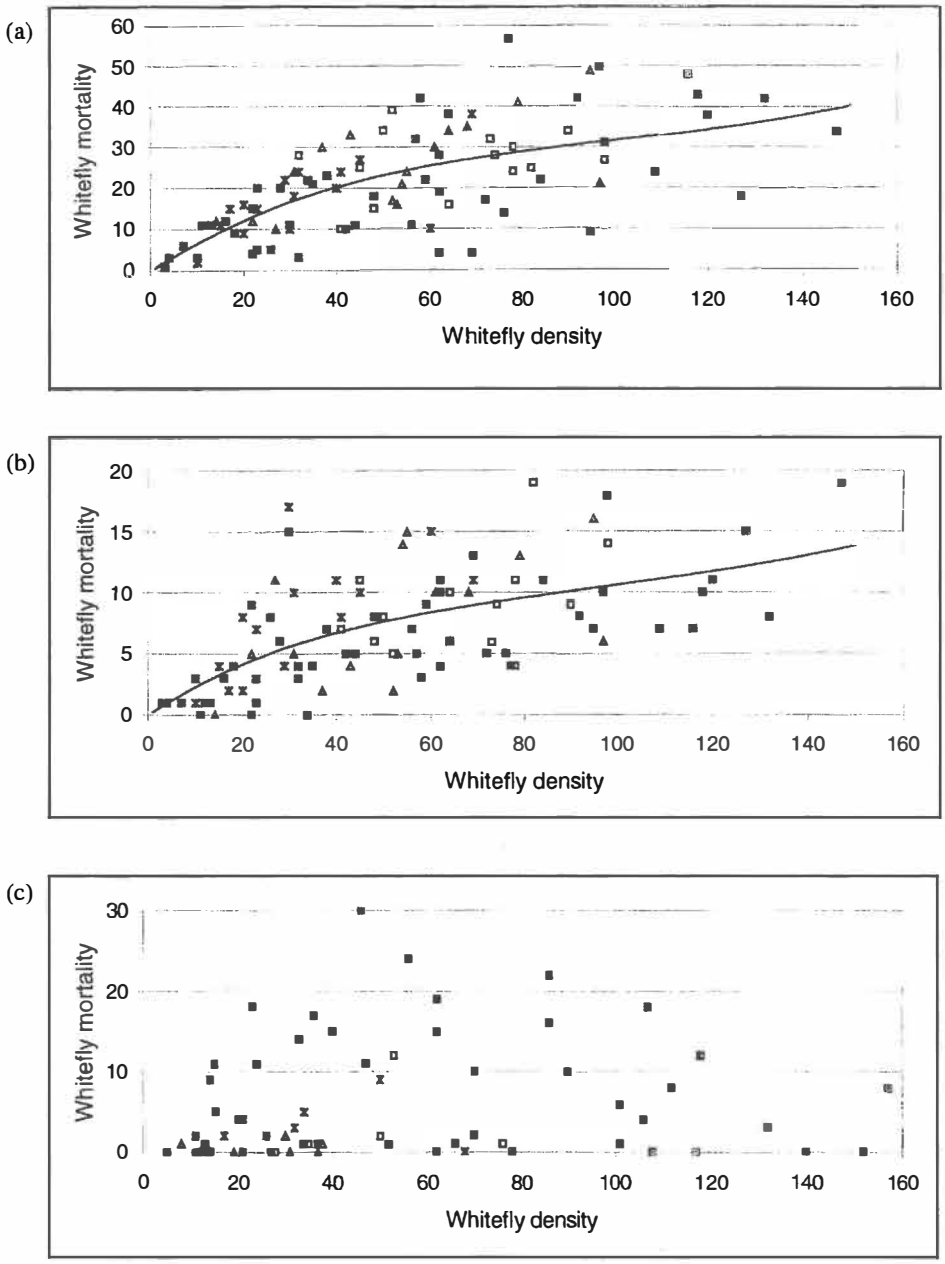

Figure 1. Responses to *B. tabaci* density of: (a) parasitism by *E. formosa* (with fitted response curves, see text); (b) host feeding by *E. formosa* (with fitted response curves) and (c) parasitism by *E. eremicus*. Whitefly mortality is the number of scales parasitised (a & c) or host fed (b) and whitefly densities are the number of scales per plant on five cultivars of poinsettia. Sonora Red (■); Sonora Pink (□); Freedom Pink (▲); Freedom White (△) and Marble Star (*).

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Effect of tomato conditioning on *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) population growth

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Abstract: Control of *Tetranychus urticae* on tomato with *Phytoseiulus persimilis* can be improved by conditioning the predators to tomato plants. Over several generations standard bean-reared (non-conditioned) predators produced fewer eggs per day and had reduced offspring survival than predators that had been conditioned on tomato. Calculations showed that after three generations, the population of conditioned predators could be 2.6 times that of non-conditioned predators. There is evidence to suggest that adaptation to tomato is a result of selection of a genotype within the original population.

Key words: *Phytoseiulus persimilis*, *Tetranychus urticae*, tomato, host plant conditioning

Introduction

Control of *Tetranychus urticae* Koch (Acari: Tetranychidae) (two-spotted spider mites) on UK protected tomatoes is based on the combined use of the predatory mite, *Phytoseiulus persimilis*, and the acaricide, fenbutatin oxide. The strategy is difficult to manage due to the slow establishment of bean-reared *P. persimilis* on tomato plants and this has resulted in too much dependence on fenbutatin oxide. There are indications that some *T. urticae* populations are becoming resistant to the acaricide (Jacobson & Croft, 1999) and methods of improving the performance of *P. persimilis* on tomatoes are required.

The tomato plant (*Lycopersicon esculentum* Mill) provides a hostile environment for invertebrate herbivores through a combination of glandular trichomes on leaves and stems (Luckwill, 1943) and toxins within leaves (Farrar & Kennedy, 1991). Some of these features also affect dispersal and searching efficiency of natural enemies (Van Haren *et al.* 1987). It has been shown, however, that the performance of some biocontrol agents can be improved by priming or conditioning them to the crop or pest upon which they will be released. *Phytoseiulus persimilis* are normally mass-reared on bean plants and conditioning to tomato plants prior to release can improve its establishment within this crop (Drukker *et al.* 1997). The present studies examined the process of tomato conditioning in *P. persimilis* and consider the effect on the population growth of the predator over three generations.

Materials and Method

Conditioned and non-conditioned *P. persimilis* were reared on tomato (cv Spectra) and French bean plants (*Phaseolus vulgaris nanus*) respectively in glasshouses (22 ± 4°C) for at least 20 generations. Synchronised populations of adult females were produced from each culture for use in these experiments. Individual 4-5 day old females were placed in large Petri dishes (14 x 2.5cm) containing either bean or tomato leaves on damp filter paper, and left for five days at 21 ± 2 °C, 16L:8D. Large numbers of *T. urticae* were maintained on the leaves throughout the experimental period. Adult survival, number of offspring produced and offspring survival were recorded. The procedure was repeated twice, using first and second

generation adult females. Figure 1 shows how adult predators from each generation were variously transferred to bean or tomato giving 16 treatments in all. Twenty individuals were recorded for each treatment.

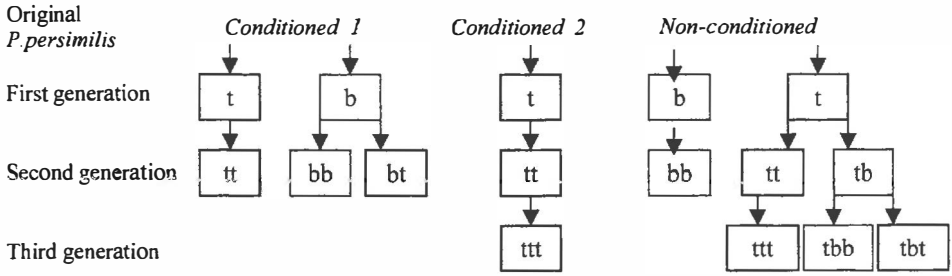

Figure 1. Transfer of conditioned and non-conditioned *P. persimilis* between tomato (t) and bean (b) leaves for three generations.

Statistical methods

Analysis of variance was performed on the all variates after suitable transformation. The results from the analysis were used to construct a simple model of generation size.

Results and Discussion

The survival of adult female conditioned and non-conditioned *P. persimilis* on tomato and bean leaves, are summarised in Table 1. The mean numbers of eggs per day and offspring survival from these individuals are shown in Table 2. When non-conditioned adult *P. persimilis* were transferred to tomato there was no significant effect on adult survival but there were significant declines ($P<0.05$) in the mean numbers of eggs produced per day and offspring survival compared with the other treatments. Conditioned predators transferred to tomato performed similarly to predators (conditioned or non-conditioned) transferred to bean.

It is not immediately apparent how the reduced fecundity and offspring survival of non-conditioned predators transferred to tomato would affect their establishment in the crop. Using the data presented here a model has been produced to predict the size of each of the populations over three generations. Starting with 100 adult females the model assumes that a proportion p_1 survive over five days. If each of the survivors lays an average of n_1 eggs per day, then a total of $5n_1$ eggs are laid in five days and a proportion p_2 are assumed to survive to maturity. From these inputs the mean number of adults emerging in the second generation is estimated as $500n_1p_1p_2$. The standard deviation is estimated using the approximate formula:

$$var(500n_1p_1p_2) = (500n_1p_1)^2var(p_2) + (500n_1p_2)^2var(p_1) + (500p_1p_2)^2var(n_1)$$

where var (short for variance) is the square of the standard error or se. In fact, only the third term of the above makes a substantial contribution to the variance, the variances of p_1 and p_2 being very small. Approximate 95% confidence limits are then obtained as:

$$se(500n_1p_1p_2) \approx 500p_1p_2se(n_1)$$

Calculations show that the population growth for conditioned *P. persimilis* transferred to tomato plants may be 2.6 times that of non-conditioned predators for up to three generations (Table 3). At more extreme differences, numbers of conditioned *P. persimilis* are 7.7 times greater.

There is now evidence to suggest that the adaptation to tomatoes is through genetic selection. The variability for non-conditioned *P. persimilis* transferred to tomato is

considerably greater than that observed for other treatments (Table 2), primarily because some females produced eggs at a much lower rate than others. This suggests that within a population of *P. persimilis*, a proportion have the genetic ability to adapt to tomato more readily than others, so that over time there will be selection for these more fecund individuals. Drukker *et al.* (1997) also found that a proportion of the population was not affected by tomato. Furthermore, it was demonstrated in the present experiment that adaptation to tomato is not readily lost; there were no reductions in fecundity or survival when conditioned *P. persimilis* were put on bean for one generation and their offspring transferred back to tomato.

It is hypothesised that three aspects of the plant and pest can affect the performance of *P. persimilis* on tomatoes; a direct plant effect via plant trichomes (Van Haren *et al.* 1987), a direct prey effect (Drukker *et al.* 1997) and an indirect plant effect through an accumulation of plant toxins in the prey (Gillespie & Quiring, 1994). Additional experiments, using the same method as above failed to show a relationship between trichome density on leaves and predator fecundity (unpublished). This suggests that at the leaf level the reduced performance of non-conditioned predators was probably either an indirect effect of the plant or a direct effect of the prey. From the present results this would seem to require genetic selection.

There is perhaps also a learned component to tomato adaptation as a response to a direct effect of the plant trichomes on the stem. Drukker (pers. comm.) reported that the predators learn to walk over the trichomes on stems.

Adaptation may possibly require both genetic selection and learned behaviour as a response to indirect plant/direct prey and direct plant effects respectively. These relationships are being investigated further. Whatever the mechanism of adaptation, it is clear that *P. persimilis* that are properly conditioned to tomato could provide considerable benefits in the IPM of *T. urticae* on tomato. However, the increased cost of mass producing *P. persimilis* on tomatoes compared to beans is a constraint to the widespread adoption of the technique.

Acknowledgements

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Table 1. Survival of adult conditioned and non-conditioned *P. persimilis* when transferred to bean or tomato plants for five days at 21 ± 2°C, 16L:8D.

P. persimilis	Moved to:	Adult survival	
		Percentage	S.D.
Conditioned	Tomato	98.4	0. 003
	Bean	93.3	0. 021
Non-conditioned	Tomato	94.5	0. 006
	Bean	94.5	0. 006

Table 2. Numbers of eggs and percentage offspring survival of conditioned and non-conditioned *P. persimilis* on bean (b) or tomato (t) plants for five days at 21 ± 2°C, 16L:8D. All S.D.s have approximately 19 df.

P. persimilis	Treatment	Eggs/day		Percentage offspring survival	
		Mean	S.D.	Mean	S.D.
Conditioned 1	t	3.75	0.324	94.7	6.2
	tt	3.78	0.324	91.4	11.7
	b	3.83	0.396	92.6	8.5
	bb	4.02	0.333	91.3	9.5
	bt	3.6	0.287	93.6	7.5
Conditioned 2	t	3.67	0.313	92.8	9.0
	tt	3.62	0.305	94.0	9.6
	ttt	3.93	0.163	92.8	8.7
Non-conditioned	t	2.90	0.971	87.7	12.8
	tt	2.52	0.863	93.0	11.6
	ttt	3.02	0.805	68.3	16.4
	b	3.75	0.345	93.9	6.3
	bb	3.70	0.407	93.3	9.5
	tb	3.60	0.315	92.5	6.8
	tbt	2.88	0.616	80.8	19.3
	tbb	3.63	0.354	93.1	8.3

Table 3. Estimated size of second and third generation populations (in thousands) of conditioned and non-conditioned *P.persimilis*, starting with 100 adult females.

P. persimilis		Treatment	Number of *P.persimilis*		95% limits	
			Mean	S.D.	Lower	Upper
Generation 2	Conditioned	t	1.7	0.14	1.4	2.0
		b	1.6	0.16	1.3	1.9
	Non conditioned	t	1.1	0.32	0.4	1.7
		b	1.6	0.15	1.3	1.9
Generation 3	Conditioned	tt	21.9	2.52	17.0	26.9
		tb	20.7	2.65	15.5	25.9
		bb	19.5	2.72	14.1	24.8
		bt	20.7	2.65	15.5	25.9
	Non conditioned	tt	8.4	3.51	1.6	15.3
		tb	12.5	3.87	4.9	20.1
		bb	18.5	2.53	13.5	23.5
		bt	12.5	3.87	4.9	20.1

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Effects of fungicides on a *Fusarium* sp. biological control agent of *Botrytis cinerea* stem infections in the perspective of an integrated management of fungal diseases in greenhouse tomatoes

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Abstract: Hexaconazole and difenoconazole have a strong effect on radial and germ tube growth of the *Fusarium* sp. biocontrol agent across the all tested concentrations. The active ingredients (vinchlozolin, bupirimate, pyrimethanil) and the mixtures of cymoxanil + mancozeb and diethofencarb + carbendazim have little or moderate effect *in vitro*. *Fusarium* sp. strain was still efficient to protect the pruning wounds against *B. cinerea* and survived well on the plants treated with these active ingredients. This biological *Fusarium* sp. strain could be used in alternation or in combination with these fungicides in an integrated management of gray mold and fungal diseases in tomatoes greenhouse.

Key words: gray mold, biological control, chemical control, integrated control

Introduction

Suppression of stem infections by *B. cinerea* can be achieved by an integrated approach combining chemical treatments with other control measures (Elad *et al.*,1995; Nicot & Baille, 1996). However, disease management by fungicides is complicated by the development of resistant strains. Integration of fungicides with biocontrol agents could provide an opportunity to reduce the pressure of fungicides on the pathogen and so the risk of development of resistant strains. This alternation would also reduce pesticide residues in foods. Moreover, the alternation or combination of fungicides with antagonists may provide better control than the antagonist alone (Elad *et al.*, 1996).

Recently, we isolated a fungal antagonist, provisionally identified as *Fusarium* sp., for its efficiency to protect the pruning wounds on tomato plants (Nicot *et al.*, 1996; Decognet *et al.*, 1997). This paper examines the effects *in vitro* and *in vivo* of fungicides on *Fusarium* sp. in order to combine biological and chemical methods to control *B. cinerea* stem infections in an integrated management of fungal diseases in tomato greenhouses.

Material and methods

The sensitivity and efficiency of *Fusarium* sp. was tested against active ingredients registered in France for use on tomato to control *B. cinerea* (vinchlozolin, pyrimethanil, dichlofluanid, diethofencarb + carbendazim), *Alternaria* spp. (difenoconazole), *Oïdium lycopersicum* (bupirimate, hexaconazole), *Phytophthora infestans* (cymoxanil + mancozeb, dichlofluanid).

Sensitivity of Fusarium sp. to fungicides

The commercial formulations were added to molten Potato Dextrose Agar (PDA), except for pyrimethanil for which the medium described by Leroux & Gredt (1996) was chosen. The tested concentrations of active ingredients are indicated in table 1a and 1b. To assess the effect on the hyphal growth, Petri dishes were inoculated in the centre with a 4,5-mm diameter disk from a 13 days-old colony of *Fusarium* sp. After seven days of incubation at 21°C, the diameter of the colony was measured to express the percent of reduction in growth compared to the untreated control.

The sensitivity of the conidia was tested by plating 0,1 ml of a 5 x 10^3 conidia suspension per ml over the surface of the media. Elongation of the germ tube of 60 spores was determined by microscopic examination after 24 hours of incubation at 21°C and the effect of the fungicide was expressed by the reduction of germ tube growth compared to the untreated control.

Effect of fungicides on the efficiency and survival of Fusarium sp.

Shake cultures of the fungal antagonist were prepared in Yeast-Malt extract broth. Prior to utilisation, the cell suspensions were filtered to remove mycelium fragments, centrifuged and resuspended in water. Spores of *B. cinerea* were obtained after 10 days of incubation on PDA by washing the cultures with sterile water. To obtain development of gray mold on pruning wounds treated with botryticides, resistant strains of *B. cinerea* were chosen. The strain BC1 (isolated from tomato) resistant to vinchlozolin was inoculated on plants treated with this active ingredient or with hexaconazole, difenoconazole, bupirimate or the mixture of cymoxanil + mancozeb. Strains resistant to dichlofluanid (SAR 3182) and to diethofencarb + carbendazim (SAR 11092) were provided by Dr F. Faretra. A strain resistant to pyrimethanil (28xsp, isolated from grapevine) was supplied by Dr P. Leroux.

Leaves on 8-week old tomato plants, cv "*Monalbo,* were removed by cutting the petioles at 5-cm from the stem. The wounds were burned before depositing *B. cinerea* and the antagonist as drops of 10 µl of spore suspension (10^4 spores per wound of each fungus). The commercial formulations of fungicides were sprayed one hour later at the rates used in greenhouse tomatoes (Table 2). Plants were incubated in a growth chamber under a plastic film to promote *Botrytis* infection. The progression of lesions on the petiole stubs was measured daily. The areas under the disease progress curves (AUDPC) were used to calculate the percentage of protection provided by the antagonist (Nicot *et al.*, 1993).

The survival of the *Fusarium* sp. was evaluated after 14 days by grounding petiole stubs in phosphate buffer with an Ultra-Turrax (20500 tours/min). Serial dilutions were plated on peptone-PCNB-agar medium (Nelson *et al.*, 1983). The results were expressed as colony forming units (CFU) per gram of fresh tissue.

Results

Sensitivity in vitro of the Fusarium sp.* strain *to fungicides

Among all tested active ingredients, the *Fusarium* sp. strain was most sensitive to difenoconazole (Table 1). The percentage of inhibition of the radial growth and of the germ tube elongation was superior to 80% at the lowest tested dose, 5 mg/l. Elongation of the germ tube was completely inhibited by concentrations above 25 mg/l. The *Fusarium* strain was also strongly affected by hexaconazole. This active ingredient reduced both radial and germ tube growth at 5 mg/l by 59,7 and 47,2%. Percentages of inhibition above 80% were observed for concentrations at 50 and 100 mg/l. Lower sensitivity to dichlofluanid was observed. Radial growth was slightly affected by concentrations lower than 10 mg/l and reduced by 80% at 50 mg/l. However, elongation of the germ tube was strongly inhibited at 10 mg/l (90,1%) and completely inhibited for the highest tested concentrations. Vinchlozolin inhibited moderately the radial growth (27,2 to 48,2%) and germ tube elongation (65,0 to 73,9%). The *Fusarium* sp. was little sensitive to bupirimate. The highest percentage of inhibition was observed for the radial growth which was reduced by 51,2% for a concentration of bupirimate of 100 mg/l. Pyrimethanil and the mixture of diethofencarb + carbendazim had a slight effect on radial growth (0-16,7% of inhibition) and germ tube elongation (0-37,4% of inhibition) (Table 1a and 1b). Radial growth was little affected by the mixture of cymoxanil + mancozeb for the concentrations of the fungicide (Remiltine pepite) below 100 mg/l (up to 47,1%) and completely inhibited for a concentration at 200 mg/l (Table 1b). An inhibition of the germ tube elongation superior to 80% was measured whatever the tested concentration of Remiltine pepite.

Effect of fungicides on the efficiency and survival of Fusarium sp.

The progression of *B. cinerea* was slowed or completely inhibited by the antagonist applied on petiole stubs of plants not treated with fungicides (Table 2). High rates of protection (67 to 100%) were provided according of the *B. cinerea* strain inoculated on these petiole stubs.

The protection afforded by the botryticides was moderate (diethofencarb + carbendazim), low (vinchlozolin) or null (dichlofluanid) due to the inoculation with strains of *B. cinerea* resistant to these active ingredients (Table 2). At the opposite, no infection of the pruning wounds was recorded on plants treated with pyrimethanil although the strain of *B. cinerea* inoculated was resistant *in vitro*. When the antagonist was applied on pruning wounds of plants sprayed with water or pyrimethanil, 100% protection was observed. The application of *Fusarium* sp. on plants treated with diethofencarb + carbendazim, vinchlozolin, dichlofluanid allowed to obtain high rates of protection (82 to 100%). For vinchlozolin, synergism may have occurred because the protection provided by *Fusarium* sp. on plants treated with vinchlozolin was higher (89%) that on the petiole stubs protected by the antagonist alone (67%) or by vinchlozolin alone (12%).

The four other active ingredients tested are not registered botryticides. However, hexaconazole and difenoconazole provided a moderate protection against infection by *B. cinerea* (about 50%). High rates of efficiency were observed when the antagonist was combined with a spray of these active ingredients (about 90%). Bupirimate and cymoxanil + mancozeb failed to inhibit the development of *B. cinerea*. The level of protection provided by the antagonist was similar on plants treated with bupirimate than on plants not treated. At the opposite, the spray of cymoxanil + mancozeb reduced the efficacy of the antagonist from 67% to 34%.

The population of *Fusarium* sp. was monitored 14 days after its application in petiole stubs. No or limited changes were observed between the population size in petiole stubs on plants treated with fungicides or not (Table 2)

Conclusion

Although effects of fungicides were observed on agar tests, the efficiency of *Fusarium* sp. to protect the pruning wounds on tomato plants was not or only moderately affected by the fungicide treatment. Moreover, the application of the antagonist allowed restoring a high protection when the efficacy of the botryticides was reduced because of the use of resistant strains. These results are very promising because this biological agent could be used in alternation or combination with fungicides in an integrated management of fungal diseases on tomato crops. These results are also interesting in the perspective of a development of this biocontrol agent in viticulture because all tested active ingredients (except bupirimate) are used on this crop.

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Tables 1: Effect of active ingredients (Table 1a) and mixtures of active ingredients (Table 1b) on radial growth and germ tube elongation of conidia of *Fusarium* sp antagonist of *B. cinerea.*

Table 1a

Active ingredient (Fungicide)	% inhibition of the radial growth					% inhibition of the germ tube growth				
	Concentration of active ingredient (mg/l)									
	5	10	25	50	100	5	10	25	50	100
hexaconazole (Anvil)	59,7	67,8	79,5	81,6	80,9	45,2	52,9	73,2	80,3	80,0
difenoconazole (Score)	80,9	83,6	88,1	89,1	90,4	95,5	97,5	100,0	100,0	100,0
dichlofluanid (Euparene)	11,3	17,3	66,1	88,3	91,5	13,2	90,1	100,0	100,0	100,0
vinchlozolin (Ronilan DF)	27,2	48,4	45,9	47,3	39,9	67,7	73,9	71,7	70,9	65,0
bupirimate (Nimrod)	3,9	10,2	20,8	31,1	51,2	0	0	17,8	34,7	44,6
pyrimethanil (Scala)	0	4,2	*	14,6	18,8	2,5	0	*	0	37,4

*: not tested

Table 1b

% inhibition	Concentration of fungicide (mg/l)									
	diethofencarb + carbendazim (Sumico)					cymoxanil + mancozeb (Remiltine pepite)				
	0,08	0,2	0,4	1	2	10	25	50	100	200
radial growth	17,3	12,0	11,3	15,2	14,8	9,6	21,2	31,1	47,1	100,0
germ tube growth	19,2	34,4	26,5	22,5	18,9	80,2	100,0	100,0	100,0	100,0

Table 2: Effect of fungicides on efficiency of *Fusarium* sp. to protect the petiole stubs on tomato plants against infection by *B. cinerea* and on its survival in these petiole stubs.

Active ingredient (Fungicide)	Field rate (mg/l) *	Percentage of protection			Log $_{10}$ (CFU/g tissue)	
		Fusarium alone	Fungicide	Fusarium + Fungicide	Fusarium alone	Fusarium + Fungicide
hexaconazole (Anvil)[a]	30	67	45	98	6,3	6,4
difenoconazole (Score)[a]	125	67	49	92	6,3	6,1
vinchlozolin (Ronilan DF)[a]	750	67	12	89	6,3	6,4
bupirimate (Nimrod)[a]	500	67	- 5	68	6,3	6,2
cymoxanil + mancozeb (Remiltine pepite)[a]	2500	67	- 1	34	6,3	6,2
dichlofluanid (Euparene)[b]	1250	100	-12	82	5,3	5,6
diethofencarb + carbendazim (Sumico L)[c]	2	100	46	100	6,3	6,4
pyrimethanil (Scala)[d]	800	100	100	100	6,1	5,5

*: Field rate concentration of active ingredient or fungicide (for mixture of active ingredients) assuming a spray volume of 1000 l/ha;

a, b, c, d: plants inoculated with BC1 strain of *B. cinerea* resistant to vinchlozolin (a); with SAR 3182 resistant to dichlofluanid (b); with SAR 11092 resistant to diethofencarb + carbendazim (c); with 28xsp resistant to pyrimethanil (d)

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Protection of stem wounds against *Botrytis cinerea* in heated tomato greenhouses with a strain of *Fusarium* sp.

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Abstract: An antagonistic strain of *Fusarium* sp. significantly reduced the incidence of stem lesions on tomatoes in 16 greenhouse trials conducted between 1996 and 1998 in southern France. Its efficiency in protecting pruning wounds was established for two tomato cultivars, and over a variety of fluctuating environmental conditions and levels of disease pressure. Delivery of this biological agent with a sprayers or with pruning shears bearing a small spray device was evaluated. Delivery of the antagonist via pruning shears did not seem to reduce the efficacy of wound protection. This result offers an interesting potential as the pruning-spraying shears allow simultaneous leaf removal and preventive wound protection. They present the additional advantage of delivering minute amounts of active ingredient in an optimally targeted fashion, reducing potential impact on the environment, including pollinators and other beneficial organisms.

Key words: grey mould, pruning shear with spray device, integrated and chemical controls

Introduction

Stem lesions, resulting mainly from infection of pruning wounds by *B. cinerea*, are one of the most devastating symptoms in heated tomato greenhouses. The incidence of the disease can be reduced by cultural practices, by physiological and climate controls and by the application of fungicides (Elad & Shtienberg, 1995; Nicot & Baille, 1996). Biological control could provide an alternative control measure. Recently, we selected from the epiphytic microflora of greenhouse tomatoes a fungal antagonist, provisionally identified as *Fusarium* sp., for its efficiency to protect pruning wounds on tomato plants (Nicot *et al.*, 1996; Decognet *et al.*, 1997).

This paper presents the results of greenhouse trials conducted in conditions close to a commercial situation to evaluate the efficiency of the antagonist to protect pruning wounds against *Botrytis*. Three means of delivering the antagonist were tested.

Material and methods

Inoculum production

Shake cultures of the fungal antagonist were prepared in yeast-malt extract broth. Prior to utilization, the cell suspensions were filtered to remove mycelium fragments, centrifuged and resuspended in water. *B. cinerea* (BC1 strain) was grown on Potato Dextrose Agar. After 10 days of incubation, spores were obtained by washing the cultures with sterile water. Spore concentrations were determined with a haemocytometer and adjusted as desired.

Experimental design

Three tests were conducted between 1994 and 1996 on plants (cv. "*Rondello* ") approximately 5 months old in a glasshouse at INRA. Treatments were arranged in a block design with four replicates (10 plants per replicate, 5 leaves per plant). Similar tests were then conducted (1996 to 1998) in a glasshouse at CTIFL on 3-month old plants (cv "*Trust*"). The treatments were distributed randomly in a block design. The cultural practices in all trials were kept as close as possible to a commercial situation.

Inoculations and disease rating

Five leaves were removed from each plant and spores of *B. cinerea* were applied on pruning wounds as a suspension in water (most experiments- see Table 1) or as dry airborne inoculum. Different inoculum levels were used to obtain different conditions of disease severity (Table 1). Spores of *Fusarium* sp. were applied on pruning wounds as a suspension in water ($5x10^5$-$5x10^6$ CFU/ml). The efficiency of the strain of *Fusarium* sp. was compared to that of Sumico L and water was applied on the untreated control plants.

Stem infection was monitored twice weekly for 10-12 weeks after inoculation. The efficiency of a control method was estimated by the reduction in percentage of pruning wounds developing stem lesions for treated plants relative to the untreated control plants. The statistical analyses were performed on these figures.

Three means of application of the antagonist and the fungicide treatments were tested: (i) by localized spray with a hand-held sprayer to ensure that the treatment was evenly distributed on all wound surfaces, (ii) application on the whole deleafed area of the plants with a back-pack sprayer and (iii) with pruning shears equipped with a small spray device.

Results and discussion

In all the trials, substantial disease development was observed on the plants inoculated with *B. cinerea* alone, and varied according to the level of inoculum applied to the pruning wounds (Table 1). Symptoms were first detected within 1-2 weeks after inoculation and the incidence of stem lesions progressed over the growing season (Fig. 1-A).

Efficacy of the antagonist delivered as a spray treatment

On wounds sprayed with the fungicide or with *Fusarium* sp., the incidence of stem lesions increased slowly until 40-50 days after inoculation and remained very low throughout the trials (Fig. 1-A). The antagonist provided significant, long lasting protection of the pruning wounds, despite wide differences in environmental conditions and severity of inoculum pressure among the different trials. The protection level, often close to that afforded by the fungicide, did not appear to be affected by the tomato variety (Table 1-A-B).

Efficacy of the antagonist applied with pruning shears bearing a small spray device

On wounds inoculated with *B. cinerea* alone, disease development was similar to the previous trials (Fig. 1-B), although maximum disease incidence appeared to be overall lower on trials where the pruning shears were used (Table 1-C). In all trials, the antagonist delivered with the pruning shears provided highly significant, long lasting protection of the pruning wounds.

The antagonistic strain of *Fusarium* sp. isolated from the epiphytic microflora of greenhouse tomatoes significantly reduced the incidence of stem lesions on tomatoes in 16 greenhouse trials conducted between 1996 and 1998 in southern France. Its efficiency in protecting pruning wounds was established for two tomato cultivars, and over a variety of fluctuating environmental conditions and levels of disease pressure. It appeared to retain

efficiency over several months even in the most severe of the tests, where disease incidence reached nearly 80% on the control plants.

Delivery of the antagonist via pruning shears bearing a small spray device did not seem to reduce the efficacy of wound protection. This offers an interesting potential for tomato growers. Such pruning tools are generating increasing interest among greenhouse tomato growers in southern France as they allow simultaneous leaf removal and preventive wound protection. They present the additional advantage of delivering minute amounts of active ingredient in an optimally targeted fashion, reducing potential impact on the environment, including pollinators and other beneficial organisms.

Work is in progress to further evaluate the potential of this micro-organism as a biocontrol agent on other host-parasite systems.

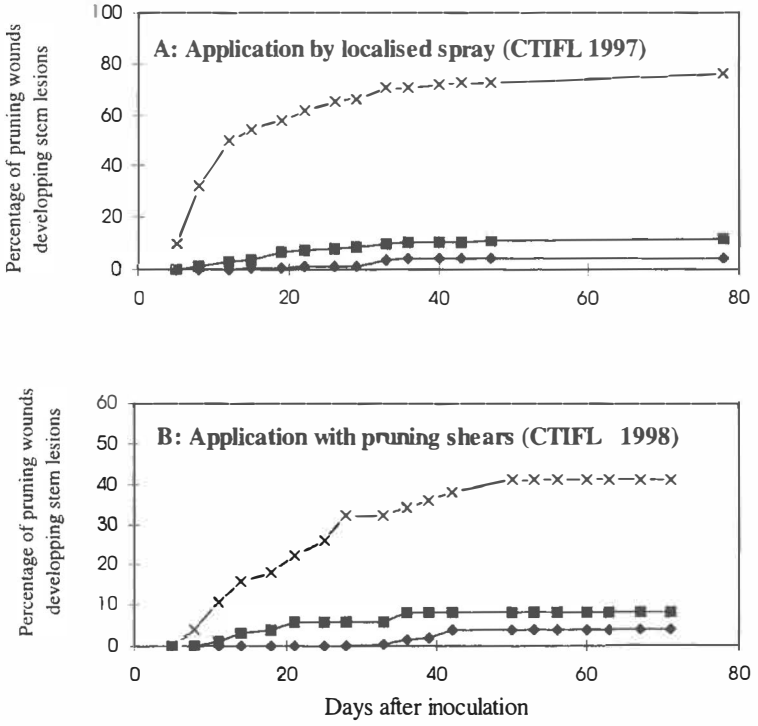

Figure 1: Protection of pruning wounds on tomato plants against *B. cinerea* in greenhouse trials in 1997 and in 1998. The treatments, water on non protected plants (×), the antagonist (■) and the fungicide (◆) were applied by localised spray (A) or with pruning shears with spray device (B).

Table 1: Efficiency of *Fusarium* sp. to protect the pruning wounds in heated tomato greenhouses. The experiments were conducted in experimental greenhouses with cultural practices close to a commercial situation.

Year	*B. cinerea* inoculum[1]	% pruning wounds developing stem lesions on control plants	% protection[2]	
			Fusarium sp.[3]	Fungicide [4]
A: Application of the antagonist and fungicide by localized spray at INRA (i) and CTIFL(c)				
1994-1995 (i)	500	78,5	48*[2]	70*
1994-1995 (i)	airborne	26,5	30	77*
1995-1996 (i)	50	56,0	47*	87*
1996 (i)	airborne	43,3	62*	82*
1996 (c)	50	42,3	79*	100*
1997 (c)	5000	50,6	95*	100*
1997 (c)	5000	73,4	82*	96*
1997 (c)	5000	76,1	85*	94*
B: Application of the antagonist and fungicide with a back-pack sprayer at CTIFL				
1998	5000	15.0	80	93
1998	5000	71.0	82*	97*
1998	5000	78.0	78*	99*
1998	aerial	65.7	83*	100*
C: Application of the antagonist and fungicide with pruning-spraying shears at CTIFL				
1997	5000	58,0	62*	99*
1998	5000	37,2	97*	100*
1998	5000	45,5	74*	100*
1998	5000	41,0	81*	90*
1998	aerial	60,6	82*	99*

[1]: number of spores of *B. cinerea* applied on the pruning wounds; airborne: no spray inoculation of *B. cinerea;* aerial: spores of *B. cinerea* were blown on pruning wounds

[2]: % reduction in the number of stem lesions at the end of the growing season on treated plants compared with the untreated control plants; An asterisk (*) indicates that the treatment was significantly different at the 5% level from the control untreated plants

[3]: $5\ 10^5$ to $5\ 10^6$ spores of *Fusarium* sp. applied per pruning wound

[4]: Sumico L applied at 2 l/hl (tests A and C) or 0.2 l/hl (test B)

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Factors influencing the adoption of biological control technologies in floriculture under glass

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Abstract: The factors that affect the adoption of biological pesticides in floriculture were investigated in Germany.

Key words: Factor Markets, Biopesticides, Biologicals, Technical Change, Adoption, Diffusion, Ornamental Plants

Introduction

Since World War II, agriculture has evolved into a highly efficient sector: Mechanization, the spread of the use of chemical pesticides, together with synthetic fertilizers and improvements in genetic material are technical changes since then that have lead to increased efficiency.

Chemical pesticides are rapid, reliable and provide a high rate of control. Undesirable secondary effects, however, such as pollution of air and water, as well as development of resistance of numerous diseases/pests against chemical pesticides are increasingly discussed world-wide. These harmful effects of pesticides in the environment are attracting the attention of politicians, farmers and the general public and have led slowly to a change in pesticide application.

The major assumption is that there will be an increase in overall environmental concerns, including chemical pesticides. These concerns will very likely continue to result in legislation which will directly impact on research and development and on use of pesticides. Agricultural production with less impact on the environment is a concern requiring technical changes. To achieve environmental and sustainable production biological control technologies are considered one of the most promising and suitable methods for pest control. Yet, the implementation of biological controls in Germany has been slow.

Little research has been published on possibilities and limits of production and application of biological control technologies (Albert, R. 1989-1991; Erfurth, P. & Hofmann, G. 1989; Grunewaldt-Stöcker, G. 1990; Lenteren, J. C. van 1992; Rodgers, P. B. 1993). However, a systematic analysis is missing to this day.

A research project was initiated to investigate biological control technologies, supported by the Deutsche Forschungsgemeinschaft (DFG). A description and judgement on structures and behaviours of the suppliers will be analysed. Analysis of the market for biological control technologies will done with diffusion research, to investigate the dynamic adoption process and its influence on hindering and supporting factors. The research deals with floriculture production under glass in Germany.

Method

Based on a review of the literature and specialist interviews, a set of hypotheses about the determinants of the demand of biological pesticides has been compiled. Based on this set of hypotheses, a list of statements and questions was compiled and tested in a pre-survey.

The mail survey consisted of 520 German horticulturists engaged in floriculture. Forty percent returned a complete questionnaire. An additional 37 were given the same questionnaire *(with supplementary questions)* in face-to-face interviews. The data was analysed with the computer programmes SPSS/SAS. This paper gives preliminary results of the survey.

Preliminary Results

First, information about the use and judgement on biological control technologies were ascertained on my initiative by the survey by Grundstedt (economic survey of the horticultural situation in Germany) in the Spring of 1995 (table 1).

Table 1. The use of biological control technologies in Germany.

	Old States			New States		
	Implemented biological methods	Change planned	Too many problems	Implemented biological methods	Change planned	Too many problems
	% of enterprises			% of enterprises		
All Enterprises	27.5	16.9	5.8	16.1	19.4	3.2
Nurseries	48.3	13.8	3.5	-	-	-
Enterprises with						
direct sales	33.5	19.4	4.8	12.7	14.9	3.3
indirect sales	21.1	14.7	7.2	19.7	32.8	1.6
. cut flowers	9.9	14.9	3.0	-	-	-
. pot plants	23.3	11.6	11.1	-	-	-
Glasshouse area:						
<2,000 qm	28.8	19.0	4.4	12.1	17.9	2.4
2-5,000 qm	29.4	17.3	5.2	26.2	11.9	9.5
5-10,000 qm	23.1	15.1	7.5	16.7	33.3	-
>10,000 qm	27.9	15.6	6.6	38.9	44.4	-

Source: Grundstedt 1995, p. 9.

The results show that 27.5 % of the respondents in the former Federal Republic of Germany "old states" and 16.1 % in the former German Democratic Republic "new states" use biologic control technologies. Nurseries for young plants had the greatest interest with 48.3 %. Pot plant enterprises (23.3 %) use biological control methods more than cut flower enterprises (9.9 %). Five and eight tenth percent of the enterprises in the west and 3.2 % in the east changed to "traditional" control methods because of implementation problems. The assessment of biological control technologies by pot plant enterprises is better than the one for cut flower enterprises. Thus, cut flower enterprises are more cautious in adopting these innovations.

The second step was a mail survey of 520 enterprises in floriculture. This survey gathered information on the present willingness to adopt biological control technologies and the incentives and hindrances influencing the process.

The diffusion theory (cf. e.g. Rogers, 1983) was used to formulate several demographic, cultural, social, psychological and economic grounds influencing the speed of the adoption process.

The survey addressed 4 different groups and was therefore divided into 4 main parts:

1. the non-adopters
2. the adopters
3. the temporary adopters having abandoned the innovation
4. the adopters having partly abandoned biological control technologies

Demographic and socio-cultural factors examined were the size, the number of employees and the turnover of the enterprise, as well as age and education of the grower. Psychological characteristics were the attitude to zero-tolerance for pest organisms in ornamental crops, the assessment of biologicals and the grower's behaviour to information. The knowledge of biological control was also an important factor with regard to the adoption of biologicals. Further examples of investigated factors which influence their adoption are listed in table 2.

Table 2. Examples of factors influencing the adoption

Negative	Positive
• the service that growers obtain from the producers of biologicals and/or from advisory service is insufficient	• the advisory service during and/or after the introduction of biologicals
• the grower experiences difficulties in the total system of biological/integrated control	• the grower's knowledge of biologicals
• lack of information	• consumer demand for environmental produced products
• the competition of biologicals with customary methods of cultivation and chemical pesticides	• the aroused interest as a result of the growing ecological awareness in society
• the zero-tolerance for pest organisms in ornamental crops	• resistance problems against chemicals
• too much different cultures in one greenhouse	• pests where no chemical pesticides are available
• lack of specialisation in production	• no toxic, growth-inhibiting effects of biologicals on plants
• the belief that there are insufficient evolved methods for the implementation of biologicals	• the assessment of switch-over difficulties in the enterprise

The results show clearly that non-adopters consider biological control strategies more negative than adopters. The temporary adopters having abandoned the innovation and adopters having partly abandoned biological control strategies are in-between. The major problem seen by non-adopters is the zero-tolerance of ornamental plants. In contrast, adopters reported no big problems. Nevertheless, they stated some problems of importance: 1. complicated total system of plant protection, 2. little choice of biologicals, 3. high prices of biologicals, 4. too many different cultures in one greenhouse, 5. time-lag between application and effect. The biggest problem of adopters having partly abandoned the innovation were 1. lack of effectiveness of biologicals, 2. little choice of innovation, 3. too many different cultures in one greenhouse, 4. and 5. complicated total system and time-lag. That means that the reason to abandon some of the biologicals was mainly due to lack of effectiveness. The temporary adopters having abandoned all biologicals stated firstly the problem of time-lag

between application and effect, secondly too many different cultures in one greenhouse and thirdly the low effectiveness of biologicals. As fourth, the total system of plant protection was too complicated and fifth, the service that growers obtain from advisory service was insufficient as well as little choice of biologicals were mentioned. The results seem to confirm the hypothesis that risks of biologicals are overestimated by non-adopters. This might be due to lack of information and/or risk aversion.

Further results indicating significance and importance of the different factors are provided in the oral presentation.

Discussion

After establishing the factors affecting demand as well as (in a further step) supply, the characteristics of the diffusion process of biologicals are determined. Finally, using the first two steps, a concept for market participants is developed, which enables a more rapid and more extensive implementation of biological control technologies and contributes to the protection of the environment.

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Use of insect growth regulators to reduce rates of *Eretmocerus eremicus* needed for biological control of whiteflies on poinsettia

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Abstract: To reduce the parasitoid release rate of *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) required to control whiteflies (Homoptera: Aleyrodidae) on poinsettia, we evaluated the integration of this natural enemy with two mid-crop applications of either buprofezin or fenoxycarb, both insect growth regulators. In small greenhouses (holding 90 poinsettias), we found that a low parasitoid release rate (one female per plant per week), if combined with applications of either of these materials, provided the same level of whitefly suppression as a parasitoid release rate of 3 females per plant per week. In a separate trial in 7 commercial greenhouse ranges holding 800-2340 poinsettias, we confirmed that low parasitoid release rates plus buprofezin were equivalent to the high parasitoid release rate, but were significantly cheaper.

Key words: whiteflies, biological control, *Eretmocerus eremicus*, integrated control, buprofezin, fenoxycarb, insect growth regulators

Introduction

In previous work, we have shown that a weekly release of three females of *Eretmocerus eremicus* Rose and Zolnerowich provides effective control of whiteflies on commercial poinsettia (Hoddle *et al.* 1998ab, Hoddle & Van Driesche 1999, Van Driesche et al. in press). However, the cost of use of this parasitoid at this rate is not competitive with chemical control. Here we present results of trials in poinsettia in which we examined the efficacy of lower rates of parasitoid release (compared to the previously assessed rate of three females per plant per week) when combined with limited mid-crop use of insect growth regulators. The intention of the trial was to assess efficacy and cost of this integrated treatment for control *Bemisia argentifolii* Bellows and Perring.

Materials and methods

Two trials were run to compare the efficacy of integrated treatments (parasitoids plus IGRs) to parasitoids alone (or IGRs alone, work not reported here). One trial was at the scale of small research greenhouses at Cornell University and the other in commercial greenhouses under a grower's management. Trials were run in fall of 1997 during the normal period of production of poinsettia for the Christmas market.

Research Greenhouses Test

Trial one was run at Cornell University in greenhouses (5 X 4 X 3.5 m) holding 90 poinsettias in 15cm pots from mid August to early December, using normal commercial poinsettia production practices. There were four treatments for whitefly control: (1) release of 3 female *E. eremicus* per plant per week, (2) release of one female *E. eremicus*, (3) release of one female *E. eremicus* plus two applications (in weeks 6 and 7 of the 14 week crop) of buprofezin (a 70% WP formulation mixed at 0.2 grams active ingredient per liter of water and applied to runoff) and (4) release of one female *E. eremicus* plus two applications (in weeks 6 and 7 of the 14 week crop) of fenoxycarb (a 25% WP formulation mixed at 0.75 grams active ingredient per liter of water and applied to runoff).

Live whitefly nymphs were counted weekly on sets of marked poinsettia leaves that corresponded to canopy strata. Stratum one consisted of the leaves originally present on the newly potted cuttings. For stratum one, one leaf on each of 15 randomly selected plants was tagged in each greenhouse and inspected weekly. After 5-6 weeks of plant growth, one leaf in the top portion of an additional 15 randomly selected plants was tagged in each greenhouse. These leaves were designated stratum two, and whitefly counts were made in both stratum one and two each week thereafter. After an additional 4-5 weeks of growth (around week 10-11 of the trail) another 15 plants in each greenhouse had one leaf tagged at the top of the plant and inspected weekly. This uppermost leaf layer was designated stratum three. At this time, 15 leaves were being examined weekly in each of three strata, for a total of 45 leaves, one leaf on each of 45 plants in each greenhouse.

Parasitoids were purchased from Koppert Biological Systems and allowed to emerged in the laboratory. The desired number of newly emerged adult females were then released weekly.

Commercial Greenhouses Test

Trial two was run at a commercial poinsettia producer's greenhouses in Methuen, Massachusetts, in 7 free standing greenhouses of similar size and construction (each being 50 m long by 5.5 to 7.3 m wide, holding 800 to 2340 plants, of various poinsettia varieties). Treatments were (1) *E. eremicus* used alone at 3 females per plant per week, (2) *E. eremicus* at an intermediate rate of two females per plant per week, combined with mid-season use of buprofezin (two applications, spaced one week apart, applied in weeks 9 and 10), and (3) *E. eremicus* at a low rate of one female per plant per week, combined with mid-season use of buprofezin, applied as in treatment two. Each of these three treatments was replicated twice, each in a separate greenhouse. In addition, whitefly densities were also monitored in a seventh greenhouse at the same site in which the grower controlled whiteflies with applications of conventional pesticides. To estimate numbers of live whitefly nymphs, three leaves (1 from the bottom, 1 middle, and 1 top) on 90 plants in each greenhouse were inspected weekly and numbers recorded.

Parasitoids were purchased from Koppert Biological Systems and released as pupae. Calculations of numbers of pupae needed to achieve the intended number of emerged females were made using assumptions of 50% female and 60% adult emergence (with also recognition that the supplier adjust numbers of pupae sent by 142% to compensation for partial emergence, which they view as 70%).

Results and discussion

In trial one (small research greenhouses) high (3 females per week) and low (1 female per week) parasitoid release treatments performed as seen in past trials. The high release rate provided effective control for the duration of the crop, but the whitefly population in the low parasitoid release rate treatment escaped control at about week 12 of the trial and began exponential growth to unacceptable levels (Fig. 1). Of interest relative to this trial was the fact the this whitefly escape did not occur when this low parasitoid release rate was combined with mid season use of an insect growth regulator. Both materials (buprofezin and fenoxycarb) integrated well with the parasitoid and maintained the whitefly under control. In a succeeding trial (reported elsewhere), neither insect growth regulator used alone provided as good control as when combined with a low release rate of *E. eremicus*, and, of the two materials used alone, only buprofezin provided adequate control (Sanderson et al. unpublished). This finding led to the choice of buprofezin as the material to test in trial two, in commercial greenhouses.

In trial two (commercial greenhouses), all treatments produced poinsettia with fewer than 2.0 live nymphs (all instars) per leaf and plants from all treatments were acceptable in the local commercial markets. The only treatment that differed from the others was the chemical control (df =3, F value = 8.63, $P = 0.0001$, Tukey's Studentized Range Test). The high rate of parasitoid release did not differ from the lower rates supplemented with buprofezin (Table 1).

Table 1. Densities per leaf of live whitefly nymphs in test greenhouses at time of harvest.

Treatment	Ave. nymphs per leaf (SE)
3 parasitoids/ pl / wk	1.49 ± 0.20 [a]
2 parasitoids plus IGR	1.83 ± 0.24 [a]
1 parasitoid plus IGR	1.41 ± 0.23 [a]
chemical control	0.28 ± 0.08 [b]

[a] Values in rows with the same letter were not significantly different in a Tukey's Studentized Range Test at $P = 0.05$

These results indicate that no loss of efficacy occurs to the whitefly biological control program when buprofezin is used to replace two thirds of the parasitoids that would otherwise be required. Cost analyses are pending, as the company planning to market buprofezin in the United States has not yet determined a price at this time. However, considering the parasitoid costs only, cost per plant would be approximately $0.34 (US), given applications in 14 or 16 cropping weeks (no parasitoids being applied in weeks of IGR applications). Whether rates even lower than 1 female wasp per plant per week has not been examined and might be a means of reducing costs even further.

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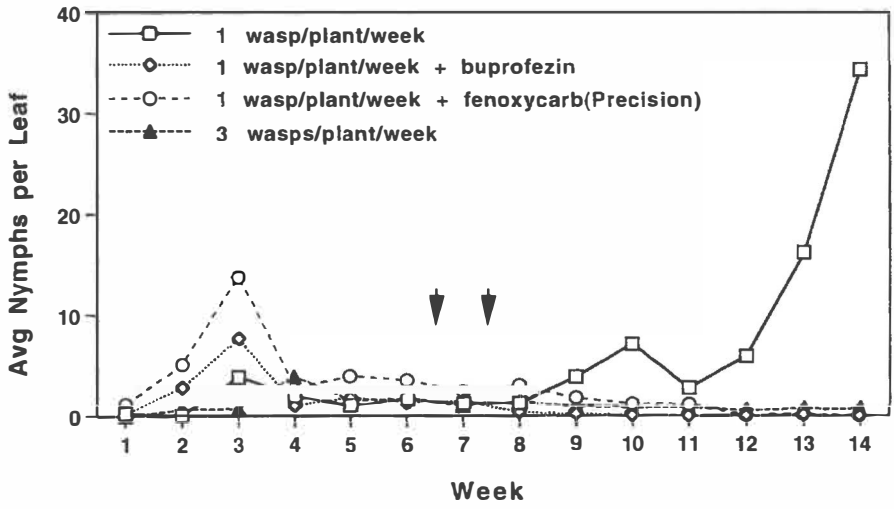

Fig. 1. Population trends of silverleaf whitefly nymphs on 14 week poinsettia crops, where whiteflies were managed with a high or a low release rate of *Eretmocerus eremicus* female wasps, or a low release rate in combination with one of two insect growth regulator (IGR) pesticides. Arrows indicate when IGR sprays were applied.

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Present use and future potential for biological control of pests and diseases in Danish glasshouses

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Abstract. In Denmark biocontrol of insect and mite pests is used routinely in glasshouse production of vegetables whereas it is used on only 30-35% of the area with ornamental production. Successful use of biocontrol of diseases in both vegetable and ornamental production is limited. Consequently there is a large future potential for biocontrol of pests in ornamentals and for biocontrol of diseases in both vegetables and ornamentals. Factors influencing the realisation are discussed.

Key words: beneficials, antagonists, biological control agents, BCAs, status, perspectives

This article is based on a report presented to the Danish Environmental Protection Agency (DEPA) in 1998 by national groups of scientist describing various scenarios for future ecologically sound control of pests and diseases in Denmark.

Present use

Pests

Since the first introduction of biocontrol of pests in Danish glasshouses in the 1970's the number of commercially available 'micro'- and 'macro'-biological agents has steadily increased. Presently Danish growers have access to 50-60 species of beneficials that can be used to give control of most of the important glasshouse pests.

Biocontrol of pests is now used routinely in Danish glasshouse vegetables, i.e. on >98% of the area. Occasionally, chemical control of pests may be needed. However, in ornamentals (mainly pot plants) biological pest control is less used – approx. 30-35% of the area is treated biologically in some way (Steen Borregaard, Borregaard BioPlant, pers. comm.), i.e. one or more pests are controlled with one or more beneficials in one or more cultural phases. Further, not all pests in a crop are necessarily controlled biologically. Pesticides still have to be used against pest invasions from outside and some pests where existing biocontrol methods are inadequate. The least harmful pesticides are chosen whenever possible but biocontrol often has to be discontinued due to the negative side effects of the pesticides. Biological pest control is used in crops such as Poinsettia, Hibiscus, Chrysanthemum, Gerbera, rose, Kalanchöe and Hedera.

Diseases

Biological control of diseases is a relatively recent concept and has only recently been applied in practice. Fungicides remain the major disease control strategy. Nevertheless internationally,

about 30 microbial products are available commercially, of which approximately 20 have been developed for use in glasshouses against various diseases. The products are often based on antagonistic fungi – especially *Trichoderma* and *Gliocladium* species. Fewer are based on bacteria – primarily of the genera *Streptomyces*, *Bacillus* and *Pseudomonas*.

In Denmark, 6 microbial products based on 4 species of micro-organisms (*Trichoderma harzianum, T. polysporum, Streptomyces griseoviridis, Phlebiopsis giganta*) are marketed. In practice the focus is mainly on the *Trichoderma*-products which are used for control of grey mould (*Botrytis cinerea*) and root diseases caused by *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani* and *Thielaviopsis basicola* in glasshouse vegetables and ornamentals. The products are primarily used as 'insurance' treatments to prevent various diseases irrespective of the fact that the antagonists may not necessarily control them fully. It is estimated that biocontrol of diseases is used on 50% of the area with tomato, 30% of the area with cucumber and 30% of the area with ornamentals (Lene Pedersen, Danish Association of Horticultural Producers, pers. comm.). However, results are variable and, often, not reproducible. In addition, the outcome of preventive applications is difficult to interpret – is lack of disease symptoms due to effective biocontrol or simply due to a lack of pathogens?

Future potential

Pests

Use of biocontrol of pests in Danish glasshouse vegetables is almost universal. Pests that can not be controlled efficiently with biological means occur occasionally and development of biocontrol against these is desirable. Implementation of such methods will, however, not increase the use of biocontrol significantly. Likewise, the future potential for increasing the use of biocontrol in vegetables is limited – unless 'new' pests become established and cause crop losses. In that case, biocontrol is likely to be extended to include these pests – depending, however, on the pest species and existing knowledge on useful beneficials. Implementation of efficient biocontrol of diseases will lead to a minor increase in the use of biocontrol of pests as this, then, is no longer limited by the occasional use of fungicides that are harmful to biocontrol agents.

The present use of biological control of pests in ornamentals is less than the actual potential in spite of the fact that beneficials against all major Danish glasshouse pests are available. The major reason for this is that biocontrol in ornamentals is very difficult. Ornamentals encompass several hundred plant species grown under widely different climatic and cultural conditions which, together with the host plant, influence the biology and interactions between pests and beneficials and thus the outcome of biocontrol. Biocontrol methods successfully applied against pests in one crop are therefore not necessarily equally successful against the same pests in other ornamental crops. Crop-specific knowledge on the many pests and beneficials in the diverse ornamental flora is still limited. In addition, it is difficult, sometimes even impossible, and expensive, to keep pest populations below the very low damage threshold of ornamentals. Finally, ornamentals are in general attacked by a large number of pest species – which makes biocontrol complicated, labour intensive and costly.

Other aspects contributing to the present less-than-possible level of biocontrol are:

- Some pests (e.g. aphids and thrips) are very difficult to control biologically in some crops e.g. roses
- Some growers refrain from biocontrol because the price is higher than for pesticides. This holds true especially when controlling difficult pests or many pest species, simultaneously
- Biocontrol is still something new and unfamiliar for many growers who often consider it more difficult to use than pesticides

- Some growers have had poor results with biocontrol which may keep them from trying again
- Growers producing for export must comply with demands for completely clean plants made by some importing countries – something very difficult to achieve when using biocontrol

Finally, a major reason for the present use of biocontrol being less than the potential use is the lack of essential knowledge and practical experience in a number of important areas.

Initiatives to remedy these aspects would immediately increase the use of biological control of pests in ornamentals.

It is evident that there is a large future potential for increasing the use of biocontrol of pest in ornamentals. Within the next 10 years, biocontrol is expected to be used by more growers on a larger area and in more crops than today. In addition, growers that already use biocontrol to some extent will use it against more pests in more cultural phases. Furthermore, biocontrol is expected to increase as a consequence of marketing 'new', especially macro-biological, beneficials.

Diseases

Much is still to be learned about the micro-organisms used for biocontrol of diseases, their mode of action, and the ecology of antagonists and pathogens in the glasshouse environment. In addition, insufficient knowledge and documentation on the practical use of biological control agents (BCAs) might lead to varying, and perhaps negative, experiences among Danish growers. The present successful use of biocontrol of diseases in Danish glasshouses is therefore still limited and below the actual potential that could be attained with proper use of the products.

It is expected that additional microbial products for biocontrol of diseases will be marketed in Denmark within the next few years especially against serious pathogens in glasshouse crops such as *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*, *Thielaviopsis* spp., *Fusarium* spp., *Botrytis cinerea* and mildew pathogens. A more detailed knowledge of the various diseases in different cropping systems will help to optimise biological control. It is expected that improved biocontrol with products based upon *Trichoderma* spp. will be achieved within 1-5 years.

Some existing microbial products may have an effect against other diseases found in few crops (e.g. *Didymella bryoniae* in cucumber). It is, however, not likely that new products will be developed against diseases where only limited sale can be expected. Use of microbial products against diseases or in crops for which they have not been developed will only rarely result in a satisfactory control. Zero-tolerance diseases (e.g. *Puccinia horiana* in Chrysanthemum) still need to be controlled through exclusion and technical/chemical eradication according to legislation. Microbiological control is not likely to be successfully developed against these types of pathogens that also have low priority among scientists and producers.

Factors that may influence realisation of future potential

There are several factors that may influence the degree to which the future potential for biological control of both pests and diseases will be realised:

Price. Reduced price for biocontrol will enhance the likelihood of its use, making it more profitable and more predictable. Cheaper beneficials would allow for preventive/repeated introductions to make up for the low damage thresholds. Competition between producers may lead to price reductions on certain, especially well-known, products. However, newer products that may play an important role against 'difficult' pests or pathogens are often expensive. Thus, if prices are determined exclusively by market mechanisms it is likely that biocontrol, in some cases, will continue to be perceived as unacceptably expensive.

Availability of biocontrol products. New biological beneficials are marketed continuously. However, marketing of new microbial products is very limited as the expenses incurred until registration exceed the profit to be obtained from the relatively small market. Less restrictive procedures for registration or dispensations could lead to an increased use of microbial control. Microbial products are badly needed for biocontrol of diseases. In addition, they are often easy to use and thus more quickly accepted by growers. Consequently, they will pave the way for the use of other BCAs in general.

Flora and fauna in ornamentals. The expansion of biocontrol may be delayed if 'new' pests or diseases, which are difficult to control biologically, become established in Danish glasshouses. Such organisms can in most cases only be controlled with pesticides that may have negative side effects on other BCAs in the crop.

Availability of pesticides. Until all major pests and diseases can be controlled efficiently with biological means there will be a need for pesticides reinforcing biocontrol to expand within an IPM-framework. Political initiatives to ban pesticides will influence this. On the other hand, expansion of biocontrol may be delayed or impeded if new broad-spectrum pesticides are marketed.

'Pesticide-minimisation'. Increased information to consumers and increased possibilities for marketing ornamentals grown with minimum pesticide use may in the future result in higher market prices and thus increase the growers' motivation for expanding the use of biocontrol.

Research, development and advice. The extent of the Danish and international efforts is crucial for the speed with which biocontrol will expand. There is a great need for research, development and advice especially in terms of:

- development of efficient and profitable methods against 'difficult' pests
- development of new methods to replace/supplement existing methods, including non-chemical control measures (forecasting, resistant varieties, mechanical/technical methods, crop rotation, sanitation and use of crop residues, compost and soil suppressiveness)
- increase knowledge about the crop-specific biology of pests, diseases and BCAs, the efficacy of BCAs and interactions between organisms in the cropping system
- development of crop-specific, tailor-made IPM-programs where biocontrol BCAs are combined to be optimal under the given climatic and cultural conditions
- education of growers to ensure the correct use of BCAs against pests and diseases

Political initiatives. Biological control of pests and diseases may be increased via political initiatives influencing the above-mentioned factors, e.g. subsidies, research grants, promote marketing of microbial products, influencing consumer choices etc.

Initiatives that have been taken recently:

In Denmark several initiatives to support the exploitation of biological control in the future have recently been taken. For example a research programme initiated in 1996 on biocontrol includes projects on both pests and disease control. Another research programme is addressing the environmental impact of introducing microbial BCAs in agriculture. The review for DEPA in 1998 (see introduction) is expected to result in further initiatives to support the use of biocontrol.

Theoretical and experimental courses are offered on biological control (MSc and PhD level) and courses training extension officers and growers have been ongoing in recent years. International collaboration between research groups focusing on the use of microbial inoculants including BCAs in agriculture has been strengthened through international programmes such as the EU Cost actions, especially Cost action 830, and the OECD research programme: Biological resource Management for Sustainable Agriculture Systems. These programmes have an important influence on Danish research and development and are important for guiding decision-makers in relation to registration and commercialisation of microbial products.

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Laboratory rearing of the predatory gall midge *Feltiella acarisuga*

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Abstract: A laboratory rearing method was developed for *Feltiella acarisuga* with cucumber as host plant, spider mites as prey and honeydew as food source for adults. The synchronised one-generation rearings were done in sealed plexi glass cages at 22±2°C, 80-90% r.h., L:D 16:8. In optimal rearings, production was about 15 pupae or 18 juveniles per female. The sex ratio of offspring was 0.6.

Key words: biological control, spider mites, honeydew, production, cucumber, glasshouse crops

Introduction

Biological control of spider mites (*Tetranychus* spp., Acari: Tetranychidae) in greenhouses with the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) is well developed and documented. However, biocontrol with *P. persimilis* may fail in certain crops or under certain conditions and there is, consequently, a need for finding other beneficials to supplement or replace *P. persimilis*.

The predatory gall midge *Feltiella acarisuga* Vallot (Diptera: Cecidomyiidae) is such an alternative (Vacante, 1985; Wardlow & Tobin, 1990; Gillespie *et al.*, 1994). A prerequisite for investigations on the biology of *F. acarisuga* – and thus ultimately for an effective commercial use – is an easy, space-efficient and productive rearing method.

This paper presents a method for rearing *F. acarisuga* in the laboratory in small sealed cages with cucumber as host plant, spider mites as prey and honeydew as food source for adult gall midges. Honeydew improves *F. acarisuga*'s oviposition (Brødsgaard *et al.*, 1999).

Materials & Methods

Peach-potato aphids (*Myzus persicae* Sulzer (Homoptera: Aphididae)), used as source of honeydew, were reared on sweet pepper plants (*Capsicum annuum* L., cv. California Wonder) in plexi glass cages (45×45×58 cm) in a climate room at 22±2°C, 80-90% r.h., L:D 16:8.

Two stock cultures of spider mites (*Tetranychus urticae* Koch) were established on kidney beans (*Phaseolus vulgaris* L., cv. Bon-bon) and broad beans (*Vicia faba* L., cv. Cargo), respectively, in net covered cages (85×69×100 cm) in a glasshouse at 18-26°C, 50-80% r.h, L:D 16:8. Spider mites reared on kidney beans were used to inoculate the cucumber plants (*Cucumis sativa* L. cv. Danora F.1) used for rearing *F. acarisuga*. However, replenishment of prey to an already established rearing was from the broad bean culture as kidney bean has an adverse effect on the gall midge larvae: many larvae that crawled from cucumber plants to added kidney bean leaves were found dead (Stig Jacobsen, pers. obs.; Gillespie & Quiring, 1995). A similar phenomenon was not observed with broad beans. Broad beans was, however, not chosen as main host for rearing spider mites as this plant grows more slowly, requires more space and supports smaller populations of mites than kidney beans.

F. acarisuga was reared in plexi glass cages (45x45x58 cm) with forced ventilation in a climate room (conditions as above). The rearings were all of a synchronised one-generation type. In the process of developing a rearing procedure for *F. acarisuga*, the following elements were varied: lower leaves of cucumber plants intact vs. removed; inoculation with spider mites from kidney beans vs. pieces of cucumber leaves; initiating rearing with both adult and pupae of *F. acarisuga* vs. adults only. Production was scored at the end of each rearing as pupae and/or juveniles (pupae and larvae) per introduced female.

Results

Rearing method

The following is a commented description of the optimal rearing procedure for *F. acarisuga*. Diapause was never observed during the more than 2 years continuous rearing.

1. Preparation of host plants. The 2-4 lower leaves of a 6-8 weeks old cucumber plant, 30-35 cm high with a total of 7-12 leaves, were removed and the remaining leaves made into a horizontal 'canopy' by supporting them with thin sticks. The horizontal 'canopy' made it easier to place spider mite-infested kidney bean leaves on the cucumber leaves. The plant was placed in a cage in a watering dish with fertilised water.

The lower cucumber leaves were removed because initial observations showed that these leaves only supported about 20% of the total juvenile population of *F. acarisuga* at the end of one generation (top(t):bottom(b) relationship t/(t+b)=0.82 (*s.e.*: 0.02, n=9)). This presumably reflected an upward movement of spider mites and either a similar movement of gall midge larvae or premature death of larvae on the lower leaves due to starvation.

2. Supply of honeydew. All leaves except the 3-4 top leaves were removed from a 30-40 cm high sweet pepper plant. Leaves were removed in order to reduce humidity in the cage and to provide more space for swarming of the adult gall midges. The plant was inoculated with peach-potato aphids and placed in the cage in a watering dish with tap water.

3. Inoculation with spider mites. Spider mite-infested kidney bean leaves were placed on the cucumber 'canopy' covering the leaves completely. This inoculation period lasted 2-3 days and ensured the presence of a sizeable spider mite population as well as of spider mite eggs at the time of introduction of adult gall midges. Although the knowledge of oviposition dynamics of *F. acarisuga* is still scarce we believe that presence of spider mite eggs positively affects the egg laying behaviour of the gall midge.

Attempts were made to inoculate with spider mites on pieces of cucumber leaves. However, these wilted more slowly (2-3 days) than kidney bean leaves (1 day) resulting in a longer period of reduced photosynthesis in the cucumber 'canopy'. This, combined with the heavy infestation with spider mites, caused some of the cucumber leaves to wilt before the gall midges had completed their development and thus reduced the efficacy of the rearing.

4. Introduction of gall midges. A synchronised one-generation rearing of *F. acarisuga* was initiated by introducing 35-110 adult gall midges (1-2 days old) at a sex ratio of 0.5-0.7 (♀/(♀+♂)) into the cage. Initial attempts to initiate rearings with both adults and pupae gave poor production results (see below) and this method was abandoned.

5. Replenishment of spider mites. If needed, spider mite-infested broad bean leaves wer placed on the cucumber 'canopy' to ensure a surplus of food for the developing larvae.

6. Removal of pupae. The major proportion (>90%) of the gall midge larvae pupated 10-14 days after the introduction of adults. Leaves with pupae were removed in this period and transferred to another cage ('emergence cage') for adult emergence.

7. Adult emergence. The 'emergence cage' (30×30×45 cm) contained a chicken net platform (20×25 cm, 0.8 mm mesh, 20 cm high legs) on which the cucumber leaves containing the pupae were placed directly. The platform arrangement ensured that adults could emerge equally well from pupae on both sides of the leaves.

An aphid-infested sweet pepper leaf, a supply of honeydew for emerging adults, was placed in an 8 cm long water filled test tube mounted on the side of the cage.

Eight layers of felt was placed in a 4 cm high dish (20 cm ∅) and soaked in water. A plaster-of-Paris plate, 2 cm high, mixed with 1:7 active carbon:gypsum (to prevent fungal growth) was soaked in water for 10 min and placed on top of the felt. A hole in the plaster-of-Paris allowed for monitoring and replenishment of water. This watering arrangement gave adult gall midges access to water from a surface where they would neither drown nor get entangled. Furthermore, the arrangement helped maintain an adequate humidity (80-90%) in the emergence cage (Gillespie & Quiring, 1995). The 'emergence cage' was placed in a climate room at the same conditions as above.

The majority of adults emerged over an 8-9 day period. Adults emerging over a 1-2 day period were collected and transferred to new rearing cages. By isolating emerged adults with access to water and honeydew and with time and space to swarm, mating and subsequent oviposition in the new rearing were optimised. In addition, this procedure resulted in synchronised egg laying and larval development.

Production in the rearing

Preliminary rearing trials in which both adults and pupae of *F. acarisuga* were used to initiate the rearing gave a low production, estimated to be about 2-7 juveniles per introduced female.

In rearings where only adults were used as inoculum, production varied between 3-23 pupae or 5-28 juveniles per female. The lower values represent early attempts in rearing where various problems caused a suboptimal production, e.g. early withering of cucumber leaves; inadequate supply of spider mites; problems with watering. The production in the top 40% of the rearings exceeded 11 pupae or 14 juveniles per female, the average (±*s.e.*) being 15.1±0.8 pupae (n=17) or 17.9±1.4 juveniles (n=10). Total numbers produced per rearing was 405±61.9 (n=17) pupae and 400±49.3 (n=10) juveniles. There was no correlation between the number of initiating females (or adults) and pupae (or juveniles) produced per female (P>0.05). The sex ratio (♀/(♀+♂)) (±*s.e.*) in the rearings was 0.64±0.03, n=10.

Cost of rearing

The amount of time involved in establishing, supervising and harvesting one rearing of *F. acarisuga* – apart from actually producing the cucumber, sweet pepper and bean plants – was approx. 2 h. Using Danish wages for a technician this is equivalent to US$41, i.e. approx. US$0.1 per pupa produced. Rational production schemes and simultaneous production in several cages could reduce the cost even further.

Discussion

Initiating rearing with both pupae and adults resulted in a low production. We attribute this to poor synchronisation in juvenile development of *F. acarisuga* due to the continuous emergence of new adults from the introduced pupae. As a consequence, egg laying lasted for

1-1½ weeks resulting in large age differences between the larvae. The older, and more mobile, larvae may have a competitive advantage over younger ones, some of which may be unable to complete their development. Initiating rearings with adults ensured synchronisation in the larval development due to the very short lifespan of adult gall midges (2-4 days at the present rearing conditions (Gillespie *et al.*, 1994; Gillespie & Quiring, 1995; Stig Jacobsen, pers. obs.)) combined with the 1-2 days the adults spent in the 'emergence cage' without egg laying possibilities. In addition to contributing to an increased production, a synchronised development ensures a homogeneous production from which individuals of uniform age can be harvested for experiments or for use in biocontrol.

The optimal production of juveniles obtained with the method described here was about 3 and 9 times higher than with the 2 methods developed by Gillespie and Quiring (1995) ('staged rearing' and '*in situ* rearing', respectively). The '*in situ* rearing' took place on spider mite-infested cucumber plants in a greenhouse. It was not space-efficient, occupying over 20 m^2. In addition, the rearing was vulnerable to invasions of both predatory mites (*P. persimilis*) and gall midge parasitoids (*Aphonogmus* sp.). In our rearing, this was prevented by the use of sealed cages. In the more productive 'staged rearing' spider mite-infested cucumber plants sprayed with a sugar solution were placed in oviposition cages for 3-7 days. Afterwards leaves were removed and transferred to sealed boxes to which cucumber leaves with spider mites were added until pupation was complete. The major problem in this method was that leaves in the boxes became damp and rotted, causing loss of larvae and pupae.

In addition, the two methods of Gillespie and Quiring (1995) differed from the one we have developed by lacking a continuous fresh supply of honeydew for the adults during oviposition. Honeydew has been shown to have a significant effect on the fecundity and innate capacity for increase of *F. acarisuga* (Brødsgaard *et al.*, 1999) and may be more advantageous in this respect than the single application of sugar solution used in the 'staged rearing' of Gillespie and Quiring (1995).

We suggest that biocontrol with *F. acarisuga* may improve if adults have access to honeydew and water and are given space and time to mate before being released in a large culture. This could be achieved by letting adults emerge from the pupae in a cage similar to our 'emergence cage' and only releasing them at certain intervals, e.g. once a day.

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Evaluation of Pheromone Concentrate for Control of Tomato Pinworm in Greenhouse Tomatoes

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Abstract: A micro-encapsulated formulation of the tomato pinworm (TPW), *Keiferia lycopersicella* (Walsingham) (Lepidoptera: Gelechiidae) pheromone was evaluated in two commercial greenhouse tomato crops to determine its effects on tomato pinworm populations. Percentage damaged plants, counts of males in traps, and of larvae on plants were significantly lower in greenhouses treated with the pheromone. No phytotoxic effects, nor any adverse effects on the activity of beneficial insects were observed throughout the duration of the trial.

Introduction

The TPW has become an increasingly serious pest in Ontario, Canada, since its most recent occurrence in greenhouse tomatoes in 1991. This pest causes damage to both foliage and fruits. In 1994, one grower reported estimated fruit loss of 32,000 kg. Because tomato growers largely depend on use of beneficials for arthropod control and pollination, it was necessary to seek an effective, IPM-compatible method for managing TPW populations. Mating disruption using TPW pheromone offered such a method. Several workers (Jenkins et al. 1990; Trumble & Alvarado-Rodriguez 1993) have already shown that the use of sex pheromone for mating disruption of TPW can be effective in field tomatoes. Application of a micro-encapsulated formulation of the TPW pheromone was therefore tested to determine its effect on TPW populations in greenhouse tomatoes in Ontario.

Materials and methods

Two commercial trials using micro-encapsulated tomato pinworm pheromone, ((E)-4-Tridecen-1-yl acetate (19.4%), (Z)-4-Tridecen-1-yl acetate (0.6%)) were carried out in Ontario, from January to June, 1998. On Farm A, there were three treatments, each treatment being allocated to a separate greenhouse area. The three treatments were as follows:

1. **High-volume Applicator (0.4 ha)** - application of the pheromone at the rate of 40 g a.i. per hectare using high volume hydraulic spray equipment. The measured concentrate was diluted in 300-450 litres of water per ha; the volume of spray material varied with the size and maturity of the crop. The pressure of spray delivery was 400 psi.
2. **Low-volume Applicator (1.2 ha)** - application of the pheromone at the rate of 40 g a.i. per hectare using low volume fogging equipment. The measured concentrate was applied in 10 litres of water for the entire 1.2 ha.
3. **Control (0.3 ha)** - not treated for TPW

The three greenhouse areas were separated by physical barriers. The physical barrier between the low-volume and high-volume applicator treatments was a wall made up of a double layer of polyethylene. The treatment closest to the control was the high-volume, and these two were separated by a 0.4 ha section of greenhouse pepper. This latter crop was enclosed in a manner as previously described.

Tomato seedlings (cv. Trust) were transplanted into a hydroponic system from January 5 to January 13. The planting density was approximately 10,000 plants per 0.4 ha or 1.0 ac. Tomato pinworm was present on this farm at the start of the spring crop in January 1998, due to carryover populations from the fall crop. Monitoring for TPW began on January 21 and continued weekly until June 10. Twelve pheromone traps (Pherocon II traps, Trece Inc.) per hectare were used to monitor male TPW. The traps were randomly placed and the minimum distance between two adjacent traps was at least about 30 metres. The rubber lures in the pheromone traps were changed at 4-week intervals. The plants were observed for presence of larvae and larval damage. Weekly observations taken included the following:

a. number of males caught in pheromone traps
b. number of larvae per plant
c. percentage plants with TPW damage

Twenty percent of the total number of plants in each of the treatments were inspected. Plants to be inspected were selected using a V-shaped pattern for each greenhouse section within a treatment. The position of the bottom point of each V-pattern was alternated in the greenhouse sections for each treatment. Some randomness was introduced by selecting plants randomly in the vicinity of the three points of the AV@ shape. Applications of the pheromone in both high- and low-volume applicator treatments began on January 22. A total of five applications were made and carried out at approximately 4-week intervals.

On Farm B, only a high-volume applicator treatment was carried out at this farm because of the absence of physical barriers within the greenhouse range. The hydraulic spray equipment delivered 300-450 litres per ha at 400 psi. Monitoring for TPW began on January 22 and continued weekly until June 11. The protocol for monitoring TPW populations was the same as that for Farm A. The first pheromone application was made on January 23, and four subsequent applications were made at 4-week intervals. A high population of TPW was present on this farm at the start of the trial in January 1998 due to a carryover from populations that infested the fall crop.

Results & Discussion:

Phytotoxic Effects

No phytotoxicity was observed on either the plants or fruits throughout the duration of the trial.

Compatibility with Beneficials

Based on the observation of parasitized whitefly scales throughout the crop=s duration by the growers and researchers involved in this project, it appears that the pheromone did not exert any adverse effects on *Encarsia formosa* released for whitefly control. Markings on the stamen cones of flowers throughout the crop=s duration also indicated that there were no adverse effects of the pheromone on pollination activity by the bumble bees introduced for this purpose.

Efficacy of pheromone on TPW

Table 1 shows that all the pheromone treatments resulted in significantly ($P < 0.05$) lower TPW populations and less damage when compared with the untreated control.

Table 1. Mean (" SE) numbers of TPW males per pheromone trap per week, larvae per plant per week, and percentage of plants damaged by TPW larvae per week on TPW pheromone treated and untreated greenhouse tomatoes in 1998.

Treatment	Mean no. of males/trap/week[a]		Mean no. of larvae/plant/week[a]		Mean % damaged plants/week[a]	
Farm A – high volume	0.2 " 0.1d	120	0.5 " 0.1bc	840	9.7 " 2.5b	20
Farm B – high volume	0.5 " 0.1c	270	0.4 " 0.0c	1512	7.6 " 2.5b	18
Farm A – low volume	1.2 " 0.3b	260	0.8 " 0.1b	2158	13.3 " 3.9b	20
Farm A – control	2.4 " 0.6a	59	31.5 " 2.7a	540	53.3 " 7.8a	20

[a]Within the same column, means with different letters are significantly different at $P<0.05$ (Duncan=s multiple range test). Data were arcsine or square root transformed before ANOVA. Untransformed data are presented. Means were calculated from weekly monitoring from January 28 through June 10, 1998.

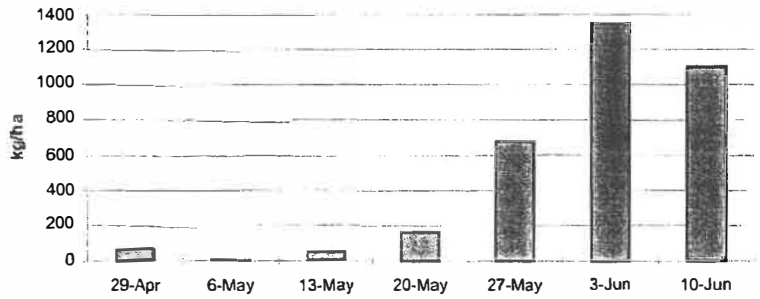
Trap Counts: The mean trap count in the control treatment was at least twice that in any of the treated greenhouses. Mean counts per trap in the treatments ranged from 0.2 in the high-volume applicator treatment at Farm A, to1.2 in the low-volume applicator treatment at the same farm, whereas the mean count in the control was 2.4.

Larval Counts: The highest mean number of larvae per plant (based on 20 sample weeks) in any of the treatments was 0.8, whereas that in the control was 31.5 (Table 1). Larval counts in the control remained relatively low until early May, or about 16 weeks after the beginning of the trial. This indicates that the larval population of TPW takes about four generations to appreciably increase between January and May under greenhouse conditions in Essex County, Ontario.

Crop Damage: The mean percentage damaged plants, or plants with damage due to larval feeding, was approximately 10-13% in the treated sections, whereas in the control, damage was over 50% (Table 1). Such damage refers to mines with and without actively-feeding larvae. During the first two months of the trial, the level of damage increased and subsequently decreased in all treatments. Thereafter, the number of damaged plants only in the control increased rapidly. By early May, when larval counts in the control were also high, 100% of the plants exhibited TPW damage, and this damage level was maintained for the remainder of the trial duration. Damage in the treated greenhouses started to increase slightly towards the end of the trial in early June and this was attributed to immigration of mated females from the control section at Farm A, and from neighbouring infested greenhouses at Farm B.

Figure 1 gives an indication of the impact of TPW on direct yield. Figure 1 shows the weight of fruits rendered unmarketable because of feeding by larval TPW at Farm A in the control treatment between April 29 and June 10. No damaged fruit were observed in any of the pheromone-treated greenhouses.

Figure 1: Weight (kg/ha) of unmarketable fruits due to feeding by larvae of tomato pinworm (*Keiferia lycopersicella*) in greenhouse tomato in the control treatment



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Biological Control of Lettuce Aphids with the entomopathogenic fungus *Verticillium lecanii* in Greenhouses

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Abstract: The aim of our research was to identify a pesticide-free treatment to prevent aphid outbreaks in greenhouse lettuce crops. In the laboratory, we first determined the effectiveness of two strains of the hyphomycete fungus *Verticillium lecanii*, DAOM 198499 and Vertalec®, on three lettuce aphid species. Estimated LC50 and aphid net reproductive rate (Ro) indicated that both strains of *V. lecanii* had a significant impact on each of the aphid species. Under greenhouse conditions, we next assessed the potential of *V. lecanii* and compared it with three other treatments: BioNeem®, a plant extract; Safer's®, an insecticidial soap; and Malathion®, an organophosphate. All treatments significantly reduced aphid populations. Vertalec® was more efficient than strain 198499 in inducing mortality of all three aphid species. The potential of using *V. lecanii* for the biocontrol of lettuce aphids is discussed in relation to aphid species and crop management.

Key words: lettuce aphid, entomopathogenic fungus, *Verticillium lecanii*

Introduction

Aphid infestation of greenhouse lettuce crops represents a serious problem for growers. Lettuce is characterized by high quality cosmetic standards, and even a few aphids on lettuce plants renders them unmarketable. Three aphid species are currently found on lettuce: the potato aphid, *Macrosiphum euphorbiae*; the green peach aphid, *Myzus persicae*; and the lettuce aphid, *Nasonovia ribisnigri*. The latter species is the major pest, as it feeds on the youngest leaves and rapidly colonizes the lettuce head (Forbes & Mackenzie, 1982).

In Eastern Canada, growers must apply insecticide from May to October in order to prevent aphid outbreaks. However, due to a growing resistance to pesticide use and consumer pressure for safer food products, growers are increasingly turning to biological control methotds. Although parasitoids and predators have been proven effective against aphids in greenhouses, their use on lettuce crop is less appealing. They leave exuvia, mummies or prominent cadavers on plants which may lead to lettuce downgrading. In this context, bio-insecticides such as entomopathogenic fungi, plant extracts and insecticidial soaps appeared to be promising options.

Verticillium lecanii Zimm. (Viegas) is a widespread hyphomycete that has drawn interest since the early seventies as a biocontrol agent of insects, especially homopterans (Hall, 1981). Under laboratory conditions, we first studied the pathogenicity of two strains of *V. lecanii*, strain 198499 and Vertalec® on the three aphids species. Strain 198499 shows promising as a versatile biocontrol agent of pests and fungal pathogens (Askary *et al.*, 1998), and Vertalec® is already commercialized in Europe by Koppert Ltd. (Ravensberg *et al.*, 1990). We next tested the efficacy of those strains under greenhouse conditions and compared them with three other products: BioNeem® (Ringer Corp.), a Neem extract (*Azadirachta indica* A. juss.), Safer's® (Safer Ltd.), an insecticidial soap, and Malathion® (Plant Products Co).

Materials and methods

Laboratory Bioassay

The three aphid colonies, established from individuals collected in lettuce crops in Quebec, Canada, were reared on lettuce plants *Lactuca sativa*, cv. Flandria. Vertalec® and strain 198499 of *V. lecanii* were maintained on potato-dextrose agar (PDA; Difco) in petri dishes. Liquid cultures in yeast-malt-peptone-dextrose broth (YMPD, Difco) were prepared for bioassay. Suspensions from 4-day-old shake cultures were centrifuged and then filtered to obtain a pure spore mixture. Spores were counted with a hemacytometer and diluted in a 0,04% Triton X-100 (Rohm & Haas Company), a wetting agent.

Pathogenicity of *V. lecanii* was determined using a range of five spore concentrations: 10^4 to 10^8 conidiospores/ml. For each aphid species, 15 third-instar nymphs were placed on lettuce leaves and sprayed until run-off with the appropriate spore concentration. Control consisted of aphids treated with 0.04% Triton X-100. Each treatment consisted of three replicates with 15 aphids/replicate. Aphids were then reared in a growth chamber at 22 $\pm$ 0.5°C under a 16L:8D photoperiod. High levels of relative humidity (99 $\pm$ 1%) were maintained in order to promote spore germination. Aphid mortality and nymph production were recorded daily for 12 days. Details of the experimental procedure are described in Askary *et al.* 1998.

The median lethal concentration required to achieve 50% aphid mortality (LC50 value) was determined by probit analysis on day 10 using Proc Probit (SAS Institute Inc., 1989). The net reproductive rate (Ro) over the 12 days following treatment was estimated as $Ro=\bullet\ l_x m_x$, where l_x is the probability of surviving from day x to day $x+1$ and m_x is the average number of offspring produced by an individual on day x (Stearns, 1992).

Greenhouse Trials

Experiments under commercial conditions took place during the summer of 1998 at Hydroserre Mirabel Inc., Quebec, the largest grower of hydroponically grown lettuce in Canada. Five treatments were assessed to control aphids: Vertalec® ($2x10^6$ spores/ml, commercial product), strain 198499 of *V. lecanii* ($2x10^6$ spores/ml, suspension obtained as described above), BioNeem® (25ppm), Safer's® (2ml/L), and Malathion® (3ml/L). Doses of *V. lecanii* were chosen from laboratory trials (they are substantially higher than those recommended by Koppert Ltd.), whereas doses of the other treatments were based on commercial specifications. Trials for each aphid species were performed separately and scheduled as follows: *M. persicae* in June, *M. euphorbiae* in July, and *N. ribisnigri* in August.

Experiments were established in a $900m^2$ hydroponical tank for a period of 18 days, which is the average time to grow lettuce from nursery to harvest. Each treatment consisted of five replicates with 18 lettuces/replicate. Each experimental unit was individually covered with an insect cage. On day 1, each lettuce was inoculated with four aphids (third to fifth instars) from laboratory colonies. All treatments were applied twice on days 4 and 7, except for Malathion® that was sprayed once on day 4. Sampling was carried out on days 4, 7, 15 and 18. Four lettuce plants per experimental unit were randomly selected and carefully examined for aphids. Following logarithmic transformation on the total count of aphids per lettuce, data were analysed by ANOVA and differences among treatments were tested using Fisher's Protected Least Significant Difference (LSD) test (alpha = 0.05) (SAS Institute Inc., 1989).

Results

Laboratory Bioassay

All aphid species were susceptible to both *V. lecanii* strains under laboratory conditions. LC50 values for each aphid species treated with Vertalec® vary from $2.0x10^4$ to $1.8x10^6$ spores/ml (Figure 1A). Based on LC50 fiducial limits (Tabashnick & Cushing, 1987), *N. ribisnigri* is significantly more susceptible to Vertalec® than *M. euphorbiae* and *M. persicae*. For aphid species treated with strain 198499, LC50 values ranged from $3.7x10^5$ to $3.1x10^7$ spores/ml (Figure 1B). Based on the LC50 fiducial limits, *M. euphorbiae* is significantly more susceptible to strain 198499 than *M. persicae*, whereas *N. ribisnigri* is not significantly different from these two species.

Furthermore, the pathogenicity of strain 198499 to aphids is similar to that of Vertalec® for *M. euphorbiae*. *Nasonovia ribisnigri* and *M. persicae* were more susceptible to Vertalec than to strain 198499. Our results also indicate that both strains of *V. lecanii* have a significant impact on aphid reproduction. Treatments with *V. lecanii* significantly reduced the net reproductive rate of aphids (Vertalec® vs *N. ribisnigri*, F=32.28, P<0.0001; vs *M. persicae*, F=9.13, P=0.0233; vs *M. euphorbiae*, F=21.78, P<0.0004. Strain 198499 vs *N. ribisnigri*, F=9.56, P<0.0086; vs *M. persicae*, F=7.39, P=0.0237; vs *M. euphorbiae*, F=7.62, P=0.0162). The mean daily fecondity of aphid significantly decreases as spore concentration increases.

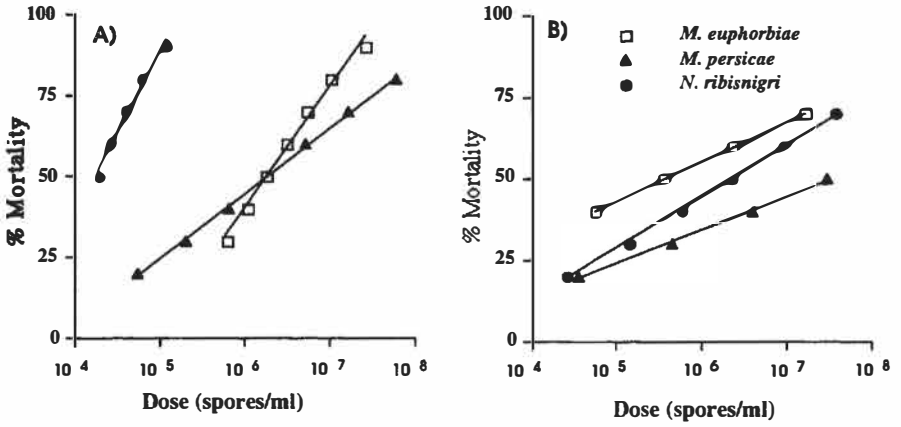

Figure 1. Susceptibility of aphids to Vertalec® (A) and strain 198499 (B) of *V. lecanii*.

Greenhouse Trials

At the end of the experiments (day 18) all treatments had significantly reduced aphid populations, except for the association strain 198499 vs *N. ribisnigri*. The Malathion® treatment appeared to be the most effective for *N. ribisnigri*, followed by BioNeem®, Safer's®, and Vertalec® (F=55.62; P<0.0001). For *M. euphorbiae*, the Malathion® treatment was also the most effective, followed by Vertalec®, BioNeem® and Safer's® ex-eaquo, and strain 198499 (F=92.51; P<0.0001). In the case of *M. persicae*, Vertalec® was the most effective, followed by BioNeem®, Malathion®, and Safer's® and strain 198499 ex-eaquo (F=41.91; P<0.0001).

Discussion

Laboratory trials clearly demonstrated that both strains of *V. lecanii* have a significant effect, not only on aphid survival, but also on the overall population dynamics of aphids. However, under greenhouse conditions, the performance of *V. lecanii* strain 198499 at reducing aphid density was poor compared to Vertalec®, probably because spores were sprayed without any formulation. The efficacy of Vertalec® was equivalent to that of Safer's® and BioNeem®. However, lettuce leaves treated with Vertalec® were often covered with white deposits. These deposits were probably due to the high dose applied ($2x10^6$ spores/ml). Moreover, aphid cadavers covered with mycelium were often found on lettuce. According to the grower, these problems would have prevented crop commercialization. Nevertheless, our greenhouse trials demonstrated that hydroponically grown crops provide favourable conditions for entomopathogenic fungi.

Laboratory bioassays and greenhouse trials revealed differences in the susceptibilities of aphid species to *V. lecanii*. For instance, under laboratory conditions *N. ribisnigri* was the most susceptible to Vertalec®, whereas under greenhouse conditions, *N. ribisnigri* appeared to be the least susceptible one. These variations may have resulted from aphid distribution, as each of the three aphid species tested exploit different microhabitats on the lettuce plant.

Further work is also needed to better assess the optimal dose of Vertalec® that should be used under greenhouse conditions, as well as the optimal time for application during the lettuce growth cycle. Each of the three 'environmentally friendly insecticides' tested showed a delayed effect compared to Malathion®. This may suggest that it would be preferable to apply those insecticides earlier, for instance during the nursery stage.

Acknowledgements

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Biological control of *Botrytis cinerea* on tomato stem wounds with *Ulocladium atrum*

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Abstract : Infection of stems by *Botrytis cinerea* is an important problem which can cause severe economic losses in greenhouse tomato crops. Greenhouse experiments have been realised to test the efficacy of a bio-control agents (BCA) *Ulocladium atrum* to protect pruning wounds from attacks by *B. cinerea*. The efficiency of *U. atrum* has been compared with that of a commercial fungicide Sumico L (diethofencarb + carbendazime). Treatments were applied after an artificial inoculation with *B. cinerea*. *U. atrum* showed a real potential to protect stem wounds from *B. cinerea*. The level of protection provided by the BCA was as good as that provided by the fungicide both in heated glasshouse and in unheated plastic tunnels.

Key words : Biological control, grey mold, *Ulocladium atrum*, tomato plants

Introduction :

Infection of tomato stem wounds by *Botrytis cinerea* is a severe problem in tomato greenhouses. Diseases on stems are often linked to leaf removal: the wounds created by this intervention represent ways of entrance for the pathogen which is able to infect stems directly but also indirectly by colonising petiole stubs. The development of a stem canker through the pruning wounds can cause severe yield lost by leading to the death of the plant (Nicot *et al*, 1996). Several methods are used to reduce the incidence of stem infections (Elad *et al*, 1995), but they do not give the guaranty to avoid the appearance of lesions on stems. Studies have been realised to test the efficacy of bio-control agents (BCA) to prevent infections of stems (O'Neill *et al*, 1996; Decognet *et al*, 1998), but only few products are already available.
We studied the efficacy of a potential BCA, *Ulocladium atrum*, to protect stem wounds from *B. cinerea* infection in different greenhouse tomato crop systems. The antagonist was tested in two representative quasi commercial systems: heated glasshouse and unheated plastic tunnel. In each case the effect of *U. atrum* was compared to the protection provided by a commercial fungicide (Sumico L).

Material and methods:

Strains:
Isolate Bc1 of *B. cinerea* was used throughout these trials. It was originally isolated from tomato plants and is sensitive to diethofencarb + carbendazime. Cultures were realised in petri dishes on oat meal agar (20 g oat meal, 15 g agar, 1000ml tap water). Conidial suspensions were prepared using 10 day old cultures by washing the culture with tap water.
Inoculum of *U. atrum* (isolate 385 originally isolated from necrotic leaf tips of onions) was produced on oat grains according to the technique proposed by Köhl *et al*,1998.

Experimental design:
Heated glasshouse: tomato plants cv "*Raissa*" were used in six individual plastic compartments (5x5m), each containing 6 rows of 9 plants. Two compartments were used for each of the three treatments: Fungicide, Antagonist, Control.
Unheated plastic tunnels: tomato plants cv "*Felicia*" were used in three tunnels (16x8m) each divided in three compartments (6 rows of 12 plants), allowing 3 replicates of each treatment. Replicates were arranged in a latin square design.
The trials were conducted both in 1997 and in 1998.

Inoculation and treatment applications:
In all trials, leaves were removed from the lower part of three month old plants (6 leaves removed per plant) leaving short petiole stubs (5 to 10 mm long). *B. cinerea* was inoculated once on each pruning wound just after leaf removal as a localised spray with a spore suspension (10^4 conidia / ml). Thirty minutes later, treatments were applied the same way: *U. atrum* sprayed as a conidial suspension (10^6 conidia / ml), the commercial fungicide Sumico L as a 2% solution; for control plots tap water was applied.

Disease assessment:
In all trials, symptoms were assessed weekly on 16 plants per compartment. The number of petiole stubs infected, and the appearance of lesion on stems (stem cankers) were recorded.

Results:

As results were similar in 1997 and 1998, only data from 1998 will be presented.

Glasshouse experiments:
The first symptoms on petiole stubs were observed about 12 days after inoculation with *B. cinerea* (Figure 1A). They appeared at the same time in control and *U. atrum* treated plots. Disease incidence for control plants was around 10%, whereas less than 5% of the petiole stubs treated with *U. atrum* were infected. On plants treated with the fungicide, the first symptoms appeared 20 days after inoculation (DAI). The number of infected petioles increased sharply with time from day 12 to day 50, principally in control plots. The maximal level of infection was obtained around day 55 and reached a value of 33% for the control plot. For plants treated with *U. atrum* or with the fungicide, the maximal level of infection (10%) was also obtained around day 50. No difference was observed between these two treatments.
Stem cankers were only observed on control plants (Figure 1B).
At the end of the experimentation, 25% of the plants in control plots were dead (data not shown).

Plastic tunnel experiments:
The first infections on petiole stubs were observed two weeks after inoculation of *B. cinerea* (Figure 2A). Symptoms on petioles were present in all tunnel compartments whatever the treatment was. Disease incidence reached 40-45% for *U. atrum* and fungicide treated plants, and about 60% for control plants. No differences were observed between the three plots.
Cankers were observed only in control compartments (Figure 2B). In two weeks, 25% of infected wounds developed cankers, and 45 DAI the disease incidence on stem reached 40%.
At the end of the experimentation, there was no dead plant in plastic tunnels.

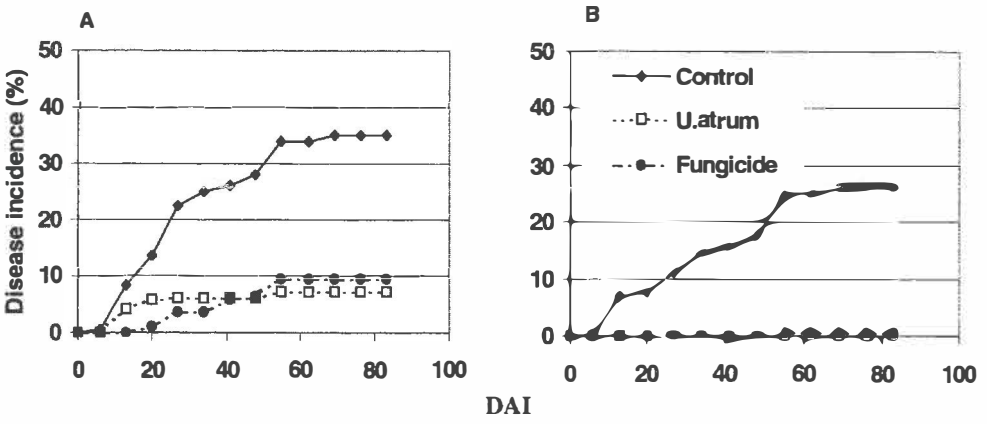

Figure 1 : Disease incidence in glasshouse for the three treatments : (A) disease incidence on petiole stubs, number of petiole stubs infected per plant (B) disease incidence on stem, number pruning wounds infected developing cankers as a function of Days After Inoculation (DAI) of *B. cinerea*

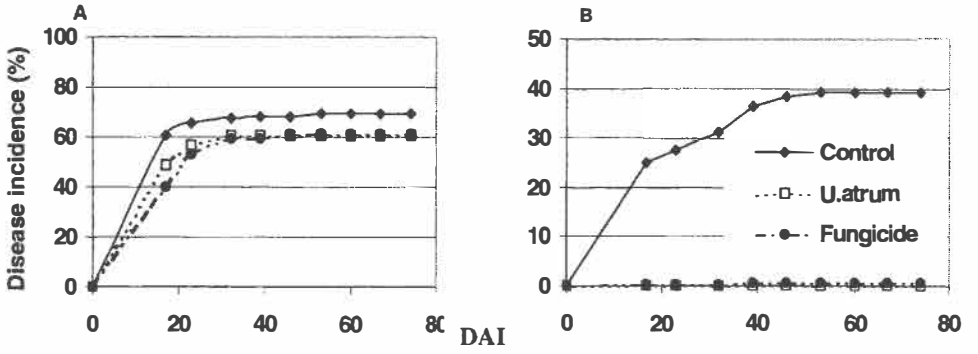

Figure 2 : Disease incidence in plastic tunnels for the three treatments : (A) disease incidence on petiole stubs, number of petiole stubs infected per plant (B) disease incidence on stem, number pruning wounds infected developing cankers as a function of Days After Inoculation (DAI) of *B. cinerea*.

Discussion:

Tomato stem infections caused by *B. cinerea* constitute an important problem which can lead to the death of numerous plants in the greenhouse. The four experiments realised in greenhouses with plants grown close to commercial conditions showed the efficacy of *U. atrum* to protect pruning wounds from development of *B. cinerea*. This efficacy was good in both cropping systems tested, which differed widely in terms of microclimate (data not shown). *U. atrum* provided equally good protection on both tomato varieties tested. The level of protection provided by the BCA appeared in all tests to be the same as the one provided by the commercial fungicide. *U. atrum* was able to prevent development of stem lesions in all cases tested, but not to protect petiole stubs (same result for the fungicide) which highlights the sensitivity of this type of plant tissue to *B. cinerea* (Verhoeff, 1967). *U. atrum* was already known to be able to suppress spore production of *Botrytis* spp (Köhl *et al*, 1995) on necrotic tissue. It appeared here to also be able to act on living tissue.

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Naturally occurring populations of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in tomato greenhouses

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Abstract: Results of a survey in IPM tomato greenhouses provide field evidence that suggests that the exotic facultative autoparasitoid *Encarsia pergandiella* interferes with the primary parasitoid *E. formosa* released for control of whitefly (*Trialeurodes vaporariorm*).

Introduction

The greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) is an important pest of greenhouse tomato crops. In order to control it, seasonal inoculative releases of the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) are successfuly used in many parts of the world (van Lenteren *et al.*, 1995; Gabarra and Besri, 1999). The exotic species *Encarsia pergandiella* Howard was introduced into Italy in the late 70s with the objective to complement whitefly control in winter unheated greenhouses (Viggiani and Mazzone, 1980). Since then, *E. pergandiella* has been recorded parasitising *T. vaporariorum* and *Bemisia tabaci* Gennadius in greenhouses and open field in other Mediterranean areas like southern France (Onillon *et al.*, 1994) and northeast Spain (Videllet *et al.*, 1997). *E. pergandiella* is a solitary arrhenotokous autoparasitoid that can primarily parasitise several whitefly species and, to produce males, needs to hyperparasite larvae of its own or other *Encarsia* species. As a consequence, there is concern whether *E. pergandiella* may interfere with other parasitoid species released for whitefly control. In northeast Spain, successful greenhouse whitefly control is also based on seasonal releases of *E. formosa* (Albajes *et al.*, 1994), and *E. pergandiella* has also been recorded from IPM tomato greenhouses since the early 90s. The objective of this paper is to present results from a preliminary survey on the presence and effects of *E. pergandiella* on Integrated Pest Management (IPM) tomato greenhouses during 1995 and 1996.

Material and methods

Encarsia formosa released in IPM greenhouses came from one private producer and from our own experimental rearings. In order to assure that there were no contaminations by *E. pergandiella*, ten cards with black pupae from each origin were selected from each release and kept for 15 days in the laboratory in transparent plastic boxes. The top was glued in order to stick emerging adults and parasitoids were identified under a stereomicroscope.

IPM greenhouses were located within 90 km in the coastal area around Barcelona and applied the IPM programme as described by Albajes *et al.* (1994). Crops lasted from mid February and early March to mid June or mid July. Inoculative releases of *E. formosa* were initiated when average density of whitefly adults on edge rows was one per plant, and four to six releases of two black pupae per plant were made. To evaluate the incidence of *E.*

pergandiella, at the end of the cropping season (from June 15 to July 15) 20 leaflets were collected in each of 29 and 22 greenhouses (1995 and 1996 respectively). Leaflets were randomly selected from the stratum of the plant where whitefly adults were emerging. Only leaflets with whitefly pupae were collected and taken to the laboratory to assess parasitism. Differences in pupal color were used to identify the primary parasitoid: *E. formosa* turns whitefly pupae black, and pupae parasitised by *E. pergandiella* are yellow. Leaflets were examined and the number of unparasitised whitefly pupae, and of black and yellow parasitised pupae was recorded. Percentage parasitism for both black and yellow pupae was calculated for each greenhouse pooling data from all leaflets. In order to evaluate field hyperparasitism of *E. formosa* by *E. pergandiella*, 20 black pupae were collected in 1995 from each of 15 greenhouses and individually kept in gelatin capsules at laboratory temperature (20 to 25 °C) until emergence of adult parasitoids which were then identified. Only samples with more than 5 emerged adults were considered.

Results and discussion

Only *E. formosa* was recovered from sample cards studied from both producers. This result discards the possibility that *E. pergandiella* found later in the greenhouses could have been accidentally introduced from contaminated releases. Naturally occurring *E. pergandiella* was thus the only possible source of the parasitoid.

Yellow pupae were present in 86.4 and 89.7% of the greenhouses in 1995 and 1996 respectively. In the rest of greenhouses only black pupae were recorded. These high percentages at the end of the cropping period in spring greenhouse tomatoes confirm our concern that this parasitoid is already well established in the area. We had previously recorded abundant *E. pergandiella* in field tomatoes and cucumbers during summer. We have also found high numbers of *E. pergandiella* in greenhouses in the Basque Country, on the atlantic coast of north Spain. Onillon *et al.* (1994) also concluded that *E. pergandiella* is well established in southern France on *Bemisia* on ornamental and vegetable crops. *E. pergandiella* is also common in Italy (Giorgini and Viggiani, 1994), but was not found in Crete after intensive searching (Kirk *et al.*, 1993).

Figure 1 shows percentages of black and yellow pupae recorded in those greenhouses where yellow pupae were found. Results show a wide range of parasitism levels among greenhouses. On average, only 30.5%±2.9 and 36.9%±5.5 (±s.e., n=29 and n=22 greenhouses for 1995 and 1996 respectively) of whitefly pupae were parasitised either by *E. formosa* or *E. pergandiella*. This contrasts with higher parasitim levels usually found when *E. pergandiella* had not yet been recorded in IPM tomato greenhouses and successful whitefly control was achieved by releases of the exotic *E. formosa* (Albajes *et al.*, 1994).

Figure 1 also clearly indicates a predominance of yellow pupae in most greenhouses: an average of 72%±5.5 and 63.7%±7.4 (±s.e., n=26 and n=19 greenhouses for 1995 and 1996 respectively) of all parasitised whitefly pupae were yellow. Moreover, 76.0%±8.5 (mean±s.e., n=11 greenhouses) of adults that emerged from black pupae collected in 1995 were male *E. pergandiella*. Seven greenhouses had more than 80% secondary parasitism by *E. pergandiella*, and in 4 greenhouses only *E. pergandiella* males emerged.

The results of this survey suggest that spontaneous colonization by the naturalized exotic parasitoid *E. pergandiella* is able to prevent successful establishment and almost replace inoculative releases of another exotic, *E. formosa*. Two factors may explain this ability of *E. pergandiella* to compete with *E. formosa*: higher tolerance to low temperatures than *E. formosa*, and the hyperparasitic male production by *E. pergandiella* females.

We have recorded *E. pergandiella* pupae in IPM greenhouses early in the season, before releases of *E. formosa* were initiated. At low temperatures, as found during early spring in our area, developmental rates are more favourable for *E. pergandiella* than *E. formosa* (Vet and van Lenteren, 1981). Therefore it seems that *E. pergandiella* is able to overwinter outside greenhouses and colonizes crops inside early in season. Onillon *et al.* (1994) have recorded *E. pergandiella* from Lantana bushes and potato crops, two species that may also be grown near to our greenhouses during early spring. On the other hand, we have found *E. formosa* on some weeds but have never observed it in greenhouses before releases are initiated.

Because of the sex determination of heteronomous hyperparasitoids, initial lack of synchrony between female and male emergence has been regarded as limiting establishment of *E. pergandiella* in the greenhouse (Hunter, 1989a). Pedata and Hunter (1996) found that *E. pergandiella* had no preference for *E. formosa* or conspecifics when selecting host for production of males, and *E. pergandiella* tend to lay more male eggs than would be expected from the proportion of secondary hosts available (Hunter, 1989b). It therefore seems plausible that females of the facultative autoparasitoid *E. pergandiella* are exploiting the abundant host resource provided by the established primary parasitoid *E. formosa* in order to produce necessary males for population growth. Del Bene and Landi (1991) document interference of *E. formosa* by *E. tricolor* Foerst., but we have never observed it. *E. tricolor* is a native european arrhenotokous species that can also be found in greenhouses, and that tends to hyperparasite alternative secondary hosts when available (Avilla *et al.*, 1991). Further evaluation will clarify the real impact of *E. pergandiella* in our IPM greenhouse systems, and specially confirm seasonal patterns of replacement of *E. formosa* by *E. pergandiella* as suggested by our results.

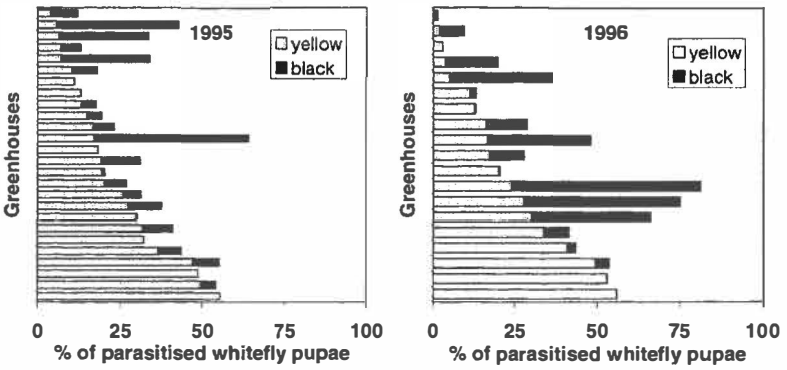

Figure 1. Establishment of *E. pergandiella* and *E. formosa* in IPM tomato greenhouses. Percentages of greenhouse whitefly (*Trialeurodes vaporariorum*) pupae that were yellow (primarily or secondarily parasitised by *E. pergandiella*) or black (primary *E. formosa* or secondary *E. pergandiella* males).

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Evaluation of *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) for biological control of *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) in greenhouse vegetable crops in British Columbia

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Abstract:
Releases of *C. marginiventris* in commercial pepper greenhouses resulted in parasitism of "sentinel" cabbage loopers ranging from 0 to 100%. Parasitized larvae were recovered from a greenhouse where no releases had occurred, indicating that wasps invaded from an adjacent greenhouse where wasps were released. In measurements in small greenhouses, *C. marginiventris* exhibited a type II functional response as the density of cabbage loopers increased. Mean searching time on frass-contaminated leaves was higher for *C. marginiventris* females with oviposition experience in the presence of frass and damaged leaves than for naive females, but this difference was not significantly different. Mean number of eggs oviposited by plant-experienced females was significantly higher than for naive females when females were held in cages with larvae on plants for 2 hours. When females were held in cages for 6 or 24 hours, there were no differences between mean number of eggs oviposited between naive and experienced females.

Keywords:
Cotesia marginiventris, *Trichoplusia ni*, greenhouse vegetables, functional response, associative learning

Introduction

Cotesia marginiventris (Cresson) is a solitary, generalist endoparasitoid of Lepidopteran larvae that attacks many species of Noctuidae, including the cabbage looper, *Trichoplusia ni* (Hübner) (Marsh 1979). Females typically oviposit into newly-hatched larvae (Ruberson and Whitfield 1996). After completing development, *C. marginiventris* larvae emerge from the host caterpillar, and pupate in cocoons on plant surfaces (Boling and Pitre 1970). *C. marginiventris* utilizes both plant and host odours in order to locate hosts (Cortesero *et al.* 1997, Turlings *et al.* 1990).

The cabbage looper is the main lepidopteran pest of greenhouse-grown vegetables in British Columbia (Portree 1993). Since 1996, we have been evaluating the potential of *C. marginiventris* for biological control of cabbage loopers. Here, we report: (1) the levels of parasitism of "sentinel" larvae recorded after releases in commercial pepper greenhouses, (2) a measurement of the functional response (Holling 1965) *vs.* cabbage loopers, and (3) the results of laboratory experiments designed to determine if searching efficiency increases after pre-release oviposition experience in the presence of frass and damaged plant material. Pre-release conditioning has been advocated as a strategy to increase the efficacy of parasitoids released for biological control (Loke & Ashley 1984, Lewis & Martin 1990).

Materials and Methods

Insect rearing
Individuals to initiate the colony of *C. marginiventris* were obtained from W.J. Lewis (USDA, Tifton, Georgia, USA). *C. marginiventris* were maintained on cabbage loopers reared on a standard artificial diet for noctuid larvae.

Releases in commercial greenhouses
All releases were conducted at the same commercial farm in greenhouse compartments planted with mature pepper crops. Two *ad hoc* releases of 1-2000 adult *C. marginiventris* were made in a 0.5 ha greenhouse during the 4 week period before monitoring of parasitism began. In a second greenhouse (0.5 ha), 3000 *C. marginiventris* were released weekly for 4 weeks during the period of monitoring (August 8 to August 29, 1996). A control greenhouse (1 ha) adjacent to this second greenhouse was monitored for parasitism, but received no releases of *C. marginiventris*. Parasitism was monitored on four dates (August 8, 16, 23, and 29) by placing 5 pepper plants (40-50 cm in height) each harbouring 100 "sentinel" cabbage looper larvae into each of the three compartments. After 5 days, the plants were removed, and larvae were dissected in the laboratory to determine whether they were parasitized.The percentage of larvae parasitized in each greenhouse was recorded for each sample date.

Functional response measurement
Three 80 m^2 greenhouse compartments were planted with 32 cucumber plants, grouped in 4 rows of 8 plants. Freshly hatched cabbage looper larvae were placed onto young leaves at a rate of 2 per leaf on 8, 16 or 32 plants, such that greenhouses received a total of 16, 32, or 64 caterpillars. Twenty-four hours later, 20 female and 60 male *C. marginiventris* were released into each greenhouse. After 5 days, larvae were recovered and dissected to determine whether they were parasitized. The number of larvae attacked was recorded for each greenhouse. The experiment was replicated three times with larval-density treatments assigned randomly at each replication.

Pre-release conditioning
Two to five-day-old female *C. marginiventris* were held individually in gelatin capsules, or small vials, containing first-instar cabbage loopers, frass and a section of damaged pepper leaf. Wasps were observed continuously until they located a larva and oviposited. These "pre-conditioned" wasps were then used in trials. Control wasps were held in empty gelatin capsules or vials until use. In one experiment, individual wasps were released onto an excised pepper leaf contaminated with cabbage looper frass and observed continuously until they left the leaf. Time spent in searching and resting activities was recorded for pre-conditioned and control wasps. In a second experiment, individual females were released into cages (50 x 40 x 40 cm) containing a pepper plant 30-40 cm in height that was infested with 20 first-instar cabbage looper larvae. Females were allowed to search and oviposit for 2, 6 or 24 h. At the end of the exposure time, the larvae were removed and dissected to determine whether parasitoid eggs were present. The number of eggs oviposited by pre-conditioned and control females was recorded.

Results & Discussion

Releases in commercial greenhouses

Parasitism of sentinel larvae was detected on plants placed in all three greenhouses (Table 1) indicating that *C. marginiventris* can locate and parasitize cabbage loopers in commercial pepper greenhouses. Parasitized larvae were recovered from the greenhouse where previous releases were made indicating that *C. marginiventris* had established a breeding population in this greenhouse. Parasitism was also detected in the control greenhouse indicating that adult females had invaded from the adjacent release greenhouse.

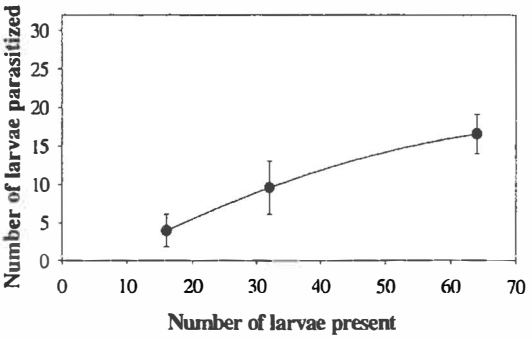
Table 1. Percent parasitism of cabbage loopers on sentinel larvae in three greenhouses

Sample date	Previous-release	Release	Control
August 8	0	19	0
August 16	100	75	22
August 23	0	9	0
Autust 29	100	60	100

Functional response measurement

C. marginiventris exhibited a Type II functional response (Holling 1965) *vs.* cabbage loopers in the greenhouse (Figure 1). The number of attacks on hosts increased with host density but at a gradually decreasing rate. Our result is similar to a previous measurement of the functional response of *C. marginiventris vs. Spodoptera frugiperda* (Riggin *et al.* 1994).

Figure 1: Functional response of *C. marginiventris*



Pre-release conditioning

In the first experiment, searching time on leaves was higher for pre-conditioned wasps (235 seconds ± 71) than for control wasps (140 s ± 30) but this difference was not statistically significant (Mann-Whitney test: U=40, p=0.45, n=10). Resting time on leaves was higher for control wasps (503 s ± 136) than for pre-conditioned wasps (216 s ± 56), but again this difference was not statistically significant (U=68, p=0.17, n=10). Pre-conditioned wasps oviposited more eggs than control wasps after 2 hours of exposure to larvae, but there were no differences in oviposition between pre-conditioned and control wasps after 6 or 24 hours (Table 2). These results indicate that there is little benefit to pre-conditioning wasps with

oviposition experience in the presence of damaged plant material because inexperienced wasps gain enough experience to "catch up" within 6 hours of release.

Table 2. Number of eggs oviposited by pre-conditioned and control females

Exposure time (h)	Pre-conditioned	Control	U	p	n
2	0.7 ± 0.3	0	25	0.01	10
6	0.5 ± 0.5	1.6 ± 1.0	65	0.15	10
24	1.4 ± 1.2	1.1 ± 0.5	61	0.37	10

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Control of watermelon insect pests by the use of multiple natural enemies

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Abstract : Greenhouse experiment was carried out during 1998 to investigate the effect of multiple natural enemies on control of three major insect pests on watermelon. Barley seedlings infested with bird-cherry aphid, *Rhopalosiphum padi*, was employed as banker plant to enhance the effect of *Aphidius colemani*, and *Phytoseiulus persimilis* and *Encarsia formosa* were simultaneously applied by direct releasing method. The experiment demonstrated that combination of *A. colemani*, *P. persimilis*, *E. formosa* could effectively control *Aphis gossypii*, *Tetranychus urticae* and *Trialeurodes vaporariorum* at the same time. This study also indicates that application of insecticides is not needed in cultivation of watermelon when combination of these natural enemies is effectively applied.

Keywords : *Aphidius colemani*, *Phytoseiulus persimilis*, *Encarsia formosa*, watermelon, multiple natural enemies

Introduction

Watermelon is usually cultivated in the greenhouse as one of major greenhouse crops in Korea. Cotton aphid and two-spotted spider mite are the most severe insect pests in cultivation of watermelon, and greenhouse whitefly is occasionally problematic in local greenhouses. Other pests, however, are little important in the watermelon greenhouse.

It is very difficult to control these insect pests since heavy application of synthetic insecticides has brought about the development of their resistance to them. Increasing concern over adverse effects of these insecticides have brought the need for the development of biological programs. Accordingly, much attempts are being made in Korea to include *A. colemani, P. persimilis* and *E. formosa* in biological control program against these insect pests (Goh and Yoo, 1998; Han et al. 1997), .

The objective of this study is to determine the efficacy of combination of these natural enemies in control of the insect pests on watermelon.

Materials and Methods

Experimental set-up: Forty watermelon plants were transplanted on April 22 in 100m^2 greenhouse. No chemicals were treated during the crop growing. Both sides of the greenhouse were covered with fine screen to prevent insects and natural enemies entering the greenhouse from outside. There were two fans in the both sides to ventilate airs.

A. gossypii and *A. colemani*: Three adults of *A. gossypii* per 3 hills of watermelon were infested on May 21. Banker plants were used to introduce natural enemy. Thirty seven and seventy seven mummies of *A. colemani* were introduced on the barley seedlings as banker plant in May 8 and 21, respectively. The number of the aphid and its parasitoid in the banker plants was counted at one month after introduction of the parasitoids.

T. urticae and *P. persimilis*: Ten individuals of *T. urticae* nymphs and adults per 1 hill were

infested on May 7. Two adults of *P. persimilis* per 1 hill were introduced on May 21. The number of mites was counted at every 10 days.

T. vaporariorum and *E. formosa*: One hundred adults of *T. vaporariorum* per 40 hills were infested on May 7. Two hundred thirty mummies of *E. formosa* were introduced twice on May 21 and 25. The number of *T. vaporariorum* and *E. formosa* was observed at 10 day-intervals.

Results and Discussion

Population of cotton aphid, *A. gossypii* was significantly lowered in the greenhouse watermelon when parasitoid, *A. colemani*, was introduced in the banker plant. A total of 114 mummies of *A. colemani* introduced on May 8 and 21 was increased to 3,930 individuals. The number of aphid increased highly in the parasitoid-absent greenhouse, whereas that of aphid did not increase in the parasitoid-treated greenhouse. Within one month after infestation of cotton aphid and its parasitoid, aphid numbers began to decline after a little increase on June 10, followed by 73.3% parasitism on June 20 (Table 1). This data means that the aphid is adequately controlled by the introduction of parasitoid. However, density of aphids considerably increased in the control plot. All plants in the parasitoid-untreated plot were dead due to the heavy infestation of cotton aphid with 2 % parasitism due to immigration of aphid from the wild population.

Table 1. Change in the population density of *A. gossypii* and *A. colemani* in the watermelon greenhouse

Dates	Natural enemies introduced		Without natural enemies	
	No. Aphid / 10 leaves	Parasitism (%)	No. Aphid / 10 leaves	Parasitism (%)
May 28	8.5	3.4	26.2	0
June 10	16.2	31.9	1,751.0	0.9
June 20	2.4	73.3	1,710.9	2.2

Two-spotted spider mite was well controlled when spider and predatory mites were introduced at the ratio of 5 : 1 (Table 2). In treated plot with predatory mites, the number of spider mites decreased after short increase in the beginning of experiment. Two hundred mites were observed per 100 leaves on May 28, and none on June 23. On the other hand, 25 predatory mites were observed on May 28, and 112 on June 10. The number of spider mite in the control plot was 39 on May 21, followed by 3,066 on June 10, eventually resulting in damage to most of the watermelon plants. However, on June 23, the spider mite as well as predatory mite was not found in the treated plot, and a few spider mites were observed only in the control plot. This indicates that the predatory mite, *P. persimilis,* is effective for controlling two-spotted spider mite in the watermelon greenhouse. In the control plot, considerable decrease of the spider mite seems to result from high temperature in the greenhouse.

Table 2. Change in the population density of *T. urticae* and *P. persimilis* in the watermelon greenhouse

Dates	No. mites / 100 leaves		
	Natural enemies introduced		Without natural enemies
	T. urticae	*P. persimilis*	*T. urticae*
May 21	37	0	39
May 28	200	25	676
June 10	52	112	3,066
June 23	0	0	32

The parasitism of whitefly by *E. formosa* reached to 83.9% in the treated plot, whereas 13.2% resulted in the control plot due to immigration of parasitoid from outsides (Table 3). This data reveals that released rate of *E. formosa* in the experiment was effective in lowering population of whitefly.

The most serious insect pests in the watermelon greenhouse were effectively controlled by the above three natural enemies without application of any insecticide. The watermelon can be harvested within 2 months after transplanting. Due to the limited number of insect pests occurring on watermelon plants, they are easily controlled by application of natural enemies rather than chemicals. For the better efficacy in natural enemy-treated greenhouse, greenhouse has to be screened to prevent immigration of insect pests and emigration of introduced natural enemies, and harmful effect of high temperature on natural enemies should be resolved.

Table 3. Change in the density of *T. vaporariorum* and *E. formosa* in the watermelon greenhouse

Dates	Natural enemies introduced		Without natural enemy
	No. *T. vaporariorum* /10 leaves	Parasitism (%)	No. *T. vaporariorum* /10 leaves
May 11	0.9		1.3
May 28	1.1		0.6
June 10	10.4	35.8	13.2
June 20	16.0	83.9	-

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The effect of a neem-based insecticide on three important greenhouse pests

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Abstract: The botanical insecticide Bionim®, based on a neem extract, was registered in Sweden in 1997 for use against pests in ornamental plants. The effect of Bionim® on population development of the western flower thrips *Frankliniella occidentalis* Pergande, the green peach aphid *Myzus persicae* Sulzer, and the two-spotted spider mite *Tetranychus urticae* Koch was studied. Spraying with Bionim® at the tested concentrations was found to significantly reduce populations of these pests.

Key words: thrips, aphids, spider mites

Introduction

The neem tree *Azadirachta indica*, fam. Meliaceae, has its origin in India but is now grown in subtropical and tropical parts of Asia, Africa, Australia and South and Central America. Many parts of the tree contains substances with insecticidal activity. Insecticides are produced primarily from the seed kernels. The kernels contain neem oil, which is rich in azadirachtin, the most important active ingredient (Schmutterer 1990).

Neem extracts function as insect growth regulators, preventing the moulting of larvae and nymphs. In some cases, treated insects live as "permanent nymphs/larvae" for the remainder of their lives. Neem-based products also disrupt mating and sexual communication, inhibit egg development, and have repellent, antifeedant and fitness-reducing effects (Schmutterer 1990).

Three sprays with the neem-based insecticide Margosan-O significantly reduced the number of larval instars of *F. occidentalis* (Lindquist & Casey 1990). According to Schmutterer (1995) the effect on thrips varies a lot. Although some species can be controlled in their nymphal stages, adults and eggs are usually not susceptible.

Foliar application of neem-based products results in disturbances in the moulting process and extends the nymphal period in some homopterans (Schmutterer 1990). According to Saxena (1995) neem extracts cause growth-disruption in homopterans, and fecundity is strongly influenced.

According to Schauer & Schmutterer (1980) neem treatments resulted in females of *T. urticae* laying fewer eggs, and repellent effects were recorded. Neem treatment of eggs resulted in increased mortality of larvae and nymphs. In addition, later research also showed that adult mortality could be increased (Mansour & Ascher 1995).

Neem extracts are short-lived under field conditions. The residual effect of neem-based products is generally restricted to five to seven days. Thus, applications may have to be repeated at frequent intervals (Schmutterer 1990).

Bionim® was registered in Sweden in 1997 as a class 2L insecticide for use in ornamentals only. In the experiments presented here, we studied the effects of Bionim on the population growth of three of the most important greenhouse pests: the western flower thrips

Frankliniella occidentalis Pergande, the green peach aphid *Myzus persicae* Sulzer and the two-spotted spider mite *Tetranychus urticae* Koch.

Material and methods

Bionim®, produced by Nim Distribution Center AB, is an alcoholic extract produced from seed kernels of the neem tree. The product contains 1500-3000 ppm of the active ingredient azadirachtin. The recommended dosage of Bionim on ornamentals is 0.25-0.5%.

Each treatment consisted of four replicates. Each replicate consisted of three plants of chrysanthemum cv. Regal Davis or one cucumber cv. Farona containing the target organisms. The former species was used for the thrips and aphid tests, while the latter was used for the two-spotted spider mite.

The pests came from our greenhouse where thrips and aphids lived on the same cultivar of chrysanthemum, and the spider mites lived on cucumber. One or two days before the start of each experiment the pests (24 thrips, 20 aphids and 16 spider mites respectively per treatment) were moved to the experimental plants with a fine brush.

Two concentrations of Bionim, 0.25% and 0.5%, were used in all experiments except those involving *F. occidentalis*. Schmutterer (1995) indicated that neem extracts was not very effective against thrips; therefore the concentrations chosen were 0.33% and 0.5%. The applications were made twice at about 10-day intervals. The plants were sprayed with a hand-sprayer to obtain full coverage. In the untreated plots the plants were sprayed with water.

The temperature was 19 °C at night and 20-25 °C during the day. The natural daylength (long days) was used during the experiments with *F. occidentalis*, and the daylength was 14 h during the other two experiments. The relative humidity was 50-70%.

Larvae and adult thrips, aphids and spider mites were counted once a week for four weeks starting a week after the first treatment, but a couple of days before the second treatment.

Results

Effect of Bionim® on Frankliniella occidentalis

Analysis of variance revealed a significant difference between untreated and Bionim-treated plants (Fig. 1, ANOVA p=0.994).

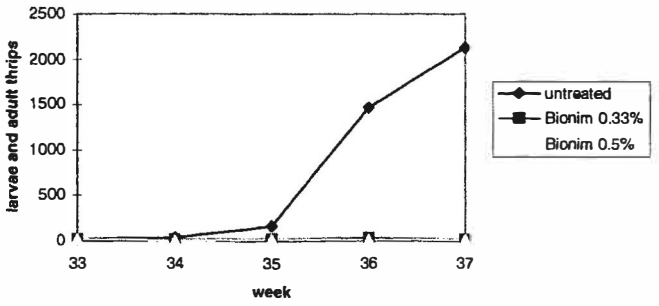

Figure 1. The effect of Bionim on *Frankliniella occidentalis*. Applications were made after the first counting of thrips and between the second and third counting.

During the last week of the experiment there were 94-97% fewer adult thrips on the Bionim-treated plants and the reduction of larvae was more than 99.5% compared with the control. Damage to the control plants was obvious. On the treated plants, the only damage observed was very mild and restricted to a few older leaves.

Effect of Bionim® on Myzus persicae

Analysis of variance showed that there was a significant difference (ANOVA p=0.997) between untreated and Bionim-treated plants after two applications (Fig. 2). There was also a significant difference between the two Bionim concentrations (SNK test at 5% level). At the end of the experiment the number of aphids was 98% and 66% lower respectively on the Bionim-treated plants. In the treatment with 0.5% Bionim there were still a few aphids on the plants, but they did not seem to reproduce.

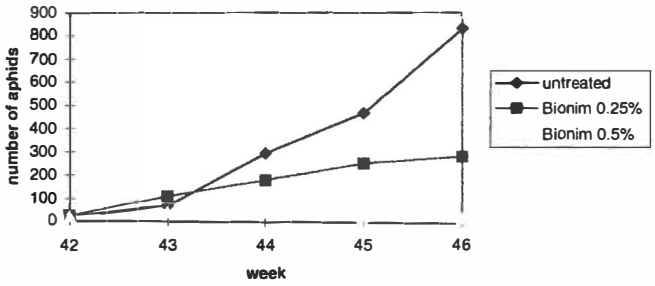

Figure 2. The effect of Bionim on *Myzus persicae*. Applications were made after the first counting of aphids and between the second and third counting.

Effect of Bionim® on Tetranychus urticae

A significant difference was found between untreated and Bionim-treated plants after two applications (Fig. 3, ANOVA, p=0.999). No significant difference was found between the two Bionim treatments.

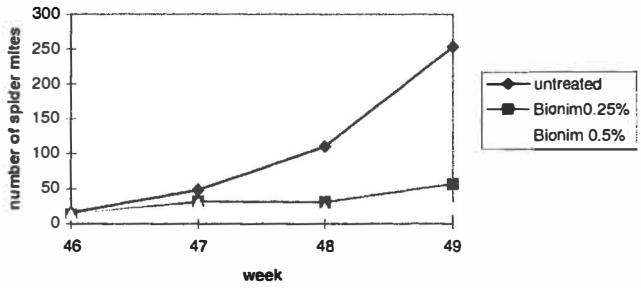

Figure 3. The effect of Bionim on *Tetranychus urticae*. Applications were made after the first counting of spider mites and between the second and third counting.

By week 49 the reduction of spider mites was 77% and 91% for the low and high Bionim concentration respectively. After only two weeks had elapsed since the second Bionim application the number of spider mites started to increase strongly. The populations increased strongly in all three treatments in week 50, when most of the mites counted were young instars (not shown in figure).

Discussion

Bionim had a significant suppressive effect on the population development of the three pests tested. The applications were made twice because neem-based insecticides break down quickly. Two or three weeks after the second treatment, we noted that populations of both aphids and spider mites began to increase. The time it takes before populations start to rebound following the second treatment depends on the length of the life-cycle of the pest in question.

The suppressive effect that Bionim had on populations of *F. occidentalis* was impressive since this thrips normally is very hard to control. Schmutterer (1995) stated that the effect on thrips varies a lot, depending on both the thrips species targeted and the type of neem extract used. Based on the results of this study it can be concluded that the formulation of Bionim used here is suitable for controlling *F. occidentalis*.

Neem-based insecticides have relatively weak contact effect on insects and mites, and therefore usually have little or no adverse impact on important natural enemies of the pests (Isman 1997, Schmutterer 1990, 1995). As a consequence of this characteristic and the short persistance of these insecticides, they are often suitable for use in combination with biological control. More research needs to be conducted on the effects of neem-based insecticides on the beneficial arthropods, especially with regard to repeated applications.

Acknowledgements

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Development of Biological Control Methods for Use in Southwestern U.S. Greenhouses and Nurseries

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Abstract: Growers producing crops in states located along the western and northern edges of the Gulf of Mexico are challenged by short periods of sub-freezing weather in the winter and prolonged periods of hot and humid weather in the summers. In this climate ornamental crops (primarily potted and bedding plants) tend to move through production in approximately 6 weeks, and vegetable crops are grown during periods of time when field-grown vegetables are unavailable to local markets (fall through spring). Biological control in these conditions must provide rapid results due to the short cropping cycles and the premiums placed on high aesthetic quality. In potted chrysanthemums, optimal methods for releasing *Aphidius colemani* for biological control of *Aphis gosypii* and *Myzus persicae* were examined. Pest control in tomatoes relies heavily on cultural practices due to the apparent failure of *Beauvaria bassiana* to control *Trialeurodes vaporariorum* or *Bemisia argentifolii* (= B strain of *B. tabaci*).

Key Words: tomato, chrysanthemum, aphids, whiteflies, *Aphidius colemani*, *Thripinema nicklewoodii, Beauvaria bassiana*

Introduction

While it defies the common perception of agriculture in the states along the Gulf of Mexico, ornamental horticulture is an important business to the economies of these states. In Texas greenhouse and nursery production is the fastest growing segment of agriculture in the state, and ranks fourth in state agricultural products behind cattle, cotton, and dairy products. The Texas greenhouse and nursery industry has grown at the annual rate of 9.6% during the 1990's, with cash receipts of 1.05 billion in 1997 (Texas Agricultural Statistics Service, 1997).

While this industry has its economic rewards, it is frequently threatened by even small amounts of arthropod feeding that dramatically reduce the aesthetic quality of these crops and, thus, reduce their marketability. Not surprisingly, a great deal of pesticide use is commonly associated with the management of these pests. In 1993, Texas nursery crop growers applied approximately 0.68 kgs of active ingredient of insecticide/acre. By comparison, Texas cotton and corn growers applied approximately 0.14 kgs of insecticide/acre to their crops during 1992. Although these insecticide-based methods for maintaining pest-free and damage-free plants are virtually universal, they are also currently being challenged by an array of regulatory agencies.

The appeal of biological control tactics applied to ornamental plants can be demonstrated by the many reviews published on this topic and by the exponential growth of the augmentation biological control industry. The growth trend is demonstrated by the three-fold increase in the numbers of natural enemies and companies supplying these organisms within North America over the last 15 years (Hunter 1997).

Despite this growth, examples of successful biological control of arthropod pests on ornamental crops are especially rare, partly because of the industry's inability to manage insecticides, and partly due to the scarcity of applied research to show if, how, and when

natural enemies should be released. While there is no shortage of information regarding the identity and basic biology of these natural enemies, recommendations of introduction methods, release rates, and subsequent monitoring techniques are infrequent for most of the commercially available natural enemies. We report on attempts to remedy some of these problems in potted chrysanthemums and tomatoes.

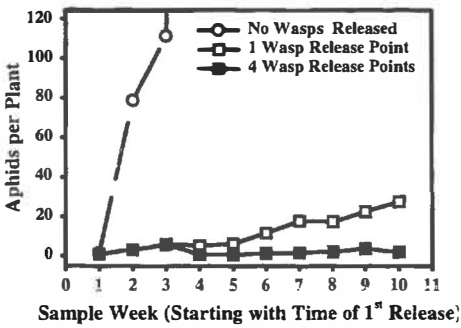
Potted Chrysanthemums

Aphids (esp. the green peach aphid, *Myzus persicae*, and the melon aphid, *Aphis gossypii*) rank as one of the most serious pests of greenhouse floricultural crops (Rabasse & Wyatt 1985). Difficulty in controlling this pest could be due to the development of insecticide resistance (Cross et al. 1983) or it could be due to the development of superior biotypes or races (Wyatt 1966). Hence, complete reliance on chemical control is a risky proposition. In addition, it is becoming necessary to find aphid control strategies that are compatible with less disruptive control measures for florist crops.

Successful biological aphid control in potted chrysanthemum requires parasitoids and predators to locate and contain aphid patches when they are relatively scarce. Further, natural enemies must respond to a rapidly changing landscape of aphid patches. It has often been suggested that biological control can be enhanced if the natural enemies inflict some form of density-dependent mortality on the target pest. However, in crops grown for their aesthetic value, the issue is not simply whether natural enemies inflict density-dependent aphid mortality but how rapidly they do so. For example, while natural enemies may aggregate to patches of high aphid density, they may do so at such a slow rate that they are overwhelmed by the reproduction of the aphids. To generate much needed information on natural enemy release strategies, studies were initiated to assess the influence of spatial structure on aphid – natural enemy dynamics. Studies focused on the use of *A. colemani,* which has been reported to be the most suitable natural enemy for use in aphid control (van Steenis 1993).

Replicated studies conducted in glasshouses revealed that *M. persicae* and *A. gossypii* spread over an area of 11.2-m^2 per day after infesting an single potted chrysanthemum. By comparison, studies with *A. colemani* demonstrated that it could spread over an area of 13.7-m^2 per day after being released from a single potted chrysanthemum. From these results, it was determined that the most effective biological aphid control could be obtained by releasing *A. colemani* from points no greater than 3.7-m apart within a potted chrysanthemum greenhouse (Heinz 1998). Subsequent trials were conducted to test the influence of *A. colemani* release strategies on their ability to biologically control green peach aphids in research greenhouses. Wasps were released at the rate of three per pot per week from 4 points, 3.7-m apart, or from one central point within 112-m^2 greenhouses filled with potted chrysanthemums. Additionally, each greenhouse contained a screened cage, which covered a bench of chrysanthemums, and prevented wasps from accessing aphid-infested plants. Comparisons between aphids densities within the cages to those outside the cages (into which *A. colemani* were released) provided an experimental method for assessing the impact of parasitoid releases. At the beginning of the trial, every third pot within the greenhouse was infested with three green peach aphids. Four replicated trials using similar methods and generating similar results were conducted at commercial operations.

Both *A. colemani* treatments (released from 1 or from 4 points) yielded significant suppression of green peach aphids. Densities in the cages from which wasps were excluded exceeded 5,000 aphids per plant by week 6 of the trials. Aphid densities climbed to 27.5 per plant at week 10 in greenhouses where wasps were released from one central point. By comparison, aphid densities reached a maximum of 5.8 per plant at week 3 in greenhouses where wasps were released from four points.



Plant Quality At Harvest

	Rank		Acceptable for Purchase as a Gift	
Wasp Release Points	1	4	1	4
Treatment				
Uninfested	2.0	2.9	30%	50%
Plants	1.6	2.9	65%	73%
Infested Plants -	5.5	5.5	0%	0%
No Wasps	5.4	5.5	0%	0%
Infested Plants -	3.6	2.1	0%	77%
With Wasps	3.1	2.1	20%	85%

The quality of the potted chrysanthemums harvested at the completion of each trial were ranked in order of quality with 1 representing the highest quality and 6 the lowest quality. In addition, judges were asked to identify pots acceptable for purchase as a gift. Pots from the aphid-infested cages were judged completely unacceptable, always ranking the poorest in quality and never being acceptable for purchase as a gift. Pots from greenhouses where wasps were released from four sites always ranked higher in quality than pots from greenhouses where wasps were released from a single location. Further, the percentages of pots deemed acceptable for gift giving from the 4-release point greenhouses were greater than the percentages of gift quality pots never infested with aphids. Thus, biological control is not only an effect method of aphid control, but facilitates production of high quality potted chrysanthemums.

Tomatoes

In Texas, greenhouse tomatoes are frequently infested by the silverleaf whitefly (*B. argentifolii* = B strain of *B. tabaci*) and the greenhouse whitefly (*T. vaporariorum*). Whitefly populations are capable of rapidly attaining unmanageable numbers in the Texas climate. In addition to a reduction of plant yield through extraction of resources and potential transmission of plant diseases, silverleaf whitefly causes irregular ripening of tomatoes.

Whiteflies enter the greenhouses in the fall, coincident with the harvest of 2.1 million hectares of Texas cotton, the summer host for whiteflies. This potential influx of whiteflies is avoided by covering vents with 0.6 x 0.6-mm mesh screening. These screens reduce airflow an estimated 23%, but they also exclude insects the size of whiteflies and larger.

In the event growers fail to use screening properly, attempts to use *Beauvaria bassiana* to manage whiteflies on tomatoes were evaluated in several locations throughout central Texas. In each of the three trials, weekly applications of the pathogen provided no detectable measure of whitefly suppression. We are currently evaluating two hypotheses for these failures. Temperatures within tomato greenhouses during the trials were frequently in excess of 40°C for 12 hours. Studies conducted with *Verticillium lecanii* and *Metarhizium anisopliae* suggest an optimal temperature for maximum pathogenicity of 23°C, and reduced levels of pathogenicity as temperatures deviated from the optimum (Vestergaard *et al.* 1995). In addition Costa & Gaugher (1989) showed that solanine and tomatine have fungicidal effects on *Beauveria bassiana.* 'Vidator', the variety of tomato grown in Texas greenhouses, contains high amounts of tomatine that may inhibit infection of whiteflies by *B. bassiana.* This hypothesis is supported by the work of Hare and Andreadis (1983), who determined that *Leptinotarsa decemlineata* (Colorado Potato Beetle) was less susceptible to *Beauveria bassiana* when after it had consumed tomato foliage.

Acknowledgements

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Surveying for non-diapausing predatory bugs for biological control of thrips pests in greenhouses during winter

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Abstract: Some arthropod natural enemies used for the biological control of greenhouse pests are inactive during winter because they enter diapause under short-day conditions. A solution to this problem is to survey for non-diapausing natural enemies in tropical and subtropical regions. Two approaches for thesurvey are: (1) to seek subtropical or tropical natural enemy species which differ from the natural enemy species in question, and (2) to seek non-diapausing geographic races of the natural enemy species in question in subtropical or tropical regions. Usefulness of these two approaches is supported by the results of surveys of non-diapausing bugs predaceous on *Thrips palmi* in Thailand (tropical region) and the Ryukyu Islands (subtropical region).

Key words: *Orius* spp., *Wollastoniella rotunda, Piocoris varius*, augmentative biological control

Introduction

In biological control of greenhouse pests, a critical problem is that arthropod natural enemies used for the control are inactive during winter because they enter diapause under short-day conditions. Forexample, predatory mites and midges and anthocorid bugs are known to have this problem as biological control agents in greenhouses, and solutions to the problem have been suggested (e. g., Gillespie and Quiring 1993). A solution to the problem is to survey for non-diapausing arthropod natural enemies in tropical and subtropical regions because arthropod natural enemies in these regions are often non-diapausing. There are two approaches for the survey: (1) seeking tropical or subtropical natural enemy species which differ from the natural enemy species in question, and (2) seeking non-diapausing geographic races of the natural enemy species in question in subtropical or tropical regions if their ranges extend to these regions. The second approach was suggested by Gillespie and Quiring (1993).

Thrips palmi Karny (Thysanoptera: Thripidae) is one of the most important pests of greenhouse vegetables in Japan. Kawai (1995) experimentally showed that *Orius* spp. native to Japan effectively control this pest on greenhouse eggplant. These *Orius* spp. were not identified to species, but they probably were *Orius sauteri* (Poppius) and/or *O. minutus* L. (Hemiptera: Anthocoridae). Both *Orius* species from Japan enter diapause under short-day conditions (Kohno 1997). Thus, non-diapausing natural enemy species other than the diapausing *Orius* spp. areneeded for more effective control of *T. palmi* in Japanese greenhouses during winter.

This paper summarizes the results of our surveys of non-diapausing bugs predaceous on *T. palmi* in Thailand (tropical region) and the Ryukyu Islands (subtropical region),

emphasizing the importance of surveying for non-diapausing arthropod natural enemies in tropical andsubtropical regions for successful biological control of thrips pests in greenhouses during winter.

The surveys

T. palmi native to the Malaysian-Indonesian region invaded Japan in 1978. Thus, we conducted initial surveys of non-diapausing bugs predaceous on *T. palmi* in eggplant gardens in its native range, Thailand in 1987-1988 (Hirose *et al.* 1993). An additional survey was made in 1994, using the same methods as the previous surveys.

The Ryukyu Islands, belonging to a subtropical region, is located near southernmost part of Japan. These islands were invaded by *T. palmi* around 1981. Surveys of indigenous and non-diapausing bugs predaceous on this non-indigenous pest in the Ryukyu Islands were conducted in 1994-1998 (Hirose *et al.* in press). Unlike Thailand, the Ryukyu Islands have no many eggplant gardens but many wax gourd gardens. Therefore, wax gourd was often chosen as a target crop.

Results and discussion

In Thailand, five species of bugs predaceous on *T. palmi* were found (Table 1). Of these five, *Wollastoniella rotunda* Yasunaga et Miyamaoto (formerly referred to as *Billia* sp.) (Hemiptera: Anthocoridae) was the commonest species as a predator of *T. palmi*in Thailand, and this predator was evaluated to be effective against *T. palmi* (Hirose *et al.* 1993).Laboratory experiments showed that *W. rotunda* is a non-diapausing species (Shima *et al.* unpublished). Temperature effects on the development and survival ofthis species have no problem in its use in Japanese greenhouses during winter (Shima and Hirose in press). A preliminary test of releasing *W. rotunda* on eggplant infested with *T. palmi* in greenhouses in winter was successful (Urano *et al.* unpublished). In addition, mass rearing methods of *W. rotunda* are developed by Nagai *et al.* (unpublished). It was observed that *W. rotunda* also preys on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Shima, personal observation). Thus, this non-diapausing predatory bug is a promising biological control agent against *T. palmi* and *F. occidentailis* in greenhouses in Japan during winter.

In both Thailand and the Ryukyu Islands, *Orius tantillus* (Motschulsky) (Hemiptera: Anthocoridae) was found (Table 1). The role of *O. tantillus*as a predator of *T. palmi* in Thailand is not known, but Rejesus *et al.* (1986) (cited from Bernardo 1991) reported that *O. tantillus* was the most predominant natural enemy preying on nymph and adult thrips on watermelon in the Philippines. A laboratory study by Mituda and Calilung (1989) showed the great potential of *O. tantillus* as a biological control agent against *T. palmi*. Although the Ryukyu Islands are almost northern most for the range of *O. tantillus*, this predator was common in this island. However, *O. tantillus* was rarely found in gardens of wax guard infested with *T. palmi* and abundant on grass weeds infested with thrips other than *T. palmi*, suggesting a habitat or crop preference of *O. tantillus*. This predatory bug was experimentally shown to be non-diapausing species (Nakashima and Hirose 1997a). Temperature effects on the development and survival of *O. tantillus* have no problem in its use in greenhouses in Japan during winter (Nakashima and Hirose 1997b). Nagai *et al.* (1998) developed a mass rearing method of this predator. Thus, *O. tantillus* is also a promising control agent against *T. palmi* in greenhouses in Japan during winter.

Piocoris varius (Uhler) (Hemiptera: Lygaeidae) was not found in Thailand but in the Ryukyu Islands (Table 1). This species was more common than *O. tantillus* in wax gourd gardens infested with *T. palmi*. A laboratory observation (Nakashima unpublished) suggests the great predatory capacity of an adult of this bug as a predator of *T. palmi*. *P. varius* is also common in temperate areas, such asHonshu, Shikoku and Kyushu, in Japan (Yasunaga personal communication). From these areas, however, *P. varius* has never been recorded as a predator of *T. palmi*.It remains unclear whether *P.varius* is a non-diapausing species. If *P. varius* in the Ryukyu Islands is non-diapausing, its great predatory capacity suggests the possibility of using this predator as a biological control agent against *T. palmi* in greenhouses in temperate areas of Japan during winter.

Neither *Orius strigicollis* (Poppius) (Hemiptera:Anthocoridae) nor *Campylom machinensis* Schuh (Hemiptera: Miridae) was found in Thailand but in the Ryukyu Islands (Table 1). *O. strigicollis*was collected from grass weeds together with *O. tantillus*, and much fewer than *O. tantillus* in the islands. In Japan, it is commonly found on eggplant in Kochi, Shikoku as a predator of *T. palmi* (Takai 1998). In Taiwan, a more southerly island than the Ryukyu Islands, *O. sauteri* was reported as a predator (Wang, 1995), butit has recently been identified as *O. strigicollis* by Dr. Yasunaga (Yasunaga personal communication). *C. chinensis* is most commonly found on *T. palmi*-infested eggplant and cucumber in Taiwan and its predatory capacity exceeded that of *O. strigicollis* in a laboratory test (Wang, 1995). Thus, if populations of both species in the Ryukyu Islands or Taiwan are non-diapause, they could be used in the Japanese greenhouses during winter. There would also be a possibility of such a use of *O. minutus* from Thailand if Thai population of this species is non-diapausing.

Table 1. A list of predatory bugs as natural enemies of *T. palmi* in Thailand, the Ryukyu Islands and temperate areas of Japan, confirmation of non-diapause of these bugs, and approaches to their urvey.

Family and species of Predatory bugs	Distribution of predatory bugs in:			Predatory bug species where non-diapause is confirmed	Approach to The survey of Non-diapausing Bugs (see text)
	Thailand	The Ryukyu Islands	Temperate areas of Japan		
Anthocoridae					
Wollastoniella rotunda	X			x	1st
W.parvicuneis	X				1st
Orius minutus	X		x		2nd
O.strigicollis		x	x		2nd
O.tantillus	X	x		x	1st
Lygaeidae					
Piocorisvarius		x	x		2nd
Miridae					
Campylommachinensis		x	x		2nd
*C.*sp.	x				Indeterminable

Conclusions

To solve the problem of reproductive diapause in arthropod natural enemies of thrips pests, such as *T. palmi* and *F. occidentalis*, seeking tropical or subtropical natural enemy species which differ the natural enemy species in question is a useful approach (Table 1). This first approach is supported by finding effective and non-diapausing predatory bugs, such as *W. rotunda* and *O. tantillus*, in tropical and subtropical regions. As the second approach, seekingnon-diapausing geographic races of the natural enemy species in subtropical or tropical regions could also be useful for solving the problem in the biological control of thrips pests, such as *T. palmi*, in greenhouses during winter if *P. varius*, *O. minutus*, *O.strigicollis* and *C. chinensis* from Thailand or the Ryukyu Islands are non-diapausing races of these species extended to temperate Japan (Table 1).

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An overview of natural enemy explorations and evaluations for *Bemisia* in the U.S.

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Key words: *Bemisia spp., Eretmocerus, Encarsia, Amitus, Delphastus, Nephaspis, Serangium, Chrysoperla, Beauveria, Paecilomyces*, taxonomy, evaluation

Introduction

Following the introduction into the U.S. of *Bemisia argentifolii* Bellows & Perring (= *B. tabaci* (Genn.) strain B) in the late 1980's, concern about widespread whitefly management problems in numerous crops led to the formation of a federally-coordinated research and action plan to develop management methods for whiteflies (Faust, 1992). This ongoing plan brings together many researchers from federal and state agencies and universities for annual research review meetings and provided a framework for directing research on whitefly management. Part of this action plan included efforts to find and evaluate biological control agents. Although various *Eretmocerus* and *Encarsia* spp. (Hymenoptera: Aphelinidae) and many different predators and pathogens were previously reported to attack *Bemisia* species, no systematic survey for natural enemies had been conducted covering widespread regions of the world. After initiating U.S. surveys and explorations elsewhere, it was apparent that few of these taxa were adequately characterized taxonomically, nor was their impact on *Bemisia* populations well understood.

The U.S. Program

Overseas Exploration

Surveys in areas heavily impacted by *Bemisia* indicated that many whitefly natural enemies indigenous to North America also attacked *Bemisia* in these newly-colonized regions (e.g. Hoelmer & Culver, 1997; Hoelmer *et al.*, 1998; Simmons, 1998). However, native species were not effective enough to prevent severe whitefly problems in many crops, and foreign exploration was initiated to search for potentially more effective species. Attempts were made to match climatic conditions of the regions of origin with three "target" zones in the U.S. comprising major whitefly-affected crop production zones in Florida, Texas and desert Arizona and California (Lacey *et al.*, 1993). Between 1992-1998, scientists from the U.S. and elsewhere searched in over 25 different countries in Africa, Central and South America, the Mediterranean basin, the Indian subcontinent and Southeast Asia for parasitoids, predators and pathogens of *Bemisia* and co-occurring *Trialeurodes* spp. Most of the parasitoids collected were from *Bemisia* spp. found on crops in the families Cucurbitaceae/Solanaceae, ornamental plants of various families and weedy hosts in Compositae/Solanaceae (Kirk *et al.*, 1996).

Several hundred isolates of the fungal pathogen *Paecilomyces fumosoroseus* were

collected from *Bemisia* spp. in India, Malaysia, Nepal, Pakistan and Taiwan. These were deposited at the ARS entomopathogen repository in Ithaca, NY (Lacey *et al.* 1996). In the U.S., other collections of pathogens, principally *Beauveria bassiana* and *Paecilomyces fumosoroseus*, were isolated and tested extensively in the laboratory and field for their efficacy against *Bemisia.* In several cases, isolates have been developed commercially for whitefly control.

The majority of arthropod collections were sent to the USDA-APHIS quarantine facility at Mission Biological Control Center in Texas. At this laboratory, over 80 shipments of natural enemies were processed during this period, which included 235 different collections of predators and parasitoids. Following initial identification, using a combination of morphological and molecular techniques, fifty-six of these were maintained in culture for varying lengths of time in support of evaluations conducted at different locations in the U.S. (Goolsby *et al.*, 1999). Based upon environmental assessments prepared for species of *Encarsia, Eretmocerus* and *Serangium*, APHIS issued permits for release from quarantine culture for those species that were associated with *Bemisia* spp. at the collection site.

Identification and Evaluation

Four relatively specific non-indigenous whitefly predators were collected: the coccinellids *Serangium parcesetosum* Sicard from India, *Serangium* n.sp. from Malaysia, and *Clitostethus arcuatus* from Spain; and the drosophilid *Acletoxenus formosus* from Crete. Laboratory evaluations of these (e.g. Legaspi *et al.*, 1996 for *S. parcesetosum*) were conducted, and *S. parcesetosum* was eventually released in Arizona and California but has not been recovered to date. Other promising whitefly predators indigenous to the U.S, including several *Delphastus* spp. (Coccinellidae), *Nephaspis oculatus* (Blatchley) and *Semidalis flinti* Meinander (Coniopterygidae), have also been the subjects of continuing studies. Interest in *Delphastus* as a predator of *Bemisia* led to a major revision of the genus (Gordon, 1994). Research also indicates that a number of generalist predators occurring in field crops, and species frequently used in protected culture, such as *Chrysoperla,* can also have a considerable impact on whitefly populations.

The majority of overseas collections were of parasitic Hymenoptera in the genera *Encarsia* and *Eretmocerus*. From the much larger number of foreign collections, forty-one cultures were maintained. To characterize these collections until definitive identifications could be provided, RAPD-PCR markers were developed at the Mission laboratory. These markers helped to identify different cultures and aided in the identification of field recoveries (Goolsby *et al.*, *in press*). Series of specimens were also sent to collaborating taxonomists, with the eventual revision of key groups and description of new indigenous and introduced species (e.g. Rose & Zolnerowich, 1997; Zolnerowich & Rose, 1998). At least seven distinct species of *Encarsia* and six distinct *Eretmocerus* were eventually determined to comprise the collections from outside the U.S. Biological differences which have been recorded between several morphologically inseparable cultures may indicate that additional cryptic species occur. One new platygasterid, *Amitus bennetti* Viggiani & Evans, was described from other collections in the Caribbean.

Various teams of researchers have been involved in laboratory and field evaluations. For example, many of the imported cultures and several indigenous parasitoids were evaluated in the laboratory at Mission, TX, on several of the key crops impacted by *Bemisia*. The results of these lab screenings were used to prioritize cultures for field cage tests (Goolsby *et*

al., 1996). Further evaluations under field or semi-field conditions identified candidates which performed best against *Bemisia* on key crops such as alfalfa, broccoli, cantaloupe and cotton (e.g. Goolbsy *et al.*, 1998; Hoelmer, 1998). Field evaluations identified several species which appear to be better adapted to specific regional climates. Colonization efforts have resulted in the establishment of *Eretmocerus mundus, E. hayati* and *E. emiratus* in several areas of the U.S. However, it is still too early to assess the full impact of these establishments. In the desert southwestern U.S., surveys of native whitefly species are currently underway to look for potential non-target impacts of the introduced species.

In addition to classical (establishment) biological control efforts, research on augmentative uses of predators and parasitoids has demonstrated that there is considerable potential for further use of introduced and indigenous North American species. For example, in greenhouse poinsettia systems, Hoddle *et al.* (1998) demonstrated the importance of host-feeding by *E. eremicus* as an added mortality factor on *Bemisia*; and Heinz & Nelson (1996) showed that additive effects of using multiple enemies were more important than interspecific interactions. In field crops, four years of field trials using introduced *Eretmocerus* spp. in seasonal inoculations have demonstrated that they are more effective than the native *E. eremicus* and are potentially useful in *Bemisia* IPM programs (Simmons *et al.*, 1997, 1998).

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Alternative food sources for thrips predators on cucumber: also a delicacy for the Western Flower Thrips *Frankliniella occidentalis*

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Abstract: In a life table study the effect of alternative food sources on life table parameters of the Western Flower Thrips were studied. All tested food sources had a positive effect on the thrips, with pine (*Pinus sylvestris*) pollen showing the biggest impact. The median development time from larva to adult dropped from 8.9 to 8.0 days, the median longevity increased from 24 to 28 days and the median fecundity rose from 64 to 252 eggs when comparing cucumber leaf without and with pine pollen. This resulted in an increase of the intrinsic rate of increase from 0.175 to 0.256. Two other potential alternative food sources for predators: *Ephestia kuehniella* eggs and an entomophage diet, showed a significant increase of the oviposition rate of the thrips to 8.8 and 7.8 eggs/day, respectively, compared to 5.3 eggs/day on the mere cucumber leaf.

Key words: cucumber, pollen, entomophage diet, *Ephestia kuehniella* eggs, *Orius* spp.

Introduction

Biological control of the Western Flower Thrips *Frankliniella occidentalis* (Pergande), an important pest of several greenhouse crops, with predatory mites and bugs has proven quite successful, but varies in relation to the greenhouse crop. On cucumber, predator populations invariably declined after predator release, whereas on sweet pepper they remained constant even in the absence of thrips. This may be explained by the presence of pollen as an alternative food source for the predators (van Rijn & Sabelis, 1990; van den Meiracker & Ramakers, 1991), suggesting that adding for example pollen to cucumber might enable preventative introduction of oligophagous predators.

So far most studies concentrated on the effect of pollen on the predators (e.g. van Rijn & Takabashi, 1999), although the pest can benefit from the pollen, too (Van Rijn & Sabelis, 1993; Trichilo & Leigh, 1988). In our study the effect of different additional food sources on the development time, longevity and fecundity of the Western Flower Thrips were compared. The aim was to find out whether there are alternative food sources that do not give a big benefit for the thrips. Such food sources could be used for preventative introduction of predators without the risk of giving the pest a reproductive advantage that might result in its population build-up beyond the control capacity of the predator. The results showed that all tested food sources have at least some positive effect on the increase capacity of the thrips.

Material and methods:

Determination of development time, longevity and fecundity
Five pollen species: common cattail (*Typha latifolia*), pine (*Pinus sylvestris*), birch (*Betula pubescens*), hazel (*Corylus avelana*) and rosebay willowherb (*Epilobium angustifolium*), all

collected by hand, and a milk powder/yeast mixture were tested as additional food sources by applying them to cucumber leaf. Additionally, the pollen of rosebay willowherb collected by bees were included.

Development time: Thrips larvae (0-4h old) were confined individually on pieces of cucumber leaf (cv. 'Jessica') in mini-chambers formed by a Petri dish inverted over the leaf and secured in place between two plexiglass rectangulars that were held together with rubber bands. Between the leaf and the lower plexiglass a filter paper and a cotton-string were inserted. The free end of the string extended to water, keeping the filter paper constantly moist to prevent the leaf from drying. Every third day the thrips were transferred to a new leaf to keep the leaf fresh and to prevent the food source from moulding. Upon replacing the additional food was applied in portions of 9 mg on the cucumber leaf. The mini-chambers were placed in a growth chamber at 25°C (±1°C), 80 % RH and L16:D8 and the development of thrips was checked twice a day.

Longevity and fecundity: Using the mini-chamber method, batches of even aged larvae were raised to the pupal stage on their respective diets. Upon adult eclosion, chambers with males were discarded. The longevity of non-mated females was determined by checking them once a day until they died. Fecundity was recorded by retaining the replaced cucumber leaves for one week after their removal and by counting the hatched larvae.

The intrinsic rate of increase (r_m) of the thrips fed with the different food sources was calculated with the following formula: ($\bullet e^{(-rm\ x)} l_x m_x = 1$), where l_x and m_x denote the age-specific survival and age-specific fecundity, respectively.

Thrips ovipostion rate on other potential alternative food sources

The effect of two other possible alternative food sources for predators: *Ephestia kuehniella* eggs and an entomophage diet at the oviposition rate of thrips was tested. The latter is a semisolid artificial diet for mass rearing of larvae of the green lacewing *Crysoperla rufilabris* (Cohen & Smith, 1998). In preliminary tests it enabled the rearing of *Orius insidiosus* (Cohen, personal communication).

From a greenhouse thrips population 45 female thrips were confined individually in the mini-chambers and assigned at random to one of the following treatments: cucumber leaf, *E. kuehniella* eggs, or the entomophage diet, both offered on a cucumber leaf. The leaves were replaced with new ones in three consecutive days. The oviposition rate was determined as the fecundity in the former experiment, i.e. by counting the larvae that hatched from the leaves of the second and third day.

Statistical analysis

The development time and longevity data were analysed by using Cox proportional hazards models. Generalised Linear Mixed models were used for the fecundity data (SAS Institute Inc., 1989).

Results & Discussion

Determination of development time, longevity and fecundity

Except the rosebay willowherb bee-collected pollen and the powder milk/yeast mix, all treatments had a significant effect on the development time. For birch and hazel pollen and the mixture of dried milk and yeast, a significant effect on the longevity was found. When thrips were offered the mixture of powdery milk and yeast, their longevity decreased

Table 1. The median development time from larvae to adult, median adult female longevity, median fecundity of unmated females and the r_m intrinsic rate of increase of *F. occidentalis* when additional food sources were applied to a cucumber leaf (cv. ' Jessica') at 25 °C, 16L:8D. qd =quartile deviation ((q3-q1)/2). Treatments marked with an asterisk differ significantly from the control (development time and longevity: Cox proportional hazards model; fecundity : Generalised linear mixed model)

Treatment	median development time (days) (qd;n)	median adult female longevity (days) (qd;n)	median fecundity (qd;n)	r_m ($days^{-1}$)
Birch	8.4(0.3;41)*	33(8;23)*	156 (56;23)*	0.214
Common cattail	8.9(0.4;34)*	26 (3;18)	135 (35;18)	0.208
Cucumber leaf (control)	8.9 (0.5;57)	24 (9;32)	64 (9;32)	0.175
Rosebay (bee-collected)	8.9 (0.6;47)	22 (7;15)	178 (62;15)*	0.224
Rosebay (hand-collected)	8.6(0.6;48)*	27 (8;28)	99 (52;28)	0.206
Hazel	8.3(0.5;37)*	37(9;23)*	170 (23;23)*	0.226
Milk powder/yeast mix	9.4 (0.7;46)	14(6;19)*	29 (56;19)	0.151
Pine	8.0(0.7;39)*	28 (6;19)	252 (64;19)*	0.256

considerably. The fecundity was significantly higher than the control for birch, fireweed bee collected, hazel and pine pollen (table 1). A reduced development time, increased longevity and fecundity was also found when *F. occidentalis* was offered cotton and sweet pepper pollen (Trichilo & Leigh, 1988; van Rijn & Sabelis, 1993).

The intrinsic rate of increase shows that pine pollen have the biggest impact on the thrips. For the mix of milk powder and yeast the intrinsic rate of increase is even smaller than when leaf served as food, but this is probably explained by the fast moulding of the food source as observed during the experiment.

Thrips oviposition rate on other potential alternative food sources

The increase of the oviposition rate of the thrips by 69 and 50% with the *E. kuehniella* eggs and the entomophage diet as the food source, respectively, is comparable to the stimulating effect of hazel and rosebay willowherb hand-collected pollen (72 and 37% increase, respectively). Earlier Trichillio & Leigh (1988) showed that the Western Flower Thrips was able to prey upon spider mite eggs. The nutritional effect of the spider mite eggs was lower than that of cotton pollen.

Table 2. Mean oviposition rate of *F.occidentalis with* food sources applied to a cucumber leaf (cv. ' Jessica') at 25 °C, 16L:8D. n=15 for each treatment. p is the statistical difference in comparison to the control (Generalised linear mixed model for repeated measurement).

	day 2	day 3	
Treatment	mean (±s.d.)	mean (±s.d.)	p
Cucumber leaf	4.9 (2.8)	5.6 (3.0)	
Ephestia eggs	9.1 (3.8)	8.6 (5.3)	0.028
Entomophage diet	7.6 (2.9)	8.1 (2.7)	0.025

All alternative food sources tested, except the mixture of powdery milk and yeast, had a positive effect on the thrips. This should be taken into account when choosing additional food sources for predators, since the range of different pollen species that are suitable as food for predators seems to be more narrow than that of thrips (van Rijn & Takabashi, 1999).

The impact of the alternative food sources on the population growth rate of thrips in this study was calculated in the absence of predators. The potential benefit for the pest might not be as pronounced when predation is included as a factor affecting the pest's population growth. Our recent studies with *Orius laevigatus* showed that the bugs prefer thrips over sweet pepper pollen in a two-choice situation. Compared to leaves with pollen, starved bugs even preferred to stay on leaves showing mere thrips damage (Hulshof & Jurchenko, 1999). However, van Rijn and Sabelis (1993) showed that the thrips predation rate of *Neioseiulus cucumeris* was reduced when ample pollen were present. Therefore, our further studies with *O. laevigatus* aim at revealing whether attraction of pest and predator to the same food source really results in enhanced predation.

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Scatella stagnalis Fallen (Diptera: Ephydridae): Towards IPM in protected lettuce crops.

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Abstract: The development of IPM against the major pests of protected lettuce will eliminate routine use of insecticides against aphids and caterpillars but allow other pests such as *Scatella stagnalis* (shore flies) to survive. *Scatella stagnalis* do not damage lettuces directly but their presence on foliage at harvest may result in the rejection of whole batches of produce. Both adults and larvae feed on green algae growing on the soil surface, particularly in non-cropped areas such as paths in growing beds and around the periphery of the crop. The growth of algae and development of flies may be partially controlled by minimising the quantity of water applied but additional action is often required in the insect's main feeding and breeding sites. These studies demonstrated that IPM of *S. stagnalis* may be based upon the reduction of the insect's food source by applying the algaecide, quinoclamine, in non-cropped areas of the glasshouse. In more difficult situations, the treatment may be supplemented by applications of the insect growth regulator, teflubenzuron, or the entomopathogenic fungus, *Beauveria bassiana*. Physical removal by suction of adult flies from lettuce heads just before harvest can also contribute to the overall control of *S. stagnalis*.

Key words: *Scatella stagnalis*, IPM, *Beauveria bassiana*, quinoclamine, teflubenzuron, lettuce.

Introduction

Growers of protected lettuce crops in the UK have traditionally depended on routine, and often intensive, applications of insecticides to control their major pests but are now under pressure from food retailers to reduce this pesticide usage. The development of Integrated Pest Management (IPM) for protected lettuce presents a formidable challenge due to sudden invasions of aphids and moths that are difficult to combat biologically, and to the customer's low tolerance for insects on produce. Studies have shown that pest invasion can be much reduced by screening glasshouses (Jacobson, unpublished) and this could form the basis of an IPM strategy. However, in the absence of routine insecticide usage, *Scatella stagnalis* Fallen (shore flies) survive and large populations develop. Although *S. stagnalis* do not damage plants directly, their presence on lettuce heads at harvest causes marketing difficulties, and sometimes results in the rejection of whole batches of produce. Furthermore, *S. stagnalis* can spread root diseases (Goldberg & Stanghellini, 1990). This paper summarises the results of a survey that investigated the activity of *S. stagnalis* in protected lettuce crops, and the potential of control strategies that combine measures to reduce the insect's algae food with other physical and biological control measures.

Materials and methods

Surveys of lettuce crops

Thirty lettuce crops were examined at five sites during 1994 and 1995. Soil type, soil moisture retention, growth of algae, species of algae and size of *S. stagnalis* infestation were recorded. In

addition, growers were questioned regarding their watering and crop nutrition regimes, and the history of *S. stagnalis* infestations on their nurseries.

Control of S. stagnalis – preliminary bioassays

Many potentially useful products, including algaecides, biological control agents and insect growth regulators, were screened using a simple bioassay. Algae was grown on rockwool sheets, soaked in liquid plant feed (Miracle-Gro), and housed in cages in a controlled environment room (21 ± 2°C, 16L:8D). Treatments were applied to the rockwool surface. Twenty adult *S. stagnalis* of known age were released in each cage and allowed to lay eggs for two days. The success of each treatment was quantified by the number of adult flies produced 14 days after the end of the egg laying period.

In a separate study, the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Naturalis-L), was applied at rates equivalent to 6.7ml product/1.7 litre water/m^2 to batches of 60 second/third instar *S. stagnalis* larvae on algae covered rockwool. Samples were taken from each spray mixture and numbers of viable spores determined by culturing on growth media. The number of emerging adults were recorded and compared to untreated controls.

Control of S. stagnalis – crop scale experiments

Some promising control measures identified by bioassay were evaluated in two experiments in autumn 1996 and summer 1997. In each experiment, 500m^2 lettuce crops were grown according to normal commercial practice with water applied evenly through overhead irrigation lines. Each plot was the width of a lettuce bed (approximately 3m) with a typical longitudinal access path. The plants were artificially infested with *S. stagnalis*.

The first experiment evaluated the efficacy of seven control strategies, based on various combinations of two algaecides (quinoclamine [Mogeton] and benzalkonium chloride [Fargro Algaecide]) and an insect growth regulator (teflubenzuron [Nemolt]), relative to an untreated control (Table 1). There were four main treatments; quinoclamine applied to the whole growing bed one day before planting at 14g product/litre water/10m^2, benzalkonium chloride applied to the whole bed one day before planting at 5ml product/0.5l water/10m^2, teflubenzuron applied to the path four weeks before harvest at 3.5ml/5l water/10m^2 and the untreated control. Each main treatment was applied with and without an additional application of teflubenzuron to the path two weeks before harvest, in a split plot experiment in four replicate blocks. Numbers of adult *S. stagnalis* were assessed at harvest using four sticky traps laid on the path in each sub-plot. Each sub-plot measured 3 x 3.8m.

The second experiment evaluated the efficacy of three control strategies based on applications of quinoclamine and physical removal of adult flies using a Dietrich Vacuum Insect Net (Table 2). Quinoclamine was applied either to whole beds before planting or to paths at the rate of 14g product/litre water/10m^2. Numbers of adult *S. stagnalis* were assessed at harvest using seven sticky traps laid on the path in each plot. The experiment was a randomised complete block with four replicates. Each plot measured 3 x 12m.

Data for both experiments were analysed by analysis of variance; a square root transformation was used in the second experiment to stabilise variances.

Results and Discussion

Survey results

Scatella stagnalis infestations were associated with growth of unbranched filamentous green algae, usually dominated by *Hormidium* spp. and *Ulothrix* spp., which provided food for both

adult flies and larvae. Numbers of *S. stagnalis* were roughly in proportion to the moisture content of the soil surface and growth of algae, ranging from less than one fly per 20 plants at the driest site to over 20 per plant at the wettest site. All the growers watered and applied fertiliser to their crops by overhead irrigation. Surface wetness was affected by the quantity of water applied and soil moisture retention. Lighter soils drained freely, so that water infiltrated quickly and the surface remained quite dry, while heavier soils retained water for longer, allowing algae to colonise the surface and *S. stagnalis* to breed. Improvements were made by minimising the quantity of water applied but there remained some moist patches in all greenhouses regardless of the soil characteristics. For example, the narrow compacted paths in the crop beds and the uncropped strips around the periphery of the crop always had growth of algae and were the main *S. stagnalis* breeding sites.

Preliminary bioassays

The most promising control measures identified by biosassay were the algaecides, quinoclamine and benzalkonium chloride, and the growth regulator, teflubenzuron. All three were further evaluated in crop scale experiments.

In the separate bioassay with *B. bassiana* (3.6×10^7 viable spores/ml), numbers of *S. stagnalis* were reduced to approximately 20% of the untreated controls. This treatment seems to have considerable potential and will be further evaluated.

The parasitic nematode, *Steinernema feltiae* Filipjev, was shown to invade and kill *S. stagnalis* larvae and to produce hundreds of progeny within the dead insects. However, the juveniles did not appear to be released in their infective stage and the overall control of *S. stagnalis* was poor. The predatory mites, *Hypoaspis miles* Berlese and *Hypoaspis aculeifer* Canestrini, fed on *S. stagnalis* larvae in Petri dishes but did not significantly reduce populations of the flies in bioassays. The results with these three biological control agents are consistent with reports by Lindquist *et al.* (1994).

Evaluation of control strategies

The most successful treatment in the 1996 experiment (Table 1) was the pre-planting application of quinoclamine to the whole crop bed. This reduced growth of algae, thus offering fewer opportunities for *S. stagnalis* to feed and lay eggs. Numbers of flies in these plots were reduced to 32% of untreated controls ($P < 0.05$). No benefit was found in combining such treatments with an application of teflubenzuron to the path 2 weeks before harvest. However, two sprays of teflubenzuron, 2 and 4 weeks before harvest, reduced numbers of flies to 50% of untreated plots ($P < 0.05$). Benzalkonium chloride used alone or in combination with a single application of teflubenzuron, did not significantly reduce numbers of *S. stagnalis* compared to untreated plots.

At over £500/ha, the cost of quinoclamine applied to the entire soil surface proved to be prohibitively expensive. However, an application to the main *S. stagnalis* breeding sites (*i.e.* paths in crop beds and the periphery of the crop) was £75/ha, which was acceptable to growers and formed the basis of the control strategies evaluated subsequently.

In the 1997 experiment, there were significantly fewer *S. stagnalis* in the three quinoclamine treatments than in the untreated controls ($P < 0.05$) (Table 2). Where quinoclamine applications were restricted to paths, the numbers of flies were reduced to 50% of untreated plots. This was not significantly improved by the additional pre-planting treatment. Physical removal of adult flies by suction combined with applications of quinoclamine to paths, reduced *S. stagnalis* numbers to 32% of untreated plots but this additional control was not significant. However, this physical method could be modified to provide better results and should be further evaluated.

IPM of *S. stagnalis* in protected lettuce crops could be based upon quinoclamine applied to non-cropped areas but the product must not be applied directly on to plants as it can be phytotoxic. This is broadly consistent with studies that have since showed that shore fly numbers could be reduced on cucumber seedlings by controlling algae with hydrogen peroxide (Vanninen & Koskula, 1998). In some situations the use of quinoclamine would have to be supplemented by physical removal of flies at harvest or additional treatments with teflubenzuron or *B. bassiana*. Like all IPM, these methods will require careful management and fine-tuning to suit individual production systems.

Table 1. Mean numbers of *Scatella stagnalis* adults recorded per sub-plot in each of seven treatment programmes in autumn 1996.

Main treatment	Mean numbers of flies in sub-plot treatments:	
	no additional teflubenzuron	teflubenzuron 2 wks pre-harvest
1. Untreated control	452	346
2. Quinoclamine pre-planting	147	170
3. Benz. chloride pre-planting	443	399
4. Teflubenzuron 4 wks pre-harvest	342	224
LSDs: 191 (12 df) for comparisons of sub-plots from the same main plot; 221 (17 df) otherwise		

Table 2. Mean numbers of *Scatella stagnalis* adults recorded per sub-plot in each of three treatment programmes in summer 1997.

Treatments applied	Mean number of flies per plot	Square root transformation of mean
1. Untreated control.	181	12.72
2. Quinoclamine to whole bed pre-planting.	78	8.24
3. Quinoclamine to paths pre-plant plus 10 & 20 d pre-harvest.	90	9.43
4. Treatment 3 plus physical removal of flies 5 & 10 d pre-harvest.	58	7.42
LSD (9 df)		2.31

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Biological Control of aphids in ornamentals: importance of plant quality.

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Introduction

Aphids are among the most important pests in commercial greenhouses today and ornamental growers use a wide variety of chemical insecticides to protect their crops from damage. One natural step in our endeavour to move towards sustainable growing systems would be to substitute insecticides with biological control. However, in spite of several years of intensive work with biological control the use of natural enemies in commercial greenhouses has not been an unequivocal success. Many growers still rely mainly on chemical pesticides to control aphids.

One possible reason for the poor results of biological control of aphids in some cases could be the influence of plant quality (such as water and nutrition supply) on the aphids and their natural enemies. If this is the case then it is also a factor that the grower can easily control in a modern greenhouse. Our aim is to evaluate the influence of plant fertilisation on the success of biological control of aphids in ornamentals.

Influence of the plant quality on the aphid

Almost 50 years ago Leonard Haseman (1950) urged applied entomologists to study how plant quality influences the risk of pest attack. He mentioned fertilisation as one factor that can influence plants and through them herbivores. Since then many studies have been conducted to evaluate the influence of nutrition on growth rate and reproduction of herbivorous insects (Summarys in Larsson 1989, Waring & Cobb 1992 and Koricheva et al. 1998). There is substantial consensus about the importance of nitrogen on herbivore performance (Mattson 1980, Scriber & Slansky 1981).

Aphids sucking directly from the phloem sap are considered to be particularly influenced by the plant condition. However, in spite of many studies on aphid reproduction and growth no unambiguous relations have been confirmed. Van Emden (1966) concluded that in 41 % of the studies he reviewed aphids responded positively to nitrogen fertilisation, in 36 % they responded negatively and in 23 % there was no response. In a later review Waring & Cobb (1992) showed that in approximately 55 % of the studies the response was positive to nitrogen fertilisation and in 25 % there was no response. High levels of potassium also influenced aphid reproduction, in most cases negatively (50% in van Emden , 40% in Waring & Cobb) or not at all (25% in van Emden, 45% in Waring & Cobb). One reason for the different results may well be that the relation between fertilisation and aphid growth may not be linear.

Influence of the plant quality on the natural enemies

Natural enemies can also be influenced by the condition of the host plant via the herbivore. Variation in the plant's nutrient content and chemical composition can be of great importance to the reproduction and growth of natural enemies, especially parasitoids (Fox et al. 1990).

Parasitation of a whitefly (*Bemisia argentifolii*), also with sucking mouthpaths, was higher on plants without extra fertilisation (Bents et al. 1996).We do not know of any studies where the importance of plant nutrition has been studied on aphid parasitoids. Studies of effects of other plant qualities on aphids have been done however. Russian wheat aphids, and also their parasitoids, were smaller on resistant compared to susceptible wheat cultivars (Reed et al. 1992). Differences in secondary metabolites (hydroxamic acids) in different cultivars of cereals had only a marginal effect on two different parasitoids of cereal aphids (Fuentes-Contreras et al. 1996).

In conclusion it is known that aphids are affected by stress and the quality of their host plants. However, it is not quite clear how a certain change in quality will act on the aphids. The response of parasitoids of aphids has hardly been studied at all.

A system to study the interactions between plant-aphid-parasitoid

We are using the parasitoid wasp *Aphidius ervi* and the aphid *Macrosiphum euphorbiae* on Petunia x hybrida Grandiflora as a model system to study the effects of fertilisation on the performance of an aphid and its parasitoid. We chose these insects because *Macrosiphum euphorbiae* is one of the most common species in ornamentals in greenhouses and *Aphidius ervi* is a wasp that is commercially available to the grower for controlling this aphid. We are growing the plants in the greenhouse at approximately 22°C and water the plants with different levels of fertilisation (low fertilisation, high with low nitrogen content and high with high nitrogen content) and study how the aphids and the parasitoids perform. We keep the aphids in cages on leaves of approximately the same age and study their performance for two generations, measuring development time, reproduction rate and size.

Both development time and reproduction rate are key-factors for the performance of aphids. The size of the adult aphid, measured as dry mass can be a good estimate of it's potential fecundity (Stadler & Mackauer 1996).

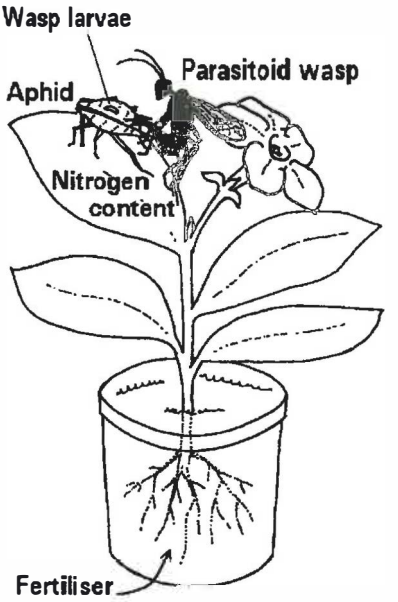

Figure 1. Schematic picture of the plant-aphid-parasitoid system.

We plan to let mated parasitoid females parasitise aphids of known size and study the development of their offspring on plants fertilised as described above. We will measure their development time, reproduction rate, size and sex ratio.

With help of the results we hope to be able to make predictions as to how fertilisation of the plant influences the success of the use of *Aphidius ervi* for biological control of *Macrosiphum euphorbiae.*

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Biological Control of Tomato Pests in the Netherlands

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Abstract: In 1996 a group of Dutch growers initiated an experiment growing insecticide-free tomatoes. After three years of experience we have drawn up the balance sheet. Most pests can be sufficiently controlled by natural enemies. The trend towards lower temperatures in the greenhouse, however, will make the biological control of whitefly more difficult. Research resulted in an *E. formosa* strain better adapted to lower temperatures. Spider mite is the biggest problem and is not sufficiently controlled by *Phytoseiulus persimilis*, so chemical intervention is sometimes necessary. This, together with the lack of interest of the market to pay higher prices for an insecticide-free product resulted in a setback of the experimental project. In 1998 not a single grower reached the end of the crop cycle without using a certain amount of insecticides.

Key words: tomato, the Netherlands, biological control, *Encarsia formosa, Macrolophus caliginosus, Diglyphus isaea, Phytoseiulus persimilis, Pseudococcus affinis*

Introduction

In the Netherlands tomatoes have been produced in greenhouses for around thirty years. Over the years growing techniques, growing conditions and cultivars have changed, and in pest management a great deal of progress has been made. Since the first commercial application of natural enemies in greenhouses in 1968 there was only slow progress, until 1987, when bumblebees replaced hand pollination of the tomato flowers. As a result, growers had to reduce the use of chemical insecticides. Because of the competition from Southern Europe some growers wanted to distinguish their product in the market, and in 1995 decided to start the production of insecticide-free tomatoes which were sold on the vine. In 1996 they began with 145 ha.

Recent developments in the production of tomatoes will have their consequences for

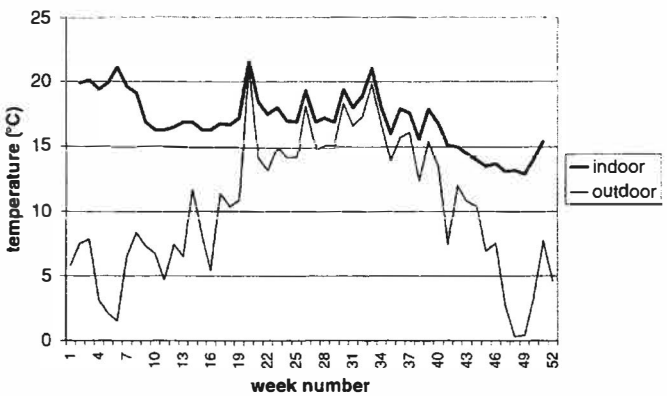

Figure 1 : Average temperatures per week over 1998 for one complete crop cycle of the cultivar 'Tradiro' in a Dutch tomato greenhouse, compared to outdoor temperature

the use of natural enemies against pests. New cultivars require a lower temperature in the greenhouses for maximum production yields, which for certain cultivars drops down to an average day temperature of around 16 °C in spring (see fig.1). Not all natural enemies are adapted to these low temperatures. There is a trend to remove the lower leaves of the plants, in order to obtain a higher production of tomatoes per plant. This is also labour saving when harvesting the vine ripe tomatoes. However important leaf picking is for quantity, this practice is hindering the biological control as it gives natural enemies, especially whitefly parasitoids no chance to build up a population. Furthermore many growers export their tomatoes to countries with very strict import restrictions. For some pests and also beneficials there is a zero-tolerance. This is especially a problem when tomatoes are sold on the vine, as there is a higher chance then that there are some insects remaining.

The most important pests in tomato are whitefly, leaf miner and spider mite. Aphids are an increasing problem and caterpillars and mealybugs are also occurring. An overview will be given below of the whitefly, leaf miner, spider mite and aphid control in the recent years.

Whitefly control

Tomato is a very good host plant for the greenhouse whitefly *Trialeurodes vaporariorum,* a major pest in tomato. Although expected, so far there has not been an outbreak of the tobacco whitefly *Bemisia tabaci* in the Netherlands. Since 1972 the parasitic wasp *Encarsia formosa* has been used for the control of the greenhouse whitefly. After the first problems with the occurrence of *Bemisia tabaci* in 1993, the search for more effective natural enemies against this pest resulted in the development of the application of the predatory bug *Macrolophus caliginosus* and the parasitic wasp *Eretmocerus eremicus.* With this complex of natural enemies whitefly can be completely controlled. However, as a result of the trend to grow tomatoes at lower temperatures and to early remove the lower leaves, the biological control, especially early in the season, is becoming more and more difficult.

The development of the natural enemies at low temperatures is very slow. Drost et al. (1996) found a development time of 48.3 days for *E. formosa* and 58.3 days for *Eretmocerus sp.* at 15 °C on *B. tabaci.* In many cases leaves are removed prior to completion of the development of the parasitoid. Leaves are left on the floor or even removed from the greenhouse. As a result only a small percentage of the parasitised pupae will emerge. A new strain of *E. formosa*, better adapted to lower temperature is in research. It has been determined already the fecundity of this strain is higher than the fecundity of the traditional strain, both at 18 °C and 22 °C. At this moment the behaviour of both strains is being analysed with digital imige analysis techniques, comparing walking speed and turning rate of the two strains.

The development time of *M. caliginosus* at 15 °C is 57.8 days (Fauvel, 1987). An early introduction is necessary, as at least two generations are required before numbers are high enough in the greenhouse. At low temperatures this will take 3 – 4 months. However, although *M. caliginosus* is able to survive host-free periods, it will not reproduce without sufficient whitefly. This can be overcome by releasing eggs of the flour moth, *Ephestia kuehniella* until the first whitefly is observed (Van Schelt *et al.*, 1997).

Another phenomenen is influencing whitefly control. *M. caliginosus* is very sensitive to infection by a fungus from the group Entomophtorales. This is mainly occurring during summer when the density of *M. caliginosus* is high. It can be a disaster for whitefly control as the population of *M. caliginosus* can collapse completely and chemical intervention is sometimes required.The situation is not clear yet and further research is necessary.

Leaf miner control

Leaf miner is one of the main pests in tomato, especially the tomato leaf miner *Lyriomyza bryoniae*. *L.huidobrensis* and *L.trifolii* are also occurring. Leaf miners have been controlled for many years by the parasitic wasps *Dacnusa sibirica* and *Diglyphus isaea*. For a long time it was believed that *D. isaea* could not be applied early in the season, as it is more adapted to higher temperatures and a high density of leaf miner larvae.

Exported tomatoes on the vine are in some countries rejected when leaf miner pupae are present. Growers were thus forced to find a solution and reduce the number of pupae on the tomatoes without the use of chemicals. Introduction of a higher rate of *D. isaea* earlier in the season can solve the problem, as this parasitic wasp kills leaf miner larvae either by egg laying or host feeding and leaf miner larvae will not pupate anymore. *D. isaea* is now used more frequently early in the season, as soon as there is a sufficient number of leaf miner larvae present (1 per 1 – 10 plants), and this appears to be successful as the parasitic wasp builds up a population sooner. Research of Sampson and Walker (1998) confirmed this idea, as they proved *D. isaea* will establish during winter, providing there are sufficient hosts.

The parasitic wasp *Opius pallipes* is indigenous to the Netherlands and is regularly found in greenhouses spontaneously, giving a good contribution to leaf miner control.

M. caliginosus also plays a role in leaf miner control. Once there is a high population of the predator bug present in the greenhouse in summer, and whitefly is under control the bugs predate on leaf miner larvae as well. As leaf miner larvae are also killed by host feeding of *D. isaea* it is difficult to determine the exact contribution of predation by *M. caliginosus* in the field.

Spider mite control

The two-spotted spider mite *Tetranychus urticae* is still one of the most difficult pests to control with natural enemies in tomato. Predatory mites such as *Phytoseiulus persimilis*, though very successful in other crops, cannot keep the spider mite under control because their mobility is hampered by the trichome exudates on the tomato leaves and stems. These exudates are also toxic for many natural enemies. Drukker *et al.* (1996) found that *P. persimilis* reared on tomato give better results in tomato than do predatory mites reared on beans. This has been proved by field trials (Van Schelt and Altena, 1997).

In some situations, spider mite control is supported by spontaneously occurring larvae of the gall midge *Feltiella acarisuga*. Especially later in the season they can be present in high numbers and contribute to spider mite control. However, trials with commercially reared *F. acarisuga* showed varying results. Further research is necessary to fully understand the possibilities and limitations of this beneficial. *M. caliginosus* will also predate on spider mite when the density of whitefly is low, thus contributing to spider mite control as well.

Despite the use of tomato adapted *P. persimilis* and the occurrence of *F. acarisuga* during the last seasons many growers had to spray chemicals against spider mite as control by natural enemies was not sufficient. As selective acaricides were used, this did not affect the activity of other natural enemies. At this moment possibilities for the application of *Amblyseius californicus* in tomato are in research.

Aphid control

There is only one species of aphids causing problems in tomato: *Macrosiphum euphorbiae*, the potato aphid. Control by the parasitic wasp *Aphidius ervi* can be successful, but must be initiated preventively as an infestation by *M. euphorbiae* can increase rapidly when the

release of parasitoids is started too late. A good method for application of *A. ervi* is the banker plant system, which makes use of the grain aphid *Sitobion avenae* on barley. Additionally, *Aphidoletes aphidimyza* is applied when the density of aphids is high or when hyperparasitoids hinder the reproduction of the parasitic wasp.

Caterpillar control

The chief moth pest in tomato is the golden twin spot moth *Chrysodeixis chalcites*. Occasionally, the tomato moth *Lacanobia oleraceae* occurs. Small instars are controlled by spraying or dusting *Bacillus thuringiensis*. In greenhouses where *M. caliginosus* is present, its predation of eggs and small instars contributes to caterpillar control.

Mealybug control

For some years the mealybug *Pseudoccus affinis* has been causing problems in tomato.These mealybugs always occur spotwise on the lower stem parts of tomatoes, appearing each year in the same place. The ladybird beetle *Cryptolaemus montrouzieri* was tested to control the mealybug without success, as the adults failed to remain in the lower parts of the plants. Growers now try to reduce the damage by mechanically removing the mealybugs, or killing them by gently burning the stems with a flamer. Chemical control is hindered by the waxy coverings of the mealybugs.

Conclusion

Production methods of protected tomato crops have changed, as a result of which biological control has had to evolve with it. Although different developments are slowing the biological control of pests in tomatoes, most problems can be overcome. Some chemical intervention is necessary when the population of *M. caliginosus* collapses due to infestation by Entomophtorales. Spider mite is still causing serious problems, and can also be reason for chemical intervention, but it is a matter of tolerance as well. Results elsewhere moreover, have proved that when growers are willing to tolerate a higher number of certain pests, intervening with chemicals is not necessary.

Market factors also play a role: The vast majority of consumers do not seem to be ready yet to pay extra for an insecticide-free product, thus decreasing the motivation of some growers to avoid the use of chemicals as much as possible. Nevertheless, despite the need for some insecticide use, the complete acreage of 1200 ha. of protected tomatoes in the Netherlands is using IPM, and growers are still committed to it.

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The role of *Macrolophus caliginosus* (Het. : Miridae) in controlling the two-spotted spider mite in greenhouse tomato under North-European conditions

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Abstract: In a study conducted in 13 commercial tomato greenhouses in Finland, *M. caliginosus* was used in combination with *Phytoseiulus persimilis* to study the potential contribution of the bugs to the control of spider mites (*Tetranychus urticae*) in tomato. The bugs (1.2-1.6 per m^2) were applied in one, two or three batches in February-March 1997. Predator and pest numbers were recorded from leaf samples every two weeks starting in April and ending in September. In the end, there were hardly any spider mites left in the ten greenhouses where the two predator species were used in combination. In the three greenhouses where *P. persimilis* was used alone, the predatory mites suppressed the pest population to a low level by July, too, after which pest numbers re-increased until in September the plants were heavily infested again. The numbers of *M. caliginosus* began to increase notably only from the end of May onwards. The application of *Ephestia kuehniella* eggs in February-April, to feed the predators after their release, could not be shown to be connected with increased predator or decreased pest numbers in May-September.

Key words: biological control, *Ephestia kuehniella* eggs, Finland, *Phytoseiulus persimilis, Tetranychus urticae*

Introduction

The predatory bug *Macrolophus caliginosus* Wagner (Het.: Miridae) is known to develop and reproduce on whiteflies, thrips, aphids, and butterfly eggs and larvae (Alvardo et al. 1997; Barnadas et al. 1998, Bérengère et al. 1996; Schelt et al. 1997). Some reports exist on the side-effects of *M. caliginosus* against the two-spotted spider mite (*Tetranycus urticae*) when using the bugs for controlling whiteflies, suggesting that the predator could contribute to spider mite control (Sampson & King 1996; Schelt & Altena 1997). However, experimental studies on the feasibility of using the bugs as an additional biocontrol agent of spider mites in tomato and on their population dynamics under North European conditions were lacking.

Finnish tomato growers use either the "tomato" or the "cucumber" strain of *Phytoseiulus persimilis (Pp)* to control the two-spotted spider mite, but the control efficacy is often unsatisfactory. In this study, *M. caliginosus (Mc)*, used in combination with *P. persimilis*, was tested as a preventative method for spider mite control under Finnish conditions. Simultaneously, the establishment and population growth of the bugs in the absence and presence of additional food, the eggs of *Ephestia kuehniella (Ek)*, was studied to get more insight into the importance of this food source in practical conditions. According to Cocuzza et al. (1997) and Vacante et al. (1997), the eggs support the development and reproduction of *Orius* spp. Experiences with *M. caliginosus* are contradictory. Schelt & Altena (1997) reported that the population of *M. caliginosus* grew faster when *E. kuehniella* eggs are applied, but some producers of beneficials claim that supplementary food is not necessary for good establishment of *M. caliginosus*.

Material and Methods:

Eight tomato growers, with a total number of 13 rooms (size 600-2500 m^2), participated in the trial in the Närpiö area, South Ostrobothnia, western coast of Finland, in 1997. There were three treatments: 1) *P. persimilis* (total of 18.8/m^2, applied in several batches between March and May; three rooms until week 29, after that 2 rooms, as one grower started chemical treatments); 2) *M. caliginosus* (1.2/m^2) + *P. persimilis* (11/m^2) (four rooms); 3) *M. caliginosus* (1.6/m^2) + *E. kuhniella* eggs + *P. persimilis* (17.2/m^2) (six rooms). The releases of predatory mites were started when the first spider mites were detected, and continued until May at three to five weeks intervals. The bugs were released in one, two or three batches in February-March. Eggs of *E. kuehniella* were applied in the crop at the rate of 10-20 g per 600-1070 m^2 (depending on the size of the room) 2-5 times between February and March (but one grower applied a batch still in June).

Control efficacy was monitored by taking leaf samples every two weeks from April (week 17) to September (week 39) 1997. After the appearance of spider mites, ten infested plants at maximum were assigned as sampling units in every room (in some rooms less than 10 plants were eventually infested). At sampling, the number of *M. caliginosus* nymphs and adults was first counted from one leaf in the top, middle and lower part of the follow-up plants, i.e. from a maximum of 30 leaves per room. These leaves were then detached and taken to the laboratory, where the number of eggs and moving stages of spider mites and *P. persimilis* were counted under the microscope. If the number of either eggs or moving stages of spider mites exceeded 150, this was used as the maximum number. The difference between treatments in spider mite numbers in the 10 sampling weeks was analyzed with the non-parametric median test and those for *M. caliginosus* numbers with the Kruskal-Wallis non-parametric test, using SAS (SAS Institute Inc., 1989).

Results and discussion:

Macrolophus caliginosus established in all the 10 rooms where the bugs were released. In two rooms not infested with spider mites or other pests at all (not included in the analysis of the present results), the bugs also established and multiplied equally well. At 22°C, the development from egg to egg of *M. caliginosus* takes 35-40 days (Bérengère et al. 1996). In Finnish conditions, bug numbers began to increase notably only from the end of May onwards (week 23) (Fig. 1), coinciding with warming up of the weather and the increase in pest numbers (Fig. 2). Peak densities were reached at the turn of August and September (week 33-37).

In all sampling occasions except one (week 39), the number of bugs per leaf was similar in rooms with and without the application of *E. kuehniella* eggs; the one time difference in week 39 was in the favour of the treatment without *E. kuehniella* eggs. No damage to the crop was observed with the numbers of *M. caliginosus* (maximum mean number four per leaf) recorded in this study in any of the experimental greenhouses.

The lowest number of pests was observed in rooms with treatment 2 (*Pp+Mc*), and the highest in rooms of treatment 1 (*Pp*) as well as in rooms with treatment 3 (*Pp+Mc+Ek* eggs). In all treatments, pest numbers were brought to low level by July (week 29) However, in the rooms where *P. persimilis* was used as the only control agent, pest numbers began to increase again from the beginning of August onwards. No reintroductions were made anymore, but there were some predatory mites present in the crop, however they were too slow to respond adequately to increasing pest numbers.

In rooms where *M. caliginosus* was applied, the initial and peak median pest densities were either significantly lower or there was a tendency of the peak median pest densities to occur later in the summer than in rooms where only *P. persimilis* was used (Fig. 2). Furthermore, no re-increase took place in the end part of the season. As in these rooms the predatory mites practically disappeared (data not shown) from the crop after July, while the numbers of *M. caliginosus* continued to increase, the bugs were very likely solely responsible for preventing the pest from re-increasing during August and September.

The median number of spider mites per leaf in the rooms with *E. kuehniella* eggs was not connected with reduced pest numbers in April-September (Fig. 2). Thus if there was any benefit from feeding the bugs with the eggs during their establishment, it was not detectable any more at the time when pest numbers began to increase. We cannot say if the eggs had an influence on the number of bugs in weeks that followed immediately the release of the bugs and the application of eggs, as leaf sampling was started only in April.

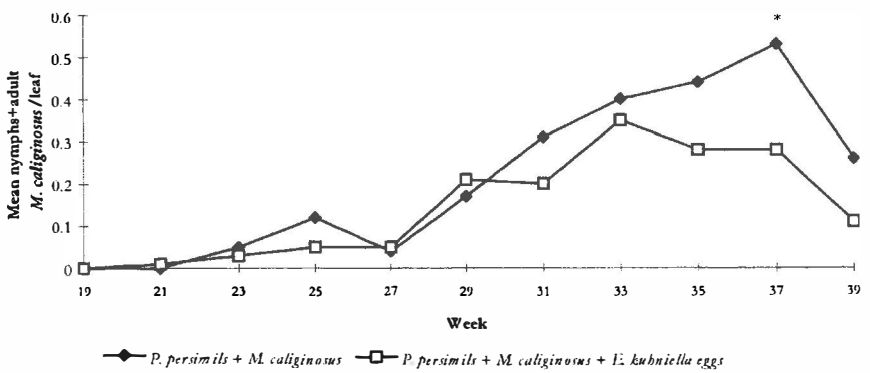

Figure 1. Number of *M. caliginosus* (nymphs plus adults) per tomato leaf in rooms with and without *E. kuehniella* eggs. Values are means from a maximum of 30 leaves per room. The asterisks (*) indicate statistical differences between the treatments (Kruskal-Wallis non-parametric analysis of variance at $P<0.05$).

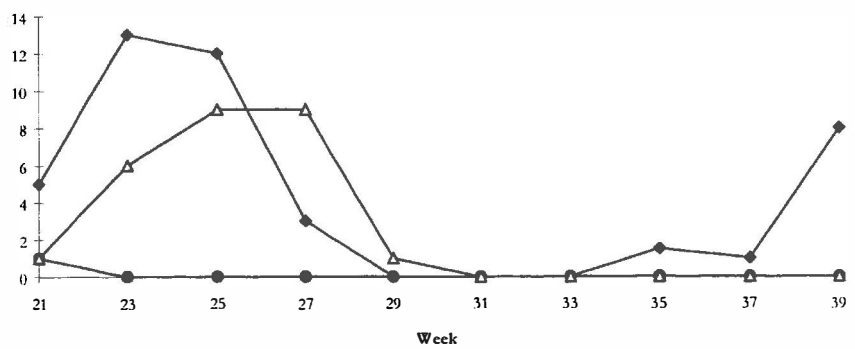

Figure 2. The effect of *P. persimilis* and of the combination of *P. persimilis* and *M. caliginosus* on the number of spider mites in tomato in Finnish tomato greenhouses. Values are medians and are based on a maximum of 30 leaves per room. Treatments marked with same letters do not differ significantly in the respective sampling week (non-parametric median test at $P<0.05$).

The results allow us to conclude that *M. caliginosus* is a good additional control agent when used in combination with *P. persimilis* to control spider mites on tomato under North European conditions. The bugs can enhance spider mite control by delaying the pest's population growth and by lowering the peak number of pests in the crop. As the bugs can build-up their population even in the absence of spider mites and other pests relying on plant material as food, they form a barrier that prevents the pest from re-increasing or reappearing in the end of the growing season. This is important when aiming at spider mite-free start of the crop in the next season, and forms an additional benefit that compensates for the initial slow population build-up of the predator.

The benefits of feeding the bugs with *E. kuehniella* eggs were not discernible in this experiment. The effect of an additional food source should be looked in more detail in situations when no natural prey is available at the time when the bugs and the extra food are applied. If no natural prey is available at the time of establishment, the bugs might clearly benefit from the additional food source.

Acknowledgments

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Control of two-spotted spider mite with *Amblyseius californicus* (Oud.) on croton.

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Abstract: The phytoseiid predator *Amblyseius californicus* was evaluated as biological control agent of two-spotted spider mite (*Tetranychus urticae* Koch) on croton. Predators were introduced at two different release rates: 1 *A.californicus*/5 *T.urticae* and 1 *A.californicus*/20 *T.urticae*. Both rates of *A.californicus* reduced the number of *T.urticae* to a very low level. Differences in control between the two rates were statistically not significant ($P<0.05$). However, on the majority of experimental plants the young leaves were malformed as a result of spider mite damage. This indicates that preventive release of predators into a glasshouse with croton plants is a necessity.

Key words: croton, *Tetranychus urticae*, *Amblyseius californicus*, biological control

Introduction

Spider mites are among the most serious pests of croton (*Codiaeum variegatum*) plants. At present, predatory mites *Ph.persimilis* and *A.californicus* are advised for biological control of spider mites on foliage plants (Koppert 1996, Piątkowski, 1996). According to our observations, even at relatively low populations spider mites can cause severe injuries which leads up to the defoliation of the plants (Piłko in press). The question arises whether in this situation biological control of spider mites is reliable and the predatory mites introduced into crops can suppress the development of the pest to such an extent that the symptoms of injury will not occur.

Material and methods

Trials were conducted in an experimental glasshouse at the Warsaw Agricultural University. Experiment I was carried out from the beginning of June until the end of October 1997. Four cultivars of potted croton (Golden Sun, Excellent, Norma, Petra) were infested with 5 females of *T.urticae* per leaf. Spider mites used in the experiment originated from the laboratory colony maintained on croton. After 5 weeks, plants were divided into three groups, each consisting of 6 plants of tested cultivars. On two groups of plants predatory mites were released at a ratio of 1 predator :20 *T.urticae* (set 1) or 1 predator : 5 *T.urticae* (set 2). The third group of plants was infested only with spider mites. Number of pests was assessed *in situ* by counting the mites on each plant with a hand lens (10x) at weekly intervals. Observations were conducted during 16 weeks. In order to get information on the long lasting effect of predatory mite presence, additionally in the spring of 1998 from experimental plants leaves were collected (60 leaves /cultivar) and inspected for the presence of mites.

In experiment II, carried out from July 3 until the end of September 1998, the procedure was similar to the previous trial, however with a few modifications. Three cultivars of croton (Excellent, Golden Sun, Norma) were used, being represented by 30 plants of each. Plants

were infested with the same number of *T. urticae* females as in experiment I. *A.californicus* was released at a density of 0.8 predators/leaf one week after the introduction of spider mites. From then during 5 weeks population dynamics of prey and predator were monitored at weekly intervals. The results were elaborated using analysis of variance (ANOVA) procedure.

Results and discussion

Obtained data showed evident differences in the suitability of tested croton cultivars as host plants for *T.urticae* (Fig. 1 & 2). The highest mite population developed on cvs Norma and Petra, whereas cvs Excellent and Golden Sun were found much less suitable ($P < 0.05$). On the latter cultivar in both experiments, mites needed about six weeks to adapt to the host. After that time their population started to build up but did not reach the density comparable to the other tested cultivars. The decrease in spider mite density observed on cvs. Petra and Norma from the beginning of September onwards was a result of plant defoliation.

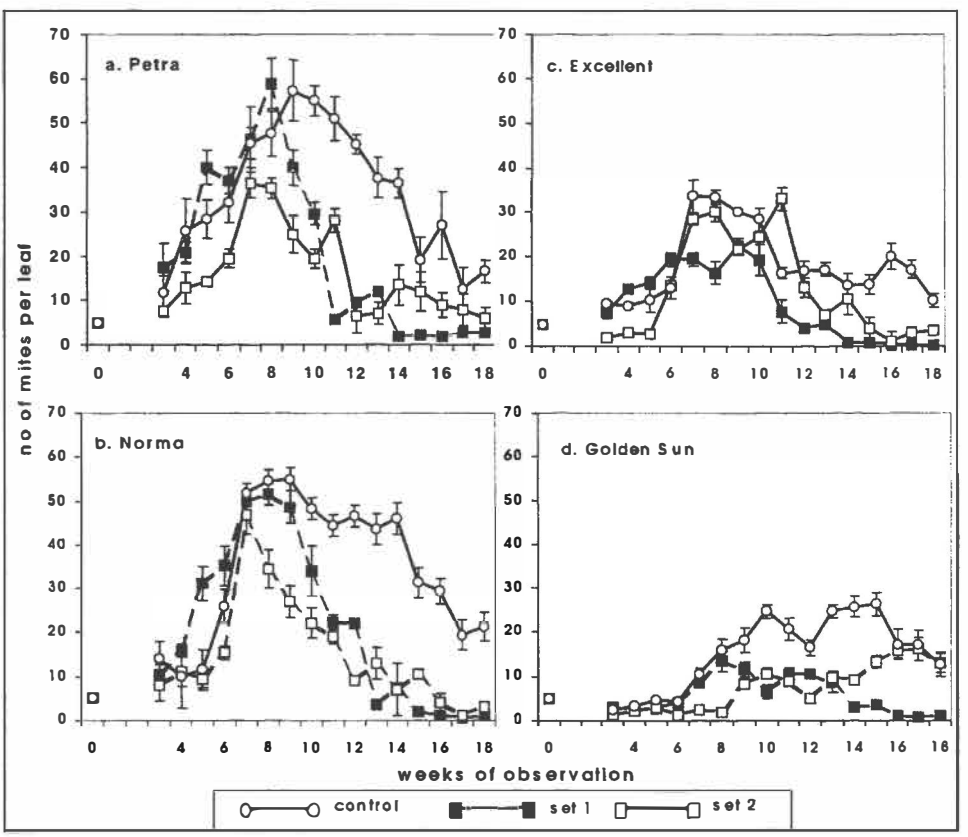

Figure 1. Population dynamics of *T. urticae* after introduction of *A. californicus* on four croton cultivars, 1997.

In the experiment I, effective spider mite control was given by *A. californicus* irrespective of prey predator ratio on cultivars on cvs Norma, Petra and Golden Sun. Pests were adequately controlled within 6 weeks after predator introduction (Fig. 1.). On cv Excellent differences in spider mite density on plants with and without predator were not statistically significant.

Early spring counting showed that the population of *A.californicus* survived during the winter on plants in the glasshouse where temperatures ranged between 8-15°C. The predators were found only on cultivars infested with spider mites (Tab.1).

Table 1. Mean number (± SE) of spider mites and predators on croton leaves after overwintering.

Cultivar	Mean no of *T.urticae*/leaf ±SE	Mean no of *A.californicus* /leaf ±SE
Excellent	7,71 ± 2,209	0,65 ± 0,12
Norma	8,59 ± 2,896	0,29 ± 0,1
Petra	16,03 ± 5,003	0,37 ± 0,08
Golden Sun	0	0

The results of experiment II indicate that the control of spider mite population could be achieved much quicker if a natural enemy was introduced shortly after the occurrence of *T. urticae* and if the initial ratio was 1 predator: 5 prey. On all three tested cultivars the high rate of *A. californicus* maintained the spider mite population on very low level from two weeks after introduction until the end of the season (Fig. 2). Differences between controlled and uncontrolled spider mite population were significant on all tested cultivars ($P < 0.05$).

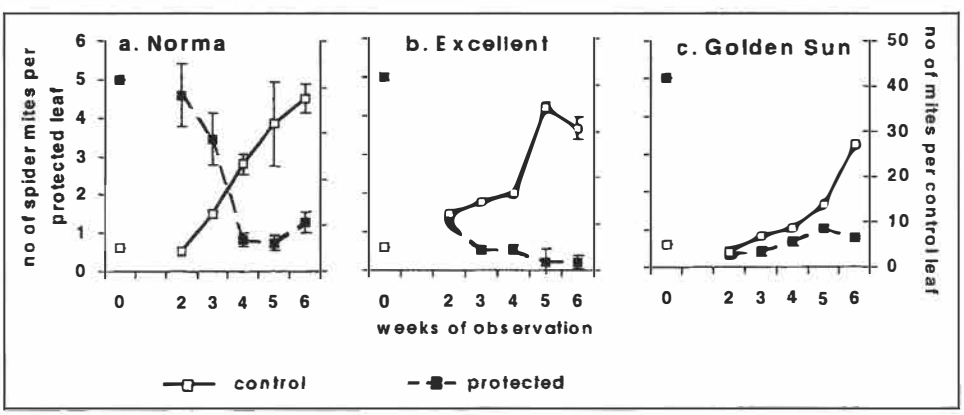

Figure 2. Population dynamics of *T. urticae* after introduction of *A. californicus* on three croton cultivars, 1998.

In all trials the introduction of *A. californicus* prevented the defoliation of the plants. Unfortunately on all plants another type of spider mite damage occurred. We observed that

mites had a tendency to concentrate on the growing tips of croton plants. A few mites feeding on the growing tip caused the malformation of the young leaves. This indicates that the preventive release of predators into the glasshouse with croton plants is a necessity.

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Damage assessment caused by *Frankliniella occidentalis* (Pergande)(Thysanoptera) on strawberry in plastic tunnels in southern Italy

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Abstract : The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is considered a major pest in tunnel-grown strawberries in southern Italy. We monitored the WFT population density and evaluated the harvest wastes of strawberries on a farm in Campania, in order to calculate the economic injury level of the thrips and verify the effectiveness of the control measures. Under the studied conditions, WFT did not reach economic injury level. Based on this, we suggest that 15-20 mobile forms/ flower/week, rather than 8-10/flower/week, represent a more realistic economic threshold.

Key Words : Western flower thrips, economic injury level

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is considered a major pest in tunnel-grown strawberries in southern Italy, including Campania. In particular, in the highly intensive farming system along the coastal areas of this region, the use of commercial cultivars of perpetual fruiting strawberry enables numerous times for harvesting from late fall to spring. Under these conditions, chemical control is very difficult to apply for both economic and ecological reasons.

In the present study, we monitored thrips population densities and evaluated the harvest wastes of strawberries in order to calculate the economic injury level of the thrips and verify the effectiveness of chemical measures.

Material and methods

The study was performed in four commercial plastic tunnels on a farm located at Eboli (SA), during the growing season from November 1995 to June 1996. The tunnels were 30 x 5 m in size in a farm located at Eboli (SA) The commercial cultivars Payaro (tunnels 1 and 4) and Chandler (tunnels 2 and 3) were used and grown according to commercial practices. In tunnels 1 and 2 no insecticide was applied, but only fungicides and acaricides; in addition, in tunnels 3 and 4 , six insecticide applications were also made (Fig. 1).

Relative humidity and temperature were registered every day. The population density of thrips was monitored weekly, using the shaking flower method (Laudonia & Viggiani, 1998) selecting at random one flower every ten plants along one planting in double rows per tunnel.

At harvest, strawberries were classified and quantified in top quality and second best, the latter chosen as hypothetically representative of the harvest waste caused by WFT.

Results and discussion

During the experimental period the daily mean temperatures ranged, in the tunnels, from 2.8°C to 38°C and RH from 31.2 to 99 %. The data on mobile forms recorded by the shaking flower method are represented in fig. 1. The mobile forms/flower were 2.56 and 2.74 respectively on insecticide-sprayed Payaro and Chandler, and 7.40 and 4.22 on insecticide-free Chandler and Payaro.

The highest population density, on average 22 thrips/flower, was recorded on Chandler in tunnel 3 in April-May. Statistical significance was detected only for Chandler (treated and untreated tunnels) by ANOVA ($P \leq 0.05$).

No correlation between the angular values of the percentage of harvest wastes and population density of WFT was detected ($P \leq 0.1$).

Wastes on harvests (as monthly percentage) (Fig.2) were on average 17.29 and 13.89 % for Chandler and Payaro unsprayed, and 16.94 and 13.06 % for their sprayed counterparts.

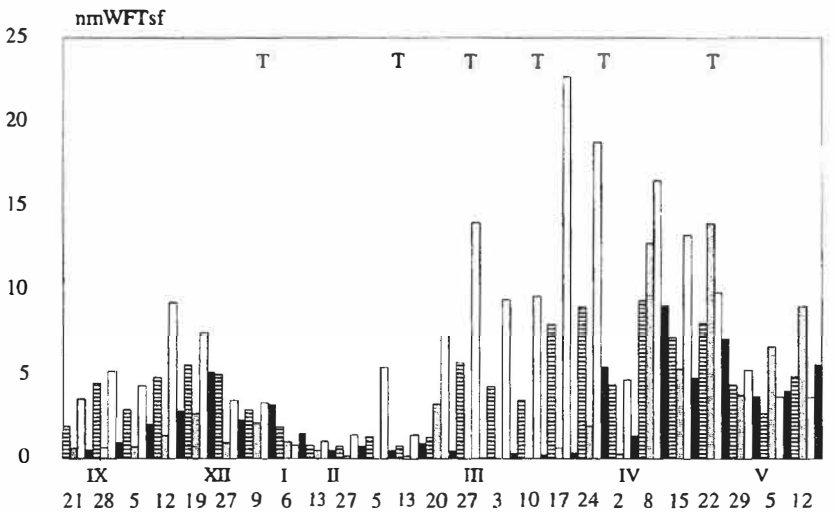

Figure 1. Mobile forms recorded by the shaking flower method (nmWFTsf) in treated and untreated tunnels. T= treatment ; ▤ = tunnel 1 Payaro untreated; =tunnel 4 Payaro treated; ▢ =tunnel 2 Chandler untreated; ■ = tunnel 3 Chandler treated .

There are no statistically significant differences between total production in unit weight, top quality and best second ($P \leq 0.05$).

Economic losses (Tab. 1) were calculated by applying the formula :

$$Pv = [(PrSS \times PIS) - (PrSS \times PSS)]$$, where :

Pv = economic losses ; PrSS= amount of the second best ; PIS = the prices of the top quality ; PSS = the prices of the second best.

Quantities and prices for top quality and second best were supplied by the grower. Moreover, in tunnels 3 and 4 , six insecticide treatments were made, with a global cost of £102,986 ≅ 53€/tunnel.

Tab.1. Values of losses and global costs of chemical treatments

Commercial Cultivar	losses (Italian Lire and Euro)	Global cost of treatments (Italian Lire and Euro)
Payaro untreated Tunnel 1	£ 163,235 ≅ 84.1€	
Chandler untreated Tunnel 2	£ 148,140 ≅ 76.3€	
Chandler treated tunnel 3	£ 141.475 ≅ 72.9€	£ 102,986 ≅ 53€
Payaro treated tunnel 4	£ 153,800 ≅ 79.2€	£ 102,986 ≅ 53€

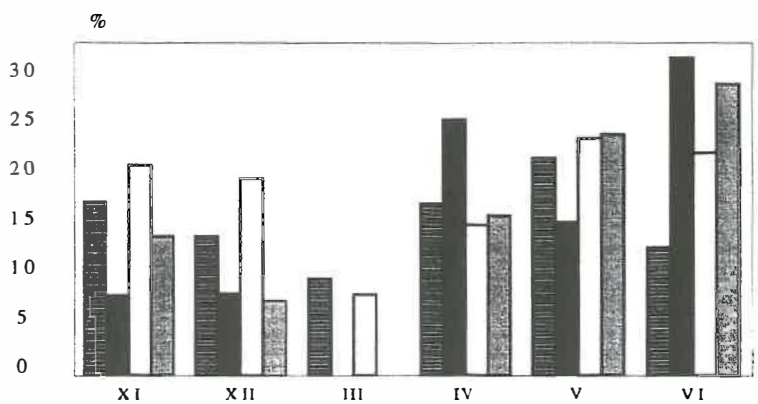

Fig.2. Harvest wastes. = tunnel 1 Payaro untreated; = tunnel 4 Payaro treated; =tunnel 2 Chandler untreated; = tunnel 3 Chandler treted.

In conclusion, the results of the present study show that under the above-mentioned conditions WFT did not reach economic injury level. These data confirm that the number of 15-20 mobile forms/flower/week suggested by Viggiani (1997) reflects a more realistic economic threshold level than 8-10/flower /week (Grasselly, 1995 ; Gremo et al, 1997).

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Biological control of thrips: how far are we?

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Abstract. *Thrips tabaci* Lindeman was the most prevalent thrips pest in Europe, but since its accidental introduction in 1983, western flower thrips (*Frankliniella occidentalis* (Pergande)) has become the number one pest in European greenhouses. Today, Europe is faced with introductions of other serious thrips pests, like *Echinothrips americanus* Morgan and *Thrips palmi* Karny. To control thrips pest, growers are forced to intensively apply chemical pesticides, thus upsetting commercially successful greenhouse IPM programs. Chemical control of thrips often proves to be difficult. Although a large variety of predators (anthocorids, mirids, thrips and mites), entomopathogenic fungi, thrips attacking nematodes and parasitoids are known, biological control of thrips is not sufficiently reliable in all crops. Predatory mites and anthocorid predators have provided adequate control of thrips in greenhouse crops like sweet pepper and cucumber worldwide, while performance in floriculture was less satisfactory until recently. Pathogenic fungi might be useful as additional control agents. Parasitoids, though the only specific natural enemies of thrips, have not shown much potential for control to date.

Key words: thrips, natural enemies, biological control, evaluation of natural enemies

Introduction

During the last decades thrips have become pests in many cultivated crops throughout Europe and elsewhere in the world. Until the early eighties *Thrips tabaci* Lindeman was the most prevalent thrips pest in Europe, but caused problems only occasionally. Since its accidental introduction in 1983, western flower thrips (*Frankliniella occidentalis* (Pergande)) has become the number one key pest in European greenhouses and, under Mediterranean conditions, also caused problems in field crops and orchards. *Echinothrips americanus* Morgan is now spreading through Europe and there is a risk of introducing *Thrips palmi* Karny. *F. occidentalis* started its expansion in Europe, *T. palmi* did so in the Far East and the Pacific (Loomans & Vierbergen, 1997). Currently, *T. palmi* is an important pest throughout large parts of tropical and subtropical vegetable and flower producing areas. The exchange of horticultural products all over the world makes this quarantine pest a serious threat to Europe as well. Interceptions from vegetables and cut flowers imported from the Caribbean and Asia have increased in numbers over recent years. Apart from greenhouse crops in temperate areas, it is an important potential problem for the horticultural industry in the Mediterranean Region. In 1996 *E. americanus*, found before occasionally on bedding plants since 1993, became a pest in sweet pepper in The Netherlands. In 1997 it occurred on more than 70 sweet pepper holdings and is now hampering IPM in this crop.

To control thrips pest outbreaks caused by native or inadvertently introduced exotic species, growers are forced to apply chemical pesticides intensively, thus upsetting commercially successful greenhouse IPM programs (van Lenteren, 1995) and resulting in rapid development of resistance to insecticides. When *F. occidentalis* arrived in Europe, it was already resistant to many insecticides and the same problem will be experienced when *T. palmi* establishes.

Chemical control of thrips often proves to be difficult, because (1) a large proportion of the juvenile stages escapes treatment (eggs and pupae are concealed during most of their development), (2) resistance to a range of commonly used insecticides, and (3) limited availability of active ingredients for control (several insecticides cause phytotoxic effects). Application of organo-phosphates may even enhance outbreaks of thrips pests, because they destroy natural enemies and leave the pest unharmed. Although a large variety of predators (anthocorids, mirids, predatory thrips and mites), entomopathogenic fungi, nematodes and parasitoids of thrips are known, proper stock taking and in depth pre-introductory evaluation of natural enemies has not occurred yet.

At the start of the 1990s, several European research groups initiated a collaborative project funded by the EC to develop effective methods for the biological control of thrips. In this project (1) the literature on thrips pests and the control capacity of already studied natural enemies was evaluated, (2) field surveys were performed for native natural enemies (predators and parasitoids) in Europe, and for parasitoids outside Europe, (3) rearing methods were developed for thrips and their natural enemies, and (4) new natural enemies were evaluated under laboratory, greenhouse and field conditions (Loomans *et al.*, 1995).

Natural enemies of thrips

A thorough analysis of the relative importance of predators, parasitoids and pathogens under natural conditions in the field is not available yet. Major groups of natural enemies are summarized in van Lenteren & Loomans (1998), van Driesche *et al.* (1998) and in Table 1.

Many arthropods are known to be predators of thrips. Most predators of thrips are generalists and do not restrict their predatory activities to thrips; some species of predators even use food of plant origin. The best-studied families of predators are the Anthocoridae and Phytoseiidae. For control of *F. occidentalis*, several *Orius* species have been evaluated (Tommasini & Maini, 1995). In Europe, *O. laevigatus* adapted well to protected environments. It can survive without thrips prey on pollen, and strains collected in southern Italy do not show diapause, which means that they can also be used in winter. *O. laevigatus* is now the most common biocontrol agent for *F. occidentalis* on vegetable crops in Europe.

Phytoseid mites have several predatory genera. *Amblyseius (=Neoseiulus) cucumeris* (Oudemans) is a cosmopolitan species and preys on several thrips species, as well as on phytophagous mites. There have been successes and failures in introductions in greenhouses in different countries. The success rate has been lower on cucumber than on peppers (where *Amblyseius* can use pollen as an alternative food source), and it has been lower for the control of *F. occidentalis* than for *T. tabaci*. Very high numbers of predators need to be released to obtain control. The most recent addition to predators of this group is *Amblyseius limonicus* Garmon and McGregor, which originates from New Zealand and performs much better on vegetables in greenhouses than *A. cucumeris*. Further, this species does not enter diapause at short day length, and, therefore, it is expected that this species will replace *A. cucumeris*.

Loomans & van Lenteren (1995) and Loomans *et al.* (1997) reviewed the biology of thrips parasitoids and concluded that information on parasitoids is presently very incomplete. Attempts to control thrips pests by parasitoids in greenhouses have been relatively few. In experimental releases of *Ceranisus menes* (Walker) and *C. americensis* (Girault) to control *F. occidentalis* infestations in sweet pepper, cucumber and roses in Dutch greenhouses, parasitoids spread readily and established themselves throughout the crops but did not reduce thrips numbers. Entomopathogenic nematodes and fungi can be used for additional control of thrips populations, but not as the major control agents (Loomans *et al.*, 1997; van Driesche *et al.*, 1998).

Table 1. Natural enemies of thrips.

	Prey/host*	Generalist/Specialist	Type of cropping system	Mass production
Predators				
Heteroptera				
Orius spp.	Fo Tt Tp	generalist	protected, field	possible, expensive
Anthocoris nemorum	Fo	generalist	protected	possible, expensive
Geocorus spp.	Fo	generalist	field	not yet developed
Nabis spp.	Fo Tt	generalist	field	not yet developed
Dicyphus spp.	Fo Tt	generalist	protected, field	not yet developed
Macrolophus spp.	Fo	generalist	protected	possible, expensive
Thysanoptera				
Aeolothrips spp.	Fo Tt	generalist	field	not yet developed
Franklinothrips spp.	Tp Hh Pd	generalist	protected	possible, expensive
Acari				
Amblyseius/ Neoseiulus spp.	Fo Tt Tp	generalist	protected	possible, cheap
Hypoaspis spp.	Fo Tt	generalist	protected, field	possible, reasonable
Various predators				
Neuroptera, Diptera	Fo Tt Tp	generalist	protected, field	available for some
Parasitoids				
Ceranisus spp.	Fo Tt Tp	specialist	protected, field	difficult
Thripobius semiluteus	Hh	specialist	protected, field	possible, expensive
Pathogens				
Thripinema sp.	Fo	unknown	protected	not yet developed
Verticillium lecanii	Fo Tt Tp	generalist	protected	possible, cheap

*Fo = *F. occidentalis*, Tt = *T. tabaci*, Tp = *T. palmi*, Hh = *Heliothrips haemorrhoidalis*, Pd = *Parthenothrips dracaenae*

Biological control of thrips under practical conditions

Orius and *Amblyseius* spp. are used commercially for biological control of thrips in vegetables (mainly sweet pepper and cucumber). *Orius* spp. are introduced as a single seasonal release (in pollen producing crops like sweet pepper) or twice (in crops without pollen). *Amblyseius* spp. have to be introduced in the form of inundative releases either regularly into crops without pollen (for instance every two weeks in cucumber) or in "slow release systems" in which food for the predatory mites is provided. In pollen producing crops one release of *Amblyseius* is sufficient. Usually very high numbers *of A. cucumeris* have to be released. *Amblyseius degenerans* Berlese is a more efficient predator of thrips, but its mass production is difficult. Ramakers & Voet (1996) developed on open rearing system where *A. degenerans* is reared on potted pollen bearing *Ricinus communis* L. plants. These banker plants can be put in the greenhouse to establish early colonies of the predator in a crop that does not yet have pollen, or even in plant propagation houses where biological control is becoming increasingly popular. In winter, it is important to release non-diapause species or strains of *Orius* and *Amblyseius*. Releases of the predatory mites *Hypoaspis* and the fungus *Verticillium* have some additional control effect on thrips.

In ornamentals, thrips are considered the most problematic pest to control. In roses, several cultivars are very sensitive to thrips and frequent chemical control is applied. Other cultivars need very few chemical applications or none at all. Chemical control against thrips makes biological control of other pests very difficult as most thrips pesticides have a long lasting negative effect on natural enemies. Biological control of thrips can be started very

early by release of the soil inhabiting predatory mite *Hypoaspis miles* (Berlese) which kills soil visiting thrips stages. For thrips control on the plant, *A. cucumeris* is advised. Results with *Orius* and *A. degenerans* are not satisfactory. *Orius* is able to significantly reduce thrips in chrysanthemum. Usually thrips are not a serious problem in poinsettia, such that a quite high thrips density can be tolerated before control is needed.

Conclusions

Although far from all options for biological control of thrips have been tested, several natural enemies have been found that are good enough to control thrips, but only under specific conditions. Biological control of thrips in vegetables is much more common than in ornamentals. Most attention has been paid to phytoseid predators, but recently studies on *Orius* have strongly increased. Phytoseiidae were studied initially more because of ease of mass rearing than because of control efficiency. The predacious mites and anthocorid predators have provided sufficient control of thrips in greenhouse crops like sweet pepper and cucumber worldwide, while performance in floriculture was less satisfactory until recently. Pathogenic fungi have been used as additional control agents. Predatory thrips and parasitoids, though the only very specific natural enemies of thrips, have not shown much potential for control to date.

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Management of whiteflies: new natural enemies and host-plant resistance

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Abstract
Problems to manage whiteflies with parasitoids and predators that are currently mass produced, initiated research on evaluation of new natural enemies and host-plant resistance at the Laboratory of Entomology of Wageningen Agricultural University. This paper summarizes the various ongoing research projects.

Key words: whiteflies, *Trialeurodes*, *Bemisia*, parasitoids, entomopathogens, biological control, evaluation of natural enemies

Introduction

Since the introduction of *Bemisia* sp. *1* strains in Europe at the end of the 1980s, research was initiated to identify effective natural enemies of these whiteflies (van Lenteren & Martin, 1999). We intend to evaluate new natural enemies of *Bemisia* partly with the aid of an individual based simulation model (van Lenteren & van Roermund, 1998), and to be able to do so, we needed to adapt the model for greenhouse whitefly by van Roermund et al. (1997), so it also properly simulates dispersal and population development of *Bemisia*. Therefore, we reviewed life-history parameters of different biotypes of the whitefly *Bemisia tabaci* (Gennadius) species complex (Drost et al., 1998).

There is a vast amount of literature on *B. tabaci* from which we have selected studies on life-history parameters in relation to temperature and separated the data according to host plant and biotype. For each group temperature-dependent development rate, mortality, adult longevity, sex-ratio, pre-oviposition period and fecundity were modelled by linear or non-linear functions. Where possible the life-history of *B. tabaci* was compared to that of *T. vaporariorum*. The review includes the B-biotype of *B. tabaci*, identified as *Bemisia argentifolii* (Bellows & Perring). The relationship between development rate, immature mortality, adult longevity, sex-ratio, pre-oviposition period and fecundity to temperature were modelled by mathematical equations that will be used to run the simulation model on biological control of whiteflies. Comparisons were made among host plants and biotypes. Biotypes discussed are A, B, Indian and biotypes of the Old World group. The next step in this research project was to evaluate new natural enemies of *Bemisia*.

Biology of *Amitus bennetti*, a parasitoid of *Bemisia argentifolii*.

Most parasitoids used in biological control of whiteflies (Homoptera: Aleyrodidae) belong to the Aphelinidae (van Lenteren et al., 1997). Parasitoids in the genus *Amitus* Haldeman of the Platygasteridae also attack different species of the Aleyrodidae. *Amitus fuscipennis* MacGown and Nebeker has been recovered in large numbers from the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), on bean crops in the Andean mountains of Colombia and Equador

in areas where insecticides are used heavily. The biology and control efficacy of this species is under study (see Manzano and De Vis, elsewhere in these precedings). *Amitus bennetti* Viggiani & Evans is a solitary internal parasitoid of *B. tabaci*. The parasitoid was discovered in Puerto Rico by F.D. Bennett from a biotype of *B. tabaci,* and according to J.K. Brown (pers. comm.) it is very likely that this was the so-named 'Sida Race' of *B. tabaci*. We studied the temperature-dependent development time of immatures, the longevity of adult females and the fecundity and the oviposition behaviour of adult *A. bennetti* females on *B. argentifolii*, also referred to as the B-biotype *of B. tabaci* (Costa & Brown, 1991), with poinsettia (*Euphorbia pulcherrima*) as host plant (Drost et al., 1999a).

The 1st nymphal instar of *B. argentifolii* is preferred by the parasitoid, but the 1st through 4th instar may be parasitised. Females first investigate hosts with their antennae, then walk over the host, and eventually step with their front legs on the leaf and insert their ovipositor inside the host facing away from the host, while the hind legs are still on the host. The time from encounter to oviposition is shortest on the 1st instar. Oviposition duration (mean = 39 s) comprises 50 % of the handling time. Development time from egg to adult decreases from 72 days at 15°C to 42 days at 20°C to 28 days at 25°C. Adult longevity in the absence of hosts was 29, 26 and 19 days and with hosts present 8, 8 and 5 days at 15, 20 and 25°C, respectively. *A. bennetti* is proovigenic and oviposits most eggs shortly after adult emergence. During the first day of their adult lives females laid 1, 31 and 49 eggs at 15, 20 and 25°C, respectively. Compared with other parasitoid species, the development time of *A. bennetti* is very long, and the implications of this for management of *B. argentifolii* will be evaluated with the earlier mentioned simulation programme.

Searching strategies of parasitoids of *Bemisia argentifolii*

Amitus bennetti, *Encarsia formosa* Gahan (two strains), *Eretmocerus eremicus* Rose & Zolnerowich, *Er. mundus* Mercet and *Er. staufferi* Rose & Zolnerowich, parasitoids of *B. argentifolii* were compared with respect to their searching behaviour as part of a pre-introduction evaluation programme of whitefly parasitoids (see also Qiu et al., these precedings). The probability of a randomly searching parasitoid to encounter a host depends to a large extent on the size of the insect, the size of the host and the walking speed of the insect (van Roermund et al., 1997). In the tritrophic system tomato - *T. vaporariorum* - *E. formosa* these parameters had a critical effect on the suppression of the whitefly (van Roermund et al., 1997). Therefore, when comparing different parasitoid species for their efficiency these might be essential parameters to use. Within a 5 cm arena, host-finding time was independent of the release distance from the host. Before oviposition, *A. bennetti* walked fastest, the *E. formosa* strains walked slowest and the *Eretmocerus* species intermediate. Leg length was not the most significant factor determining the differences in walking speed. After oviposition, *A. bennetti* and *Er. eremicus* had a lower walking speed and higher turn rate, which is an indication of area, restricted search. The effect was strongest for *A. bennetti.* All species showed preference for counter-clockwise turns. Based on the walking speed alone, it is expected that the *A. bennetti* will be the most efficient natural enemy of *B. argentifolii*, the *Eretmocerus* species intermediate and the *E. formosa* strains the least (Drost et al., 1999b).

The use of entomopathogenic *Aschersonia* fungi for the control of whiteflies

Entomopathogenic fungi of the genus *Aschersonia* are specific for whitefly and scale insects, and can be used as a biological control agent against silverleaf whitefly, *B. argentifolii* and greenhouse whitefly, *T. vaporariorum*. More than 40 isolates belonging to this genus were

tested on their ability to sporulate, germinate and infect both whitefly species (Meekes et al., 1996). Seventeen isolates either sporulated poorly, or were not able to infect the whiteflies. After selection based on spore production and infection, virulence of the remaining isolates was evaluated. Infection levels varied between 2% to 70%, and were positively correlated between *B. argentifolii* and *T. vaporariorum*. On basis of sporulation and pathogenicity, six isolates were selected, originating from southeast Asia (3), South America (2) and Africa (1). These isolates were tested for their rate of infection and dose mortality response on both whiteflies. Several isolates showed consistently good results in their capacity to infect both whitefly species. The influence of host plant (cucumber, gerbera, poinsettia and tomato) on the interaction between insect and fungus was also investigated. The interaction between whitefly and pathogens was highly host plant dependent: infection on poinsettia was very low, while infection was high on the other host plants.

Analysis of resistance against the greenhouse whitefly through electrically monitored and visually observed probing and feeding behaviour

The effects of resistance factors located in different tissues of the host plant were compared by measuring the electrically monitored (the Electrical Penetration Graph (EPG) system) and visually observed probing and feeding behaviour of whiteflies on resistant and susceptible tomato lines, and a host plant of very poor quality for whitefly, sweet pepper. On sweet pepper, whiteflies displayed very short first probes, very long pathway probing and spent much time on non-feeding activities like walking and standing still. Also, a high percentage of whiteflies rejected sweet pepper without ingesting substances from the phloem vessel. These data suggest a strong resistance that is based on the factors present in surface/epidermis and/or mesophyll layers of this plant. The behaviour of whiteflies on the resistant tomato was very different from that on sweet pepper: whiteflies apparently did not perceive resistance factors on the leaf surface and in the mesophyll. Resistance factors appeared to be present in the phloem tissue, because a higher number of phloem phases, longer phloem salivation periods and shorter phloem ingestion periods were observed when compared with the susceptible tomato cultivar. EPG waveforms have been analysed and where correlated with whitefly probing and feeding behaviour. Using EPGs to judge plant resistance against whiteflies has not been previously reported (Lei et al., 1998).

Discussion

The host-searching efficiency of natural enemies is an important parameter in the evaluation of their potentials for biological control of insects. Because host densities are typically low when parasitoids are released in a greenhouse and should remain low under a succesfull biological control programme, the probability of encountering hosts for parasitization is extremely important. Species with high fecundity but inefficient host-searching behaviour may never encounter hosts to deposit their eggs. This of course could be compensated by releases of large numbers of parasitoids, which increases the probability that at least some parasitoids will encounter the pest insects, but this is expensive. But it is economically more efficient to select for parasitoids with good searching capacities. Therefore, different species and strains of parasitoids of immature *B. argentifolii*, also referred to as the B-biotype of *B. tabaci*, are compared with respect to their searching behaviour and developmental biology.

If whitefly densities are very high, release of parasitoids might not result in timely control. For biological control of whiteflies at high densities, entomopathogenic fungi could offer better possibilities. Many strains/species of the entomopathogenic fungus *A. aleyrodis*

have been evaluated for control of *T. vaporariorum* and *B. tabaci*. Several effective strains have been identified.

Another option to reduce whitefly population development is to develop host plants that are (partly) resistant to whiteflies. Evaluation of host-plant resistance is a very time consuming process, and, therefore, we aim to develop a method that is less time consuming based on the Electrical Penetration Graph (EPG) system that monitors the probing behaviour of whiteflies.

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Insectivorous Birds for Biological Control of Pests in Glasshouses

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Abstract: In most cases biological control of insects and mites is carried out with other insects and mites as natural enemies. Larger pests such as caterpillars, but also phytophagous bugs can be very noxious in glasshouse crops. It would be most convenient to find a generalist natural enemy to control these pests. Biological control is widened from an entomological to a more general ecological approach. Because there are many species of typical insectivorous birds, the obvious thing to do is to try species that would fit well in a particular glasshouse habitat. General considerations are given to be able to make the best choice of an insectivorous bird. The first results with *Alcippe brunnea* (Passeriformes: Timaliidae) against caterpillars are very promising.

Key words: insectivorous birds, *Alcippe brunnea*, Timaliidae, Aegithalidae, Maluridae, Zosteropidae, biological control

Introduction

Damage by noxious moths in glasshouses is a permanent threat. So far, there is little experience with research on biological control of caterpillars in glasshouses. In most cases *Bacillus thuringiensis* is used in IPM programs, but this agent is not equally effective against all species. A review of noxious moth species and possible natural enemies has been presented earlier (van der Linden, 1996).

Many fruit growers augment the bird fauna in their orchards by installing nest boxes. Some growers do the same in commercial glasshouses. The birds that breed in glasshouses sometimes are *Montacilla alba*, *Parus major* and *Turdus merula*. Particularly during the breeding season these birds are able to destroy large numbers of insects. However, native birds are being protected by law. They should have free choice to come and to go. This makes it difficult to rely on them. The application of insectivorous birds, such as *Lamprotornis purpureus, Sturnus malabaricus* and the Peking Robin, *Leiothrix lutea*, is also a subject of research in pig houses (Roelofs, 1999). The Peking Robin is a rather popular insectivorous exotic bird in aviaries. The species is now on the list of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Birds were effective in farrowing rooms, rooms for weaned piglets and rooms for growing pigs, while the costs are low. In glasshouses several growers have used zebra finches *Poephila guttata* for some years. These birds are a common pet and they are cheap. However, it is a seed eating species and will mainly supply its young with insects during breeding, while insectivorous birds eat also insects when they are not breeding. Insectivorous birds are therefore a better option for glasshouses. But not any insectivorous bird would fit in the glasshouse environment. An up going sweet pepper crop may be very well regarded as an equivalent of dense undergrowth. It is obvious that bird species should be searched that are bound to this type of habitat. Species that search for food in dense cover and on the ground give the least risk of flying away via the ventilators. But there are more considerations before a bird species can be chosen. Birds that

are protected by law, convention or some kind of regulation are no candidates. To avoid crop damage only smaller bird species can be used. Further the birds should not forage on the crop or fruit itself. Offering soft fruit or berries should prevent possible damage of fruit. The birds have to be social and not aggressive, even during breeding. Species that potter about searching for food in flocks are probably the best candidates. An important matter is also that it should be possible to rear the birds. It must be ruled out that glasshouse horticulture would depend on large numbers of birds caught in the wild, in case biological control with birds in glasshouses is a success. Starting with birds in the glasshouse at the time the ventilators do not open during the day, and arranging foraging sites will most probably stimulate a bond with the glasshouse environment.

The first results with the Brown-capped Fulvetta, Rufousheaded Tit-Babbler or Dusky Fulvetta *Alcippe brunnea* (Gould) (Passeriformes: Timaliidae) in a sweet pepper crop are given.

Material and methods

Literature survey for insectivorous birds

Literature was examined of insectivorous birds and of the natural habitats they live in (Ali & Ripley, 1972; Cramp & Perrins, 1993; Grzimek, 1974; King & Dickinson, 1976; Mackinnon & Phillipps, 1993; Rowley & Russell, 1997; Smythies, 1953).

Predation by Alcippe brunnea in glasshouse sweet pepper

Four *Alcippe brunnea* were bought (EUR 20.40 / couple) and released in a glasshouse compartment of 76 m^2 with a sweet pepper crop. Fine meshed insect screens were fixed in the ventilators. The birds were fed universal food for insectivorous birds, mealworms *Tenebrio molitor*, and raisins. They were supplied with a bath of fresh water every day. Four types of introductions of the moth *Chrysodeixis chalcites* were carried out. Caterpillars were placed singly on labelled plants; 10 pupae; 590 eggs and newly hatched caterpillars; and the release of moths. The effect of predation by the birds was established after introductions of the pest.

Results and discussion

Facts from literature

Particularly the encyclopaedia of Grzimek (1974) gives a general overview of families and details of some of the subfamilies, tribes and species of birds. Considering the requirements, the following families of the Passeriformes may have several useful species: Timaliidae (ca. 270 species) from Africa and Asia, Aegithalidae (8 species) from Asia, Maluridae (25 species) from Australia and New Guinea, and Zosteropidae (ca. 90 species) from Africa, Asia and Australia.

Further details of Timaliidae living in dense undergrowth habitats in south-east Asia is given by Ali & Ripley, 1972; Cramp & Perrins, 1993; King & Dickinson, 1976; Mackinnon & Phillipps, 1993; Smythies, 1953. Many members of the family Timaliidae are highly sociable, some being group-territorial and nesting communally. Unlike many other passerines, they have bodily contact with others in their social group when loafing or roosting. The genus *Alcippe* comprises 17-18 spp. *Alcippe brunnea* hunts in dense undergrowth, close to or on the ground and move about in small parties. They like bracken, brambles, and in winter bamboo jungle, scrub and secondary growth. They are breeding on or near the ground. *A. brunnea* is often seen in company of other birds, such as *Stachyris* spp., which is another member of the

Timaliidae. *Alcippe* spp. and probably some other genera as well may yield potential candidates.

The members of the Aegithalidae are small insectivorous species, eating small insects including aphids. They are very social, sleep together in a group, show no territorial behaviour and members of the group are helping to raise the young birds. They like dense undergrowth for breeding, but they frequent trees also. There are potential candidates, but only those which show the least need of searching for food in trees.

The Maluridae are small species that feed mainly on the ground and in low vegetation (Rowley & Russell, 1997). The birds are social and live in family groups. All members of the group help to raise the young. Some are probably potential candidates.

The Zosteropidae live mostly in flocks of 3-20 birds, sleeping in a group. Their food consists of nectar, insects and fruit. Some species are considered to be noxious in orchards and vineyards. This may also give a risk for glasshouse crops. Most species are arboreal, which makes them less suitable as candidates.

Alcippe brunnea in glasshouse sweet pepper

The birds were released in the crop in April 1998. The behaviour did not indicate that they would have left the glasshouse when the ventilators would have been without screens, because the birds always stayed in the crop and they never flew above the crop. Although the birds were fed daily, the supply of mealworms was lowered or stopped when caterpillars or pupae were used in tests. Ten pupae were introduced in a plastic bottle to prevent immediate eating by the birds. However, the next day the bottle was empty (Table 1). Some way the birds have obtained the pupae from the bottle. In a control glasshouse without birds the introduction of ten pupae resulted in an infestation of the crop that had to be treated several times during the season with formulations of *Bacillus thuringiensis*.

The results of the introductions of caterpillars are also given in Table 1. Small numbers of caterpillars disappeared in 1 to 3 days. The searching efficiency of the birds was quite impressive. They inspect plants thoroughly and probably also the droppings of the caterpillars help the birds to locate their prey.

The mass introduction of 590 moth eggs and freshly hatched caterpillars was eliminated in 17 days. None of the caterpillars became larger than 1 cm in length.

When moths were released they were chased, and progeny was never found.

Table 1. Predation of *Chrysodeixis chalcites* by *Alcippe brunnea* in glasshouse sweet peppers.

Number of *C. chalcites* introduced	**Extinct after... days**
4 late instar larvae	3
5 late instar larvae	2
5 late instar larvae	1
5 late instar larvae	1
10 late instar larvae	1
10 late instar larvae (at 2.5 m height)	3
10 pupae	1
590 eggs and first instar larvae	17
25 moths (over 4 weeks)	no crop infection

The glasshouse was also more or less free of house crickets and spiders. In large commercial glasshouses it is probably easier for the birds to gather all kinds of naturally occurring insects and spiders.

In a practical situation with natural flying in of moths it must be possible to prevent damage of the crop without using any pesticide or *Bacillus thuringiensis* formulation.

Application of beneficial insects and mites and the use of bumblebees as pollinators are already reason enough to be reluctant to use pesticides. Application of birds for biological control is another one.

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Evaluating environmental effects of *Encarsia* species (Hymenoptera: Aphelinidae) introduced for whitefly control into Europe.

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Abstract: Biological control of whitefly pests has become a key component of sustainable horticulture in Europe over the past 25 years. Nowadays, billions of exotic beneficials are produced and released seasonally or inundatively for the biological control of whiteflies. Although no clear direct adverse effects have been found up till now, the potential non-target effects of these releases have little been emphasised. Here we discuss some of the effects of releases of exotic parasitoids introduced for the biocontrol of greenhouse whiteflies and we indicate the lines of research along which we wish to assess, analyse and control any indirect or ecological effects of these introductions.

Key words: biological control, introductions, exotics, environmental risks, non-target effects, natives

Introduction

The introduction and release of exotic biocontrol agents has contributed significantly to a reliable and economic pest control during the past 100 years. During recent years, however, there is an increasing discussion, that besides the benefits of these introductions, also potential ecological risks could be involved: direct and indirect effects on non-target organisms and native natural enemies, and thus changes in biodiversity (e.g. Hokkanen & Lynch, 1995; Van Lenteren, 1997). Most of the time the effects of exotic classical biocontrol agents have been emphasised, but little or not the effects of seasonal and inundative releases in protected crops. Exotic agents introduced in the past, attack related native host species as well, or even have become the dominant species in the natural ecosystem. For instance, *Lysiphlebus testaceipes* (Cresson) (Starý et al., 1988) and *Cales noacki* Howard (Viggiani, 1994) not only attack their target pest (aphids and whiteflies respectively), but also native pest species and indifferent, non-target species, and displaced native competitors. Until recently, however, these effects were not considered as adverse, but even beneficial, allowing establishment of the exotic species. More and more countries, however, are currenty applying or making regulations for the import and release of (exotic) biocontrol agents, based on international guidelines (FAO, 1997). Here we discuss the potential ecological effects of exotic parasitoids, released for whitefly control in protected crops in the temperate and Mediterranean zones, and we explain how we want to screen for these effects on non-target organisms and native ecosystems.

Exotics introduced for whitefly control

Biological control of whitefly pests has become a key component of greenhouse IPM in Europe. Every year billions of exotic beneficials are produced and released seasonally or inundatively to control *Bemisa tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood). The worldwide production of whitefly biocontrol agents alone per year is

enormous, mainly the exotics *Encarsia formosa* Gahan (2.5-3 billion) and *Eretmocerus eremicus* R. & S. (about 1 billion) and the native *Macrolophus caliginosus* Wagner (about 1 billion) (Koppert, 1998). Eighty per cent of these are released on about a large part of the 8000 ha of greenhouses in Europe. Several other exotic and native species attack whitefly pests in protected crops (table 1, box 1). *E. pergandiella* is a nearctic species that was introduced for the control of *T. vaporariorum* in Italy in 1978 (Viggiani, 1994). It established outdoors and meanwhile has spread over Italy and occurs naturally in southern France, northern Spain and probably Tunesia. The ecological effects of these releases have been analysed only marginally.

Whitefly - Parasitoid - Autoparasitoid interactions

In greenhouse IPM systems exotic, primary whitefly parasitoids can interact and compete with endemic parasitoids that may spontaneously enter the greenhouse. On the other hand, the exotic parasitoids leave the greenhouse and attack local whiteflies in the field. In temperate areas this seems rare, in the Mediterranean more common. In Europe, there is a range of species (box 1, table 1) with different life histories and levels of attack on whiteflies, and a diversity of related, indigenous parasitoids that may interact and compete with introduced species. A number of these introduced parasitoids have hyperparasitic habits (attacking other parasitoids for male production). The whitefly-parasitoid-autoparasitoid complex allows us to:

- determine the impact of regular seasonal inoculative releases of exotic specialists on the native whitefly and parasitoid fauna, their performance and survival in different climates;
- explore how heteronomous hyperparasitoids, which are building up on primary parasitoids, affect the original relationships and to better understand their interactions;
- assess under what conditions the introduction of a hyperparasitoid is acceptable or permitted; it also allows us to test the hypothesis that heteronomous hyperparasitoids (facultative autoparasitoids), like the exotic *E. pergandiella* or the native *E. tricolor,* could disrupt biological control by primary parasitoids like *E. formosa* (Mills & Guttierrez, 1997) or will contribute to an increased control. Williams (1996), however, provides examples that facultative autoparasitoids do not always dominate the natural ecosystem complex or the outcome in biocontrol programs. In Italy, *E. pergandiella* is the dominant species on *T. vaporariorum*, attacking *B. tabaci* and others as well, both in protected crops as in the open (Viggiani, 1994). The level of 'natural' infestation is often too low (33% - 50% parasitisation, occasionally 80%) for control. Field experiments in the USA, the native area of distribution of *E. pergandiella*, indicate that the combined effect of this facultative autoparasitoid and the primary *E. formosa* increases biocontrol (Heinz & Nelson, 1996); greenhouse surveys in the Mediterranean area (Spain) on the other hand indicate that it may replace *E. formosa* and reduce its efficacy (Gabarra et al., 1999);
- test the hypothesis that exotic parasitoids originating from tropical areas cannot survive under temperate field conditions, and form, therefore, only a temporary potential risk for ecosystems they may invade. *E. formosa* occasionally has been collected from whiteflies on outdoor crops or wild plants, e.g. in Greece, Egypt, Italy and The Netherlands (on *Aleyrodes proletella* L. on *Chelidonium majus* L.), but information is about its ecology or population dynamics in nature is not available (Hoddle et al., 1998);
- provide data for modelling the effects of different kinds of natural enemies on competition between parasitoids, and the effects on endemic and alien host (whitefly) species.

Table 1. Native and introduced (accidental or *intentional*) exotic species of *Encarsia* parasitising *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) - complex in Europe (Tryapitsin et al., 1996; Woolley & Heraty, 1998; and others). □ reproduction strategy: 1 = both sexes primary (biparental), 2= thelytokous (uniparental), 3= facultative autoparasitoid (= heteronomous hyperparasitoid); * = synonym?

***Encarsia* species**	**Origin**	**□**	**Host species - genera**	**Westpalaearctic distribution**
E. formosa	*Exotic*	2	14 - 9	Westpalaearctic
E. hispida *	Exotic	3	5 - 5	France, Italy
E. meritoria *	Exotic	3	13 - 9	Italy, Spain
E. pergandiella	*Exotic*	3	20 - 9	France, Italy, Spain, Tunesia
E. inaron	Native	1	16 - 8	Westpalaearctic
E. lutea	Native	3	>20 - 11	Mediterranean
E. transvena	Native	3	11 - 10	Mediterranean
E. tricolor	Native	3	16 - 11	Westpalaearctic

Box 1: Overview of the whitefly parasitoid species complex in protected crops in Europe

The genus *Encarsia* includes over 200 species (Woolley & Heraty, 1998), about 110 of which are known from aleyrodids. More than 25 species of *Encarsia* have been recorded from *B. tabaci* (complex) and 15 species from *T. vaporariorum*. Twelve species occur in the Westpalaearctic region (table 1): 7 are native (mediterranean: *Encarsia adrianae*, *E. davidi*, *E. mineoi*, *E. transvena;* westpalearctic: *E. inaron*, *E. lutea*, *E. tricolor*) and 5 have been introduced either intentionally or by accident (*Encarsia formosa*, *E. luteola*, *E. hispida*, *E. meritoria*, *E. pergandiella*) (Tryapitsin et al., 1996; Woolley & Heraty, 1998). Although *Encarsia* species are specific to aleyrodid hosts, most species have a broad range of host species and genera (table 1). Also other *Encarsia* species, native or introduced on whiteflies in nature or field crops, and other endoparasitoids (*Amitus, Cales, Euderomphale*) or primary ecto-endoparasitoids (*Eretmocerus*) can interact within a same whitefly-parasitoid complex (Viggiani, 1994), either bidirectionally (competition) or unidirectionally (competitive displacement). All *Encarsia* species are solitary endoparasitoids, exhibiting a variety of reproductive, hyperparasitic male strategies (obligate or facultative autoparasitism, thelytokous parthenogenesis, primary or diphagous parasitism, or using other host families (table 1)), life history characteristics, dispersal abilities and overwintering strategies. Because most exotic and native species have been reared and studied on whitefly pests, little is known about the actual and quantitative host range, particularly in natural ecosystems.

Evaluating environmental risks

To evaluate competition and environmental effects of exotic and native *Encarsia* species we use standardised protocols, based on the following methodology (ERBIC, 1997): biological studies on host selection and specificity in the lab and field to determine the range of pests and non-targets; a reliable identification using traditional and molecular techniques; life-table studies to analyse the influence and changes; interspecific competition studies in the field in different climatic zones: mediterranean and temperate; dispersal studies to assess the capacity to migrate from the target crop and to invade natural habitats in time, distance and population density; physical requirement studies of to assess any limits of adaptation and survival, and implementation of the collected data in mathematical models.

In general, field and laboratory post-release studies, in combination with mathematical models, will allow us to design a conceptual, theoretical and ecological phramework for examining, understanding, and predicting population dynamics in terms of risks of biological control, in particular its effects on non-target species (see ERBIC, 1997).

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Franklinothrips : perspectives for greenhouse pest control

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Abstract: Predatory thrips are generally considered as important elements of the natural control reservoir in natural and agro-ecosystems. Their use as commercial biological control agents for greenhouse pest control, however, has gained relative little attention thus far. Species in the genus *Franklinothrips* Back (Thysanoptera: Aeolothripidae) are currently evaluated as biological control agents for greenhouse pests. Here we introduce this relatively unknown, exotic guild of predators, emphasising biological features and possible ecological and economical implications.

Key words: *Franklinothrips vespiformis, F. megalops*, biological control, exotic, predatory thrips

Introduction

Polyphagous predators play an important role in greenhouse IPM: pirate bugs, mirid bugs, chrysopids and predatory mites are widely used (Lewis, 1997; Van Lenteren & Loomans, 1998), but the role of predatory thrips as biological control agents has long been neglected. Members of the genus *Franklinothrips* Back are known as thrips predators and ant-mimics before long. Since a few years, a number of *Franklinothrips* species are produced and tested by Dutch companies: *Franklinothrips vespiformis* (Crawford), *Franklinothrips* sp. nr. *vespiformis* and *F. megalops* (Trybom). They are released for the control of thrips in internal plant-scapes (offices, tropical glasshouses), in particular *Heliothrips haemorrhoidalis* (Bouché) and *Parthenothrips dracaenae* (Heeger), infesting perennial ornamentals like *Ficus*. They are currently investigated as control agents of *Echinothrips americanus* Morgan and *Thrips palmi* Karny (Loomans & Heijboer, in press). Here we generally introduce *Franklinothrips,* a relatively unknown genus of exotic predators, their potential role as biological control agents in greenhouses, emphasising biological features, ecological and economical implications.

Identity and distribution

About 300 predatory thrips species are known (Zur Strassen, 1995). They belong to more than 24 genera, with *Franklinothrips* included. The 13 species described in *Franklinothrips* are distributed throughout the tropics (table 1). Adults are mainly black, between 1.8 and 2.9 mm long, with large antennae, a thin waist and legs with white bands, resembling ants or wasps in appearance and behaviour (Johansen, 1976, 1983). Larvae are yellowish with bright orange-red bands. *F. vespiformis* and *F. megalops* are the best known representatives. *F. megalops* is an Oriental and African species, whereas *F. vespiformis* is pan-tropical: its native distribution ranges from southern USA to Brazil (Stannard, 1952). Recently it has been recorded from Madeira, Réunion, Thailand, Taiwan, Japan, Fiji and Hawaii, where it probably was introduced. Its distribution is now questioned, however: the keys available (Stannard, 1952; Mound & Marullo, 1996) do not allow proper identification of specimens from California.

Table 1. Overview of the predatory genus *Franklinothrips* Back, including their geographic distribution and prey associations (Mound & Marullo, 1996; Lewis, 1997; Arakaki, 1998).

Franklinothrips species	distribution	thrips prey genera
atlas Hood	Zaire	?
basseti Mound & Marullo	Australia	?
caballeroi Johansen	Mexico, Costa Rica	*Leucothrips*
fulgidus Hood	Brazil	?
lineatus Hood	Brazil, Argentina, C. Rica	?
megalops (Trybom)	Southeast Africa, Nigeria, India, Indonesia	*Frankliniella, Scirtothrips, Retithrips, Parthenothrips, Diarthrothrips, Heliothrips, Caliothrips, Zaniothrips*
myrmicaeformis Zanon	Israel, Egypt, Libya, China	*Heliothrips, Retithrips*
orizabensis Johansen	Mexico	*Leucothrips*
rarosae Reyes	Philippines	?
suzukii Okajima	Taiwan	?
tenuicornis Hood	South America	*Selenothrips, Heliothrips*
variegatus Girault	Australia	?
vespiformis (Crawford)[1]	pan-tropical	*Frankliniella, Scirtothrips, Thrips Chaetanaphothrips, Caliothrips, Selenothrips, Parthenothrips, Heliothrips, Selenothrips, Hercinothrips, Echinothrips*

[1]other prey: spider mites, leafminer larvae, whiteflies, moth eggs, leafhoppers, lace bugs, mealy bugs

Prey specificity

Franklinothrips species are, like most thrips predators, general opportunistic feeders, attacking slow-moving stages of a wide range of small arthropods, including their own (Lewis, 1997). Larvae and adults are mainly known from preying on larvae and pupae of surface-dwelling panchaetothripine species (table 1). *F. vespiformis* also preys on larvae of thripine species like *T. palmi* (Arakaki, 1998; Loomans & Heijboer, in press), *Thrips tabaci* Lind. (Loomans unpubl.) and *F. occidentalis* (Norosomahefa, 1991). Attacks, however, on very active stages or those excreting anal droplets, are often unsuccessful. Adults of *F. vespiformis* and *F. megalops* also feed on eggs of various species, which are embedded in the plant tissue (Sureshkumar & Ananthakrishnan, 1987; Loomans & Heijboer, in press) or on other hidden prey, like leafminer larvae (Arakaki, 1998). Besides thrips, *F. vespiformis* and *F.* sp. nr. *vespiformis* feed on e.g. leafhoppers, whiteflies (eggs and larvae of *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius): Loomans unpubl.; *T. floridensis* Quaintance: Stannard, 1952)) and spider mites (*Oligonychus yothersi* (McGregor): Stannard, 1952; *Tetranychus kanzawai* Kishida: Arakaki, 1998; *T. urticae* Koch: Loomans, unpubl.), although areas of dense webbing are less explored. *F. vespiformis* feeds on plant exudates and honeydew and is a facultative pollen feeder as well: larvae complete their development on a diet of (pine) pollen alone, when supplied on a leaf disk on agar. Adults, however, did not reproduce on a diet of pollen (Loomans unpubl.). The knowledge on the prey range, prey preference and prey requirement level of most species in a crop, however, is still lacking.

Biological features

Biological information on *Franklinothrips* is scarce: some life-history data are available for *F. megalops* (Sureshkumar & Ananthakrishnan, 1987) and *F. vespiformis* (Arakaki, 1998; Loomans, unpubl.). The time from egg to adult takes 2-3 weeks (*F. megalops*: temperature unknown) or 3.5-4 weeks at 25°C (*F. vespiformis*), the variation of which is largely caused by the prey (thrips species, stage or moth eggs). On a diet of pollen, at 25°C it takes 4.5 weeks for *F. vespiformis* to mature. The pre-oviposition period, female longevity and fecundity depend on the type of food (prey species, stage): when supplied with eggs of *Ephestia kuehniella* (Zeller), *F. vespiformis* females start ovipositing earlier, live longer and produce more eggs (170/♀) than when supplied with *T. palmi* adults (65/♀) or larvae (32/♀; *F. megalops*: 20-40 eggs/♀). *Franklinothrips* is inactive during most of her development (egg, pupa), the active larval preying phase is relatively short: 30% (*F. vespiformis*) to 35% (*F. megalops*) of the developmental period. The sex ratio of most species is female-biased (*F.* sp. nr. *vespiformis*; *F. megalops*), reproduction in *F. vespiformis* is thelytokous, males are very rare (1♂: 1000♀).

Franklinothrips species inhabit a wide variety of host plants in nature, such as grasses, herbs, shrubs, bushes and trees in a wide variety of habitats, from roadsides to tropical rainforests, including agro-ecosystems like orchards and field crops. Like in most Terebrantia, eggs are laid inside plant tissue, mainly the veins of leaves or soft stems (*Solanum* spp., cucumber, aubergine, sweet pepper, *Ficus*). Larvae and adults are very active, foraging all over the plant, but are most frequently found on the mature leaves. On leaves were thrips have been feeding, they search the area intensively, probing the surface with their mouthcone. Prior to pupation a cocoon is spun, usually between two joining veins at the underside of a leaf, sometimes in crevices of the plant stem (potato, *Ficus*) or in the leaf litter, and there is no propupal stage. Most aspects of the biology and ecology are still unknown: e.g. the ability to disperse in a crop after eradication of local prey-patches, the ability to reproduce in a greenhouse, and whether or not the rate of population increase is sufficient to keep pace with the pest population during the season.

Ecological implications

IPM in greenhouses rapidly evolves towards a multiple biological control system, including simultaneous releases of two or more natural enemies against the same pest. The ability of *Franklinothrips* to feed on a wide variety of prey, their wide 'prey-window' (eggs, larvae, pupae as well as adults) and their wide range of host plants and climatic adaptations, makes them an interesting new group, also commercially. Although rather expensive, (alleged) mass-production problems seem to be solved for *Franklinothrips*, which makes them commercially available for releases in numbers, large enough to have a sufficient impact on a target pest. In particular for those crops and for those thrips pests, like *E. americanus* or *P. dracaenae*, that have a relative long developmental time and all prey stages present in or on the leaf, where indigenous agents are few or less suitable, they may contribute to their biological control.

The potential of *Franklinothrips* as part of a biological control strategy may be limited by feeding non-selectively on other beneficial predators or non-target organisms. Intraguild predation (IGP) could be a serious constraint to a (combined) release with other biocontrol agents in a crop. In a multiple biological control system, where *Franklinothrips* species could be subject to larger, very active stages of predators, their ant-like appearance could protect them (Johansen, 1976, 1983). They will likely exploit smaller and slow-moving stages of other predators: in no-choice tests in the laboratory *F. vespiformis* adults and larvae prey on

predatory mites (eggs and nymphs of *Amblyseius cucumeris* (Oudemans)), and are occasionally exploited by pirate bugs (*Orius laevigatus* (Fieber)) (Loomans unpubl.), but this needs to be verified in a crop. IGP and intraspecific predation ('cannibalism') will depend on the availability of prey, hunger level, spatial distribution, heterogeneity and potential refuges in the crop, and the life-style, size and activity of the prey.

Future regulation of the import of exotics by various countries, will emphasise features other than their capacity to control thrips pests, such as their ability to establish in the native environment and side-effects on non-target organisms. The tropical origin of *Franklinothrips* makes it unlikely that they will establish outdoors in temperate zones, but it may do so in the Mediterranean Area. Strict regulatory measures to avoid the import of exotics may restrict, however, the use of many beneficials prevalent in greenhouse IPM, even in temperate regions.

Conclusion

Exotic *Franklinothrips* species may present an addition to biological control applications in greenhouse IPM for certain situations and target pests. Current biological and ecological knowledge, including potential non-target effects (intraguild predation, invasiveness into natural ecosystems) is yet insufficient to decide if they can contribute significantly and safely.

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Application of *Trichoderma harzianum* by using *Apis mellifera* as a vector for the control of grey mould of strawberry: first results.

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Abstract: In 1997 and 1998 the efficacy of honeybees in vectoring *Trichoderma harzianum* to strawberry flowers and the activity against *Botrytis cinerea* of two application methods of such antagonist were tested. The results showed that the antagonist delivering system by the honeybees is efficient, with very high numbers of propagules of *T. harzianum* being detected on honeybee bodies and on strawberry flowers. However, *T.harzianum* was more effective in controlling grey mould when distributed as water suspension, while when vectored by the honeybees it provided significant rot control only during the storage of fruits after harvest. Pyrimethanil showed better or equal control.

Keywords: *Trichoderma harzianum*, *Botrytis cinerea*, strawberries, *Apis mellifera*, biocontrol.

Introduction

Trichoderma is one of the most investigated fungi which has been shown to control various plant pathogens. Although not normally associated with foliar surfaces, *Trichoderma* spp. have been widely investigated for the control of foliar diseases, such as grey mould incited by *Botrytis cinerea* (Gullino, 1992), particularly of strawberry (Tronsmo and Dennis, 1977; Gullino *et al.*, 1989; 1990). Peng *et al.* (1992) proposed the use of *Apis mellifera* for vectoring *Gliocladium roseum* to the strawberry flowers: such a technique achieved good results in terms of antagonist delivery and disease control. Our research aimed to verify the vectoring efficiency of the honeybees and to assess the efficacy of strains of *T. harzianum* selected for their activity against *B. cinerea*, applied by bees or as water suspension, under field conditions.

Materials and methods

Two experimental trials have been carried out in 1997 and 1998. Two screen houses (25x4,5x2,5 m) were built up in a strawberry field grown crop of the cv Marmolada, highly susceptible to *B.cinerea*. The following treatments were compared: *1) T. harzianum* powder preparation vectored by the honeybees; *2)* water suspension of *T .harzianum*; *3)* chemical treatment with pyrimethanil (Scala, 50% a.i; 2g/l; 0.1 l/m^2); *4)* water. In 1997, the *T. harzianum* preparation was a mixture of strains, at 4.9×10^{10} Colony Forming Units/g (CFU/g), applied at the concentration of 2 g/l (1×10^8 CFU/ml), using 0.1 l/m^2; in 1998, the preparation was at 1×10^8 CFU/g applied at a concentration of 10 g/l (1×10^6 CFU/ml), using 0.1 l/m^2. The *T. harzianum*-honeybees treatment was arranged in one screen house, and a dispenser was placed at the exit of the hive to allow the contamination of the honeybees by the antagonist. In the other screen house, the other treatments were randomly distributed, and one honeybee colony was introduced only for pollination. The treatment with *T. harzianum* vectored by the bees continued for two weeks, using ca. 5 g of the same powder preparations

in the dispenser each morning. Pyrimethanil was sprayed twice; the *T. harzianum*-spray treatment and the water treatment were applied five times, while contemporarily water was applied to the *T. harzianum*-honeybees and chemical plots. Flowers were labelled in each plot (240 in 1997; 150 in 1998). To assess the efficiency of bees as vectors of the antagonist, the amount of *T. harzianum* inoculum on different matrixes was evaluated, after reisolation on a selective medium (Elad *et al.*, 1981): i) on 5 bees captured at the exit of the dispenser; ii) on 20 strawberry flowers collected after being visited by a bee; iii) on five pieces of wax taken both from the anterior and the posterior part of the combs (only 1998). To evaluate the efficacy of *T. harzianum* in providing disease control, the incidence of grey mould was recorded by counting the number of rotted fruits: i) at the harvest; ii) after 7 days of fruit storage at 5±1°C; iii) after 1 more day of storage at ca. 20°C; iv) the cumulative damage was also measured (all fruits with grey mould counted in the three checks, on the total number of harvested fruits).

In 1997 strawberry fruits harvested from the same plot were stored mixed in commercial plastic boxes, while in 1998 each strawberry was kept isolated. Climatic conditions were registered since the flowering till the ripening of the fruits. Because of the dry weather, the plants were irrigated in the evening, by sprinkle irrigation in 1997, by hand carried pump in 1998, ($3l/m^2$) to favour disease development. Grey mould incidence, recorded for the four treatments, was analysed through a 1-way ANOVA, and Tukey test for mean separation.

Results and discussion

Honeybees efficiency in vectoring T. harzianum

In both 1997 and 1998, the simple dispenser developed resulted suitable for *T. harzianum* distribution by the honeybees. Particularly, it proved to be very efficient in selecting only the outgoing honeybees, as showed by the very low amount of *T. harzianum* conidia registered on the wax sampled from the anterior part of the hive ($1.7x10^3 \pm 2.4x10^3$) and from the posterior part ($0.6x10^3 \pm 0.6x10^3$). On the contrary, the sampled outgoing insects showed on average $2.7x10^5 \pm 6.2x10^5$ conidia in 1997, and of $4.5x10^6 \pm 4.3x10^6$ in 1998. The honeybees were successful in delivering *T. harzianum* conidia to the strawberry flowers: in 1997 the mean presence of conidia was $3.9x10^4 \pm 3.1x10^4$ and in 1998 it was of $4.1x10^5 \pm 5.5x10^4$ CFU/flower.

Efficacy of *T. harzianum* in preventing grey mould development on the fruits

In 1997, the incidence of grey mould was generally low, with very slight differences among the treatments, for many of the 7 dates of harvest (6-26 of May) (fig. 1). Moreover, the abortion of high percentages of the labelled flowers caused by the low temperatures, resulted in a high variability among the number of fruits per plot. The heterogeneity of variances (Levene test; $P<0.001$) didn't allow the data analysis through 1- way ANOVA for each date of harvest, but only for the total production. At harvest, pyrimethanil was statistically more effective in preventing grey mould development compared to *T. harzianum* treatments (both applied as a water suspension or by the bees) which showed an insufficient activity. Similar results were observed on fruits stored at 5±1°C. At the last check, after one more day of storage at 20°C, the statistical analysis resembled that of the harvest, with pyrimethanil showing the best control (fig. 2). By analysing the cumulative damage, pyrimethanil proved to be the most effective treatment, by preventing the development of *B. cinerea*; the *T. harzianum*-spray treatment also provided partial activity, significantly reducing the incidence of *B. cinerea* attacks with respect to the non treated fruits, while the *T. harzianum*-bees treatment was less effective (tab.1).

In 1998, after a very dry flowering and ripening period, very heavy rains occurred during the harvest. The incidence of *B. cinerea* on the fruits for each date of harvest is reported in figure 1, which show a very low disease incidence for all the treatments at the first harvest, and then a significant increase of the damage after the heavy rains. As in 1997, the variances homogeneity is respected only considering the total strawberry production of each plot.

At harvest, the *T. harzianum*-spray treatment was as effective as the pyrimethanil in providing protection against *B. cinerea*, while the *T. harzianum*-honeybees treatment was less effective, showing no significant difference in the percentage of damage with respect to the water treatment (fig.3). After the storage at 5±1°C, both the *T. harzianum*-spray and the *T. harzianum*-honeybees treatment showed the same activity as the fungicide, significantly but only partially reducing the *B. cinerea* damage on the fruits compared to the non treated fruits

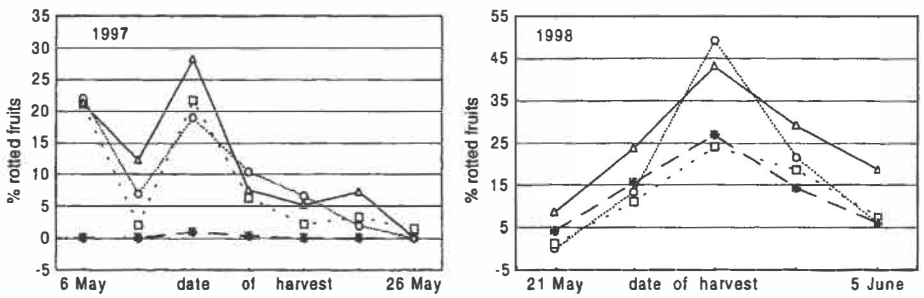

Figure 1. Incidence of grey mould on strawberry fruits at harvest in 1997 and 1998.

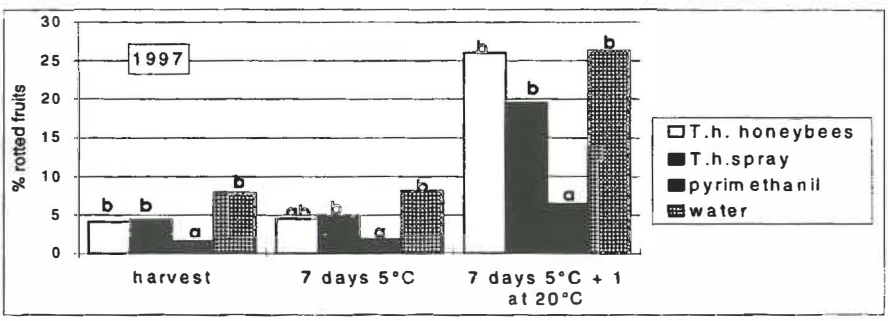

Figure 2. Efficacy of different treatments at harvest and during storage in 1997.

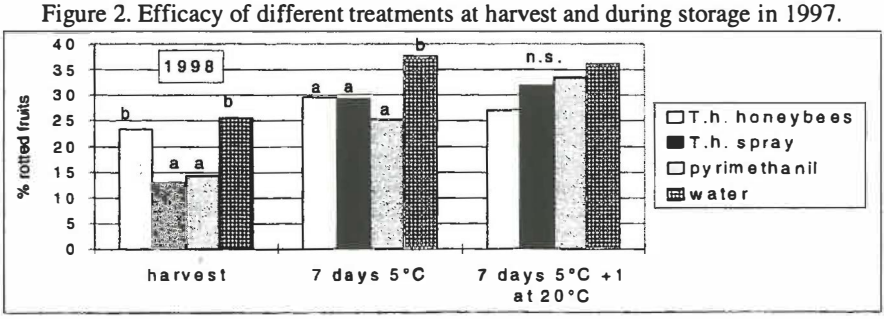

Figure 3. Efficacy of different treatments at harvest and during storage in 1998.

Table 1. Cumulative incidence (%) of rotted fruit in the two years of trials (mean±sd).

Year	*T. harzianum*-honeybees	*T. harzianum*-spray	pyrimethanil	water
1997	33.3±4.4 *bc*	27.3±6.9 *b*	9.6±4.1 *a*	39.5±5.6 *c*
1998	60.5±3.1 *a*	58.0±5.0 *a*	57.0±7.6 *a*	70.2±3.1 *b*

(fig. 3). At the last check, after one more day at 20°C, the ANOVA showed no significant difference between fruits harvested in treated and not treated plots (fig.3). By evaluating the cumulative percentage of damage, *T. harzianum* provided significant suppression of the grey mould development, both as a sprayed water suspension or delivered by the honeybees, which showed the same efficacy of pyrimethanil (tab.1).

In conclusion, the data showed that the honeybees' vectoring system works well also for *T. harzianum*, thus confirming the results obtained in Canada with *G. roseum* (Sutton and Peng, 1993). Honeybees resulted highly contaminated by the antagonist and vectored *T.harzianum* to the flowers at levels comparable to those reported by Peng *et al.* (1992). Concerning the efficacy in *B. cinerea* control, the antagonist showed only a partial activity when distributed by the honeybees, while the water suspension, expecially in 1998, provided better results, confirming the results already reported by Gullino *et al.* (1989; 1990). Pyrimethanil provided the best control in 1997, being, on the contrary, much less effective in 1998. Such reduced activity could be explained by a late occurrence of the first spray (on April 29). The efficacy of *T. harzianum* was more evident at the checks carried out on fruits after storage then at the harvest. Possibly, the unusully dry climatic condition had a negative impact on *T. harzianum* activity, which could be partially restored during storage, in the presence of a higher relative humidity. New research will be done trying an evening distribution of the antagonist and combining the *Trichoderma* treatment with honeybees at flowering, with water suspension spray during the ripening.

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The potential of *Chrysoperla lucasina* for IPM programmes in greenhouses.

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Abstract : Originating in the southern half of France, *Chrysoperla lucasina*, is a new species of lacewing. The biotic potential of this polyphagous predator is wide and not so different from *Chrysoperla carnea*.

A mass rearing unit of this beneficial insect has been created to produce enough larvae for testss in different crops. The first results show the capacity of *C. lucasina* to contain aphid populations to an acceptable level in late strawberries. The predator also gave interesting results against *Thrips tabaci* in cut flowers (Gerbera and Alstromeria) and in seedling leek.

Key words : *Chrysoperla lucasina*, *Chrysoperla carnea*, mass-rearing, biological control

Introduction

Chrysoperla lucasina (Neuroptera : Chrysopidae) is an important species of the *Chrysoperla carnea* complex and is dominant in the Mediterranean region (Thierry, 1996). Populations of the predator were collected near Toulouse (F) and were mass-reared in the laboratory of the S.R.P.V. (Phytosanitary Service Ministry of agriculture) in Brest (F).

Following an initial study on the diets and feeding behaviour of larvae and adults, the rearing unit has been successful for five years and 6 million eggs were produced in 1998.

This production has enabled experiments to be carried out on different crops (Strawberry, Gerbera, Alstromeria, seedling-leek and egg plant) with variable success in greenhouses and outdoors..

Material and methods.

Chrysoperla carnea sensu lato

Chrysoperla lucasina sensu lato was defined again in 1992 by Cloupeau and Thierry (Malet, 1994). Six different species of Chrysoperla occur in Western Europe, *ankylopteryformis* Monserrat α Diaz-aranda, *carnea* sensu stricto, *kolthoffi* Navas, *lucasina* Lacroix, *mediterranea* Hölzel, *renoni* Lacroix (Thierry, 1992).

In Western Europe, the most important species are *C. lucasina* and *C. kolthoffi* (Thierry, 1994) and can be discriminated by the claw shape (Thierry, 1998).

C. kolthoffi predominates in the north and *C. lucasina* in the south (Thierry, 1994).

Mass rearing

In 1992, M. Canardof Toulouse University provided some *C. lucasina* for our laboratory so that we could develop a mass rearing unit and carry out experiment in different crop in the west of France..

150 adults are introduced in a Plexiglass cylinder measuring 20 cm * 20 cm. A Kraftpaper's sheet is placed around the wall inside the cylinder as an egg-laying area for the females. Adults feed on a mixture of pollen, honey and yeast. Kraftpaper's sheets are collected from the cylinder three times a week for the harvesting of eggs..
Green lacewing eggs are deposited on a slender stalk which can be severed by soaking in diluted bleach. Eggs are swilled and then dried for four hours when they are then ready to be used in experiments.

A larval rearing unit consists of a 1.5 L plexiglas box which countains husks of buckwheat and cardboard sheltersto prevent cannibalism. Larvae are fed with frozen eggs of *Ephestia kuehniella* and honey. At 25 °C, 150 eggs will produce about 25-30 imagos.

Newly emerged adults are fed on the same diet and when they are 7 days old, they are moved in adult feeding and ovoposition units.

Eggs which are destined for experiments are held in a 400 ml plastic flask with buckwheat husks and eggs of *Ephestia kuehniella* to sustain hatching larvae. Because of cannibalism, it's necessary to allow three eggs of *C. lucasina* to obtain one larva. Flasks are kept for 72 hours at 20 °C, before the larvae are used in experiments.

In 1992, 30,000 eggs of *C. lucasina* will be produced in the unit.. After six years of research and improvements, the mass rearing process has become reliable and in 1998, the unit produced more than 6,000,000 eggs.

The Brest laboratory has been working on the reduction of production cost of larvae in order to make *C. lucasina* releases cheaper than chemical sprays. In 1996, the price of one larvae was 0.23 FF, in 1997 it was reduced to 0.09 FF and in 1998 it was 0.06 FF.
Work continues to automate the mass-rearing system to save time reduce cost production of *Chrysoperla lucasina* to 0.01 FF. The predator will be economical at this level.

Advantages and objections regarding *Chrysoperla lucasina*

C. lucasina is a polyphagous predator able to feed on a diversity of prey so it may be used to protect crops against a wide range of pests. In the absence of prey, *C. lucasina* may survive eating other species of Arthropods. Recorded prey include aphids of nearly all families, thrips, tetranychids, eggs and larvae of Tortricidae, Pyralidae, Noctuidae and Pieridae (Canard, 1984). In mass-rearing, larvae feed on eggs of *E. kuehniella* but it is possible to obtain fecund females from larvae fed on eggs of *Artemia sp.*, or with eggs of *Otiorrhynchus sulcatus*.

C. lucasina appears naturally in France and seems specialised to crop pests. Natural enemies of *C. lucasina* may regulate the species.

C. lucasina is acclimatised to Meditteranean conditions and larvae are not sufficiently active in low temperatures to control pests on early crops. In Bretagne (F), night temperatures in early spring are in the order of 0°C to 5°C and the mortality of larvae increases.

Experiments with *Chrysoperla lucasina* in the west of France

C. lucasina has been tested on cut flowers in greenhouses in Brest since 1994. The predator was fairly effective in the control of aphids in Alstromeria and Gerbera at 4 to 6 L1 larvae / m² in 20 releases (J.C. Maisonneuve, 1998). New research shows that there are possibilities to use a complex of *C. lucasina, Aphidoletes aphidimyza, Aphidius sp* and *Aphelinus abdominalis* to control aphid populations on Gerbera (J. Blum, 1998).

Experiments of I.P.M. on early strawberries showed that *C. lucasina* could not control aphid in Finistere (F) because of low night temperatures in spring (0°C to 5°C).
On the other hand, *C. lucasina* may be effective against aphid populations in protected late strawberries (Elsanta). In Finistere, 5 releases of 4 larvae / m² are necessary to success. (Fig. 1). This had been confirmed by other experiments made by SRPV in the Orleans area of central France.

C. lucasina shows potential to control *Thrips tabaci* in IPM experiments carried out on leek crops grown under protection for seed production near Angers (F) where it established well; after releases of larvae (L1), all the stages of the green lacewings were found (Galez, 1998).

C. lucasina in association with *Hypoaspis miles* was also effective against *T. tabaci* in leeks and no chemical sprays were neccesary to protect the crop during pollination (Franco, 1999).

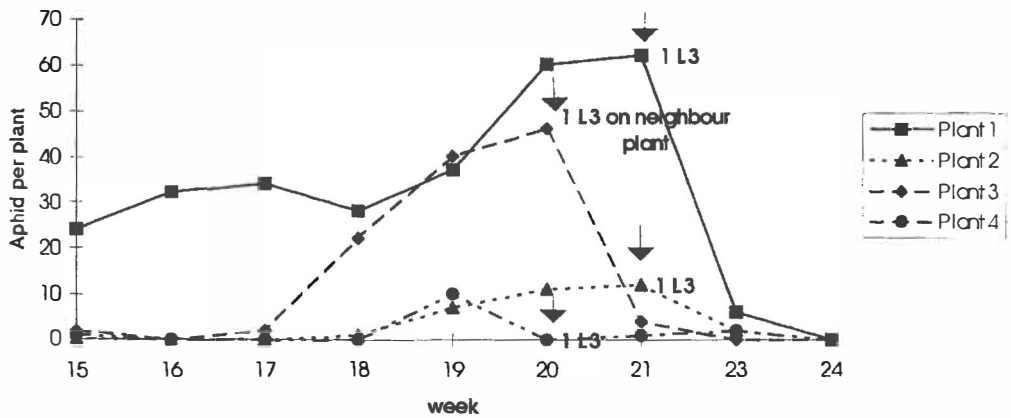

Figure 1 : Evolution of aphid populations on strawberries in 1998 in Finistère (F). Arrows represent the observation of one larvae on the plant or its neighbour .

These experiments show that *C.lucasina* can be used on a wide range of crops under protection and probably outside. One of the characteristics of this predator is that it walks a lot more than *Coccinella*; this helps to define a special strategy of releasing, alone or with other beneficials.
Its polyphagous characteristic and searching behaviour will help in the development of biological control programmes for new crops and will increase the reliability in existing IPM programmes.

Conclusion

The biological control potential of *Chrysoperla spp* is well known but there has never been any experimental work on this predator in greenhouses. Since the predator is polyphagous it has potential in different crops (seeds, vegetables, cut flowers) and can combine with other beneficials to improve efficiency of pest control.

Before the use of *C. lucasina* can be recommended, it will be necessary to decrease the its rearing-cost, which is possible. Twenty years ago, the development of *Encarsia formosa* was possible because it was cheaper than chemical control programmes, and now it is universally used. This must also be possible for *C. lucasina*..

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Some biological characteristics of *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera : Platygasteridae), parasitoid of the greenhouse whitefly

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Abstract : The parasitoid *Amitus fuscipennis* MacGown and Nebeker (Hymenoptera: Platygasteridae) is being evaluated as a potential biological control agent of *Trialeurodes vaporariorum* (Westwood) (Homoptera : Aleyrodidae) on bean crops in Colombia. Partial life history data of this natural enemy is presented here. The development and longevity of of adult parasitoids were compared under different temperature and relative humidity combinations. The intrinsic rate of increase of *A. fuscipennis* was obtained at different climatic conditions on two different bean cultivars using *T. vaporariorum* as host. Based on our results, *A. fuscipennis* is considered a good candidate for biological control of the greenhouse whitefly because a) its intrinsic rate of increase is higher than the intrinsic rate of increase of *T. vaporariorum* at hillside and lowland climatic conditions, and b) it has a broad altitudinal distribution but very dry conditions and high temperature diminish its longevity.

Key words: *Amitus fuscipennis*, *Trialeurodes vaporariorum*, *Phaseolus vulgaris*, longevity, developmental time, intrinsic rate of increase, IPM.

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera : Aleyrodidae) reaches key pest status on bean (*Phaseolus vulgaris* L) crops in Colombia. Due to its damaging effect in crop production, chemical treatment was intensively applied and pest resistance to pesticides appeared from 1978-1979. Recently whitefly resistance to a number of insecticides has been detected (Cardona *et al.*, 1998). As an alternative to chemical control, an IPM system has been implemented. It consists of a combination of cultural control practices with the application of insecticides at a pre-established action level (Prada *et al.*, 1993). The IPM system was tested in a series of trials conducted in farmes's fields and it resulted in 54% reduction in insecticide use and 18% reduction in costs, compared to the conventional pesticide spray programme (Prada *et al.*, 1993). The biological component of this IPM system is still missing.

In order to fill this gap, the parasitoid *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera : Platygasteridae) is being evaluated as a potential biological control agent of *T. vaporariorum* on bean in Colombia. *A. fuscipennis* is easily found on crops in the surrounding areas and it was able to colonise naturally a chrysanthemum crop where previously *Encarsia formosa* Gahan had been released (L. E. perez, personal communication).

The performance of *A. fuscipennis* on tomato greenhouses is now being studied (R. de Viss, personal communication). Partial results of the work carried out to collect life history

data needed to understand the capabilities and limitations of this natural enemy are presented here.

Materials and methods

Insect rearing

A. fuscipennis was reared on *T. vaporariorum* first and second instars nymphs on bean cv. ICA-Pijao plants at 22-24 °C, 40-80% RH, 12L :12D.

Longevity

Longevity of *A. fuscipennis* females was determined by enclosing parasitoids less than 24 h old in individual glass vials (11 ml). Females were fed with honey three times per week. The vials were stored in climate cabinets at different temperature and relative humidity conditions : 15 °C, 45 ± 5% RH ; 19 °C, 90 5% RH ; 25 °C, 55 ± 5 % RH. Another group of females was kept at 15, 19 and 25 °C, uniform 75 ± 5% RH. Survival was recorded daily. Differences in female longevity were analyzed using ANOVA.

Developmental time

Bean plants infested with first instar nymphs of *T. vaporariorum* were enclosed using an acetate cone and exposed to 40 or 60 parasitoid females during 24 or 48h. The plants were then transferred to climate cabinets at different temperature and relative humidity combinations as in longevity experiments. Differences in developmental time were tested using one-way ANOVA.

Intrinsic rate of increase

Small clip-cages were used to create 2 or 3 spots of first instar *T. vaporariorum*. Each spot had at least 50 nymphs. Unmated female parasioids less than 24 h old were introduced individually into a larger clip-cage that covered the nymphal spot. Every 24 h the parasitoid was removed to a new spot until she died. Parasitised whiteflies were reared until the parasitoids emerged. Twenty-three females were tested at 19 °C, 80% RH, 12L :12 D and 21 females at 22 °C, 72 6% RH, 12L :12D on the commercial bean cultivar "Chocho". Twenty females were tested on the non-commercial bean cultivar "ICA-Pijao"at 19 °C, 80% RH, 12L :12D. With data from the experiments the intrinsic rate of increase, r_m, of *A. fuscipennis* was calculated.

Results and discussion

Longevity of fed *A. fuscipennis* females was longest at 19 °C, 90 5% RH (18.1 d) and shortest at 15 °C, 45 5 % RH (3.9 d). At different combinations of temperatures and relative humidities, the longevity without hosts differed significantly (Table 1, one-way ANOVA, $F = 34.9$, $p < 0.0001$; all means are different, Duncan´s test $p< 0.05$). Mean longevity of *A. fuscipennis* found here without hosts is longer than longevity reported for *A. hesperidium* (Flanders, 1969 ; Zhanng *et al.*, 1982) and *A. bennetti* (Drost *et al* ., 1996).

Developmental time increased when temperature decreased. Developmental time of *A. fuscipennis* differed significantly between the different temperature and relative humidity combinations (Table 2, one-way ANOVA, $p < 0.0001$). The effect of humidity was significant within 15 °C and 19 °C but not within 25 °C (table 2). *A. fuscipennis* shows high longevity at high relative humidity conditions and temperatures around 15-19 °C, conditons that are

similar to the environmental conditions of hillside areas where big areas of beans are grown in Colombia and where the parasitoid is easily found.

Table 1. Longevity (days) of *A. fuscipennis* fed females in relation to temperature T , (°C) and relative humidity, RH (%).

T	15	15	19	19	25	25
RH	45 ± 5	75 ± 5	90 ± 5	75 ± 5	55± 5	75 ± 5
Mean	3.9	42.2	17.3	18.1	6.2	10.1
N	91	97	78	72	71	71
SE	0.1	1.3	0.6	0.8	0.4	0.4
range (days)	1-7	3-67	3-29	1-37	1-13	1-17

All means differed significantly (one-way ANOVA, Duncan's test $p < 0.05$).

The intrinsic rate of incrase was lower at 19 °C (0.099) than at 22 °C (0.144) on cv. "Chocho". The rm at 19° C for cv. " Ica-Pijao" was 0.085. The r_m values of *A. fuscipennis* were higher than r_m values of *T. vaporariorum* at the same climatic conditions and bean cultivars (Manzano *et al.*, unpublished results). Results suggest that *A. fuscipennis* may overcome whitefly populations at hillside areas around 1500 m (with average conditions of 19 °C, 80% RH), and lowland areas around 1000 m (with average conditions of 22 °C, 72% RH) where bean is also cropped.

Table 2. Developmental time (days) and immature mortality (%) of *A. fuscipennis* in relation to temperature, T (°C) and relative humidity, RH (%).

T	15			19			25		
RH	45 ± 5		75 ± 5	90 ± 5		75 ± 5	55 ± 5		75 ± 5
Mean	51.0	--*--	65.6a	31.3	--*--	38.1b	23.4	--ns--	24.9c
N	450		120	1030		390	260		180
SE	0.1		0.2	0.06		0.1	0.06		0.1
Range (days)	45-62		63-71	27-40		35-45	22-32		22-28

All means differed significantly (one-way ANOVA, $p < 0.0001$). Values at 75 ± 5% RH followed by different letters are significantly different (Tukeys's test , $\alpha = 0.05$, post ANOVA). The effect of humidity was significant within 15 °C and 19 °C but not within 25 °C (NS, test for independent variable, $\alpha = 0.05$).

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Potential for the biological control of *Frankliniella occidentalis* (Pergande) with a nematode, *Thripinema nicklewoodi* (Siddiqi)

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Abstract
Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is a severe pest of most floricultural crops. The allantonematid nematode, *Thripinema nicklewoodi* Siddiqi, is a potential biological control agent of WFT with features that suggest that it may be better than current means of control. The nematode parasitizes female thrips residing within the foliar terminals and flower buds, sterilizing them without killing them. Nematode-infected WFT can enter feeding sites, areas impenetrable with current control options. In other words, nematodes are vectored live into traditionally inaccessible areas of ornamental plants. Here, we provide an overview of the current status of *T. nicklewoodi* for the potential biocontrol of WFT in ornamentals.

Key words: *Thripinema nicklewoodi*, *Frankliniella occidentalis*, biological control

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is the most prevalent species of thrips attacking greenhouse crops (Robb 1989). In floriculture crops, damage resulting from WFT feeding on flowers and foliage can significantly reduces their aesthetic quality, making the plants less valuable or even totally unmarketable. Hence, early detection of WFT infestations is desirable to avoid extensive plant damage. This is however difficult due to their small size and secretive feeding behavior. Thus, although the final feeding damage is readily identifiable, by this stage irreversible damage has occurred, frequently rendering the plants of lower economic value or even totally unmarketable. In addition to direct feeding damage, indirect damage by WFT also results from their transmission of tospoviruses, both tomato spotted wilt virus (TSWV) and impatiens necrotic spot tospovirus (INSV) in ornamentals, further adding to economic crop losses. The only effective means of controlling tospovirus is the eradication of viruliferous WFT. Considering the high value of ornamental crops, the range of floriculture and nursery crops damaged and seriousness of both direct and indirect damage, it is imperative for growers to achieve effective long-term control of WFT. However, with the control options currently available, this is not always achieved or maintained. Hence there is urgent need for alternative methods of control.

Traditionally, insecticides have been relied upon to provide WFT control. However, they are not always successful due to both poor contact between the sprays and WFT and resistance having developed to many of the commonly applied insecticides (e.g, Robb, 1989; Immaraju *et al.*, 1992). Insecticide cross-resistance has also been reported (Brodsgaard, 1994). Furthermore, growing awareness of the environmental impact of insecticides has led to efforts to reduce usage. The use of arthropod natural enemies against WFT on a number of

greenhouse crops, such as green pepper and cucumber, has resulted in some success (e.g., Castañe *et al.* 1996). However, their use on ornamentals has, to date, been unsuccessful (e.g., Loomans & van Lenteren, 1996). These failures are likely due to the relatively large body size of the natural enemies, which hinders their progress into the niches that WFT life stages occupy. The effective use of other microorganisms has been limited. There are no known effective bacterial or protozoan pathogens, and the use of viral pathogens is currently not practical. Entomopathogenic fungi have displayed pathogenicity in laboratory studies, but such potential has generally not been translated to the more rigorous conditions of the greenhouse (Brownridge, 1995). The use of entomopathogenic nematodes has been limited, with bioassays conducted only against the soil dwelling stages of WFT. A number of species of *Steinernema* and *Heterorhabditis* have been tested but displayed considerable variability in their ability to control pupae of WFT (e.g., Helyer *et al*, 1995; Chyzik *et al.* 1996).

In contrast, nematodes of the genus *Thripinema* (Tylenchida: Allantonematidae) may have considerable potential for the biological control of thrips. There are currently five described species, although here we are specifically interested in *Thripinema nicklewoodi* (Siddiqi), an obligate parasite of WFT. Although there is little published information on this nematode, the potential for using *T. nicklewoodi* for WFT biological control has been noted by several authors (Nickle & Wood, 1964; Reddy *et al.*, 1982). Recently, Heinz *et al.* (1996) reported *T. nicklewoodi* as the numerically dominant common natural enemy of WFT infesting floriculture and nursery crops in California. Here, we provide an overview of the current status of *T. nicklewoodi* and its potential for WFT biocontrol on ornamentals.

Biology

Larval WFT are infected by the adult female nematode, only female WFT being infected. Infection of WFT pupae and adults does also occur. Infection by *T. nicklewoodi* does not lead to death of the WFT; the result is sterilization. The number of parasitic females infecting a thrips host varies; the highest number we have observed developing in the abdomen of WFT is eleven. Following infection, the oviparous female nematodes release their eggs into the WFT. Upon hatching, the vermiform juveniles feed within the thrips' abdominal cavity, with finally the mature male and female nematodes exiting the host via the anus in the frass. *Thripinema* fertilization and infection of new hosts probably occurs within plant structures that provide the high humidity necessary for nematode movement. Sharga (1932) suggested that *Thripinema* fertilization may occur within the host prior to emergence based on the observation that no males were observed emerging from the host. However, it is generally thought to occur outside of the host (Nickle & Wood, 1964; Siddiqi, 1986). The preferred feeding site of WFT, within the confined habitat of flowers and meristematic tissues, is an ideal habitat for such nematode activities.

Site of Transmission

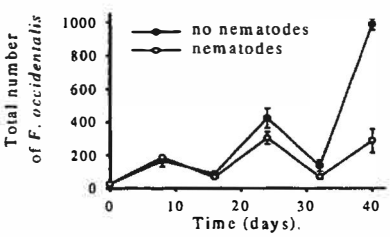
In order to determine the site of transmission of *T. nicklewoodi* we conducted a preliminary study to examine on which plant structure infection of WFT does indeed occur. Cut chrysanthemum flowers, with leaves and stems attached, were infested with both adult female WFT infected with *T. nicklewoodi* and uninfected larvae. All thrips were removed after four days and status of nematode infection assessed. Nematode-infected larval WFT were recovered only from the flowers, with those that had fed on the leaves not infected. Additionally, flowers and leaves were washed with tap water to check for the presence of nematodes. Approximately 88% of the nematodes recovered were found on flowers. The results from this study tend to confirm that infection of WFT by *T. nicklewoodi* does occur

primarily within the protected environment of the flower. However, we have also shown that, in the absence of flowers, transmission does occur on the leaves.

***Population Suppression of WFT by* T. nicklewoodi**

Thripinema nicklewoodi is a potentially promising biological control agent of WFT, with features that suggest that it may be better than current means of control. Nematode-infected WFT can enter feeding sites, areas impenetrable with current control options. In other words, nematodes are vectored live into traditionally inaccessible areas of ornamental plants. Indeed, we have already shown that it is in these exact sites where transmission of *T. nicklewoodi* occurs (see above). Such vectoring of the nematode should effect long-term WFT suppression. Can long-term suppression be achieved? Results from a preliminary study that we have conducted suggest that long-term suppression of WFT by *T. nicklewoodi* can be attained. We established populations of nematode infected and uninfected WFT by releasing nematode infected or uninfected WFT (controls) in cages containing kidney bean leaves as a food source, with transmission of *T. nicklewoodi* then allowed to proceed naturally. Numbers of WFT life stages were then recorded every eight days for a total of three WFT generations. In Figure 1, the midpoints of each of the three WFT generations for both infected and

Figure 1. The effect of *T. nicklewoodi* on *F. occidentalis* populations



uninfected are on days 8, 24 and 40. No difference in WFT numbers between the two populations is observed on day 8. By day 24, a small difference in WFT numbers are observed, with the numbers being slightly lower in the nematode infected population. However, by the middle of the third generation (day 40), there is an approximate 3.5 fold reduction in WFT numbers in the nematode infected WFT population. This reduction in WFT numbers in the nematode infected population is even more striking as the initial percentage parasitism in the nematode infected WFT was only 10%.

Conclusion

The preliminary results presented here suggest the potential of using *T. nicklewoodi* for suppression of WFT. We have shown that transmission of *T. nicklewoodi* occurs within flowers, the very feeding sites of immature WFT. In addition, the nematode-infected WFT vector the nematodes into these sites, areas currently impenetrable with sprays or arthropod natural enemies. Using a natural infection process, we have also demonstrated the potential suppression of WFT by *T. nicklewoodi*. These results suggest that use of *T. nicklewoodi* would be for long-term WFT suppression, and not for immediate reduction in population numbers. However, with development of *T. nicklewoodi* as a spray application, it may be possible that a more immediate reduction in WFT numbers can be attained.

Finally, as an obligate parasite of WFT, *T. nicklewoodi* should prove compatible with other biologically based pest management strategies currently being developed for biocontrol WFT. Host specificity is a feature of nematodes belonging to the family Allantonematidae, with each species usually restricted to one or several host genera and rarely found infecting insects of different families. To date, *Thripinema* species have been recovered only from thrips species.

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Biological pest control in cucumbers in the Netherlands

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Abstract: Biological control of pests in cucumbers in 1997-1998 has been very successful. More than 95% of the Dutch cucumber growers are using IPM. Control of cotton aphids (*Aphis gossypii)* using banker plants of wheat with *Rhopalosiphum padi* and *Aphidoletes aphidimyza* has proved to be effective. *Amblyseius limonicus* has been shown to be a good predatory mite for biological control of thrips, however, a commercial rearing system has not been found. A previously undescribed *Franklinothrips spp.* has been tested for biological control of western flower thrips (*Frankliniella occidentalis)* in cucumbers and initial results are promising. Some remaining bottlenecks in biological control in cucumber are the rapid population development of cotton aphids (*Aphis gossypii*), the appearance of the European tarnished plant bug *Lygus rugulipennis,* and biological control of caterpillars such as *Chrysodeixis chalcites.* An overview of the status of biological control in cucumbers in the Netherlands is given.

Key words: *Frankliniella occidentalis, Franklinothrips* spp., *Amblyseius cucumeris*, *Amblyseius limonicus,* banker plants, *Aphis gossypii, Aphidoletes aphidimyza*, cucumbers

Introduction

In 1998 there were about 700 hectares of cucumbers grown in glasshouses in the Netherlands. Cucumber was the first crop in the Netherlands where biological control was used back in 1967 when growers started to use *Phytoseiulus persimilis* for biological control of two-spotted spider mite. Currently, more than 95 % of the Dutch cucumber growers are using biological control in at least the first planting, which starts in December and lasts until April or June depending on the number of plantings per year. About 50% of the Dutch cucumber growers will also use biological control in the second and third planting.

The main pests occurring in cucumber in the Netherlands are cotton aphid (*Aphis gossypii*), onion thrips (*Thrips tabaci),* western flower thrips *(Frankliniella occidentalis*), two-spotted spider mite (*Tetranychus urticae*) and greenhouse whitefly (*Trialeurodes vaporariorum).* Caterpillars (*Chrysodeixis chalcites*) and plant bugs such as the European tarnished plant bug *Lygus rugilipennis* are pests for which adequate biological solutions are still missing.

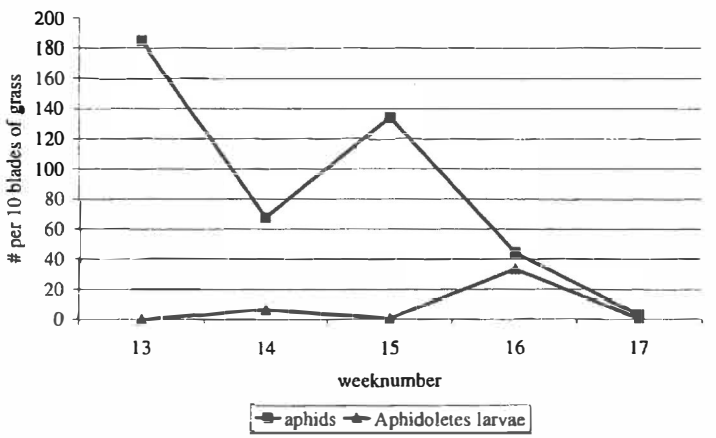
In the Netherlands growers currently tend to increase the number of plantings from two to three or even four plantings per year. Because of the increasing number of plantings per year and therefore shorter cropping periods, biological control is becoming more expensive for the growers. Every time a new crop is planted, the biological control program needs to be started up again, which increases the total annual cost of a biocontrol program. Since 1997 more and more cucumber growers have begun using the system of interplanting when beginning a new crop. In this system growers do not disinfect their greenhouse anymore between crops. This means that the natural enemies can immediately establish on the new plants. A potential disadvantage if pests and diseases are not managed well is that they can

also immediately colonise the young plants. Below we will focus on the current status of biological control in cucumbers in the Netherlands.

Aphids

Aphis gossypii can multiply very quickly in a cucumber crop (Van Steenis & El-Khawass, 1996). Growers start with weekly preventive releases of the parasitoid *Aphidius colemani* from the beginning of March onwards, when the pest pressure from outside starts to increase. Hyperparasites such as *Dendrocerus carpentri* can completely upset biological control of aphids with *A. colemani* from April on. Weekly releases of the predatory gall midge *Aphidoletes aphidimyza* can be effective when hyperparasitism appears. However, many growers do resort to cheaper, easier-to-use, systemic insecticides.

Banker plants, also called "open rearing systems", provide the aphid parasitoids with a substitute host on which they can maintain a population during periods when the target aphids are low in numbers. These banker plants consist of small trays of wheat infested with the cereal aphid *Rhopalosiphum padi*. Results with banker plants for *A. colemani* have been excellent. About 30% of the Dutch cucumber growers are currently using banker plants in their first planting. We tested whether banker plants could be used as well for the predatory gall midge *A. aphidimyza*. Two banker plants with *R. padi* were installed in a greenhouse of 14.000 m^2. Because the larvae of *Aphidoletes* pupate in the soil, wheat was sown in large trays filled with coconut fibre (two trays of 60x40cm). Weekly, the number of aphids and the number of *A. aphidimyza* larvae were assessed on 10 leaves (graph 1). To test the dispersion of *A. aphidimyza,* extra banker plants were installed at a distance of 70m from the banker plants where the gall midges were released. Within 14 days the larvae of gall midges were also observed there.



Graph 1. Number of aphids and larvae of *A. aphidimyza* per 10 blades of grass on banker plants.

Spider mites

Phytoseiulus persimilis is still used for biological control of two-spotted spider mites. The predatory gall midge *Feltiella acarisuga* is frequently observed as a naturally occurring and

very effective natural enemy of two-spotted spider mites in Dutch greenhouses. Results with releases of mass-reared gall midges have been mixed. The predatory mite *Amblyseius californicus*, known to be able to survive longer in a crop without prey, have also been released. More research into understanding the possibilities and limitations of these potentially very useful beneficials is needed, as are more studies with alternative strategies such as "pest-in-first".

Whitefly

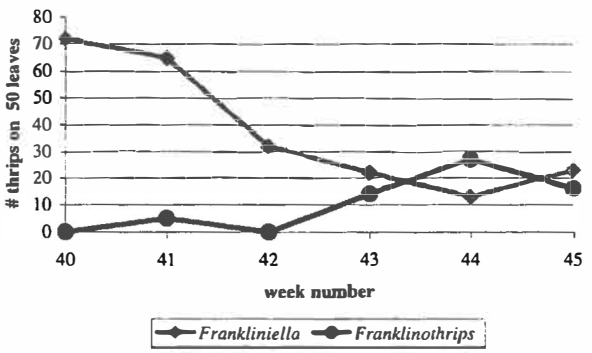
In the Netherlands only the greenhouse whitefly *Trialeurodes vaporariorum* is found on greenhouse cucumbers. It is standard practice to start with weekly preventive releases of *Encarsia formosa* immediately after planting. A mixture of *E. formosa* and *Eretmocerus eremicus* (50/50) gives very positive results. Some growers who are interplanting or growing cucumbers in a high wire system are experimenting with using *Macrolophus caliginosus*. The main problem with *M. caliginosus* is the long generation time and inconsistent establishment in cucumbers. Establishment can be favoured by providing the introduced *M. caliginosus* with a food source such as moth eggs (*Ephestia kühniella*) during the first weeks after introduction. Early in the year, before fungicides have been used for controlling powdery mildew, *Verticillium lecanii* (MYCOTAL) can be used to manage whitefly populations complementary to using natural enemies.

Thrips

Two thrips species infest cucumbers in the Netherlands: onion thrips (*Thrips tabaci*) and western flower thrips (*Frankliniella occidentalis*). Since its introduction into Europe in 1983, western flower thrips, *F. occidentalis*, has become a major pest of greenhouse grown cucumbers in the Netherlands (Van Houten & Van Lier, 1996). In contrast to most of the other important pests on cucumbers there are currently no selective insecticides available which can be used to control a thrips population without disrupting the natural enemies. Therefore, effective biological solutions for thrips are critical. In cucumbers thrips can successfully be controlled with the predatory mite *Amblyseius cucumeris* with a combination of slow release systems (paper sachets) and curative introductions, as well as *Orius* spp.
Because of the higher predation and oviposition rate of *Amblyseius limonicus* on a diet of thrips larvae reported by Van Houten et al. (1995) field trials were set up to compare both *A. cucumeris* and *A. limonicus*. Van Houten (1996) already proved that the impact on thrips populations throughout the season by *A. limonicus* was greater than the impact of *A. cucumeris*. In those experiments equal numbers of both species were introduced (it is well known that high numbers of *A. cucumeris* are needed to get satisfactory control of thrips). Both *A. cucumeris* and *A. limonicus* can use pollen as a substitute food source in the absence of prey. Unlike sweet peppers, cucumber flowers do not produce pollen. This was the main reason for the development of the controlled release sachets for *A. cucumeris*. We compared *A. limonicus* and *A. cucumeris* extensively in field trials and came to the same conclusions as Van Houten with regards to control capacity of *A. limonicus* and the positive influence of pollen on population development of both predatory mites (Altena K. & H. Hoogerbrugge, unpublished data). We found that *A. limonicus* is generally much more sensitive to fungicides than *A. cucumeris*, which is a major drawback. Fungicides are often used weekly to control powdery mildew in cucumbers. Another limiting factor for the use of *A. limonicus* has been the difficulty in developing an economical mass-rearing system for this predator.

Promising results were reported with the predatory thrips *Franklinothrips vespiformis* for biological control of *Echinothrips americanus* in sweet peppers.
We conducted trials with *Franklinothrips* spp. collected from an avocado orchard in California.

About 300 western flower thrips (*F. occidentalis)* were introduced on two rows of 9 cucumber plants of 1,5 m high with 8 to 11 leaves. One week later 50 *Franklinothrips* spp. (50% females) were released in the crop. The number of thrips and *Franklinothrips* was assessed weekly on 50 leaves (graph 2).



Graph 2. Number of *F. occidentalis* and *Franklinothrips* spp. on 50 leaves.

In preliminary trials *Franklinothrips* showed promising results for biological control of *F. occidentalis* in cucumbers and was able to reduce a thrips population to an acceptable level. One disadvantage of this *Franklinothrips* spp. is that it has its pupation sites on the ground and not on the leaves. Further research is needed to determine the possibilities and limitations of this predatory thrips species.

Conclusion

Biological control in cucumbers in greenhouses in the Netherlands is very successful in the spring crop. Cotton aphids, true bugs and caterpillars are the major challenges to biological control in the second and third crop. Current research is focussing on banker plant systems for aphid control. Timely introductions of *Amblyseius cucumeris* remain the best solution for thrips control.

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Interaction between fungal pathogens and natural enemies: Implication for combined biocontrol of greenhouse pests

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Abstract: Advancements in fermentation and formulation technologies have led, in recent years, to the widespread commercial availability of effective entomopathogenic fungi for pest control programs in greenhouses. Natural enemies have been observed to persist in the presence of one fungal pathogen suggesting both natural enemies and fungi could be employed within pest control programs. Experimental trials were conducted to evaluate the degree of compatibility between the fungus, *Beauveria bassiana* and two economically important natural enemies. Results indicated *B. bassiana* did not significantly impact populations of the parasitoids *Lysiphlebus testaceipe* or *Aphidius colmani* attacking the melon aphid, *Aphis gossypii.* Furthermore, evidence suggests that when used together, both parasitoids and the fungus contributed toward aphid suppression.

Key words: ornamentals, *Lysiphlebus testaceipes*, *Aphidius colmani*, *Aphis gossyppi*, *Beauveria bassiana*, biological control

Introduction

Effective integrated pest management (IPM) programs for ornamental crops have yet to be fully implemented. Over reliance on repeated applications of traditional pesticides have presented a number of problems for growers including the development pesticide resistance, threats to worker safety and the environment, and interference with IPM tactics such as biological control.

A major obstacle to implementing IPM in ornamental crops has been the lack of effective alternatives to conventional pesticides. In recent years, advances in fermentation technologies and formulation have made effective microbial pesticides commercially available for greenhouse and field use. One such product is the entomopathogenic fungi, *Beauveria bassiana* (Mycotech corp., Butte MT, USA). *Beauveria bassiana* has been shown an effective alternative control agent for many important ornamental pests including the western flower thrips, the melon aphid, rose and green peach aphid and whiteflies.

Preliminary studies and observations have also shown some natural enemies of key ornamental pests can persist under *B. bassiana* applications suggesting both fungi and natural enemies could be used together in pest control programs.

Here, we present the results of experimental trials designed to test the impact of *B. bassiana* applications in the presence of two important parasitoid species, *Lysiphlebus testaceipe* and *Aphidius colmani* attacking the melon aphid, *Aphis gossypii.* The trials were undertaken to examine the interaction between *B. bassiana* and parasitoid populations when used simultaneously for pest control.

Materials and methods

Caged field trial. This study was established to test the effect of fungi and natural enemies alone and in combination against *A. gossypii* populations infesting asiatic lilies. Field cages were

established in a commercial fresh cut lily greenhouse in Arcata, California, USA. Twenty eight cages measuring 2 x 1 x 3 ft were placed over asiatic lilies (17 to 25 lilies per cage). Each cage was randomly assigned to one of four treatment groups; *A. colmani* only, *B. bassiana* only, *A. colmani* plus *B. bassiana* (8 replicate cages each) and an untreated control (4 replicate cages). Fifty mixed age melon aphids (*Aphis gossypii*) were introduced to the cages and allowed to reproduce for 2 weeks uninhibited. *Beauveria bassiana* was applied at the label rate of 1.2 gm/liter to the appropriate cages. Within twenty four hours approximately 10 *A. colmani* pupae were also introduced to treatment cages. Three weeks after treatment applications, 3 leaves from each of six plants within each cage replicate were sampled and the number of aphids, parasitized aphids and fungus infected aphids were recorded.

Laboratory trial. A laboratory trial was conducted on potted chrysanthemums infested with *A. gossypii* and used to determine how the timing of parasitoid release, with respect to *B. bassiana* applications, effected aphid control. Eight caged leaf replicates containing a starting population of 25 *A. gossypii* were erected for each of 6 treatments (48 replicates total). The leaf cages were constructed using ventilated 90mm plastic petri dishes. The treatments were, 1. aphids only, 2. *L.testaceipes* only, 3. ***B.bassiana*** only, 4. aphids plus *B. bassiana* and *L. testaceipes* (parasitoids released 3 days prior to *B. bassiana* spray) 5. aphids plus *B. bassiana* and *L.testaceipes* (parasitoids released immediately after *B. bassiana* application) 6. aphids plus *B. bassiana* and *L.testaceipes* (parasitoids released 3 days after *B. bassiana* application). Aphids were allowed to reproduce uninhibited for 3 days before treatment applications. Parasitoids were released at a rate of 15 per replicate and *B. bassiana* was applied at the label rate of 1.2 gm spores / Liter. Twelve days after all treatment applications, the leaf replicates were removed and the number of aphids, parasitized aphids and infected aphids were recorded.

For both the caged field trial and laboratory trial, parasitism rates were determined by holding a subsample of aphids for 7 days and examining them for evidence of parasitism. Fungal infection was determined by disinfecting the aphid cuticle of ungerminated spores with a 0.5 percent solution of hypochlorite and plated on a selective agar medium and examined for evidence of *B. bassiana* infection after 7 days.

Statistical analyses. Results were subjected to analysis of variance using the treatment factor as the independent variable and aphid density, parasitism or fungal infection rate as the response variable. Mean comparisons were accomplished using Tukey-Kramer HSD tests to detect differences among treatment means. Multiple regression analysis using fungal infection and parasitism rates as the independent variables and aphid densities as the dependent variable were used to determine which of these factors explained aphid density.

Results and discussion

Caged field trial. The ANOVA tests showed that at the conclusion of the trial there were significantly different aphids densities among treatments ($F = 8.76$; $df = 4, 27$; $P = 0.0002$), and infection ($F = 3.46$; $df = 3,16$; $P = 0.0481$), but not for the parasitism rate ($F = 1.6$; $df = 3,19$; $P = 0.2264$). Mean comparisons tests determined that all treatments resulted in significant reductions in aphid numbers relative to the control cages (Table 1). No differences in aphid densities were seen among the individual *B. bassiana* and *A. colmani* treatments. For the fungal infection rate, the *B. bassiana* plus *A. colmani* treatment resulted in greater aphid infection relative to all other treatments including the *B. bassiana* alone treatment. In addition, a sub-sample of mummified aphids were removed from the *A. colmani* only and *A. colmani* plus *B. bassiana* treatments and held for 10 days and the number of emerged parasitoids recorded. Parasitoid emergence for the *A.*

colmani only treatment was 100%, and 70% emergence was recorded for the *A. colmani* plus *B. bassiana* treatment.

For the caged greenhouse trial, our results had shown that parasitoids and fungi caused significant reductions in aphids whether used singly or combined. The lack of difference in parasitism rate between the *A. colmani* and *B. bassiana* plus *A. colmani* treated cages did support the proposition that *B. bassiana* conserves *A. colmani* populations thus allowing their persistence when using the fungus for pest control. However, observations on parasitoid emergence did suggest some enhanced mortality may be suffered by *A. colmani* in the presence of the fungus. The enhanced *B. bassiana* infection seen in the *B. bassiana* plus *A. colmani* treatment may indicate a positive effect when both species are used together. However, the greater infection rates did not translate into greater aphid reductions. The reason may have been that aphid densities were too low to test for treatment effects.

Table 1. Replicated caged greenhouse trial comparing *A. colmani*, *B. bassiana*, and *B. bassiana* plus *A. colmani* treatments on *A. gossypii densities*, *A. gossypii* parasitism and *B. bassiana* infection.

Treatment	Aphid density*	% parasitism	% infection
Aphids only	7.0 ± 2.3 a	0.2 ± 0.2 a	2.0 ± 2.0 a
A. colmani	0.58 ± 0.4 b	35.4 ± 14.3 a	20.0 ± 20.0 a
B. bassiana	0.75 ± 0.4 b	0.0 ± 0.0 a	42.3 ± 16.1 a
B. bassiana +A. colmani	1.33 ± 1.1 b	20.0 ± 20.0 a	73.3 ± 18.6 b

*Mean separation using Tukey - Kramer HSD test.

Laboratory trial. Results of the ANOVA showed significant differences among treatment means for aphid density ($F = 25.7$; df = 5,47; $P < 0.0001$), parasitism ($F = 6.88$; df = 5,47; $P < 0.0001$), and fungal infection ($F = 5.21$; df = 5,47; $P = 0.0008$). Mean comparison tests showed that aphid densities were significantly lower among all treatments relative to the aphids only (control) treatment (Table 2). Furthermore, the *B. bassiana* plus *L.testaceipes* simultaneous release (zero day) treatment had significantly lower aphid numbers among all treatments. For parasitism rate, the *L.testaceipes* alone treatment had the highest mean parasitism rate but not significantly greater than the *B. bassiana* plus *L.testaceipes* treatments. Fungal infection was also found to be significantly greater in the *bassiana* plus *L.testaceipes* simultaneous release (zero day) than all other treatments.

The laboratory trial also showed that parasitoids and fungi caused significant reductions in aphids whether treated singly or combined. Within the combined treatments, application of both fungi and parasitoids on the same day resulted in the lowest aphid numbers thus supporting the notion that parasitoids and fungi could be used effectively for pest control. Again, no significant differences in parasitism was detected among the *L.testaceipes* and the combined treatments. Similar to the caged trial, significant increases in *B. bassiana* infection was seen, but only for the combined treatment when released on the same day. The reason for this is uncertain. One possible explanation may be that when *L.testaceipes* and *B. bassiana* are released on the same day, oviposition punctures in the host by parasitoids provide a more effective route for infection by germinating spores than when parasitoids are absent. When *L.testaceipes* is released 3 days before *B. bassiana,* most oviposition activity may have already taken place before the spores arrive. When *L.testaceipes* is released 3 days after the fungi, most fungi may have germinated and not benefited from oviposition activities.

Table 2. Replicated laboratory trial comparing *L.testaceipes*, *B. bassiana*, and *B. bassiana* plus *L.testaceipes* treatments at 3 release times on *A. gossypii* densities, *A. gossypii* parasitism and *B. bassiana* infection.

Treatment	Aphid density [a]	% parasitism	% infection
Aphids only	417.6 ± 49.2 a	1.6 ± 1.4 a	4.9 ± 1.9 a
L.testaceipes	125.8 ± 24.8 b	16.6 ± 2.8 b	8.0 ± 3.8 a
B. bassiana	144.0 ± 26.5 b	2.0 ± 0.8 a	11.1 ± 3.7 a
***L.testaceipes +B. bassiana* -3d** [b]	92.0 ± 25.2 b	7.9 ± 1.7 b	20.0 ± 4.4 a
***L.testaceipes +B. bassiana* 0d** [c]	22.9 ± 4.7 c	9.1 ± 2.4 b	31.9 ± 6.6 b
***L.testaceipes +B. bassiana* +3d** [d]	58.8 ± 15.6 b	7.8 ± 2.1 b	12.6 ± 4.2 a

[a] Mean separation using Tukey - Kramer HSD test. [b]*L.testaceipes* released 3 days before *B. bassiana*. [c]*L.testaceipes* released on the same day as *B. bassiana*. [d]*L.testaceipes* released 3 days after *B. bassiana*.

The multiple regression analysis for both the laboratory and caged field trials revealed that percentage parasitism and percentage fungal infection of aphids were both significant factors explaining aphid density for both trials (Tables 3 & 4). As parasitism and/or infection rates increased, there was a corresponding decrease in aphid densities. The lack of significance for the interaction term suggested both factors operated in an independent manner.

Table 3. Multiple regression analysis from the caged greenhouse trial using parasitism and *B. bassiana* infection as the independent variables and *A. gossypii* as the dependent variable.

Treatment	DF	SS	F ratio	Probability
Parasitism rate	1	1152.8	16.2	0.0002*
Fungal infection rate	1	531.3	67.4	0.0084*
Parasitism x infection	1	3.5	6.5	0.8257

Table 4. Multiple regression analysis from the laboratory trial using parasitism and *B. bassiana* infection as the independent variables and *A. gossypii* as the dependent variable.

Treatment	DF	SS	F ratio	Probability
Parasitism rate	1	191414.3	12.90	0.0008*
Fungal infection rate	1	156448.5	10.53	0.0022*
Parasitism x infection	1	14649.6	0.99	0.3261

Overall, our results have demonstrated that for at least some species *B. bassiana* can conserve natural enemies within pest control programs. Results of the manipulative experiments and regression analyses further indicate that both could be used in combined biological control programs with each factor contributing an independent source of mortality and thereby provide greater control of pests than either factor acting independently.

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Diglyphus isaea (Walker) and *Macrolophus caliginosus* Wagner for biological control of *Liriomyza bryoniae* (Kaltenbach) in tomato

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Abstract: Biological control of *Liriomyza bryoniae* was achieved with *Diglyphus isaea* alone or in combination with *Macrolophus caliginosus* in commercial tomato greenhouses. Where both natural enemies were used, there was a decline in activity of *D. isaea* during summer months. When numbers of *M. caliginosus* decreased during autumn, this resulted in increased survival of *L. bryoniae* in comparison to the greenhouse where *D. isaea* had been introduced alone.

Key words: tomato, biocontrol, *Liriomyza bryoniae, Diglyphus isaea, Macrolophus caliginosus*

Introduction

The parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae) is considered a reliable biocontrol agent against the tomato leafminer *Liriomyza bryoniae* (Diptera: Agromyzidae) and other leafminers in greenhouses (Benuzzi & Raboni, 1992). In Northern Europe *D. isaea* is often used in combination with *Dacnusa sibirica* and/or *Opius pallipes* (Hymenoptera: Braconidae). These are introduced for the early season control as they have better searching capacity than *D. isaea* at low levels of infestation (van der Linden, 1988).

The polyphagous predator *Macrolophus caliginosus* (Heteroptera: Miridae) has rapidly become established as a whitefly controller in tomato, both in Southern (Trottin-Caudal & Millot, 1997) and Northern Europe (Sampson & King, 1996). Swedish tomato growers are using the mirid bug for general biocontrol purposes, against whiteflies, spider mites, aphids, caterpillars and leafminers in addition to the specialized biocontrol agents. In order to find out if *M. caliginosus* could improve the control of leafminers, the development was followed in some greenhouses with both *D. isaea* and *M. caliginosus* and one greenhouse where *D. isaea* was used alone.

Methods

Four commercial tomato greenhouses (A, B, C and D) in southern Sweden with infestations of *L. bryoniae* were observed from April to September 1998. The study area was in each case a block of 3000m². For early season leafminer control *D. sibirica* and/or *O. pallipes* had been used; introduced in January, February and March but also overwintering.

Split introductions of *M. caliginosus* were made early March in greenhouse B, C and D with the rates 0.5, 0.5 and 0.3 per m² respectively. *D. isaea* was introduced from March to May. Total numbers in house A, B, C and D were 1.4, 0.9, 1.3 and 1.5 per m² respectively.

Samples of leaflets with mines were taken at random on a monthly schedule. 100 leaflets were picked from the medium plant level (1.5 - 2m), containing at least 100 mines. Dead

leafminer larvae were examined by stereomicroscope for eggs, larvae, pupae or exit holes of *D. isaea*. It was not possible to identify dead leafminer larvae used for host feeding or otherwise killed without oviposition by *D. isaea* as being different from those used by *M. caliginosus* for feeding. They were all counted as "dead without *D. isaea*".

Numbers of *M. caliginosus* (adults and nymphs) were recorded in July, August and September directly in the greenhouses on 100 randomly selected leaves at 1.5m level.

Parasitization rates of the braconids were followed in samples of puparia.

Results and discussion

Many years of experience have shown that the efficacy of the braconid parasitoids will not be adequate during summer, so Swedish tomato growers generally rely on *D. isaea* for control, and have realized that early introductions are necessary. During winter and early spring the braconids still are very useful, and in this study the parasitization rate was around or over 50% in February. In July it was 10% or less and of little significance.

Establishment of *D. isaea* was good in all cases. In April (house C and D) or May (house A and B) all stages of the parasitoid could be be found in the leaflet samples. The ratio of dead leafminer larvae with : without *D. isaea* was then around 1 (Table 1-4). According to our experience this ratio tends to be low during the establishment period, but once there is a good number of parasitoids breeding in the greenhouse, the ratio usually stays around or above 1.

Where *M. caliginosus* was introduced we expected to find an increase in numbers when the second generation had started, after 12-14 weeks (Sampson & King, 1996). In July the predators were present in quite high numbers (Table 2-4). *M. caliginosus* seemed to have a strong influence on the parasitoid as can be seen from the diminishing ratios of dead fly larvae with and without *D. isaea*. In August the parasitoid was about to disappear in greenhouse B and D (Table 2 and 4). This negative effect was slightly reduced late in the season when the

Table 1. Tomato greenhouse A, with *D isaea*, without *M. caliginosus*

	1: % dead fly larvae with *D. isaea* ± s.e.	2: % dead fly larvae without *D. isaea* ±s.e.	ratio 1 : 2
May (early)	2.0 ± 1.9	6.0 ± 3.6	0.3
May (late)	5.6 ± 3.4	7.4 ± 4.8	0.8
June	16.4 ± 6.6	10.6 ± 8.4	1.5
July	35.1 ± 7.8	28.4 ± 7.6	1.2
August	44.4 ± 5.7	34.4 ± 4.9	1.3
September	40.9 ± 18.7	34.4 ± 11.9	1.2

Table 2. Tomato greenhouse B, with *D. isaea* and *M. caliginosus*

	1: % dead fly larvae with *D.isaea* ± s.e.	2: % dead fly larvae without *D.isaea* ± s.e.	ratio 1 : 2	number of *M.caliginosus* per leaf ± s.e.
April	0.8 ± 1.3	3.0 ± 5.2	0.3	-
May	23.9 ± 6.0	19.7 ± 4.6	1.2	-
June	18.6 ± 6.2	31.9 ± 9.6	0.6	-
July	24.3 ± 6.0	69.8 ± 4.9	0.3	0.31 ± 0.38
August	1.2 ± 1.9	55.3 ± 13.8	0.02	0.17 ± 0.06
September	2.7 ± 3.8	27.6 ± 9.0	0.1	0.08 ± 0.07

Table 3. Tomato greenhouse C, with *D. isaea* and *M. caliginosus*

	1: % dead fly larvae with *D.isaea* ± s.e.	2: % dead fly larvae without *D.isaea* ± s.e.	ratio 1 : 2	number of *M.caliginosus* per leaf ± s.e.
April	32.1 ± 15.4	24.4 ± 4.3	1.3	-
May	52.4 ± 6.6	32.4 ± 14.5	1.6	-
June	44.8 ± 2.2	43.0 ± 5.1	1.0	-
July	11.9 ± 8.0	74.5 ± 11.3	0.2	0.36 ± 0.15
August	9.6 ± 5.9	65.3 ± 7.8	0.1	0.41 ± 0.12
September	16.9 ± 5.3	60.0 ± 7.6	0.3	0.11 ± 0.06

Table 4. Tomato greenhouse D, with *D. isaea* and *M. caliginosus*

	1: % dead fly larvae with *D.isaea* ± s.e.	2: % dead fly larvae without *D.isaea* ± s.e.	ratio 1 : 2	number of *M.caliginosus* per leaf ± s.e.
April	12.7 ± 9.7	20.2 ± 7.7	0.6	-
May	29.5 ± 10.8	33.9 ± 15.5	0.9	-
June	47.6 ± 15.9	52.4 ± 15.8	0.9	-
July	12.2 ± 6.4	71.2 ± 11.0	0.2	0.24 ± 0.10
August	1.6 ± 1.4	58.6 ± 6.8	0.03	0.67 ± 0.23
September	4.2 ± 3.7	34.5 ± 10.9	0.1	0.09 ± 0.07

predator numbers decreased. In house A the population of *D. isaea* was unaffected and we found little variation in ratios from June to September (Table 1).

Leafminer larvae were the main prey available to the mirid bugs in these greenhouses. No other pests were present, except for some few spots of spider mites in house B and C.

From a practical point of view it could be irrelevant whatever kills the leafminer larvae, parasitoids or predators. In this case, however, we see it as a problem that *M. caliginosus* could become the dominating natural enemy in July - August and then almost disappear in September. The reason for this might be a pathogen, as we noticed many dead nymphs in September, though no chemical pesticides had been used. *D. isaea* is still active during autumn, but would have needed more time to build up a strong population once more. Now the result was that quite many leafminers survived in autumn, specially in greenhouse B and D, creating a potential problem for the following planting.

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Integration of biological and chemical control in case of Japan

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Abstract: In Japan the biological control is coming into wide use, but more slowly than inEurope. The reason is that even biological controls have to be registered by the Governments in accordance with more rigid guidelines and the registration process takes considerable time. Only six beneficial insects have been allowed to use in Japan. This fact indicates that at this moment, the users have to make good arrangements for biological and chemical controls. The present situation in biological control in the greenhouse in Japan will be discussed in this article.

Key words: registration, Japan, chemical, biological, integrate use

Introduction

In 1995, the first beneficial insects from Holland were imported as Biological pesticides. These are *Encarsia formosa* for use in tomato and *Phytoseiulus persimilis* for strawberries in greenhouse. Following them, 4 beneficials were registered in 1997 and 1998.

Table 1. The registration situation of beneficial insects in Japan

Beneficial Insects	Crops	Target Pest
Encarisa formosa	tomato	*Trialeurodes vaporariorum* *Bemisia tabaci*
	cucumber	
Phytoseiulus persimilis	*strawberry*	Tetranychus urticae
	Japanese basil	
	cucumber	
	aubergine	
Dacnusa sibirica *Diglyphus isaea*	tomato	*Liriomyza trifolii*
Aphidius colemani	strawberry	*Aphis gossypii* *Myzus persicae*
	cucumber	
Aphidoletes aphidimyza	cucumber	*Aphis gossypii* *Myzus persicae*
Amblyseius cucumeris	*strawberry*	Frankliniella occidentalis Thrips palmi
	cucumber	
	aubergine	
	paprika	

Under these circumstances, even growers who are willing to reduce chemical pesticide use, have to make use of chemicals in some event. Accordingly, various field experiment, which integrate the biologicals and chemicals have been carried out done by research stations.

Materials and Methods

The field trials in Nagasaki Agricultural Experimental Station

An experiment was made by Nagasaki A.E. S.The object of this test is to establish an IPM program in tomato by using granules of chemical pesticides at planting firstly and beneficial insects during growing period.

The target pests were the silverleaf whitefly (*Bemisia argentifolii*), aphids, leaf-miner (*Liriomyza trifolii*) and rust-mite on tomato. Infestation of the silverleaf whitefly and tomato rust mite were high, the leafminer was infested artificially (low infestation) and any aphid has not been observed. The tomato (cultivar : Momotaro) tested was transplanted on October 24 and at the same time a granule pesticide was treated. Two kinds of beneficial insects released and some chemicals sprayed were as follows.

Table 2. Calendar of applying beneficials and chemicals

Date	Plot 1	Plot 2	No granule used
October 24	imidacloprid 5G (1gram / plant)	acetamiprid 3G (1gram / plant)	
November 1 9 18 24	*Encarsia formosa* (1card / 2 plants)	*Encarsia formosa* (1card / 2 plants)	*Encarsia formosa* (1card / 2 plants)
December 6 13 21 January 10	*Dacnusa sibirica* (25 adults / 45 plants)		*Dacnusa sibirica* (25 adults / 45 plants)
November 26 March 18	dicofol 40%EC(1:2000)	dicofol 40%EC(1:2000)	dicofol 40%EC(1:2000)

Plot 1 treated with imidacloprid granule and *Encarsia formosa*, showed good efficacy on the silver leaf white-fly. Any influence of granule on beneficial insects has not been observed. Same results was obtained in plot 2 with acetamiprid granule.

Dacnusa sibirica worked effectively. Protection from the rust-mite is most difficult problem. To protect beneficial insects, the times of spraying dicofol was limited, and the control was not satisfactory.

Table 3. The change of the silverleaf whitefly and *Encarsia formosa* population

Date of Investigation	Plot1	Plot2	No granule used
	A E L P R	A E L P R	A E L P R
10. 25	11	11	40
11. 01	3 0 12 22 27. 1	36 1 1 25 22 7. 8	131 0 41 25 9. 6
11. 14	0 0 6 3 40. 0	4 1 1 0 85. 7	50 15 30 7 11. 9
11. 29	0 0 2 0 0. 0	10 0 3 6 30. 8	5 2 30 2 6. 1
12. 13	1 0 0 0 100. 0	9 0 6 3 30. 8	2 0 30 3 0. 0
12. 2	7 0 0 0 100. 0	27 0 3 4 50. 0	13 0 0 12 30. 8
1.5	2 0 1 0 0. 0	23 0 0 0 50. 0	13 0 16 12 9. 7
1.18	0 0 0 1 50. 0	9 2 0 0 0. 0	30 1 10 17 43. 3
2.6	0 0 6 100. 0	1 13 4 0. 0	3 6 18 25. 0
2. 2	0 0 0 0. 0	0 22 6 0. 0	7 14 8 63.9
3.5	0 1 0 0 100. 0	3 5 5 0 0. 0	3 0 7 1 11. 1
3.18	0 3 0 25.0	0 2 2 0. 0	0 23 0 11. 5

A : *Bemisia* adult, E : egg, L : larva, P : pupa, R : rate of parasitism of *Encarsia*

Results and Discussion

The Integration of biological and chemical control

It is motivated by hesitation for using chemicals during harvesting time and avoidance of resistance to chemicals that growers use beneficial.

When using the beneficials, growers have to change the conventional way of growing the plants. They have to think about what kind of pesticides should be used and prepare the suitable condition for using beneficials. They have to choose the chemicals which is less harmful to the insects. Buprofezin has been used for such purpose. Pymetrozine is also expected to be beneficial-user-friendly chemical. These chemicals have a good reputation among the IPM growers.

The utilization of beneficials in Japan would be more like that in USA, a little different from that in Europe. Japanese growers will never give up using chemicals but replace some of them to beneficials. It is because the Japanese greenhouse is not always suitable for use of beneficial insects. The green houses are small, average area is 10 are (1,000m^2), and the change of temperature throughout the year and between night and day is drastic. The environment sometime becomes very harsh to beneficials. Most growers think that it is almost impossible to use beneficials whole year around. At this moment, the number of beneficials registered is the bottleneck for penetration of these products into agricultural market. However even if more beneficials become allowed to be used, the environmental obstacles still remain. Therefore, it is an important and urgent task to establish the procedure for integration of chemical and biological control.

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Implementation and development of IPM in greenhouse crops in Austria

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Abstract: A project on advisory service for implementation and development of BC/IPM in greenhouse crops has been set up in Austria in 1992. Demonstration trials which took place in 1996 and 1997 show the structure and importance of the BC/IPM advisory service.

Key words: IPM greenhouses, advisory service, beneficial arthropods

Advisory service

The second part of the pilot project on advisory service for BC/IPM in greenhouses (Blümel & Schausberger, 1996) was carried out in Austria since 1995. In 1996 two full-time BC/IPM advisers and in 1997 one full-time and one half-time adviser were engaged. In 1998 only one full-time BC/IPM adviser was employed by the Austrian Horticultural Growers Association (OGE) and funded by the Ministry of Agriculture and Forestry like in the years before.

Advisory tasks cover the implementation of BC/IPM strategies in vegetable and ornamental production, as well as on indoor plants in public buildings or private areas.
Vegetable and ornamental production greenhouses are visited regularly in intervals of 7 to 14 days depending on the crop and infestation levels. Consultations in public buildings are required especially in spring and autumn. Charges are calculated dependent from the time the adviser spends for controlling the crops and for recording of the findings and the recommendations. Approximately one fourth of the total advisory costs is covered by the growers themselves. A new system for advisory service in Austria should be arranged which could work cost-covering. However until then, during the next years the financial subsidy from the public sector stays an urgent need. The greenhouse area surveyed by the advisory service was variable during the last years (tab.1).

Table 1. Extent of BC/IPM Advisory Service in Austrian greenhouse production from 1996 to 1998

Year	1996	1997	1998
Number of growers	57	60	51
Area: vegetables	1.m5	77.700 m5	5.m5
ornamentals	2.m5	4.m5	39.000 m5
indoor	3.m5	24.400 m5	26.000 m5
total	106.950 m5	149.260 m5	140.500 m5

The average glasshouse area monitored by the BC/IPM adviser is about 4.000 m5 per grower. Actually there is a trend in Austria to renew and enlarge the protected horticultural areas and as a result new greenhouses are neighboured by obsolete ones. Height, size, arrangement and type of the greenhouses vary in every respect, which of course can influence the success of BC/IPM strategies for pest control.
The increase of the vegetable area monitored by the BC/IPM advisory service from 1996 to 1998 was due to an increase of IPM in cucumber. In contrast 1998 less greenhouses with ornamental production than in 1997 were visited, as a result of the reduced number of advisers present.The area of indoor plants extended in 1997 because some botanical gardens started with biological pest control. Detailed figures about the portion of crops surveyed in 1998 are presented in fig.1.

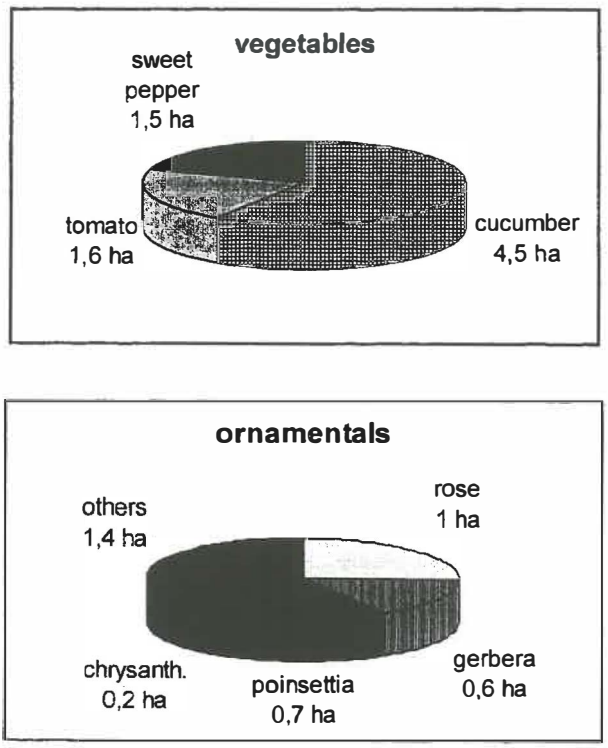

Figure 1. Area of crops regularly monitored by the BC/IPM Advisory Service in Austria in 1998

26 % of the annual working time, the BC/IPM adviser spends on consultations at the growers and 20% on travelling to the greenhouses. In contrast, other tasks like ordering and delivering beneficials without advising, organizing and giving lectures on BC/IPM, writing articles and reports, giving information at horticultural fairs and exhibitions, analyzing trial-results and exchanging informations with other Austrian and international advisers and experts require about 54% of the adviser=s capacity.

The unsolved problem of limited financial support for the advisory service was the main reason for the employment of only one adviser in 1998 and for the lack of further increase of BC/IPM in greenhouse crops in Austria (fig.2).

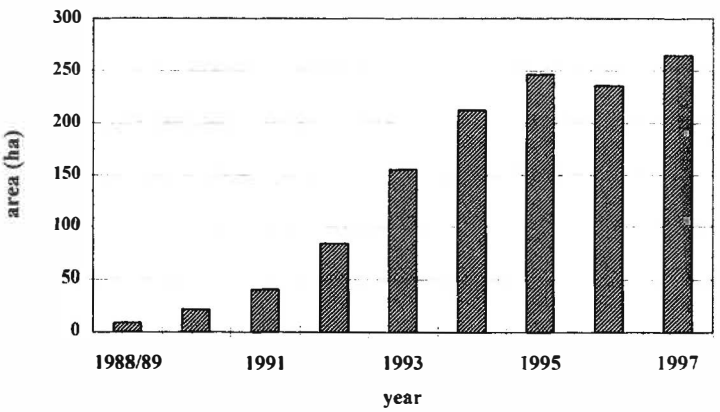

Figure. 2: Area of use of beneficial organisms in greenhouse crops in Austria (except pollinators)

Demonstration trials on the use of beneficial arthropods in cucumber

In 1996 and 1997 three demonstration trials of BC/IPM in cucumber greenhouses in Vienna were carried out as cooperation of the BC/IPM advisory service with the BFL, Institute for Phytomedicine and the Vienna Board for Agriculture. The intention was to promote and to establish BC/IPM pest control techniques on a larger scale in the Austrian cucumber production, by demonstrating the high efficacy, combined with the reliability and the feasibility of natural enemies to control greenhouse pests. The trial area covered 12.200 m5 in total. Beneficials were either released from the beginning of the growing season or after the first occurrence of infestations. An Aopen rearing system@ with *Aphidius colemani* and *Aphidoletes aphidimyza* was installed to prevent from unacceptable infestation levels of *Aphis gossypii* and other aphid species. During one growing season in two greenhouses one supporting chemical treatment with Heptenophos 0,1% on the top of the plants was necessary. The regular release of *Amblyseius cucumeris* in intervals of two weeks from the start of the crop, sufficiently controlled thrips infestation. *Phytoseiulus persimilis* was applied especially in the hot spots of spider mite infestation to achieve the highest effect. In some of the greenhouses one additional chemical treatment of spider mite infested hot spots with Acorit 0,05% had to be carried out. In both trial

periods the use of natural enemies proved to be a successful method for pest control in greenhouse cucumbers during the whole growing season. In the second trial year it was possible to reduce the amount of beneficial arthropods considerably, resulting in a cost reduction. For cost portions of the different beneficials used in cucumber see fig. 3.

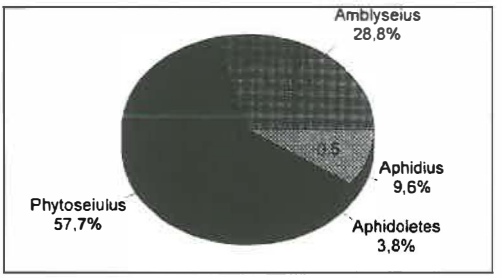

Figure 3. Average portion of costs for benficial arthropods in the demonstration trial in cucumber 1997

Development and results of the demonstration trials were presented to the growers by invitation to the greenhouses during the season and by training and discussion courses after the season. As a consequence of the successful demonstration trials, an increasing application of biological pest control/IPM in cucumber occured in 1997 and 1998 in the Vienna growing area (1996: 1,8 ha BC/IPM in cucumber; 1997: 4,2 ha BC/IPM in cucumber; 1998: 4,5 ha BC/IPM in cucumber).

Future prospects

BC/IPM advising will be installed as a service of the Austrian beneficial producer (BIOHELP). The charges for the consultations will be mainly integrated into the beneficial prices with the exception of time consuming monitoring for instance on green plants in public buildings.

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Preliminary study on interplant movement and host location rate of five parasitoids of *Bemisia argentifolii* in small greenhouse

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Abstract: Interplant distribution, horizontal and vertical movement through plant canopy as well as host location rate were compared for five parasitoid species/strains of *Bemisia argentifolii* in a small greenhouse.

Key words: host location rate, interplant movement, silverleaf whitefly, *Encarsia formosa*, *Amitus bennetti*, *Eretmocerus eremicus*, *Eertmocerus mundus*, biological control

Introduction

Bemisia argentifolii has become a serious pest in Europe for a range of vegetable or crops as well as ornamental plants in the last ten years. Chemical control is increasingly more difficult due to the development of insecticide resistance. Biological control of *Bemisia* with *Encarsia formosa*, which has been successfully used in greenhouses on greenhouse whitefly, *Trialeurodes vaporariorum*, turned out to be less effective (Hoddle et al., 1996). Selecting more efficient parasitoids for *B. argentifolii* is urgently needed.

Earlier studies showed that searching efficiency is important for evaluation of whitefly parasitoids (van Lenteren, 1986; van Roermund et al., 1997a) especially at a low pest density which is requested typically for mamental plants.

Two experiments were set up in a small greenhouse for five parasitoids of *B. argentifolii* intending to answer the following questions: (1). what is the average distance of movement for these parasitoids when they move through the canopy and is the movement of parasitoids within plant canopy guided by host related cues, (2). which parasitoid locates the hosts the quickest?

Material and methods

Host and parasitoids

Bemisia argentifolii was reared on poinsettia (*Euphorbia pulcherrima*, cv. Goldfinger). The following parasitoid species (strains) were reared on *B. argentifolii* on poinsettia or obtained from a commercial company: *Amitus bennetti* Viggiani & Evans (Platygasteridae), solitary internal parasitoid obtained form California, uniparental; *Encarsia formosa* Gahan (Aphelinidae) Dutch strain (NL), Obtained from Koppert B.V., reared on *Trialeurodes vaporariorum*, uniparental; *E. formosa* Beltsville strain (MD), obtained from Cornell University, reared for more than 7 years on *B. argentifolii*, uniparental; *Eretmocerus eremicus* Rose & Zolnerowich (Aphelinidae) obtained from Koppert B.V., biparental; *Er. mundus* Mercet obtained from Spain and reared on *B. argentifolii* for more than one year, biparental.

Greenhouse condition

All experiments were done in a small greenhouse (6.1m×3.4m) under an average temperature of 20±2°C; average relative humidity of 50±25%; photoperiod of 16:8 (L:D) and light intensity of 10,500 Lux.

Interplant movement

Poinsettia plants were cut down to four leaves in such a way that they were either opposite or vertical to each other as much as possible. Each leaf was evenly sprayed with insect glue at both sides so that parasitoids would be trapped once they land on a leaf. A square of 9 plants (3×3) were positioned at each side of the release point. One *Bemisia* infested poinsettia leaf was put in the middle of the most remote row. Number of trapped parasitoids on every leaf were counted each hour until sunset. This was continued for 3 days until no more parasitoids were being retrieved. Five parasitoid species/strains were tested in two groups since it is technically not possible to test all species at once.

Host location rate

Same set up as in the first experiment except that clean plants were used. Only parasitoids present on the whitefly infested leaves were counted and removed from the leaves every hour. The average host location time (T) is calculated as: $T=\sum T_i * N_i / \sum N_i$, where N_i is the number of parasitoids retrieved at time T_i. Host location rate (HLR) was calculated as the inverse of T.

Results and discussion

Interplant movement

The distributions of retrieved parasitoids were not homogenous among plants in each block (Chi-square test, df=8, $p<0.05$) except for *E. formosa* (NL) and *A. bennetti* at the Northeast block. Plants close to the release point caught more parasitoids than those further away. Linear regressions were found for the number of recaptures against the distance from the release point (Fig. 1). Two *Eretmocerus* species landed significantly more often at the Northeast block when first landing on plants (Chi-square test, df=1, $p<0.001$). The other species showed no difference between landings in Northeast and Southwest blocks. The results indicate no effect of chemical cues emitted from the host infested leaves on the first landing, because then a higher recapture should be expected around the site of the infested leaves. Average distance covered by horizontal movement was calculated for the five parasitoids (Fig. 2). Only *Er. eremicus* covered a larger distance than *A. bennetti* (t-test for independent samples, $p<0.01$). No further significant differences were found between the other species. Considering vertical movement it seems that with an exception for *A. bennetti*, all other species moved towards a lower position than the release point (Fig. 3). *A. bennetti* covered significantly smaller distances in their vertical movement than *E. formosa* Beltsville strain and *Er. mundus*. A sensitivity analysis of a simulation model (van Roermund et al., 1997b) for the greenhouse whitefly-*E. formosa* system showed that parasitoids with a smaller average flight distance between plants would have a greater capacity of whitefly reduction.

Host location rate

The host location rates (HLR) for five species are listed in Tab. 1. The results suggested that *A. bennetti* had the largest value, *E. formosa* Beltsville strain and *Er. mundus* the smallest value, while *E. formosa* Dutch strain and *Er. eremicus* showed intermediate HLR values. The ratio of parasitoids that located hosts at different times (pooled results from different

Tab. 1 Host location rate (HLR) of five parasitoids of *B. argentifolii.* Number of replicates and SD are given between brackets.

Species	*A. bennetti*	*E. formosa* (NL)	*E. formosa* (MD)	*Er. eremicus*	*E. mundus*
HLR(h^{-1})	0.17(2, 0.04)	0.047(4, 0.21)	0.10(3, 0.64)	0.058(4,0.35)	0.071(1, -)

replicates) after release are shown in Fig. 3. Note that the number of *A. bennetti* that found host was very low. As only one replicate was done for *Er. mundus*, the HLR value for these species is less representative than for the other species.

Three groups of parasitoids could be distinguished: a). *A. bennetti*; b). *E. formosa* (NL) and *E. eremicus* and c). *E. formosa* (MD) and *E. mundus* according to their different responses in these experiments. The difference between group b and c could be due to their different rearing conditions, especially the rearing host. These together with other life-history and behavioural parameters (Drost et al., 1999; Drost et al., 2000) will be integrated in a simulation model and an overall evaluation of parasitoid quality for *Bemisia* control is then possible.

Acknowledgements

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Fig. 1 Linear regression of parasitoids retrieved against distance from release point

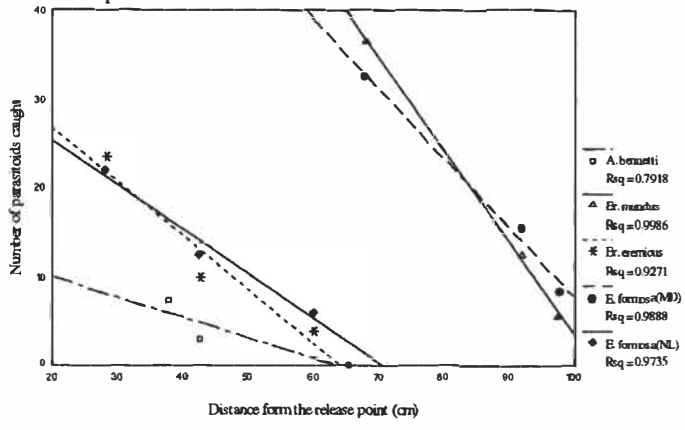


Fig. 2 Mean distance of horizontal movement for five parasitoids (different letters above the error bars indicate significant differences, t-test, p=0.019)

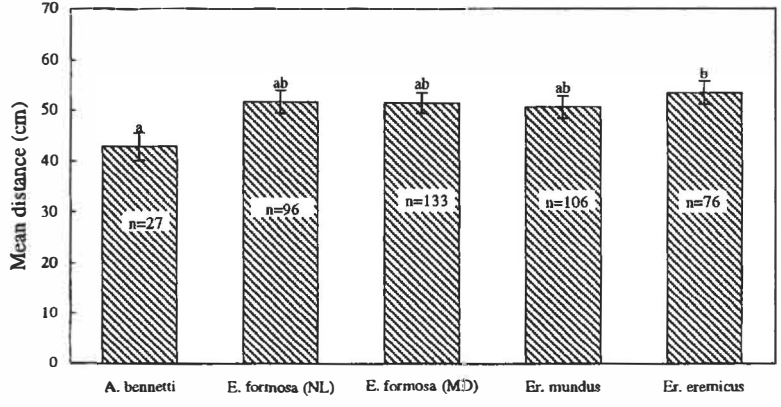


Fig. 4 Cumulative ratio of host location of five parasitoids

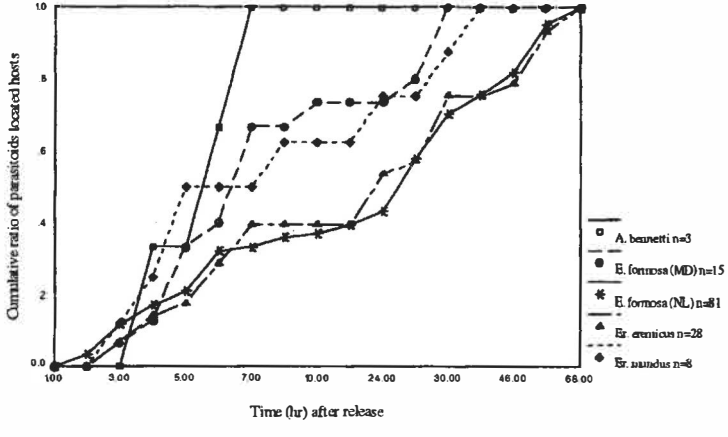
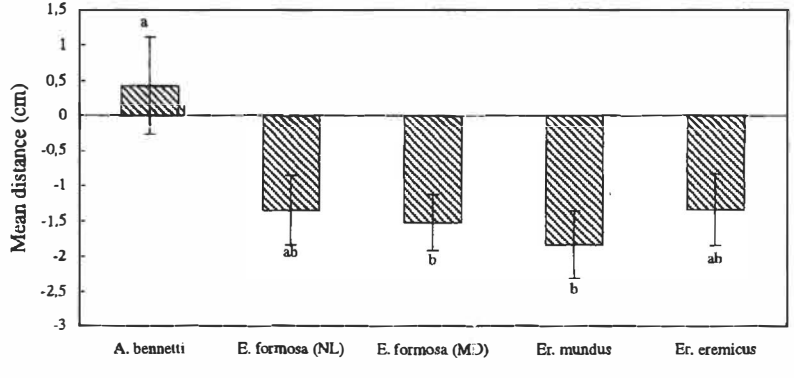


Fig. 3 Mean distance of vertical movement for five parasitoids (different letters at the error bars indicate significant differences, LSD test at 0.05 level)



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Biological control of *Thrips tabaci* on protected leek seed crops

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Abstract : Seed producers are compelled to respect favourable fauna, especially during pollination, therefore they turn towards Integrated Pest Management. In French Loire Valley, on leek seed crops under plastic shelter, biological control of *Thrips tabaci* was tested by releasing different predators whose efficiency was also studied *in vitro*. *Chrysoperla lucasina* appeared to be the most efficient in the field, while *Hypoaspis miles* and *Orius laevigatus* showed the higher predation efficiency *in vitro*. *Amblyseius cucumeris* and *A. degenerans* did not seem to be adapted to this crop.

Key words : *Thrips tabaci*, leek, seed production, bank plant, biological control, integrated control

Introduction

Seed production under plastic shelters is developing in French Loire Valley. Seed producers, as other growers, are aware of pesticide impact on health and environment. They are faced to a reducing number of registered insecticides on such "minor crops" and to the development of insect resistance. Moreover, as quantitative and qualitative yield depends mainly on pollination, using bees, bumblebees or flies, producers are then compelled to avoid insecticides before and during the flowering. Therefore, they turn towards Integrated Pest Management.

This project is carried out within the framework of GIS L.B.I.O., a working group associating growers organisations and scientists. Leek seed production was chosen as a model to carry out a first step experiment, testing biological control on the major pest, *Thrips tabaci* Lindeman (Theunissen & Legutowska, 1991), releasing beneficial auxiliaries on the crop with the help of a bank plant.

It was decided to select the beneficials among those available on French market and susceptible to control *T. tabaci*. Their efficiency was tested in field tests and *in vitro* predation tests carried out in 1997 and 1998.

Material and methods

Beneficial auxiliaries
Five predators were selected: Anthocoridae predator *Orius laevigatus* Fieber, predatory mites *Amblyseius cucumeris* Oudemans (1997) and *A. degenerans* Berlese (1998), and predator *Chrysoperla lucasina* Lacroix sold for thrips control; predatory mite *Hypoaspis miles* Berlese sold for fungus gnats (Sciaridae) control.

Bank plant
Castor-oil plant (*Ricinus communis* Linné, var. Impala) was used as a bank plant for *A. cucumeris* and *A. degenerans*.

In vitro predation tests

For each tested species, 4 *T. tabaci* larvae and one predator were placed on a leek leaf disc in a small Petri dish while only 4 thrips larvae were placed in the control dishes. The larvae came from a mass rearing on leek. The 25 dishes for each treatment were covered with a glass pan to avoid larvae escape. The Petri dishes were all kept at 20°C., in the dark for *H. miles*. Surviving thrips larvae were quoted after 24h. All the 25 leaf discs of each treatment were placed in a Berlese funnel as some larvae may hide inside the leaf tissues.

Field experiments

Four plastic shelters, 480 m^2 each, located in Ambillou-Château (Loire Valley) were used for this experiment with the same leek variety. The crop was divided in four compartments for beneficial releasing: (I) soil *for H. miles*; (II) leaves for *A. cucumeris* and *A. degenerans* and for *C. lucasina*; (III) inflorescences for *O. laevigatus*; and (IV) bank plants for *A. cucumeris* and *A. degenerans*.

Predators were released from April to August in 1997 and 15th May to August in 1998. *T. tabaci* and predators were monitored weekly from May to late August, first by visual control on the leaves and spikes, then by striking ten times the inflorescence when the bract was opening.

Results and discussion

In vitro predation tests

Predation tests have shown the efficiency of *H. miles, O. laevigatus* and *H. miles*, while in our conditions there was no difference between *A. degenerans* and control (Table 1). No thrips were found with the Berlese funnel trap.

In a previous test, *A. cucumeris* had shown no significant difference with the control. It was not found on the leek leaves and very few on the bank plant in the field (Galez *et al*, 1998). Thus, *A. degererans* was chosen for the 1998 experiment. None of the two species seem to be adapted to our conditions.

We intended to test the efficiency of the predatory thrips *Aeolothrips intermedius* Bagmall, currently found on leek crops in open field and under shelter as well (Carl, 1971). All the rearing attempts failed, the larval surviving never reached more than 5 days.

Tableau I . Predation efficiency *in vitro* of different predators of *T. tabaci* on leek leaves (Student test, p. 5%).

Auxiliaries	predation %
Amblyseius degenerans	0
Chrysoperla lucasina	63
Hypoaspis miles	89
Orius laevigatus	87

Field experiments

In 1997, *T. tabaci* was the only thrips species found during all the experiment. Thrips populations were very low up to early July (under 2 thrips/plant). The 24 thrips/plant level reached in late July was not considered as harmful by the producer, who meanwhile decided to apply an insecticide against both leek moth *Acrolepiopsis assectella* Zeller and *Thrips tabaci*.

Only 2 insecticides were applied: Talstar (bifenthrine) on *A. assectella* (21st July) and Vertimec (abamectin) + Orthène (acephate) on *A. assectella* and *T. tabaci* (8th August). Those two treatments were placed after the beehives were removed.

As a comparison, in a plastic shelter with no auxiliaries releasing, located in an other site and belonging to the same producer, 4 insecticides were applied. The 15th July, thrips population was the same in this shelter as in Ambillou-Château. In 1996, with a lower thrips population, the same producer applied 5 insecticides.

In 1998, trips monitoring and auxiliaries releasing started later (15th May). Thus thrips population (5 to 18 insect/plant) was regulated by chemical control (20th May to 5th June) down to 2/plant level similar to 1997 level: Orthène + Vertimec Lannate (méthomyl); Orthène, Vertimec. From that time, biological control of *T. tabaci* was similar to 1997 (Fig 1; Table 2).

Chrysoperla lucasina was always present on the crop at all its development stages with a higher density than the releasing (1/m^2) and even under other shelters were it was not released. *Hypoaspis miles* was found in the soil (Berlese funnel) up to one month after the first releasing but at a very low level. It is not yet known whether this low level is due to poor adaptation to the crop conditions or to a slow dispersion of the mite that we found only at a short distance from the releasing plots.

O. laevigatus was also found after the releasing. The spontaneous species *O. niger* was found mixed with *O. laevigatus* during the flowering, which provides pollen as a food supply. Flower attraction and absence of insecticide seem to favour the installation of these Anthocoridae.

Releasing of *A. degenerans* was a failure, they were found neither on the bank plants, nor on the adjacent leeks.

Table 2 . Auxiliaries releasing dates and doses on leek seed crop under shelter - Ambillou-Château , Loire Valley, 1998. (doses per m^2 on leek, per plant on *Ricinus*)

auxiliaries	14/5	19/5	28/5	04/6	11/6	18/6	26/6	02/7	10/7	16/7	23/7	30/7	06/8	13/8	20/8
A.degenerans /*Ricinus* /leek					250 3		250 3		250 3						
C. lucasina	1		1		1		1		1		1		1		1
H. miles					50		50		23				20		
O. laevigatus									2				0.5		

The total cost of biological control was around 25 FF./m^2 in 1997, it was reduced to 15 FF./m^2 in 1998, while chemical control of *T. tabaci* is usually 8.80 FF./m^2. In 1998, *C. lucasina* releasing cost 1.60 FF./m^2. It is expected to reach a similar cost to chemical control by adjusting the releasing number and doses, and by reducing the predator production cost.

Quantitative and qualitative yield were not affected by *T. tabaci* population level. In 1997, the best yield was obtained in the most infested shelter (25 thrips/plant): this level was too low to induce significant yield reduction (Galez *et al* , 1998).

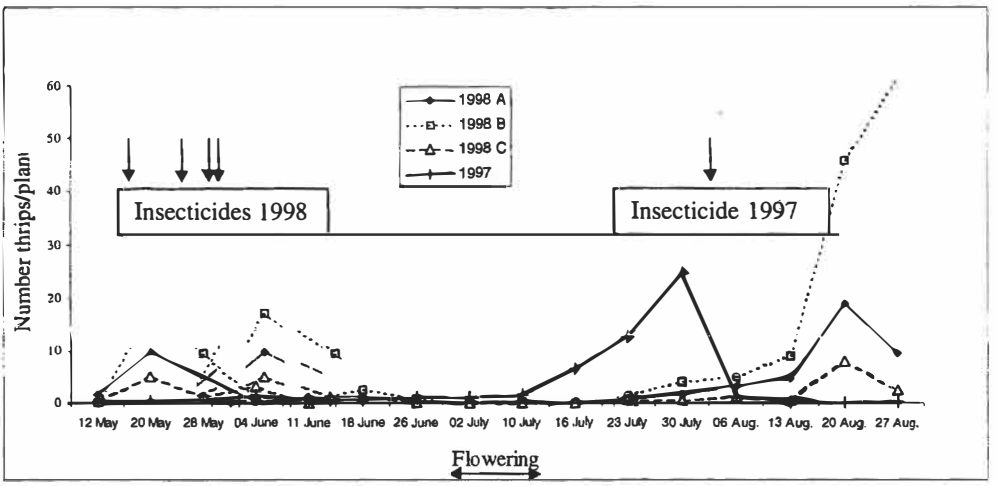

Figure 1 . Development of *Thrips tabaci* with biological control on leek seed crops under plastic shelters. 1997: 3 shelters, mean population; 1998: shelters A, B and C - Ambillou-Château, Loire Valley.

Biological control of *T. tabaci* on leek seed crops under plastic shelters was effective in our experiments. *C. lucasina* appeared to be the most efficient in field conditions. Reducing insecticide treatment before and during the flowering allowed a good pollination and spontaneous auxiliaries arrival as *O. niger*. Predator mite *H. miles* efficiency was demonstrated *in vitro* but not in field conditions. *A. cucumeris* and A. degenerans do not seem to be adapted to this crop. Therefore our further work will develop on *C. lucasina*, adapting the releasing periods and doses in order to reduce the protection cost.

Acknowledgements

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Development of an IPM system in soilless culture by using slow sand filtration and a biocontrol fungus, *Pythium oligandrum*

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Abstract: Epidemiological studies have shown that introduction and dispersal of pathogens into soilless greenhouses widely depend on the irrigation system implemented. To prevent serious problems of pathogen contamination, our group has developed an integrated disease-management system based on the use of slow sand filtration and of a biocontrol agent, *Pythium oligandrum*. First, the reliability of slow sand filtration to disinfect the nutrient solution by eliminating pythiaceous, fusarium fungi and bacteria was demonstrated; as a consequence, the introduction of pathogenic micro-organisms into the hydroponic culture was strongly reduced. Second, it was shown that the mycoparasite *P. oligandrum* successfully colonized the roots during the cultural season. This colonization was non-pathogenic and induced a slight increase in culture yield. In conclusion, the combination of slow sand filtration to disinfect nutrient solutions with the use of *P. oligandrum* to colonize and protect the roots from pathogenic attack can offer good prospects for minimizing or even preventing problems associated with pathogenic microorganisms in soilless cultures.

Key words: integrated disease management, root colonization, nutrient solution disinfection.

Introduction

Epidemiological studies, conducted in hydroponic cultivation systems, have clearly demonstrated that the introduction of pathogens inside greenhouses largely depends on the implemented irrigation system. As a consequence, preventing pathogenic infections by appropriately disinfecting the nutrient solutions used in soilless cultures has become a major challenge (Rey and Déniel, 1998b).

The slow sand filtration technique have been proposed for disinfecting nutrient solutions while preventing microorganism introduction and spread within greenhouses. Mechanical and biological factors such as microorganisms with antagonistic properties, are thought to be responsible for the effectiveness of the system. Recent studies provided evidence that zoosporic fungi like *Phytophthora* spp., pathogenic bacteria and even viruses were eliminated at substantial efficiency rates (Wohanka, 1998). However, one may consider that some of these pathogens as well as beneficial microorganisms may pass through the filter to some extent. It is, thus, likely that a few pathogenic microorganisms may penetrate into hydroponic systems even after slow sand filtration disinfection.

To prevent this problem, our group has developed an integrated disease-management system based on the use of slow sand filtration for disinfecting the nutrient solution and on the use of a biocontrol agent, *Pythium oligandrum*, for colonizing and protecting the roots from pathogenic attack (Picard *et al.*, 1998). *P. oligandrum* has been selected essentially because this fungus displays unique properties: (i) it acts as a mycoparasite against pathogenic fungi both *in vitro* and *in planta* (Benhamou *et al.*, 1997); and (ii) it induces defense-related

reactions in tomato roots (Rey et *al.*, 1998a) and plant resistance against *Fusarium* attack (Benhamou *et al.*, 1997).

The aim of the present study was to demonstrate the disinfection capability of slow sand filtration and to assess the ability of *P. oligandrum* to colonize and develop on the roots during the whole cultural season under greenhouse conditions.

Material and Methods

Filter unit

The filter unit is composed of a plastic pipe (220-cm in length and 40-cm in inner diameter) and a filter made of pouzzolan sand and gravel. To test the effectiveness of slow sand filtration, regular samplings were made from March to September, during the cultural season. Each month, three samples of the nutrient solution were collected just before passing through the sand filter, and three others were taken from the filter effluent. Detection of fungi such as *Pythium* sp. and *Fusarium oxysporum*, and of bacteria including total mesophylic aerobe bacteria and fluorescent *Pseudomonas*, was performed because these are key components of roots and nutrient solution microflora in soilless greenhouses.

Root colonization by Pythium oligandrum in soilless cultures

P. oligandrum inoculum consisted of oospores (30 000 oospores/ml^{-1}) and mycelium (8×10^3 CFU/ml^{-1}). One week after crop start, 20-ml of the inoculum were deposited at the collar level. A second inoculation was carried out four weeks later using 20-ml of the inoculum poured on each side of the plants. Tomato roots from control and *P. oligandrum*-inoculated plants were sampled from three hydroponic cultures throughout the growing season. Each greenhouse used a typical cultural system different from the other ones; they consisted of organic substrate (peat), inorganic substrate (rockwool) or the N.F.T. (Nutrient Film Technic) technique.

Root samples were collected monthly from February to August in three selected sites per greenhouse. Sixty root segments were plated per site. *P. oligandrum* thalles were counted and results were expressed as the percentage of root pieces from which *P. oligandrum* thalles were recovered.

Results and discussion

Efficiency of slow sand filtration for eliminating microorganisms

The present study showed that nutrient solutions used in soilless cultures were invaded by different fungi such as *Pythium* sp., *F. oxysporum* and bacteria. The slow sand filtration proved to be efficient by eliminating about 97-99% of the *Pythium* sp. and 96% of *F. oxysporum* from nutrient solutions (Table 1). Results obtained over two one-cultural-season experiments were similar; however, in both experiments, complete pathogen eradication could not be obtained.

Bacterial growth was also reduced by the slow sand filtration technique, although to a lower extent than fungi. The disinfection process allowed a reduction rate of 86.2% against total mesophylic aerobe bacteria while destruction of fluorescent *Pseudomonas* ranged by about 88-93%. Again, one may consider that pathogenic and/or beneficial bacteria likely passed, to some extent, through the filter.

Previous experiments reported in the literature have also shown similar disinfection rates. However, these experiments were always conducted over short periods of time (Runia, 1995). To our knowledge, the present study are the first to present results dealing with two whole

cultural seasons, and to convincingly demonstrate the properties of slow sand filtration over such a decisive period.

Table 1. Efficiency of slow sand filtration to eliminate microorganisms.

		Percentage of eliminated microorganisms			
		Pythium sp.	*Fusarium oxysporum*	*Total mesophylic aerobe bacteria*	Fluorescent *Pseudomonas*
Cultural season	1997	97.5	96.6	86.2	88.2
	1998	99.2	96	86.2	93

However, the question can be raised as to which extent the disinfecting efficiency is sufficient to completely prevent plants from pathogenic attack. Because microorganisms cannot be completely eradicated by this approach, it is reasonable to assume that few pathogenic micro-organisms are still introduced into greenhouses. Despite this limitation, filtering nutrient solutions through slow sand unit contributes to reduce and/or delay pathologic risks.

Among the possible mechanisms involved in fungal and bacterial interactions in filter unit, one may cite parasitism, antibiosis, and/or competition for nutrients. Further studies are required to support this assumption since a better knowledge of the microbial populations occurring in filters and a better understanding of the interactions between these microorganisms would lead to a higher standardization of slow sand filtration efficiency.

Assessment of root colonization by P. oligandrum in soilless cultures

As shown in Figure 1, the rate of root colonization by *P. oligandrum* varied according to the three cultural systems investigated. Fungal colonization in the roots growing in inorganic substrate (rockwool) was markedly lower than that occurring in the roots developed inside organic substrate (peat) or NFT system. Only few thalles *of P. oligandrum* were detected in the rhizoplan of plants growing in rockwool. Between the first and the third months of plant culture, this mycoparasite was isolated from only 16 to 9% of the roots.

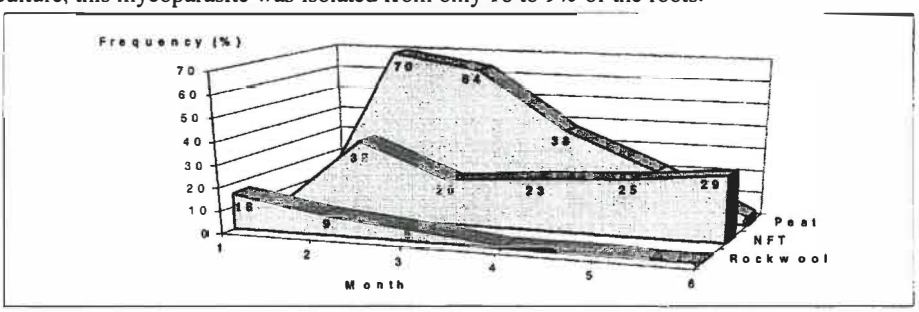

Figure 1. Assessment of *P. oligandrum*-root colonization in different culture substrates or NFT system in soilless greenhouses.

By contrast, about 70% of the roots were invaded *by P.oligandrum* thalles in the peat system, two months after plant inoculation. A progressive decrease was noticed in the following months to finally reach 4% of the roots, six months later. *P. oligandrum* development in the NFT-root system was rather stable over the whole 6-month experiment.

From one month after *P. oligandrum* inoculation to the end of experiment, the rate of colonization remained at 20 to 35% of the roots.

The present results indicate that the nature of the substrate greatly influences *P. oligandrum*-root colonization. It would now be essential to further investigate the root-*P. oligandrum*-substrate interactions in order to determine the conditions allowing optimal root colonization by *P. oligandrum*. Such a colonization is of key importance for the induction of local and systemic plant resistance as previously reported by Benhamou *et al.*, 1997. Wulff *et al.* (1998) have reported a stimulating effect of *P. oligandrum* on the root elongation of cucumber plantlets. In the present experiment, *P. oligandrum* colonization was also associated with a slight increase in culture yield (4.5 to 7%, unpublished data).

In conclusion, our results demonstrate that *P. oligandrum* along with other biocontrol agents is likely a key component of the rhizosphere microflora. Promoting such beneficial microflora in soilless cultures is a promising avenue for minimizing damages caused by pathogens. In that context, research efforts should focus on increasing the suppressive activity of this root microflora.

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Pollen improves thrips control with predatory mites

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Abstract
To achieve permanent suppression of western flower thrips in cucumber repeated introductions of predatory mites are usually needed. This suggests that the resulting thrips population levels are too low to maintain a predator population. A solution may be to provide alternative food, such as pollen, to the predatory mites. Pollen, however, is also a food source for thrips. How pollen affects biocontrol of western flower thrips, has been tested experimentally by applying cattail pollen on cucumber crops on which either *Amblyseius degenerans* or *A. limonicus* was released. In these two experiments, the predator population increased faster, and the thrips population remained smaller, in the compartments with pollen than in those without pollen. Application of *A. limonicus* together with pollen even resulted in negligible fruit damage.

Keywords: western flower thrips, *Frankliniella occidentalis*, phytoseiids, *Iphyseius degenerans*, *Typhlodromalus limonicus*, alternative food, pollen, biological control, apparent competition

Introduction

In cucumber, repeated introductions of predatory mites are required to achieve control of western flower thrips, suggesting that the resulting thrips levels are too low to maintain a predator population. This problem might be overcome by providing alternative food to the predators. Laboratory experiments and population modelling predicted that a permanent supply of pollen will reduce equilibrium thrips levels, despite the fact that both predatory mites and thrips feed on pollen. However, before the equilibrium is reached, pollen may stimulate thrips growth so as to cause a larger initial peak in thrips density (Van Rijn and Sabelis, 1993). In addition, predators might be applied that can use the available thrips more efficiently as a food source (Sabelis and Van Rijn, 1997).

These methods to improve biological thrips control have been tested experimentally by applying cattail pollen and two different phytoseiid species (*Amblyseius degenerans* or *A. limonicus*) on cucumber crops. The first species has shown to be an efficient pollen feeder (Van Houten and Van Stratum, 1995; Van Rijn and Tanigoshi, 1999), whereas the second has the advantage of a more efficient use of thrips larvae (both small and large) (Van Houten *et al.*, 1995; Van Houten, 1996; unpublished results).

Materials & Methods

Amblyseius (Iphiseius) degenerans was originally collected in 1982 in Morocco and was most recently reared on birch pollen (Van Rijn and Tanigoshi, 1999). *Amblyseius (Typhlodromalus) limonicus* was collected in 1996 in New Zealand and reared on broad bean pollen ever since (Van Houten *et al.*, 1999).

Each of the two experiments was performed in 4 greenhouse compartments with gauzed windows (2 for the treatment and 2 for the control) at the Research Station for Floriculture and Glasshouse Vegetables (PBG) in Naaldwijk, The Netherlands. Each compartment had 108 cucumber plants with initially 8-9 leaves. Female western flower thrips, *Frankliniella occidentalis*, (1-2 per plant) were randomly released one week after planting. Exactly 4 female predators were introduced on every plant two (*A. degenerans*) or three (*A. limonicus*) weeks later. In the first experiment *A. degenerans* was introduced in the 4th week of 1997. In the second experiment *A. limonicus* was introduced in the 24th week of 1997. At the same time cattail pollen (*Typha latifolia*) was applied on one high leaf of every plant in two of the four compartments. Every other week additional pollen (1-2 gram per compartment) was introduced on a leaf high up in every plant (in the first experiment) or was dusted over the top of each plant (in the second experiment). Previous experiments had shown that cattail pollen supplied on a cucumber crop retains its quality as food source for more than two weeks.

Juvenile thrips and predator populations were estimated based on *in situ* observations of 8-16 representative leaves per plant, on 10 plants per compartment (one plant randomly selected per row). Initially all, but with increasing plant size, one of every two or three leaves were sampled, always including the leaves provided with pollen. To obtain an estimation of the population size per plant, non-sampled leaves were assumed to contain a similar number of mites and thrips as the nearest (pollen-free) sampled leaf. In addition, adult thrips were monitored by two blue sticky traps that were replaced weekly.

Results

Experiment A (A. degenerans)

In the compartments with pollen the predator *A. degenerans* increased in numbers right after its introduction. However in the control compartments, its number declined to virtually zero in the first few weeks, but increased thereafter due to the increased numbers of thrips (Figure 1A1).

The number of thrips larvae increased in a similar way in all compartments during the first 4 weeks. Whereas in the control compartments larval density continued to increase until week 15, in the pollen-treated compartments its growth stopped from week 8 onwards (Figure 1A2). The adult thrips population (sticky trap results not shown) also stopped growing, but with a delay of 3 weeks. In the pollen-treated compartments, thrips densities were 20 times lower than in the control compartments (in week 9 for the larvae and in week 11 for the adults). The much lower thrips numbers in the treated compartments still gave rise to some fruit damage.

The predator population was strongly clustered on leaves with pollen, whereas the thrips concentrated on the young, top leaves.

Experiment B (A. limonicus)

With or without pollen, the predator *A. limonicus* increased in numbers right after its introduction. In the presence of pollen the populations initially increased faster, but levelled off at ca. 250 mites per plant (Figure 1B1).

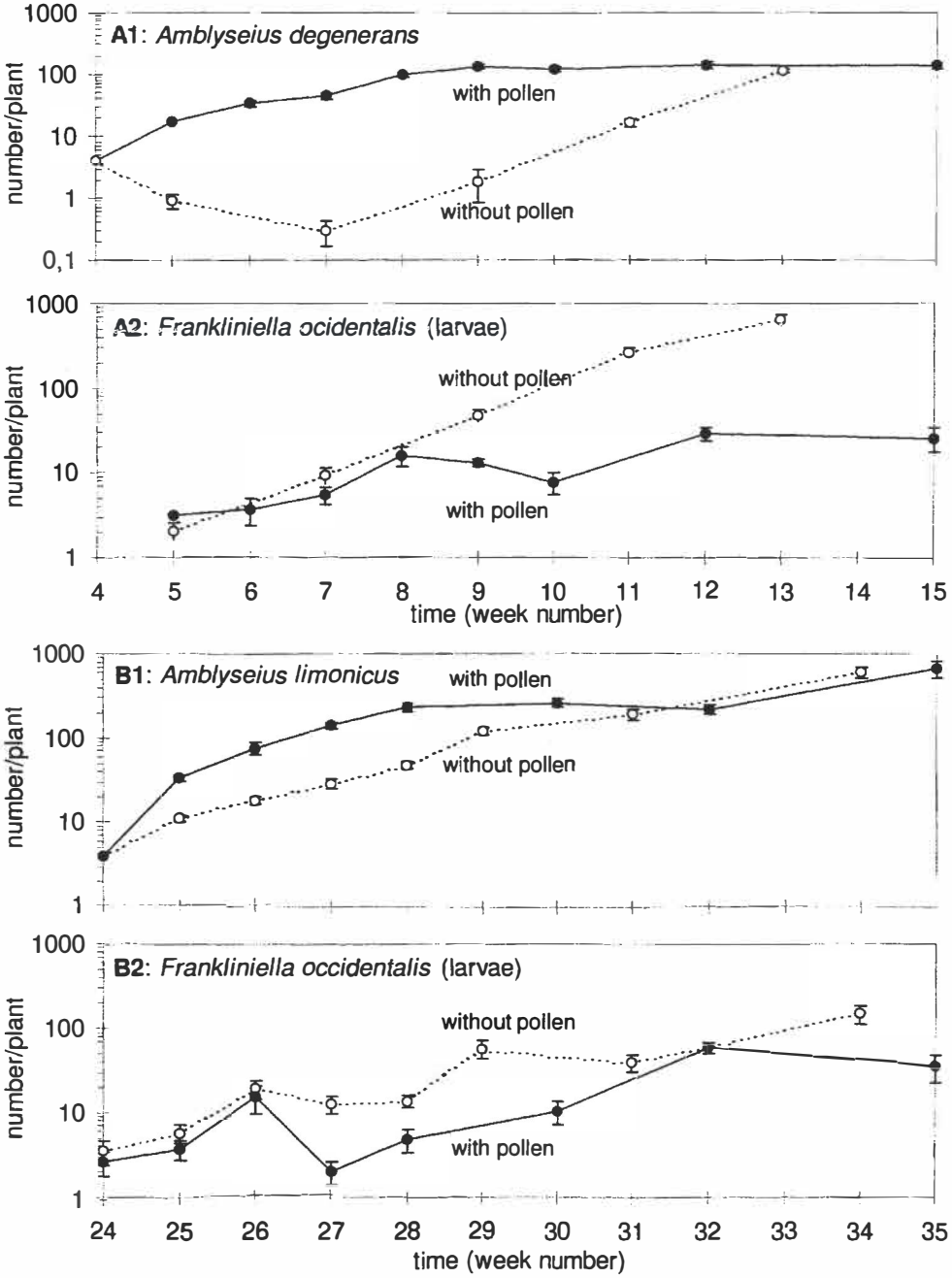

Figure 1. Population dynamics of predatory mites (nymphs and adults; **A1**, *Amblyseius degenerans* and **B1**, *Amblyseius limonicus*) and western flower thrips (larvae; **A2** and **B2**) in absence (open dots, dashed lines) and presence of cattail pollen (closed dots, drawn lines) on cucumber plants. Predatory mites were introduced in the first week indicated on the X-axis. Dots and vertical bars represent means and standard errors over 20 samples (plants) in 2 replicate experiments.

With or without pollen, the density of thrips larvae increased at the same rate during the first few weeks, but started to deviate from the fourth week onwards. After a sharp decline, the thrips populations (both larvae (Figure 1B2) and adults (results not shown)) in the pollen-treated compartments remained 3-6 times lower than in the control compartments, and fruit damage was virtually absent.

Amblyseius limonicus concentrated on leaves with pollen, but to a lesser extent than *A. degenerans*.

Discussion

The results of the two greenhouse experiments showed that thrips growth was not stimulated by pollen. Possibly, the aggregation of predators on leaves with pollen prevented the thrips from using the pollen effectively. How the pollen's distribution pattern affects foraging behaviour of thrips and predatory mites, is the subject of further study. In addition, the greenhouse experiments showed that application of pollen ultimately resulted in lower thrips numbers. The amounts of pollen needed to enhance control were still low enough (1-2 gram/100 plants/fortnight) to make practical application feasible.

In absence of pollen, the experiment with *A. limonicus* indicated much lower thrips levels as the one with *A. degenerans*, even though the first was performed in a warmer period of the year. This corresponds with the results obtained by Van Houten (1996) and Van Houten *et al.* (1999). In presence of pollen, the two experiments resulted in similar numbers of thrips per plant, but since the cucumber plants in experiment B had on average twice as many leaves, the thrips numbers per leaf were lower. In addition, the adult trap catches in experiment B were much lower, as well as the amount of fruit damage. Which elements of foraging behaviour are responsible for the differential impact on thrips populations, is subject to further study.

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Macrolophus caliginosus Wagner (Heteroptera: Miridae): A predator causing damage to UK tomatoes

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Abstract: During the second season of use in the UK, the predatory bug *Macrolophus caliginosus* was found to be causing direct damage to tomato plants. A survey of tomato crops in 1997 revealed several types of damage; the most serious being premature flower and fruit drop. Economic losses were reported in all cherry tomato crops monitored and 20% of round tomato crops. A number of factors influenced damage, including numbers of *M. caliginosus*, type and abundance of invertebrate prey, tomato cultivar and plant quality. *Macrolophus caliginosus* can make an important contribution to pest control in UK tomato crops if populations can be manipulated to avoid injury to plants. Further work is in progress to identify IPM compatible methods of regulating *M. caliginosus* populations.

Key words: *Macrolophus caliginosus*, tomato, crop damage

Introduction

The predatory bug, *Macrolophus caliginosus*, was originally reared for the control of two whitefly species, *Trialeurodes vaporariorum* Westwood and *Bemisia tabaci* Gennadius, in southern Europe (Malausa *et al.*, 1987). In addition to improving control of whiteflies, it was found to suppress populations of spider mites, aphids and caterpillars (Fauvel *et al.*, 1987; Trottin-Caudal & Millot, 1994). It was demonstrated subsequently that the predator could also make a useful contribution to Integrated Pest Management (IPM) in northern Europe (Sampson & King, 1996). *Macrolophus caliginosus* is not indigenous to the UK and was first released in tomato crops in 1995 with promising results against a range of pest species. However, in 1996, the predators were reported to be causing direct damage to tomato plants (Hayman & Jacobson, 1996). This paper summarizes the results of a survey of commercial crops and a series of small-scale studies that investigated the incidence and nature of *M. caliginosus* damage to tomato plants.

Materials and methods

Survey of commercial crops

Eight cherry tomato crops and ten round tomato crops were monitored at two to four week intervals between May and October 1997. During each assessment, numbers of *M. caliginosus*, their positions on the plants, availability of invertebrate prey and damage symptoms were recorded. At the end of the season, growers estimated economic losses due to *M. caliginosus*.

Macrolophus caliginosus damage to tomato flowers and fruit

To observe *M. caliginosus* feeding, adult females were confined individually on flowering trusses (cv Spectra) for three hours and their activity recorded on video tape. In addition,

batches of ten females were confined on single trusses of swelling tomatoes (cv Criterium) for three weeks, after which fruits were examined for damage. There were ten replicates.

Preliminary studies to explore the relationship between prey-type and damage

To investigate the effect of prey-type on *M. caliginosus* damage to tomatoes, batches of ten females, that had been reared on diets of either Mediterranean flour moth (*Ephestia kuehniella* Zeller) eggs or tomato leaf miner (*Liriomyza bryoniae* Kaltenbach) larvae, were confined without invertebrate prey on flowering tomato trusses (cv Criterium). The treatments were compared to trusses without *M. caliginosus* and there were ten replicates of each. After three weeks, the numbers of flowers and fruits that had dropped prematurely were recorded, and differences compared by analysis of variance.

In a larger scale study, tomato crops were monitored to investigate the effect of prey availability on *M. caliginosus* damage. Very large numbers of predators were present on two adjacent cherry tomato (cv Favorita) crops following severe infestations of leaf miners. In one crop, the leaf miner population was almost completely controlled and there was very little invertebrate prey remaining. In the other crop, leaf miner larvae were still numerous. Forty plants were examined in each crop. The numbers of predators per plant and the numbers of trusses with one or more fruits lost due to premature drop were recorded.

Results and discussion

Survey of commercial crops

The survey revealed several types of damage that were influenced by numbers of *M. caliginosus* on plants, size of pest infestations, type of invertebrate prey and tomato cultivar:

Types of damage - *Macrolophus caliginosus* probe leaves to feed on sap, which leads to distorted growth and down-curled terminal leaflets. This was observed in all cherry tomato crops monitored and 40% of round tomatoes but was not thought to affect yield. Excessive feeding on leaves destroyed vascular tissue, resulting in necrotic patches. The predators also fed on flowering trusses, which resulted in failed set and premature flower or fruit drop. This was observed in every cherry tomato crop monitored and in 20% of round tomatoes. Scars on the surface of maturing fruit were observed in one cherry tomato (cv Favorita) crop.

Numbers of *M. caliginosus* - All damaged crops had over 50 *M. caliginosus* per plant but this should not be considered a definitive damage threshold. There are several other factors that influence damage and the threshold will vary according to which of these occur at any time.

Pest infestations - The number of *M. caliginosus* at the peak of the population and the consequent damage were more closely related to pest numbers than to the initial number of *M. caliginosus* released in the crop. *Macrolophus caliginosus* can survive and produce offspring on a predominantly vegetarian diet but their reproductive rate is far greater when they have an abundance of insect prey (Foglar *et al.*, 1990). Large numbers of *M. caliginosus*, sometimes over 300 per plant, were associated with pest infestations in mid-summer. Damage to tomato plants usually occurred four to six weeks after *M. caliginosus* had achieved control of the pest infestation. At that time, there were still large numbers of predators but few prey.

Type of invertebrate prey - Pest type influenced damage, which was most severe after large numbers of *M. caliginosus* had developed on infestations of *L. bryoniae* or *T. vaporariorum*. There were fewer predators and less plant damage associated with infestations of *Tetranychus urticae* Koch (two-spotted spider mites), which is consistent with studies that have shown that *M. caliginosus* perform less well on *T. urticae* (Foglar *et al.*, 1990).

Plant cultivar - Cherry tomatoes were more seriously damaged than round tomatoes but this may be related to the numbers of predators found in each type of crop. For example, at the end

of July, the average numbers of predators were 65 per plant in cherry tomatoes and 30 per plant in round tomatoes. Several factors may have contributed to this. Pests tend to be more prolific on cherry tomatoes and these support larger populations of predators. Cherry tomato plants may also be more nutritious for *M. caliginosus* than round tomatoes. This is supported by unpublished data, which showed that without invertebrate prey, *M. caliginosus* lived twice as long and produced more progeny on cherry tomatoes than on round tomatoes. Furthermore, predators were found more commonly on the trusses of cherry tomatoes than round tomatoes and the narrower pedicels of cherry tomato flowers may be more vulnerable to damage.

Plant vigour - There was some evidence to suggest that the vigour of the plant affected damage. For example, young plants were less susceptible to damage than older plants and predators were more frequently observed on trusses when leaf quality was poor.

Time of damage – Although the first record of plant damage in 1997 was in a cherry tomato crop in early June, the majority of cases occurred towards the end of the season, after mid-August. Less damage would be expected earlier in the year when there is less prey.

Economic loss - All the cherry tomato growers in the survey reported an economic loss due to *M. caliginosus* damage and in the most extreme case this was estimated to be equivalent to £3 per m^2. Growers quantified their losses in different ways; one grower lost half a truss per plant over three weeks, another lost four fruits per plant, and a third reported 17% reduction in yield over five weeks compared to a similar crop that was not infested with *M. caliginosus*. Most damage observed in round tomato crops was in patches that corresponded to areas of pest activity. In the worst case, the grower reported 20% fruit loss over four weeks and an additional 2% loss due to down-graded fruit. Several growers restricted the damage by applying insecticides but indicated that this disrupted IPM and resulted in secondary problems with other pests. It was impossible to quantify the predator's contribution to pest control because there were no untreated areas for comparison.

***Macrolophus caliginosus* damage to flowers and fruit**

Macrolophus caliginosus was observed and filmed feeding on tomato flowers and pedicels. The predator penetrated the anther cone of the flower with its stylets and damaged the ovary or young fruit within. It also probed the abscission point on the pedicel and on one occasion fed there continuously for over ten minutes. The damaged flowers subsequently became detached at the abscission point. On the swelling fruit, *M. caliginosus* caused small curved surface scars, which down-graded the fruit from class 1 to class 2.

Preliminary studies to explore the relationship between prey-type and damage

Macrolophus caliginosus, which had been reared on a diet of *L. bryoniae* larvae, caused significantly ($P<0.05$) more damage to tomatoes than those reared on *E. kuehniella* eggs (Table 1); thus suggesting that predators may have learned to increase plant probing.

Table 1. Mean percentages of flowers/fruit that dropped from trusses prematurely following confinement with *Macrolophus caliginosus*.

Treatments	Predators reared on moth eggs	Predators reared on leaf miners	Trusses without predators
% dropped fruit	10.4 ± 4.8	29.7 ± 6.4	1.4 ± 1.7

In the study comparing two crops with similar predator numbers, damage was most severe where there were least invertebrate prey (Table 2). This indicates that *M. caliginosus* feed more voraciously on plants after the supply of invertebrate prey has been exhausted.

Table 2. Numbers of *Macrolophus caliginosus*, with few or many *Liriomyza bryoniae* prey, compared to premature flower/fruit drop.

Mean no. of *M. caliginosus* per plant	Leaf miner numbers	Percentage of trusses with:	
		One fruit lost	> 1 fruit lost
330	Few	81%	52%
291	Many	31%	14%

These studies have confirmed that *M. caliginosus* can cause direct damage to UK tomato crops. As the predator also contributes to pest control, its status as a beneficial or a pest species is under debate. Most growers who suffered damage in 1996, decided not to release *M. caliginosus* in 1997, but the predators survived within empty glasshouses and colonized the new crops. The predator can make a valuable contribution to IPM in UK tomatoes if the populations can be manipulated to avoid injury to plants and subsequent financial loss. Further work is in progress to identify methods of regulating *M. caliginosus* populations that are compatible with other biological control agents used in the tomato IPM programme.

Acknowledgements

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Biological Control of Sweet Pepper Pests in the Netherlands

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Abstract: In 1998 a total of approximately 1000 ha. of sweet pepper was cultivated in glasshouses in the Netherlands. The aphids *Myzus nicotianae* and *Aulacorthum solani* often disrupted the IPM program and had to be corrected with chemicals. The thrips *Echinothrips americanus* has been found in peppers since 1996. The occurrence of ants (*Lasius niger*) seems to hinder the development of *Orius laevigatus* in the crop. In a semi-field trial it was proven that the mite *Amblyseius degenerans* preys on the eggs of *Aphidoletes aphidimyza*. New possibilities to work with bankerplants for aphid control are described. The use of the gall midge *Aphidoletes aphidimyza* has been improved by leaving the pupae in the bottle with the carrier material. In a field test the combined use of *Trichogramma brassicae* and *Orius laevigatus* against eggs of the moth *Chrysodeixis chalcites* was a success.

Key words: Sweet Pepper, Netherlands, biological control, *Amblyseius degenerans, Aulacorthum solani, Aphidoletes aphidimyza, Myzus nicotianae, Orius laevigatus, Trichogramma brassicae*

Introduction

In 1998 a total of approximately 1000 ha. of sweet pepper was cultivated in glasshouses in the Netherlands. Almost all growers used an IPM system. Most pests could be controlled biologically, but the aphids *Myzus nicotianae* and *Aulacorthum solani* were especially difficult to control. Growers had to take corrective measures with imidacloprid and pirimicarb respectively. An overview is given of the pests and the beneficials used. Several laboratory and field experiments are presented which can be used to come to a more reliable control

Thrips

The two most common thrips species in Dutch glasshouses are *Frankliniella occidentalis* and *Echinothrips americanus*. *E. americanus* has been found in vegetables since 1996. This thrips species lives low in the crop and is easily overlooked. Because of this, an estimated 50 % of the growers had severe outbreaks in the summer of 1997. In 1998 the problem was less (10%), partially because of the cool summer, and because growers were more aware of the problem. As *E. americanus* pupates on the leaf, it can not easily survive when the crop is changed and the greenhouse is thoroughly cleaned between cropping cycles.

Biological control of thrips.

A combination of *Amblyseius cucumeris* and *Orius laevigatus* forms the basis of thrips control. *A. cucumeris* is introduced on the rockwool pot (25 A.c/25cc/pot) a few weeks after planting. After week 12, 0.25 *O. laevigatus* /m2 are introduced four times.
The development of an *Orius* population can vary considerably between growers. The more frequent use of sulphur, displacement by ants in the flowers, and topping practices, are all factors that may explain this. The influence of ants, which displace adult *Orius* in the flowers, seems to have the most negative impact.
To test this theory, the development of four *Orius* populations was monitored in commercial greenhouses by counting every week 25 flowers. Also the number of black garden ants

(*Lasius niger*) was assessed. Fig. 1 shows that at low ant densities the development of *Orius* was normal, however, at higher ant densities the population development was very poor. Because the number of replicates was low, this trial should be repeated, and direct or maybe indirect effects have to be investigated in more detail.

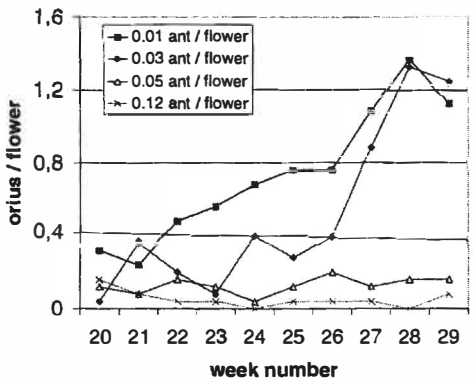

Fig. 1. The population development of four *Orius* populations at different ant densities.

Amblyseius degenerans is used by many growers for thrips control. The predatory mites are released in small numbers (0.2/m^2) when the first flowers appear and can be present in high numbers (>5 leaf) during the rest of the season. The actual benefit in thrips control is not very well quantified. Because of their possible negative impact on other beneficials (see fig. 2), their use should be reconsidered.

Aphids

The most common aphids are *Myzus nicotianae* and *Aulacorthum solani*. *M. nicotianae* is found mainly in the top of the plant. As there are no chemicals available, which can be integrated into a biological control program, the control of *M. nicotianae* is a key factor in an IPM program.

A. solani has become a very common pest in sweet pepper over the last three years. The plants can react very strong to the saliva of the aphid at already low densities. On the leaves yellow necrotic spots are found and often the top has malformations. Two to three chemical corrections with pirimicarb are currently standard practice for most growers.

Biological control of aphids

To control *M. nicotianae* the parasite *Aphidius colemani* is used. Since two to three years most growers are using "bankerplant systems". Winter wheat infested with the grain aphid *Rhopalosiphum padi*, and a small infestation with *A. colemani*, is placed in the greenhouse to create a continual parasite population. Every two weeks five bankers/ha. are added. Though the system can work well, two practical problems have been observed: the sometimes very early presence of hyperparasites, and problems with the growth of the wheat. This is especially so later in the season, when the plants are more shaded. It is now recommended to put the wheat in buckets and hang them between the top of the plants to receive maximal light. A trial with the bankerplant system *Sitobion avenae/Aphidius ervi* showed that it was not possible to put both bankerplant systems in the same greenhouse. *R. padi* quickly outcompeted the bankers with *S. avenae*. As a result the control of *A. solani* is mainly dependent on adult *A. ervi* and the gallmidge *Aphidoletes aphidimyza*.

The coccinellid beetle *Hippodamia convergens* is used as a "biological insecticide" in case of large outbreaks of aphids. Good results with larvae of the coccinellid *Harmonia axyridis* have been obtained, when released in hot spots in a ratio of 1 larvae per 10 aphids. The high production cost of this product hinders a more widespread use.

Tests with the gallmidge *Aphidoletes aphidimyza*

The gall midge *Aphidoletes aphidimyza* is used as a general predator of aphids. Unfortunately results can be unpredictable. Most growers use *A. degenerans* for thrips control, and it is known that this mite is very polyphagous. The hypothesis that this mite influences the performance of *A. aphidimyza* by predating on its eggs was posed.

This hypothesis was tested in an experimental greenhouse in two cages of 3 m^2; temp.: day 22, night: 20°C. In each cage 12 sweet pepper plants were placed and infested with *M. nicotianae*. Aphid populations were allowed to grow for one week. In one cage, *A. degenerans* mites were introduced from the start. To build up a population, a small amount of pollen was also added. In both cages, around 400 pupae of *A. aphidimyza* were placed. When the midges emerged, an average of 50 aphids and 4-8 mites were present per leaf. Three days after the emergence of the midges, 20 leaves were picked at random in both cages. The number of healthy eggs and predated eggs was assessed. Six days later, another 20 leaves were picked and the number of larvae was counted. Results are given in fig. 2.

In the cages where mites were present, around 40% of the eggs were predated, versus none in the other cage. The difference in the number of larvae found was even greater ($p<0.05$, K.W.). It was concluded that the role of *A. degenerans* in sweet pepper should be reconsidered.

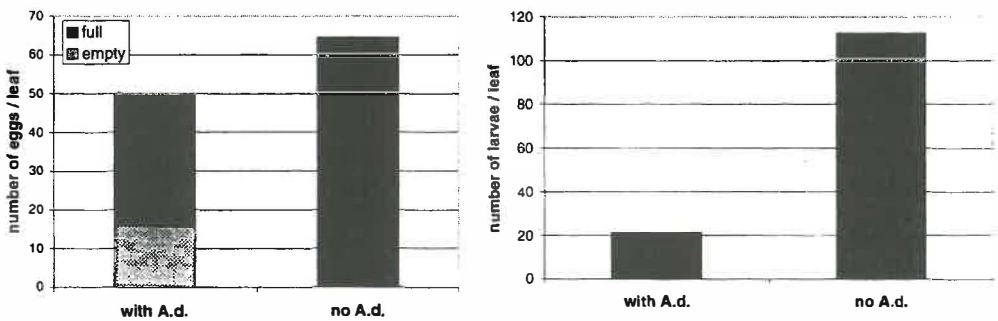

Fig 2. The predation of eggs and larvae of *A. aphidimyza* by *A. degenerans*

Another factor that can help to improve the effect of *A. aphidimyza* is the method of distribution. Until now, the recommendation was to place small heaps of the vermiculite and the pupae on the rockwool across from the dripper. Two negative aspects has been observed. Even though the material is placed on the moist rockwool, it can dry out, which has a very negative influence on the fecundity of the midges. Also it is not necessary to carry out a very fine distribution in the greenhouse because the midges, once emerged, can locate aphid colonies from long distances. This raised the idea to test if the midges were capable of emerging from the standard bottle.

A half liter bottle with 1000 pupae was placed in a cage at 24 °C. until all midges were emerged. From three depths (0-5, 5-10, 10-15 cm.) the pupae were checked for emergence. The vermiculite was checked for adult midges. From all depths the pupae were found empty and normally eclosed. No midges were found in the vermiculite. It was observed that the midges had pushed themselves through the vermiculite still in their pupal skin. Most of these

skins were found in the neck of the bottle, where the final emergence had taken place. Simply placing an open bottle of *A. aphidimyza* in the greenhouse has now become a standard procedure.

Caterpillars

The most common caterpillar species in sweet pepper is *Chrysodeixis chalcites*. *Lacanobia oleracea* and *Spodoptera exigua* are found less frequently. Beneficials which are commercially available are the egg parasite *Trichogramma brassicae* and the pentatomid bug *Podisus maculiventris*. With *P. maculiventris* good results can be obtained against aggregating caterpillar species (e.g. *S. exigua*). In several field tests, we found that it was also possible to control *C. chalcites* with *P. maculiventris*. However, this took some time and quite a bit of (non-economic) damage had to be accepted.

Test with *Trichogramma brassicae*

In a field test the performance of *T. brassicae* was tested. A population of *C. chalcites* was present in low numbers. We calculated that during this trial there were 40 – 50.000 moth eggs per ha. in the greenhouse. Every week 20 wasps/m^2 were released on cards (250 *Trichogramma* pupae/card). Every week 25 plants were checked for the number of black (parasitized) eggs, fresh lepidopteran eggs, number of *Orius laevigatus* and number of eggs predated by *O. laevigatus* . Results are presented in fig. 3.

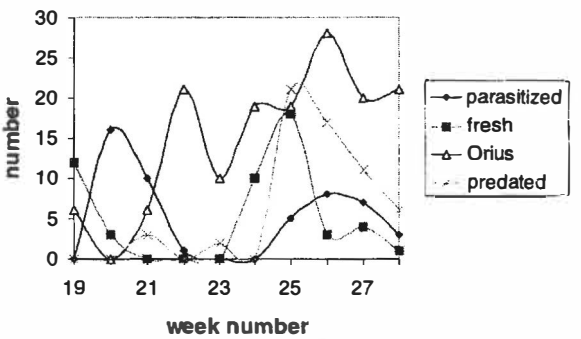

Fig. 3. The parasitization and predation of the eggs of *C. chalcites* by *T. brassicae* and *O. laevigatus*

The first three weeks most eggs were parasitized by *T. brassicae*, thereafter the majority of the eggs were predated by *O. laevigatus*. The labour involved in distributing *Trichogramma* cards every week has to be economized.

Spider mites

Spider mites are controlled with *Phytoseiulus persimilis* and *Amblyseius californicus*. When the climate is not too dry these beneficial are very effective. Some growers use "pest in first" systems for a quick establishment of *P. persimilis*. Approximately 800 plants are infested with spider mites and predatory mites per hectare. On each plant two leaves are infested with spider mites and only one of the leaves is infested with *P. persimilis*. Though *A. californicus* consumes fewer spider mites per day, it functions as a "stabiliser" because it can survive better at low prey densities and is more resistant to chemicals than *P. persimilis*.

Acknowledgements: Bart Sels for the trials with *Trichogramma*, Sandra Mulder for the trials with *A. aphidimyza* and *A. degenerans*, Hans Hoogerbrugge for the *Orius* trial, Karel Bolckmans and Carol Waddington for editorial suggestions.

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Biological Control of the Leafminer, *Liriomyza trifolii*, in Chrysanthemums: Implications for Intraguild Predation Between *Diglyphus begini* and *Steinernema carpocapsae*

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Abstract: Studies were conducted on the combined use of a parasitoid wasp, *Diglyphus begini*, and an entomopathogenic nematode, *Steinernema carpocapsae*, for control of the leafminer, *Liriomyza trifolii*, on chrysanthemums. Several factors indicate that these two agents may be suitable for combined use: (1) leafminer larvae parasitized by the wasp are less susceptible to nematode infection, and (2) nematodes show equal orientation towards paralyzed/parasitized leafminer larvae and healthy leafminer larvae. However, interspecific interference and intraguild predation (IGP) between the agents were found. IGP may be minimized through proper timing of release of the control agents.

Key words: *Liriomyza trifolii*, *Diglyphus begini*, *Steinernema carpocapsae*, entomopathogenic nematode, biological control, intraguild predation.

Introduction

The leafminer, *Liriomyza trifolii* (Burgess), is a major pest of numerous floricultural and vegetable crops. This species is a particularly serious problem on cut and potted chrysanthemum and gerbera and on many kinds of leafy vegetables. Leafminer adult feeding causes significant reductions in photosynthetic rates in greenhouse chrysanthemums (Parrella et al., 1985), whereas larval mining causes photosynthetic reductions and cosmetic damage to the leaves that results in unmarketable floral products.

Typically, near 100% control of the leafminer population is necessary to produce cosmetically marketable crops. This level of control has historically been obtained by the repeated applications of foliar chemical insecticide sprays. Over a 40 year period of insecticide pressure, populations of *L. trifolii* have developed resistance to a range of insecticides (Parrella et al., 1988). The use of biological agents has been an area of intense research for leafminer control, yet despite numerous trials utilizing parasitoids, micro-organisms, and predatory bugs, no single agent has provided control in a cost effective manner (Broadbent and Olthof, 1995). However, the combined action of two biological control agents may provide adequate leafminer larval control. Our research focuses on the combined use of two of the more successful biocontrol agents, *Steinernema carpocapsae* (Weiser), and *Diglyphus begini* Ashmead. This paper reports on experiments performed in the laboratory and greenhouse on the interactions between these agents that can either benefit or inhibit effective leafminer control.

Material and methods

Insect colonies

Colonies of *L. trifolii* and *D. begini* were maintained in greenhouses at the University of California, Davis according to methods detailed in (Parrella et al., 1989).

Nematode colony

A continuous colony of *S. carpocapsae* was maintained on greater wax moth (*Galleria mellonella* L. and *Achroia grisella* Fabr.) larvae.

S. carpocapsae orientation to leafminers

Orientation arenas were set up in petri dishes containing 2% agar. Three holes were made in the lid at the center and 25.4 mm to either side. All three holes were covered with strips of parafilm, and micropipette tips were placed through the two outer strips. Each tip was either empty or contained one healthy leafminer larva or one wasp-paralyzed leafminer larva. Three trials were set up for nematode orientation, either a healthy larvae vs. blank, a paralyzed larva vs. blank, or a healthy vs. paralyzed larva. Once the pipette tips were placed above the agar surface, a gradient of volatile host-cues was allowed to develop for 1 h. At this time, the center strip of parafilm was removed and ca. 500-2000 infective juvenile nematodes (IJs) were placed on the agar surface, and the parafilm strip was replaced. The nematodes were allowed to disperse for 1 h, after which an agar block directly beneath each pipette tip was removed and placed in a counting dish, where the number of IJs found under each tip was counted.

Nematode infection of healthy and wasp-paralyzed Liriomyza larvae

Fifty healthy 3rd instar *L. trifolii* larvae were removed from leaf mines and 10 each were placed on filter paper in five petri dishes. Fifty 3rd instar *L. trifolii* larvae that had been paralyzed by *D. begini* adults within the previous 6 h were also removed from leaf mines and 10 each were placed on filter paper in five 100 mm petri dishes. The filter paper of each dish was saturated with 1 ml distilled water containing 50 IJs. The petri dishes were held for 48 h and all larvae were dissected and examined for the presence of IJs.

Efficacy of the wasp and nematode alone or in combination

Three pots (2 plants per pot, 5 leaves per plant) of chrysanthemum containing 3rd instar leafminer larvae were removed from the insectary colony. Pots were then subjected to one of three treatments: (1) all leaves were sprayed with a suspension of 1000 IJs /ml until runoff and placed in a humidity chamber at 25 °C: 90% RH for 24 h, after which the pot was exposed to 200 adult wasps from the insectary colony for 24 h, (2) the pot was treated with IJs as in treatment 1, but was held for 48 h and was not exposed to wasps. In treatment 3, the pot was held for 24 h without any IJs and then was placed in a cage with 200 adult wasps. At the end of the 48 h (total) trial period, all leaves were removed from the plants and all mines were dissected. The larvae were scored as either being (1) healthy, (2) paralyzed with no eggs or nematodes present, (3) paralyzed with an egg but no nematodes, (4) paralyzed with nematodes but no eggs, or (5) paralyzed with nematodes and an egg.

Results

S. carpocapsae orientation to leafminers

There were significant differences in the numbers of nematodes found under the pipette tips in the healthy larva vs. blank and paralyzed larva vs. blank, but there was no significant difference in the trials with a healthy larva vs. a paralyzed larva.

Nematode infection of healthy and wasp-paralyzed Liriomyza larvae

There was a significant difference in the number of healthy (64% ± 7) vs. paralyzed (22% ± 6) leafminer larvae infected with nematodes.

Relative efficacy of the wasp and nematode alone or in combination

When leafminer larvae inside intact leaves on whole plants were exposed either to nematodes alone, wasps alone, or nematodes followed by wasps (Table 1), there were significantly more

healthy larvae in the nematode alone treatment than in either the nematode and wasp treatment or the wasp alone treatment. Although there was no significant difference between the nematode and wasp and wasp alone treatments, the fewest live leafminer larvae were found with the combined treatment of nematode and wasp.

There were significantly more eggs found in the wasp alone treatment than in the nematode and wasp treatment. There were significantly more leafminer larvae infected with nematodes in the nematode alone treatment than in the nematode and wasp treatment. Parasitoid eggs were not found on or near a nematode-infected larva.

Discussion

General biocontrol theory has stated that for terrestrial communities, if predatory species are abundant enough, they will limit herbivore populations and allow plant communities to grow until they are limited by competition (Rosenheim et al., 1993). The common wisdom has therefore been that promoting a large, diverse community of predators and parasites will ensure regulation of pest populations. One concern with the use of multiple biological control agents, however, is the possibility of intraguild predation (IGP), which is defined as when two species, that share a common host, engage in predation or parasitism of each other (Rosenheim et al., 1995).

The use of a parasitoid (*D. begini*) and a pathogen (*S. carpocapsae*) provides us with the opportunity to study detailed tri-trophic ecological interactions between a host and its two biological control agents. From our research, both positive and negative aspects of their combined use are apparent. Paralyzed leafminer larvae are less susceptible to nematode infection. This result is promising in that the wasp food source is partially protected from nematode infection. In addition, adult female wasps seem to detect and avoid ovipositing on nematode-infected leafminer larvae (Sher and Parrella, 1996). We have also found that nematodes show approximately equal propensity to orient towards paralyzed leafminer larvae versus healthy leafminer larvae. Finally, we found that using nematodes and wasps together resulted in fewer living leafminer larvae than when either agent was used alone. On the negative side, some IGP of *D. begini* larval stages by nematodes has been found, as the presence of nematodes in mines with wasp eggs decreases the chance of the wasp surviving to adulthood (Sher and Parrella, 1996).

We are presently investigating the effects of timing of release of the two agents on leafminer control. From the results reported here, it appears that the use of nematodes after wasps have been released into the crop may adversely affect the wasp population, but that nematode application before large-scale wasp releases could control a portion of the leafminer population, leaving the remainder to be utilized by the parasitoids. The timing between releases may affect control in allowing enough time for nematode penetration of leafminers before wasp searching/oviposition begins. It may be that the judicious timing of nematode applications that minimizes negative interference with the wasp population will allow for improved control of the pest over that presently seen by either agent alone.

Table 1. Percent (Mean ± SE) of *Liriomyza trifolii* larvae in each infection/parasitism category when exposed to *Steinernema carpocapsae, Diglyphus begini*, or *S. carpocapsae* and *D. begini* in combination.

Category of Larva	Nematode Alone	Wasp Alone	Nematode and Wasp
	N=39	N=37	N=36
% Healthy Larvae [1]	55.2 ± 5.3 **a**	29.4 ± 4.5 **b**	21.3 ± 4.1 **b**
% Paralyzed Larvae [1]	25.7 ± 4.4 **a**	41.6 ± 4.1 **b**	57.1 ± 3.9 **b**
% Larvae w/ Nematodes [1]	19.0 ± 3.4 **a**	(not applicable)	1.4 ± 0.8 **b**
% Larvae w/ Wasp Eggs [1]	[2]	28.8 ± 4.0 **a**	20.4 ± 2.9 **b**
% Larvae w/ Egg and Nematode	----------	----------	0.0 ± 0.0

[1] Means in rows followed by the same letter are not significantly different (P>0.05).

[2] Less than 4% of mines in the nematode alone trial (N=317) contained *D. begini* eggs, due to a greenhouse infestation.

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Economic injury levels for western flower thrips on greenhouse cucumber

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Abstract: Economic injury levels for western flower thrips, *Frankliniella occidentalis* [Pergande], on greenhouse cucumber were determined by establishing low, medium and high densities of thrips in 3 greenhouses at the Greenhouse and Processing Crops Research Centre, Ontario, Canada in 1996 and 1998. Pest densities were monitored weekly using yellow sticky cards and flower counts. Fruit was harvested weekly, graded for size and number 1, 2, or unmarketable, weighed and rated for thrips damage using 3 damage ratings. Fruit damage was significantly related to thrips density. Economic injury levels ranged from 20-50 adult thrips per sticky card per day or 3-7 adult thrips per flower depending on control costs, yield and fruit prices. These levels can be used as a guideline to assist growers in improving the timing of insecticide applications.

Key words: *Frankliniella occidentalis*, economic injury level, greenhouse cucumber, adult thrips

Introduction

Economic thresholds are an important component of a cost-effective integrated pest management program. However, few thresholds have been determined for pests of greenhouse vegetables. Western flower thrips (*Frankliniella occidentalis* [Pergande]) is an important pest for cucumber and sweet pepper, and also at times for tomato in Ontario. Precision-level sampling programs for *F. occidentalis* have been developed for sweet pepper (Shipp & Zariffa, 1991) and cucumber (Taylor et al., 1998). In addition, economic injury levels (EILs) for *F. occidentalis* on sweet pepper have been determined (Shipp et al. 1998). In Ontario, many cucumber growers still use pesticides for part of the season to control thrips. This study examined the EILs for *F. occidentalis* on cucumber.

Materials and methods

The study was conducted in 3 greenhouses at the Greenhouse and Processing Crops Research Centre and repeated over 2 growing seasons from February to June 1996 and June to November 1998. The greenhouses were 10 x 16 m with a planting density of 160 plants in 1996 and 8 x 13 m with a plant density of 112 in 1998. The commercial cultivar 'Flamingo' was used and grown according to commercial practices. Each greenhouse was exposed to a different pest density (low, medium and high) by introducing 0, 0.3-1 and 5 adult thrips per plant, into each of the greenhouses respectively, when the crop was transplanted into the greenhouses. Predatory mite, *Amblyseius cucumeris* (Oudemans) was used in the low density greenhouse and pesticide applications were used in the other greenhouses to maintain treatment levels.

The population densities of the thrips were monitored weekly using yellow sticky cards (13 x 8 cm) and flower counts. Each greenhouse was divided into 4 quadrants with 1-24 h sticky card sample per quadrant per week. Also, each week 1 plant was randomly selected per quadrant for flower counts. First, adult and immature thrips were counted *in situ* for each flower and then each flower was placed in a labelled vial for counting again in the laboratory.

With respect to fruit harvest, cucumbers were harvested 2-3 times per week. In each greenhouse, each quadrant was harvested separately and labelled accordingly. All fruit were graded individually for size (small, medium, large and extra large), grade (1, 2 or unmarketable), and weighed. The fruit was also rated for thrips damage to the whole fruit. A rating scale of 1-3 was used; where F1 = essentially no damage, F2 = moderate damage and F3 = heavy damage.

To determine the EILs, only F3 data (all fruit sizes) were analysed. Linear correlations between fruit damage, expressed as the percentages of F3 fruits in weekly fruit harvests, and thrips densities for several different time periods before fruit harvest, were determined. Economic injury levels were developed based on the best relationship between *F. occidentalis* densities and fruit damage. The formula used for calculating the EILs was based on economic loss equals control costs (Pedigo et al. 1986): $F3 = C/(V_{df} \cdot Y)$, where C is the cost of control ($/ha), Vdf is the price difference between grade 1 and 2 fruit ($/dozen), Y is yield (number of fruit/ha/wk) and F_3 is percentage of F3 fruits in weekly fruit harvests, which is a linear function of thrips density x. Thrips densities that were used to calculate the %F3 were then used as the EIL.

Results and discussion

The seasonal trends for adult *F. occidentalis* as monitored by the yellow sticky cards and flower counts were similar each growing season (Fig. 1). Adult population densities in the medium and high density greenhouses increased exponentially the first part of the seasons in 1996 and 1998. In 1998, densities then decreased rapidly due to pesticide applications. In 1998, *Orius* entered the greenhouses the latter part of August and reduced thrips densities for 4-5 weeks. The population densities were allowed to increase again in the medium and high densities greenhouses. In the low density greenhouses, thrips densities were constantly much lower than the other treatments except for the flower counts in the latter part of the season in 1996.

Frankliniella occidentalis caused substantial fruit damage with a F3 rating and resulted in the fruit being downgraded to grade 2 fruit. The difference in price between grade 1 and 2 fruit throughout the season varied from $2.00 to $3.50 per dozen fruit. Although fruit with a F2 rating also had some thrips damage, this damage did not cause any economic loss as the fruit was still graded as 1. A substantially higher percentage of F3 fruit was found in the medium (0.7%, 4.8%) and high (5.2%, 9.8%) density greenhouses compared to the low (essentially 0%) density greenhouse in 1996 and 1998, respectively.

Regression analyses of the density of thrips in each week, as monitored by the 2 sampling methods, against the percentages of F3 fruit each weekly harvest found that the best correlations were 1 week before harvest for sticky cards ($F3 = 0.0020 + 0.0055x$, $R^2 = 0.87$) and 2 weeks before harvest for flower counts ($F3 = -0.0025 + 0.0008x$, $R^2 = 0.91$). Fruit development from anthesis to harvest ranged from 12-18 days. The differences in the correlations for the 2 time periods may be due to the monitoring of different segments of the thrips population. With sticky cards, the population densities of active flying thrips were monitored which could feed on any stage of developing fruit. With flower counts, however, the damage caused by this feeding population would not show until the fruit is harvested approximately 2 weeks later.

With sticky cards, a density of 63.5 thrips per day resulted in 5% of the grade 1 yield being downgraded to 2 due to thrips damage. With flower counts, 9.5 adults thrips per flower resulted in 5% of the yield being downgraded. The EILs for *F. occidentalis* for both sampling methods based upon different control costs, weekly yields and fruit prices are presented in Table 1.

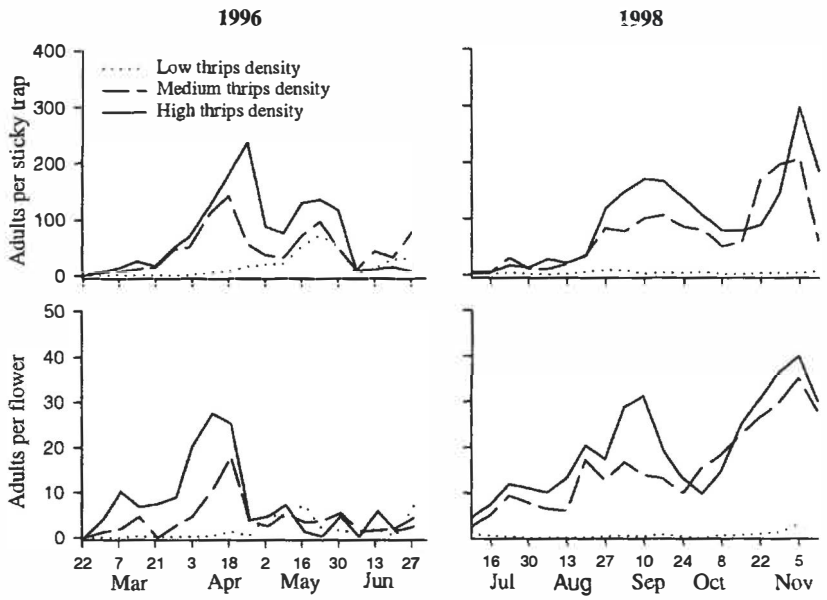

Figure 1. Mean number of *F. occidentalis* sampled weekly by sticky cards (top) and flower counts (bottom) in greenhouses that were maintained at different thrips densities in 1996 and 1998.

From Table 1, it is evident that EIL depends on many factors. Any changes in fruit yield, market price and cost of control measure will affect the EIL. Therefore, EILs should be considered dynamic and need to be recalculated when factors change. When using chemical control measures, the pesticide should be applied as soon as the EIL is reached. In addition, EILs are useful for determining the cost-effectiveness of the various control options used to manage a pest. Further analyses of the present study are also being conducted to determine the impact of *F. occidentalis* on plant photosynthesis and yield.

Table 1. Economic injury levels (EIL) for *F.occidentalis* on greenhouse cucumber based on different weekly yields, market prices and costs of control.

Components of economic injury level				Economic injury level		
Cost of control ($/ha)	Yield (# of marketable fruit /ha/wk)	Price for grade 1 fruit($/dz)	Price for grade 2 fruit($/dz)	%F3 at EIL	Adults /card /day	Adults /flower
120	25000	10.00	8.00	2.88	37.9	5.6
120	27500	11.00	8.50	2.09	28.4	4.2
120	30000	15.00	11.50	1.37	19.6	2.9
140	25000	10.00	8.00	3.36	43.7	6.5
140	27500	11.00	8.50	2.44	32.6	4.8
140	30000	15.00	11.50	1.60	22.4	3.3
160	25000	10.00	8.00	3.84	49.5	7.4
160	27500	11.00	8.50	2.79	36.8	5.5
160	30000	15.00	11.50	1.83	25.2	3.7

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Biological Control in Ornamentals: An individual-based modelling approach

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Abstract: An individual-based, spatially explicit simulation model has been developed which simulates the temporal and spatial dynamics of the interaction between *Phytoseiulus persimilis* and *Tetranychus urticae* on three ornamental crop species (*Ceanothus* 'Autumnal Blue', *Choisya ternata* and *Euonymus japonicus*). The model consists of a library of generic subroutines that describe the biology of invertebrates: development, survival, reproduction, predation and movement. The model will enable the key factors in the tritrophic plant-pest predator system that influence the efficiency of biological control to be identified, and novel introduction strategies to be determined.

Key Words: *Phytoseiulus persimilis*, *Tetranychus urticae*, individual-based, model, biological control

Introduction

The wide diversity of crop species and growing practices, and low tolerance to damage are factors which make the development of robust biological control strategies for the ornamentals industry a challenge. In an attempt to respond to this challenge, a combined experimental and modelling approach is being used to gain understanding of the key factors of the tritrophic plant-pest-predator interaction that determine the efficiency of biological control in ornamental crops. In this paper, I present a description of a model describing the interaction between *Tetranychus urticae* and *Phytoseiulus persimilis* along with a brief description of experimental data used to develop the model.

Description of the model

The model consists of a simulated square grid of 100 plants, and the model uses a time step of 24 hours. Each pest has a set of associated variables, which are its age, x position, y position and longevity. In addition to this, adult pests also include variables relating to reproductive period and fecundity. Adult predators have an additional variable that stores the amount of food eaten.

Initial distribution

The model starts by distributing the pests and predators over the grid of plants. Each individual pest and predator is assigned an x and y co-ordinate by randomly sampling from a uniform (1,10) distribution. The number of plants onto which the pests are introduced can be varied to produce a range of distributions from uniform through to highly clumped, enabling the effect of pest distribution on the efficiency of biological control to be investigated.

Development and survival

Having distributed the pests and predators, the model then calculates the development of each individual by increasing its age by the time step. If the age of the individual is greater than its longevity then it either moves into the next developmental stage, or dies, as appropriate.

Survival is simulated as a stochastic process where each individual has a survival probability; this probability is then compared with a value sampled from a uniform distribution. If the value is greater than the probability of survival, then the individual is assumed to die.

Reproduction

Each individual adult female is assigned an egg laying rate, based on either experimental data or published data. The total number of eggs laid by the pests on each plant is then calculated by summing the number of eggs laid by each individual pest on a plant. For the predators, it is assumed that the number of eggs laid is related to the amount of food eaten in the previous time step. A similar summation to that used for the pests calculates the number of predator eggs laid on each plant.

Predation

The model uses a type II functional response, based on both experimental work and published data, to calculate the number of pests consumed by each individual predator. The biomass of prey eaten is stored for use in the reproduction process in the next time step, and the pests eaten are removed from the model.

Movement

Movement is simulated using a simple stochastic process. Each individual pest and predator is assigned a probability of moving, based on experimental data. This value is then compared with a random variable sampled from a uniform distribution. If the random variable is less than the probability of moving, then the individual is assumed to move. The direction of movement is determined by which quartile of the movement probability the random variable falls into. Movement is restricted to only the four cardinal directions. All mites are allowed to move only once in a time step. The model then iterates onto the next time step

Output

On each time step the number of pests and predators in each stage is written to a file. The model ends after 100 days, unless either the pest or predator is driven to extinction. The day on which the model ends is also written to a file along with a variable, which indicates whether the pest or predator goes extinct.

Experimental Work

Initially the model was developed using published data. However, since few data were available on the biology of *T. urticae* on the ornamental crops being simulated, it was necessary to obtain information for the model via experimentation. Small scale experiments were done to determine the development, fecundity and longevity of *T. urticae* on the three ornamental crops of interest, and to investigate the movement of both spider mites and *P. persimilis*. The predatory interaction between *T. urticae* and *P. persimilis* was also determined.

The experimental work showed that the development rate of *T. urticae* did not differ in a biologically meaningful way between the three ornamental crop species (*Ceanothus* 'Autumnal Blue', *Choisya ternata* and *Euonymus japonicus*), being approximately 11 days on each species. Significant differences were found, however, in the fecundity of *T. urticae* on each of the three crop species. The greatest fecundity was on *Choisya ternata* (4.5 eggs per female per day), whilst the lowest was on *Ceanothus* 'Autumnal Blue' (1.4 eggs per female per day).

The movement experiments have shown that immature stages of *T. urticae* are sedentary, having a low probability of movement. The probability of plant to plant movement of adult *T. urticae* is related to the density of spider mites on the plant. For all *T. urticae* stages, probability of movement is affected by the crop species, although the precise reason is

unknown, it is likely that this is due to the suitability of the crop species as a food source for the mites.
The probability of movement of adult *P. persimilis* is related to the density of prey available irrespective of crop species.
The functional relationship between *P. persimilis* and its prey *T. urticae* was determined on *Choisya ternata*, using a novel method (Skirvin & de Courcy Williams (in press)). The response obtained was similar to previously published work (Takafuji & Chant 1976, Fernando & Hassell 1980, Rabbinge & Hoy 1980, Ryoo 1986), but with a lower predation rate at low prey densities.
These data were then incorporated into the modelling framework, and the model was run to simulate a range of scenarios with differing levels of prey and predator introductions.

Model predictions

The model has been run using five different levels of introduction for both *T. urticae* and *P. persimilis*. The numbers of *T. urticae* introduced varied from 100 to 500 in steps of 100, whilst the numbers of *P. persimilis* introduced ranged from 1000 to 5000 in steps of 1000. This gave 25 combinations of introduction levels, and for each combination the model was run 30 times, assuming random distribution of both mite species in all cases.
The model was also run to simulate the effect of different plant species by using different equations to describe the fecundity and development of the spider mites, and the movement of both spider mites and *P. persimilis*, for all introduction level combinations.
The predictions of the model suggest that for *C. ternata*, *T. urticae* and *P. persimilis* coexisted when between 200 and 300 spider mites are introduced initially, regardless of the number of *P. persimilis* introduced. At the lowest spider mite introduction level, and the two highest introduction levels, the spider mite population tended to build up to unmanageable levels, irrespective of the number of *P. persimilis* introduced. The time taken to reach an unmanageable population density increased as the number of *P. persimilis* introduced increased, indicating that *P. persimilis* was having an effect in reducing the number of spider mites.
For *Ceanothus* 'Autumnal Blue', *P. persimilis* tended to become extinct within 25 days for all mite introduction levels. The time to extinction increased with the number of *T. urticae* introduced. Coexistence of the predator and prey became more likely as the number of *T. urticae* introduced increased, but less likely as the number of *P. persimilis* introduced increased. The chance of *T. urticae* reaching unmanageable levels also increased with the number of spider mites introduced, but decreased as the number of predators introduced increases. The time to reach an unmanageable population decreased as the introduction level of the spider mites increased.
On *E. japonicus*, *P. persimilis* tended to become extinct at the lowest two levels of *T. urticae* introduction, with coexistence being the most prominent outcome for introduction levels of spider mites above 300 mites. The time to extinction of *P. persimilis* increased as the numbers of spider mites introduced rose. Unmanageable populations of *T. urticae* developed mainly at the lowest three levels of spider mite introduction.

Discussion

This work shows that plant-mediated differences in the biology of both *T. urticae* and *P. persimilis* can have major affects on the model predictions for the interaction between the two

species. The low reproductive rate of the spider mites on *Ceanothus* 'Autumnal Blue' causes the *P. persimilis* to go extinct, whilst the much higher fecundity of spider mites on *C. ternata* allows them to overcome predation and reach unmanageable population levels. The situation in *E. japonicus*, where coexistence of the mites becomes more likely at higher levels of pest is the situation that occurs in the edible crops, but is not so suitable for ornamentals, where damage cannot be tolerated. The next step is to obtain further information on the effect of plant species on the predator-prey interaction by examining the spatial distributions predicted by the model of both mite species. In addition, different patterns of introduction need to be investigated, as the current method of a single introduction over the whole grid of plants does not represent the natural situation. Other mite predators also need to be investigated, and the modelling work may show which predators are best suited to which plants.

This work clearly demonstrates that knowledge of the effect of plant species on the biology of both pests and natural enemies will be extremely important in developing suitable biological control strategies for use in ornamental crops. The model described here provides a useful method for investigating these effects, and will aid the development of biological control for a wide range of ornamental crops, pests and natural enemies. In addition to the spider mite model described here, similar models, using the same framework have been developed for western flower thrips and aphids.

The model is not perfect, and some further refinement of the routines describing predation and movement may well lead to much better predictions of the interaction between *T. urticae* and *P. persimilis*. In addition, models for other crops, pest species and natural enemies will be developed using the modelling framework and experimental data. Once these refinements have been made, the model should be able to be used to predict the most appropriate strategies for effective biological control of pests within ornamental crops.

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The effect of plant species on the biology of *Tetranychus urticae* and *Phytoseiulus persimilis*.

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Abstract: The influence of plant species on the population dynamics of the spider mite, *Tetranychus urticae* and its predator, *Phytoseiulus persimilis* were examined as part of a framework for the development of effective biological control in ornamental crops. Development of *T. urticae* was found not to differ with plant species in a biologically meaningful way. Plant species had significant influence on both fecundity ($p<0.001$) and movement ($p<0.01$) of *T. urticae*, but no effect on the movement of the predator.

Key words: *Phytoseiulus persimilis*, *Tetranychus urticae*, ornamentals, biological control, plant species

Introduction

The low damage tolerance and high diversity of crop species and growing practices within the ornamentals industry present a challenge to the development of effective biological control strategies for *Tetranychus urticae*. In order to develop a robust strategy for biological control, the effect of plant species on both the pest and the natural enemy needs to be understood. This paper presents a summary of experimental work showing how plant species affects the *T. urticae* - *P. persimilis* system (Skirvin and de Courcy Williams, (1999)).

Materials and methods

For a description of the methods used, see Skirvin and de Courcy-Williams (1999).

Results

***Development and fecundity of* T. urticae**
Plant species had a significant effect on the reproductive period and fecundity ($p<0.001$), being greatest on *C. ternata* (There was no significant difference between the other two plant species. Development time differed between plant species by less that a day, which is not biologically meaningful.

***Movement of* T. urticae *and* P. persimilis**
The movement of juvenile stages of *T. urticae* was not related to their density, but the movement of adult female *T. urticae* was positively correlated to their density, except on *Ceanothus* 'Autumnal Blue' (Figure 1). There was a significant difference in the movement of all stages of spider mites between the plant species ($p<0.001$).
For *P. persimilis*, there was a highly significant effect of the presence of spider mites on a stem ($p<0.001$), but no effect of plant species. The movement of adult female *P. persimilis* was negatively proportional to the density of all stages of *T. urticae* remaining on a stem (Figure 2).

Table 1: The development time, reproductive period and fecundity of *T. urticae* on the three plant species (Standard errors in brackets) [from Skirvin and de Courcy-Williams (1999)]

Plant		*Ceanothus* 'Autumnal Blue'	*Choisya ternata*	*Euonymus japonicus*
Development time (days)	Mean	10.9 (±0.59)	10.5 (±0.31)	11.7 (±0.52)
	n	11	33	31
Reproductive period (days)	Mean	8.5 (±2.45)	19.1 (±1.72)	12.6 (±1.34)
	n	11	33	31
Fecundity (eggs/female/ day)	Mean	1.4 (±0.01)	4.5 (±0.26)	2.0 (±0.20)
	n	11	33	31

Functional response

The loss of spider mites in the absence of predators was a constant proportion of the density of spider mites. These losses were corrected for by subtracting this linear relationship from the quadratic curve to give the fitted equation (Figure 3).

Discussion

The data clearly show that plant species can have a significant effect on both *T. urticae* and *P. persimilis*. The effect of plant species on the fecundity and reproduction of spider mites is marked, and this could have major implications for effective biological control. On plants where it has a high fecundity, *T. urticae* will be able to reach damaging levels very quickly. Once its presence has been identified, and by the time biological control agents are used, it may be too late. It is probably, therefore, more useful to have a prophylactic approach to biological control in these cases, where repeated natural enemy introductions are used in an attempt to prevent the pest from entering the crop, rather than being contained and controlled once it has arrived.

The differences seen in the movement of spider mites on the three plant species suggest that *T. urticae* may well be more mobile on less suitable host plants, which could be problematic when trying to achieve control. On plants where the spider mites are moving quickly from stem to stem, large patches will probably not develop and so the predatory mite may find it more difficult to locate its prey.

The relationship between the movement of adult *T. urticae* and their density is not unexpected since the more mites there are, the less food is available, and therefore the more likely it is that the mites will move. Also, the dependency of *P. persimilis* movement solely on prey density of is an intuitive result, since the predator will remain in a prey patch rather than move out of it.

The data obtained in the functional response experiments agree well with published work (Takafuji and Chant, 1976; Fernando and Hassell, 1980; Ryoo, 1986) in terms of the asymptotic value of the response, but there is greater predation at low prey densities. The novel method used in these experiments incorporates some of the spatial complexity associated with real plants, which may lead to the differences seen between this work and published data, as the method reduce the chances that the predator will repeatedly search the same area. The mortality of mites in the controls leads to the question of whether similar losses occurred in the published work. The lack of webbing on the arenas in the published work may have led to different estimates of the predation capacity of *P. persimilis*. The differing responses of both pest and predator to plant species will hamper the development of a robust biological control strategy for use in ornamental crops. Further investigation of the effect of plant species will be necessary in order to determine which effects are the most

important in influencing the efficiency of biological control. In addition to experimental work, a modelling framework (Skirvin, this issue) will be used to examine how the differing responses of *T. urticae* to plant species affects the efficiency of biological control.

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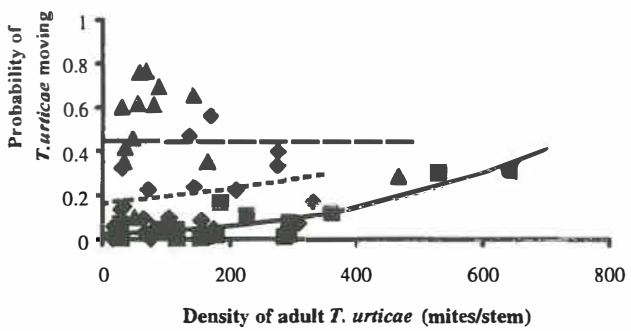

Figure 1: The effect of the density of adult *Tetranychus urticae* on their movement on *Ceanothus* 'Autumnal Blue'(• ; observed data, • • ; fitted line y = 0.45), *Choisya ternata* (■, observed data ; • • , fitted line y = [exp(0.0048x-3.7)] / [1+exp(0.0048x-3.7)], and *Euonymus japonicus* .(♦, Observed data ; – – – – ,Fitted line y = [exp(0.0022x-1.6)] / [1+exp(0..0022x-1.6)]) where y = Estimated probability of *T. urticae* movement, and x = Density of adult *T. urticae*(number per stem)}.[From Skirvin and de Courcy Williams (1999)]

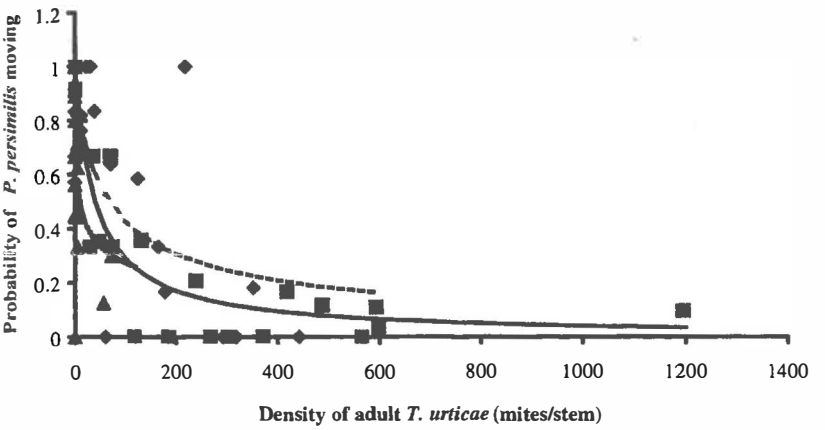

Figure 2: The effect of the density of adult *Tetranychus urticae* on the movement of adult female *Phytoseiulus persimilis* on *Ceanothus* 'Autumnal Blue' (• ; observed data, • • ; fitted line y = [exp(0.99-0.98(log(x)))] / [1+exp(0.99-0.98(log(x)))]), *Choisya ternata* (■, observed data ; • • , fitted line y = [exp(3.6-2.3(log(x)))] / [1+exp(3.6-2.3(log(x)))];) and *Euonymus japonicus* (♦, Observed data ; – – – – ,Fitted line y = [exp(3.2-1.7(log(x)))] / [1+exp(3.2-1.7(log(x)))]) where y = Estimated probability of *P. persimilis* moving, and x = Density of *T. urticae* (number per stem)}[From Skirvin and de Courcy Williams (1999)]

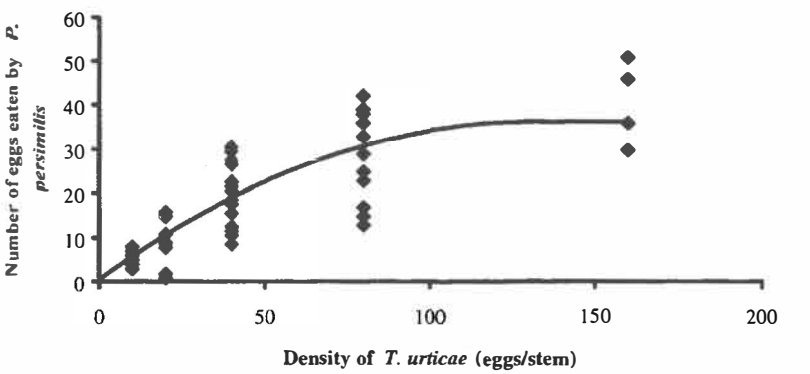

Figure 3: The number of prey eaten by an adult female *Phytoseiulus persimilis* in 24 hours at different densities of *T. urticae* eggs. ◆,.Observed data; • , Fitted line (y =0 [x =0] ; y = 0.5 + 0.546x – 0.0021x^2 [0 < x ≤ 130]; y = 36.0 [x >130] ; where y = Estimated number of prey eggs eaten, and x = Density of prey eggs (number per stem)) [From Skirvin and de Courcy Williams (1999)]

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Product control of *Phytoseiulus persimilis* (Athias-Henriot): current practice of a commercial producer

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Abstract: Quantity (numerical integrity) and fecundity criteria from the product control guidelines of the commercially mass reared predatory mite *Phytoseiulus persimilis*, were routinely practiced. Counting the number of *P. persimilis* per bottle during a packaging process revealed that the amount of predators was gradually reduced as the process advanced, due to escaping from the vermiculite carrier (loss of up to 35% of the standard contents). Lowering the temperature at the packaging room to 12°C coupled with reducing batch-size by 50% have decreased the loss of predators to 18% relative to the standard. An A-priori compensation of 20% is currently added to each bottle in order to assure numerical integrity. In 3 out of 4 periodical fecundity tests the females of *P. persimilis* exhibited egg laying capacity well above the standard of 10 eggs/female/5 days. A strong positive relationship was found between longevity and total fecundity of female predators. At present two new aspects of *P. persimilis* product control are being studied: one is searching capacity of adult females and the other is the relationship between abdominal discoloration or crystal presence in different parts of body of the mite and its performance.

Key words: *Phytoseiulus persimilis*, product control, fecundity, longevity.

Introduction

In February 1997, the final workshop of the EC concerted action group on "Designing and Implementing Quality Control of Beneficial insects: Towards More Reliable Biological Pest Control", was held in Barcelona, Spain. One of the key appointments agreed upon was that the commercial companies will use, on a regular basis, the product control guidelines designed for ca. 20 commercially available natural enemies (van Lenteren 1998).

As an experienced producer, involved in export of natural enemies as well as implementing them in the local market (Israel), Bio-Bee Biological Systems has taken a strategic decision to adopt the above mentioned guidelines and practice them regularly, year round.

Herewith we describe the experience of practicing the product control guidelines for the spider mite predator *Phytoseiulus persimilis* (Athias-Henriot), a key product in Bio-Bee's export to Europe and North America as well as a an important component in IPM programs in greenhouse and open field vegetables in Israel. Results of the most frequently tested guidelines are presented, together with comments and suggestions for further improvement in the near future.

Methods

Quantity

Routine batch-wise counts were performed to check that the quantity of *P. persimilis* per bottle (=500 ml product container) is not less than the predefined standard: 2,500 or 4,000. The counts were carried out according to the 5 sub-sample procedure described in the

guidelines (van Lenteren 1998). A special emphasis was put on counting the number of predators in bottles which were packed at different phases of the packaging process.

Fecundity

Periodical fecundity tests were run with individual predators, following closely the guidelines and checking the results against the reference of $\geq$ 10 eggs/female/5 days (van Lenteren 1998). In one test the mites were kept in the experimental units until they died naturally (old leaf discs were replaced by fresh ones every 5 days). Thus total fecundity could be determined for each predatory mite.

Results and discussion

Quantity

Bottling process of a mix of pure *P. persimilis* and vermiculite carrier for packing a batch of 50 bottles was followed by counting bottles packed at the initial, middle and final phase of the process (bottles no. 13, 27 and 39, respectively). Results are shown in table 1. There was a gradual reduction in the number of *P. persimilis* per bottle, relative to the standard 2,500, as the packaging process advanced. It reached almost 35% reduction at the final stage of the packaging. Apparently the mites escaped from the vermiculite while "waiting" in the packing machine to be bottled because of a relatively high temperature in the packaging room (15°C) and the fact that the packing process has taken too long (12 minutes).

Table 1. Quantity control of *P. persimilis* (*P.p*) in the packaging process (June 22, 1998).

	Bottle #13	Bottle #27	Bottle #39
Average no. of *P.p*±SD*	62.8±5	56.8±10.2	42.4±2.9
Weight of vermiculite (gr)	76.5	78.8	77
P.p/bottle	2,402	2,238	1,632
% reduction from 2,500	3.92	10.5	34.7

* - Calculated from 5 samples, 2 gr. each, of the vermiculite carrier.

In a subsequent count, the batch-size was reduced to 26 bottles and the standard number of predators per bottle was increased to 4,000. Six bottles were sampled: bottles number 6 and 7, 12 and 13, 19 and 20, representing the initial, middle and final phase of the packaging process, respectively. Results are presented in table 2. The amount of *P. persimilis* in the bottles packed at the first third of the packaging process met the numerical standard. However, as packaging advanced there was a loss of mites reaching ca. 18% of the standard 4,000 per bottle. Although the packing room was cooled to 12°C and the packaging process reduced to 7 minutes due to reduction in the batch-size, *P. persimilis* still tended to escape the vermiculite. It should be emphasized that only a negligible amount of dead mites was recorded in the samples. Hence the reduction of the predators during the packaging process is attributed to escape rather than mortality. Currently, an A-priori compensation of 20% is added to the bottle's standard predator contents to assure numerical integrity of the final product.

Table 2. Quantity control of *P. persimilis* (*P.p*) in the packaging process (July 7, 1998).

	Bottle #6	Bottle #7	Bottle #12	Bottle #13	Bottle #19	Bottle #20
Average no. of *P.p*±SD*	86.6±16.6	97±22.6	70.8±8.8	68.8±11.9	83±11.8	71.2±9
Weight of vermiculite (gr)	94.6	96	95.4	95.7	95.6	94.3
P.p/bottle	4,096	4,656	3,377	3,292	3,967	3,357
% reduction from 4,000	-	-	15.6	17.7	0.82	16

* - Calculated from 5 samples, 2 gr. each, of the vermiculite carrier.

Fecundity

Periodical fecundity tests have shown that in the majority of the cases the number of eggs/female/5 days exceeded by far the reference of 10 eggs/female/5 days except for the test of June the 23rd (Table 3). The reason for this exceptional reduction in fecundity is unknown but it stresses the need for periodical fecundity tests, more frequent than the seasonal tests recommended by the guidelines.

Table 3. Fecundity of individual females of *P. persimilis* recorded in periodical fecundity tests.

Date	20.5.98	23.6.98	5.8.98	15.10.98
Eggs/female/5 days±SD	14±8.5	8.2±6.3	15.3±5.5	14.7±5.3
n	27	30	26	29

In another fecundity test, when the female predators were kept in the experimental arena until they died naturally, there was a strong positive relationship between longevity and total fecundity of *P. persimilis* (Figure 1). Under the experimental conditions the female predators reached a maximum longevity of 27 days and more than 100 eggs/female.

Glenn Scriven of Biotactics, California (personal communications) challenges the IOBC recommended fecundity test and offers a DRI (Daily Rate of Increase) test, which is performed batch-wise and gives an immediate index of predator performance. Counts of active stages of *P. persimilis* are carried out on a 20-leaf sample taken from each developing culture. Sequential counts of the same culture are compared and a DRI value is determined. A value of 0.3 (30% population increase per day) or above should characterize a healthy culture of *P. persimilis* reared at 28°C.

At present two additional aspects of product control are being investigated: 1) A greenhouse bioassay for testing searching ability of *P. persimilis*. The bioassay is

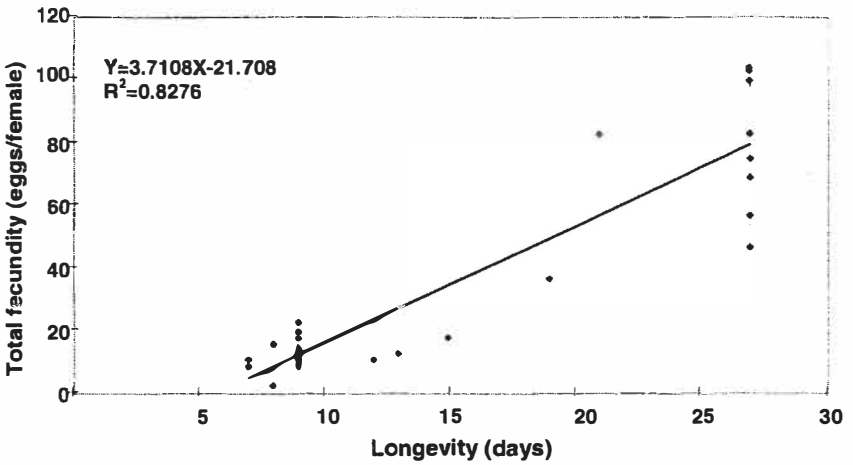

Figure 1. Relationship between longevity and fecundity in adult females of *P. persimilis*. Test performed during October 1998.

developed as a joint project of Koppert Biological Systems and Bio-Bee (Wilma Reerink and K. Bolckmans – personal communications); 2) White abdominal signs associated with crystal accumulation, indicate overall poor health in *P. persimilis*, according to Bjørnson *et al.* 1997 and Conny Schütte (personal communication). Therefore, a study is conducted to find whether there is any relationship between abdominal discoloration or presence of crystals and product control attributes of *P. persimilis*, e.g. longevity, fecundity and searching capacity. After establishing an appropriate protocol for these new aspects, they will be offered for inclusion in an updated version of the product control guidelines.

The Association of Natural Bio-control Producers (ANBP) in North America has initiated a process of standards' formation for biological control organisms. The ANBP has invited the American Society for Testing and Materials (ASTM) to facilitate the process by providing a management system for the development of the standards (Anna Luczynski - personal communication). The contact between ANBP and ASTM has stimulated an evaluation process of the EC guidelines for product control of commercially available natural enemies. Presumably this process will yield a multitude of reservations, suggestions and ideas to change/correct the guidelines. Thus it is a rare opportunity to "join forces" in order to improve and adopt the guidelines on a global basis, especially with regard to natural enemies like *P. persimilis* which are commercially used around the world.

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A simplified rearing method for *Stratiolaelaps (Hypoaspis) Miles* (Acari: Laelapidae)

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Abstract: A low input method of rearing the predatory laelapid mite *Stratiolaelaps miles* (Berlese) is described. The method utilizes the standard host, *Tyrophagus putrescentiae* (Schrank) but the rearing medium for both *S. miles* and *T. putrescentiae* has been modified to minimise handling. Thirty-five fold
increases have been achieved in 21 days.

Key words: predatory mites, rearing, *Stratiolaelaps miles, Tyrophagus putrescentiae.*

Introduction:

The laelapid mite *Stratiolaelaps* (*Hypoaspis*) *miles* (Berlese) has been reared by commercial insectaries for several years, primarily for use against fungus gnat larvae in soil in protected crops. Rearing methods generally utilise trays or boxes containing a mixture of peat and vermiculite. The predator mites are fed twice weekly with the mould mite *Tyrophagus putrescentiae* (Schrank) (Tp) or other *Tyrophagus* spp., which in turn are reared on bran as the primary food ingredient. Rearing of *Tyrophagus* is relatively straightforward so long as strict attention is paid to sanitation and humidity levels. Novices quickly run into difficulties with souring of the medium and asphyxiation of the mites from high ammonia levels.

We previously reported on a method for rearing predatory mites in small units utilising an alternative medium as host for *T. putrescentiae* (Steiner and Goodwin, 1998), and we adapted this method to rear *S. miles*.

Materials and methods:

Tyrophagus putrescentiae culture

The basic rearing unit is a 4L shallow plastic container with top-screened lid. Ventilation holes are about 3 cm diameter with 100µ screening, small enough to provide aeration, but not rapid drying out of the medium. Two layers of paper towel are placed on the bottom of the container and semi-moist dog food chunks approximately 1cm diameter are added to a depth of about 10cm. A starter culture of Tp is added and the box is kept at 25°C and about 60-70% RH until the chunks are covered with mites (1-3 weeks). Any sliminess of the chunks indicates the humidity is too high. The culture is now ready to feed to *S. miles* or to start another Tp culture.

Stratiolaelaps miles culture

The rearing unit for the predatory mites is a lidded 10L plastic box with four 3 cm-diameter screened (100µ) vents, one at each side just below the rim. The rearing medium consists of a 2:2:1 mix of spelt (whole wheat grain husks), fine (Grade 1) vermiculite and milled peat. Spelt (*Triticum spelta*) is also known as Dinkel or Farro. This medium is porous and allows ready movement of mites through it. The mix is autoclaved and stored in a freezer before use to inhibit mould and other contaminants. When ready for use, the mix is brought to room temperature and enough water added to make it moist but not wet. About 4L of the mix is added to each box.

A starter culture of *S. miles* is added to the medium and mixed in, to give an initial predator density of 2 mites/mL. The mix is levelled and a piece of flyscreen (mosquito netting) (~1.5 x 2mm mesh opening) is placed on the surface to cover it. About 10-20 Tp-infested dog food chunks are distributed over the surface of the mesh. The dog food provides a continuing food source for the Tp which in turn feeds the *S. miles*. The mesh can be stapled onto a wooden frame for easy handling. The boxes are closed, stacked, and kept at about 25°C and 60-75% RH. Initially, the boxes are checked every 3-4 four days to ensure that there is no serious mould in the medium (stirring periodically and drying out a little usually cures this), no slime on the dog-food (humidity or moisture levels are too high) and that there are good numbers of Tp. After the first week, a weekly check is all that is required to make sure there are adequate Tp and the mix has not dried out.

After 3 weeks, the density of mites is estimated by washing three x 10mL samples through two sieves - coarse e.g. mosquito netting (to remove spelt) and fine (~100µ) (to collect all mite motile stages). Live mites are pootered up under the microscope to obtain a count/10mL. The bulk population is then used to restart the culture or diluted down with rearing mix as required for distribution to growers. Surplus *S. miles* can be stored for several weeks in larger 60L containers at 10°C, using the same feeding method.

Results and discussion

Population densities of *S. miles* should reach about 35 mites/mL after two weeks and 70 mites/mL after three weeks. Normal shipping densities of commercial insectaries are 10-15 mites/mL.

The method outlined has proved very useful for maintaining large population densities of *S. miles* with minimum labour input. The main limiting factor may be the availability of semi-moist dog food. Dry dog food is not a suitable substitute.

Acknowledgements

Financial assistance from the Horticultural Research and Development Corporation and the Horticulture Stock and Nurseries Act is gratefully acknowledged.

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Influence of humidity on the functional response of larvae of the gall midge (*Feltiella acarisuga*) feeding on spider mite eggs

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Abstract: The functional response of the larvae of the gall midge *Feltiella acarisuga* preying upon spider mite eggs on *Hedera* was examined at 45% and 80% r.h. The larvae had a high predation rate with 23.4 and 27.3 eggs being eaten at high densities at 45% and 80%, respectively. The functional response was significantly higher at 80% r.h., than at 45% r.h. at two of the higher densities (16 and 32 eggs/leaf). At 80% r.h. the response could be described by both type II and type III models with the maximal predation rate estimated to 12.7 and 7.8 eggs/h, respectively.

Key words: predation rate, *Tetranychus urticae*, biological control, *Hedera*, glasshouse crops.

Introduction

The two spotted spider mite (*Tetranychus urticae* Koch (Acari: Tetranychidae)) is a serious pest in many glasshouse crops. The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) in many cases is an effective biological control agent of *T. urticae*. However, insufficient control may occur in crops or areas of crops where temperature is high and humidity low. Beneficials able to tolerate low humidities would therefore be a useful supplement to *P. persimilis*. The predatory gall midge *Feltiella acarisuga* Vallot (Diptera: Cecidomyiidae)) might be a good candidate.

Humidity influences the biology of the gall midge. Thus, fecundity and adult emergence of *F. acarisuga* is highest at humidities around 85-90% (Gillespie & Quiring, 1995). In addition, the predation of 3-day old gall midge larvae on spider mites on tomato has been found to increase exponentially with humidities between 40-90% (Gillespie *et al.*, 1994).

The influence of humidity on the predatory capacity of *F. acarisuga* larvae is important for an evaluation of the gall midge's potential as a control agent against spider mites. This article describes preliminary results with functional response of *F. acarisuga* larvae preying upon spider mite eggs at low and high humidities with *Hedera* chosen as the model host plant.

Materials & Methods

Rearings

Two spotted spider mites were reared on kidney beans (*Phaseolus vulgaris* L., cv. Montano) in net covered cages (61×66×75 cm) in a glasshouse at 25±2°C, 70±10% r.h., L:D 16:8. *F. acarisuga* was reared on cucumber (*Cucumis sativus* L., cv. Danora F1) in similar cages and under similar conditions with honeydew from aphids (*Myzus persicae* Sulzer (Homoptera: Aphididae)) on sweet pepper plants (*Capsicum annuum* L., cv. California Wonder) as food source for adult midges. For details on rearing of *F. acarisuga*, see Enkegaard *et al.* (1999).

Experiments

Spider mite females were transferred to ivy leaves (2-4 cm^2) (*Hedera helix* L., cv. Nena) placed individually on moist cotton in Petri dishes (8.5 cm ∅), with the ventral side turned upwards. The dishes were placed in a climate chamber at high (80-90%) humidity, 25±1°C and L:D 16:8 for egg laying for 1-2 days after which the females were removed. Spider mite eggs were then removed to produce densities of 2, 4, 8, 16, 32 or 64 eggs/leaf.

Large, 6-9 days old, gall midge larvae were collected from the rearing and placed in Petri dishes with a small piece of moist towel paper in the middle to ensure high humidity. After 2 h of starvation the larvae were transferred to the dishes with ivy leaves (one larva per leaf). The dishes were placed on an elevated platform in an aquarium (37×21×28 cm) at either 45% or 80% r.h. obtained by salt solutions of KCL and K_2CO_3, respectively. The Petri dishes were left open to allow the predator to depart from the set-up in response to egg density.

After 4 h the remaining eggs were counted, and the state of the larva observed. The functional response was determined for larvae being alive after the 4 h period only.

Results

The larvae of *F. acarisuga* had a high predation rate of spider mite eggs with up to 42 eggs (=10.5 eggs/h) or 46 eggs (=11.5 eggs/h) being eaten at high densities and 45% and 80% r.h., respectively. The average number of eggs (±s.e.) eaten at the two humidities at the highest density was 24.4±4.2 and 27.3±9.7 at 45% and 80% r.h., respectively (Fig. 1).

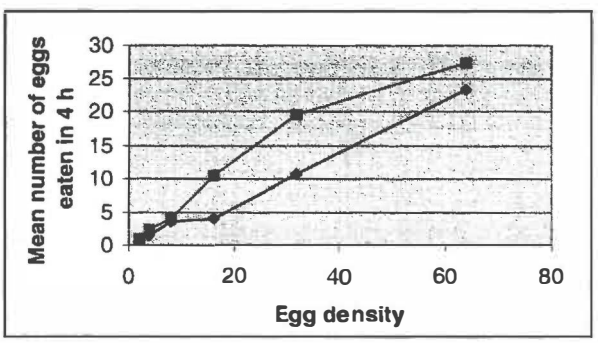

Figure 1. Functional response of *F. acarisuga* larvae preying upon spider mite eggs at 45% (◆) and 80% r.h. (■) at 25 °C. Mean (±s.e.; n) number of eggs eaten at the densities of 2, 4, 8, 16, 32 and 64 eggs/leaf was at 45%: 0.63 (±0.18; 16); 1.50 (±0.33; 22); 3.62 (±0.47; 27), 4.00 (±0.43; 33); 10.74 (±1.36; 23) and 23.44 (±4.20; 9) and at 80%: 1.00 (±0.23; 14); 2.4 (±0.43; 18); 4.00 (±0.67; 21); 10.42 (±0.61; 53); 19.63 (±1.73; 27) and 27.33 (±9.70; 3) eggs.

There were no significant differences between the number of spider mite eggs eaten by *F. acarisuga* larvae between the two humidities at densities of 2, 4 and 8 eggs/leaf (Kolmogorov-Smirnov test, $P>0.33$), but significant differences were observed at egg densities of 16 and 32 eggs/leaf ($p<0.001$) with about 2-2.5 times as many spider mite eggs eaten at 80% r.h. compared to 45% r.h.. At 64 eggs/leaf no significant difference was found, presumably because of the few replicates (n=3) at the highest humidity.

The data were fitted to models for Type II (model (1)) and Type IIIa and b (models (2) and (3)) functional response, respectively (Hassell, 1978):

$$N_a = N_t\left[\,1-\exp\left\{-a' P_t\left(T - T_h\frac{N_a}{P_t}\right)\right\}\right] \quad (1)$$

$$N_a = N_t\left[\,1-\exp\left\{-\frac{bN_t P_t}{1+cN_t}\left(T - \frac{T_h N_a}{P_t}\right)\right\}\right] \quad (2)$$

$$N_a = N_t\left[\,1-\exp\left\{-\frac{bP_t}{c}\left(T - \frac{T_h N_a}{P_t} - \frac{N_a}{bN_t P_t\left(N_t - N_a\right)}\right)\right\}\right] \quad (3)$$

where N_a is the number of prey consumed, N_t the prey density, P_t the predator density (in this case P_t = 1), a' the search rate, T the total time available for search (in this case T = 4 h) and T_h the handling time; b and c are constants. Estimates for search rate (a'), handling time (T_h) and the two constants (b and c) were obtained by non-linear regression.

The functional response of *F. acarisuga* larvae at 80% r.h. could be described by both type II (model (1)) and type IIIb (model (3)), but not by type IIIa. The estimated parameters (±s.e.; [95% confidence limits]) were a'=0.348 h^{-1} (±0.055; [0.194; 0.502]) and T_h=0.079 h (±0.018; [0.030; 0.129]) (R^2=0.976) (type II), and b=0.033 (±0.006; [0.013; 0.053]), c=0.034 (±0.021; [−0.031; 0.100]) and T_h=0.128 h (±0.003; [0.118; 0.137]) (R^2=0.996) (type IIIb), respectively. The maximum predation rates can thus be estimated as $1/T_h$ to be 12.7 (type II) or 7.8 (type IIIb) eggs/h. The data at 45% r.h. fitted neither of the three models.

The larvae of *F. acarisuga* disappeared (or, in a few cases (5.4%), died) from the experimental set up in a density dependent manner, the proportion disappearing decreasing exponentially with the density of spider mite eggs on the ivy leaves (Table 1). Thus, at the two lowest densities, between 14 and 24% of the larvae chose to leave the leaves, apparently in response to low prey availability. No significant differences in the proportion of missing or dead larvae were found between low and high humidity at any egg density (Chi^2-test, p>0.10).

Table 1. The proportion (±s.e.) of *F. acarisuga* larvae disappearing or dying in the experiment on functional response. 'n' is the number of observations.

Eggs/ leaf	45% r.h. Proportion missing (±s.e.)		80% r.h. Proportion missing (±s.e.)	
2	0.158±0.007	(n=19)	0.177±0.008	(n=17)
4	0.241±0.009	(n=29)	0.143±0.006	(n=21)
8	0.129±0.004	(n=31)	0.250±0.007	(n=28)
16	0.108±0.003	(n=37)	0.036±0.001	(n=55)
32	0.042±0.002	(n=24)	0.036±0.001	(n=28)
64	0.100±0.009	(n=10)	0	(n= 3)

Discussion

Roberti (1954) observed that *F. acarisuga* larvae may eat up to 30 prey items (*Tetranychus* sp. eggs, nymphs and adults) per day. The environmental conditions were, however, not specified. For *Feltiella* sp. larvae preying upon the spider mite *T. kanzawai* Kishida, a

predation rate of up to 80 eggs/day at 15-20°C was reported by Nakagawa (1986), corresponding to an average consumption of 3.3 eggs/h.

The predation of spider mites by *F. acarisuga* has been found to increase exponentially with humidities between 40-90% (Gillespie *et al.*, 1994). This supports the tendency we have seen to a higher predation at high humidity.

The functional response of *F. acarisuga* larvae preying upon spider mite eggs at 80% r.h. could be described both as a type II and IIIb response, whereas the functional response at 45% r.h. could not be described by any of the three models. Further experiments may make these results more clear. Opit *et al.* (1997) reported a type II functional response of *F. acarisuga* larvae preying upon both male and female spider mites at 27°C and 90% r.h.. The search rates and handling times were a'=0.34 h^{-1} and T_h=0.45 h for males, and a'=0.24 h^{-1} and T_h=1.4 h for females (Hassels model for type II functional response (Hassel, 1978) (model (1)) applied to their data), giving a maximum predation rate of 2.2 males/h and 0.7 females/h. Not surprisingly these predation rates are considerably below the predation rate we found of the much smaller eggs (Type II: 12.7 eggs/h). The search rate for larvae preying upon spider mite eggs (0.32 h^{-1}; type II response) is similar to that of larvae preying upon spider mite males, but lower than that of larvae preying upon spider mite females (Opit *et al.*, 1997).

The maximum predation rate of *F. acarisuga* larvae at 80% r.h. was 11.5 (type II) or 7.1 (type III) times higher than that of *P. persimilis* (Sabelis, 1985; approx. 1.1 eggs/h at 70% humidity, 20°C (estimated from his data)). At conditions similar to ours *P. persimilis* is likely to have a higher maximal predation rate, but it seems clear that *F. acarisuga* larvae are superior to *P. persimilis* in terms of predation upon spider mite eggs at high humidity. Even though the maximal predation rate of *F. acarisuga* larvae presumably is lower at low humidity it is still higher than that of *P. persimilis*. However, other aspects of the biology of the two predators, including their lifetime consumption, has to be considered as well, when evaluating their usefulness as biological control agents against spider mites.

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Experiences with insect exclusion screening of greenhouse vents in Ontario, Canada

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Abstract: Insect exclusion screening has proven a useful component of IPM in warm climates, but has only recently been used in greenhouses in Ontario, Canada. Even in cool climates, greenhouse vents can be an important entry point for insect pests, and growers' experiences in Ontario indicate that screening to exclude insects can enhance IPM programs and reduce dependence on chemical pesticides.

Key words: insect screening, thrips, greenhouses, ornamentals, integrated pest management

Introduction

Screens over greenhouse openings can reduce the entry of pests, and their use is seen as an important component of greenhouse IPM (Berlinger *et al.* 1993a, Berlinger *et al.* 1993b, Bethke *et al.* 1994). However, much of the work with greenhouse screening has been carried out in warm climates such as those in Israel or California. In cooler climates, such as that of southern Ontario, Canada, it was assumed until recently that screening would have minimal impact on greenhouse pest populations. Outdoor pest populations are low until summer, and movement of pests into greenhouses was noticeable primarily during late summer and early autumn.

In the early 1990's, an ornamentals grower in the Niagara area of southern Ontario installed screening over the vents in part of his greenhouses. He subsequently reported greatly reduced numbers of pests, and reduction in pesticide use. Since that time, additional growers have installed screening in areas of their greenhouses. Although the percentage of growers using screening is still small, increasing interest is being shown, and the number of growers using screening is expected to grow. In 1998, an information session was organized, in which growers who had installed screening described their experiences with it. This paper summarizes the experience of these growers, including data from screened and unscreened areas, and presents data on the entry of thrips through vent openings in an unscreened greenhouse.

Reasons for Screening

Growers who installed insect screening did so for a variety of reasons. These include:

- improvement of the effectiveness of IPM programs.
- protection of a new crop from yearly invasions of insect pests from an abandoned orchard on adjacent property.
- a desire to reduce pesticide use.
- as a preliminary measure to facilitate adoption of a biological control program

- the need of a major plant propagator to maintain virus free plant material.

There is evidence that even in the relatively cool climate of southern Ontario, vents can be an important source of insect pests. An example of thrips movement into a rose greenhouse was demonstrated in a monitoring trial in 1998. In an unscreened greenhouse, ventilated through side vents by fan-forced air movement, thrips numbers were monitored weekly using yellow sticky cards. It soon became apparent that thrips numbers were much higher in the half of the compartment adjacent to the vents, than in the half away from the vents. When sticky traps were placed in the vents, correspondingly high numbers of thrips were captured (Fig. 1). In an adjacent top-vented compartment, this difference was not seen.

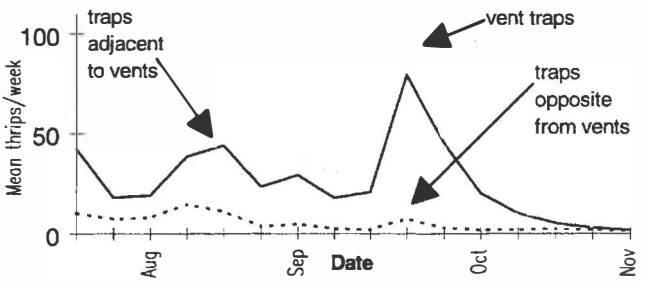

Figure 1. Weekly thrips catches in a greenhouse with side vents.

Problems with Screening

One of the most serious problems with installing screening in greenhouses is the resulting reduction in airflow, often addressed by increasing the surface area of vents (Robb 1993, Bethke *et al.* 1994). Growers who installed screening in Ontario did so in greenhouses not originally designed for use of screens, so frames were retrofitted around vent openings to increase surface area. Growers achieved this in a variety of ways, and in most cases, installed a greater surface area of screen than was recommended by manufacturers. Reduced airflow was not considered to be a problem by any of the growers.

An additional problem, unique to temperate and cool climates, is the potential for damage to screens due to snow and ice build-up during winter. A number of approaches have been taken in Ontario to overcome this problem. One grower installed frames around the inside of his vents. However, most growers either cover screening with polyethylene greenhouse cover, or remove it from the frames in the fall after outdoor pest populations have decreased.

The cost of installing screening in a greenhouse can be considerable, depending on the style of frame built. Most growers in Ontario estimated the initial costs for screen, framing material, and labour to be approximately $60-90 (Canadian) per running metre. Only one of the Ontario growers had estimated the time needed for cost recovery, which was put at about two years, based on reduced pesticide costs alone. If other, less tangible factors were included (increased plant quality, less time lost to re-entry time in sprayed areas, more efficient use of employee time), costs would be recovered in a shorter time.

Screening of roof vents in commercial greenhouses has not been done in Ontario, and there is little information on the costs and effectiveness of screening in these situations.

However, cost estimates associated with screening roof vents are far higher than costs of screening side vents. The impact of roof vents as an entry point for insect pests is not yet clear, and future research is planned to examine the relative importance of pest movement into a greenhouse through top versus side vents.

Benefits of Screening

Growers were unanimous in considering the benefits of screening to far outweigh the costs. Some of the benefits mentioned specifically included:

- greatly reduced spraying for thrips
- no pesticides required for thrips in 1998.
- successful implementation of biological control programs against thrips over several years.
- reduced pesticide use resulting in healthier, less stressed plants, and higher quality flowers.
- more efficient use of employees, with less time wasted adjusting schedules to conform to re-entry times in sprayed areas.

Two examples presented by growers demonstrate the impact of screens on greenhouse thrips populations. In one greenhouse, 100% thrips exclusion screening was installed during mid-May, prior to commencing a biological control program in cyclamen. After installation, thrips numbers immediately declined and remained at virtually undetectable levels throughout the entire season. In a nearby unscreened compartment, thrips were found on sticky cards throughout the season (Fig. 2).

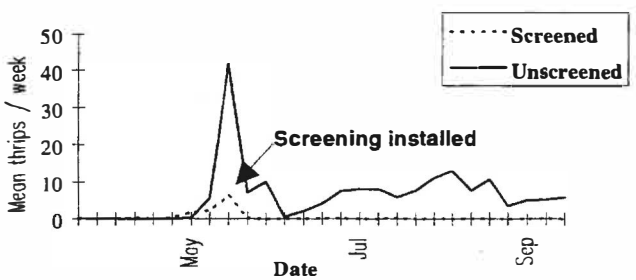

Figure 2. Weekly thrips catches in screened and unscreened greenhouses. (Data courtesy of Fernlea Flowers, Delhi, Ontario)

In another greenhouse, one compartment screened with 80% thrips exclusion screen, was situated between two unscreened compartments, each approximately 2000m^2. All three compartments were separated by walls, but connected by doors and a common walkway. The thrips population in the screened (central) compartment was maintained at a much lower level throughout the summer than in the unscreened compartments (Fig. 3). Eventually, in the late summer, thrips numbers started to rise in the screened compartment, probably due to traffic between compartments.

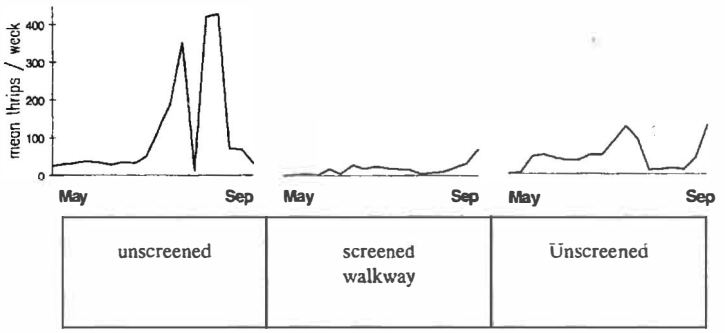

Figure 3. Schematic of screened and unscreened greenhouse compartments and mean weekly thrips catches from May to September. (Data courtesy of Jeffrey's Greenhouses, St. Catharines, Ontario)

Conclusion

Screening in warm temperate or tropical conditions has proven effective. However, even in a cool climate, screening is proving to be a very important component of greenhouse IPM programs. By greatly reducing the entry of pests into the greenhouse, screening can facilitate the implementation of biological and cultural control methods, which would otherwise have less chance of success, and can help lead to reduction in the use of chemical pesticides. Future studies are planned to determine the relative importance of side vents and roof vents as entry points for thrips and other insect pests.

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The use of plant growth promoting rhizobacteria (PGPR) to decrease the susceptibility of cucumber to spider mites

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Abstract: Effect of PGPR on glasshouse cucumber suitability for spider mites and its susceptibility to these pests was studied. It was found that bacterized plants suppressed the development of mite population by more than 40% on the leaves of susceptible cultivar Corona. The fecundity of females was also decreased after feeding on the plants with bacteria as compared to those without bacteria. The plants with PGPR were less injured in relation to the plants without bacteria. Infested leaves of cultivar Aramis showed a lower level of inhibition in photosynthesis , as compared to injured Corona leaves and the presence of bacteria had a positive effect on this process.

Key words: spider mites, cucumber, susceptibility, rhizobacteria

Introduction

In recent years there have been numerous reports of plant resistance induction, against pathogens and pests, resulting from inoculation of seeds or young plants with root inhabiting bacteria that stimulate plant growth. Several different strains of bacteria have been identified as having the ability to promote the growth of plants and are known as plant growth-promoting rhizobacteria or PGPR (Kloepper et al. 1989). The different strains of Pseudomonas and Serratia were often screened for the induction of systemic resistance of cucumber to bacterial pathogens (Wei et al. 1996). The bacterized plants were also less injured by insect pests. On cucumber, growing in the open, lower population of cucumber beetle was found in relation to nonbacterized plants. Similarly, lower number of aphids was noticed on glasshouse tomato inoculated with PGPR (Zehnder et al. 1996). The studies have shown that the metabolism of plants treated with PGPR differ from those untreated and that these differences are connected with the level of plant resistance to a pathogen or pest (Borowicz et al. 1992, Cook et al. 1995, Zehdner et al. 1996).

The purpose of the study was to check how the inoculation of cucumber seeds with PGPR would influence the plant susceptibility to spider mites as well as its suitability as a host for these pests.

Material and methods

The experiments were conducted on two cultivars of cucumber – Corona and Aramis. Seeds of plants were inoculated during 20 min with plant growth promoting rizobacteria Pseudomonas P-112, originated from the roots of cucumber seedlings. Seeds of control plants were treated with water. Inoculum of bacteria contained 3×10^9 bacteria cells in 1 cm^3. Seeds were sown into the pots, then young plants were transferred (with the soil) to their stable place in a glasshouse.

Forty plants of each cultivar were used in the experiment. At the stage of 3 leaves, half of the plants were infested with the females of *Tetranychus cinnabarinus* Boisd. The initial mite population was 5 females per leaf.

1. Tests for estimation of plant suitability to spider mites

Development of mite populations in time

The number of mites on cucumber plants inoculated with PGPR was checked during 8 weeks at 7-10 day intervals

Fecundity of females

Fecundity of females was studied under laboratory conditions using fresh leaf discs (diam. 2 cm.). The leaf discs, originated from bacterized and nonbacterized plants of each cultivar were placed on wet cotton in Petri dishes. Thirty disks were used for each plant combination. One deutonymph of *T. cinnabarinus* per disc was placed together with one male. The eggs were counted and destroyed every day to the end of female life. Leaf discs were changed every 3-4 days.

2. Tests for estimation of plant susceptibility to spider mites

level of the plant injury

The leaf damage index (LDI) for mite infested plants growing with or without PGPR was estimated 7 weeks after plant infestation, according to Hussey and Scopes (1985).

intensity of plant photosynthesis

Photosynthesis was measured in the glasshouse, for mite injured and healthy leaves of Corona and Aramis growing with or without PGPR , using LI-COR 6200. Five measurements for each group of plants were performed.

Results and discussion

As shown in Fig. 1 the development of spider mite populations on plants inoculated with PGPR , is suppressed in relation to plants without bacteria.

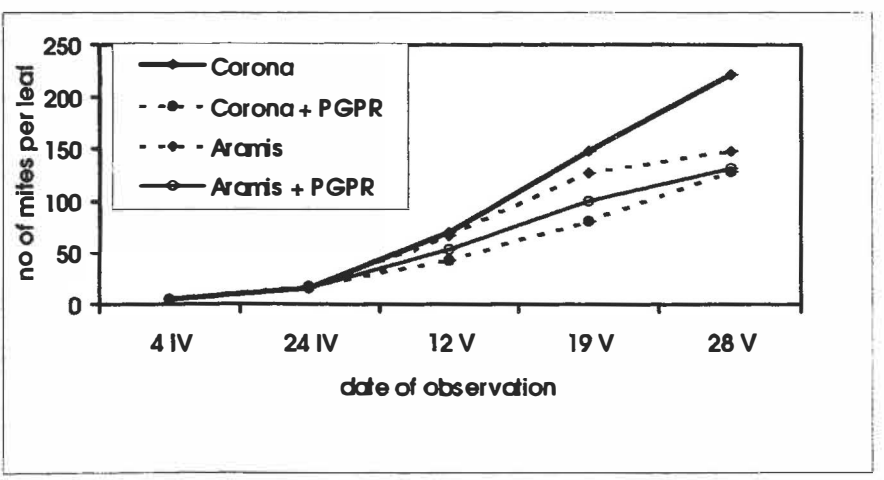

Figure 1. Development of spider mite populations on cucumber cultivated with or without bacteria (PGPR). Standard errors (SE) are presented.

The influence of bacteria is more evident on the mite-susceptible cultivar of cucumber, Corona, (suppression above 40%) as compared to the less susceptible, Aramis.

The presence of PGPR in the soil during plant cultivation had a negative influence on the total fecundity of *T. cinnabarinus* females, feeding on the leaves of both cucumber cultivars (Table 1). It was much more evident in the case of cv. Corona (decrease by 18%) as compared to Aramis. Time of oviposition and female longevity were shorter on leaf discs originated from the plants treated with bacteria.

Table 1. Fecundity, oviposition time and longevity of *T. cinnabarinus* females feeding on cucumber cultivated with or without PGPR

Cultivar	Oviposition time (days) ± SE	Total fecundity (egg per female) ± SE	Fecundity netto (egg per female per day)	Longevity (days)
Corona	14.7 ± 0.32	89.2 ± 3.31	6.1	15.3
Corona + PGPR	13.6 ± 0.93	76.4 ± 5.42	5.6	14.5
Aramis	13.9 ± 0.68	73.2 ± 3.05	5.3	13.1
Aramis + PGPR	12.1 ± 0.26	72.9 ± 4.02	6.0	12.8

Data on plant injury and its photosynthesis intensity are presented in Table 2.

Table 2. Influence of PGPR on leaf damage index (LDI) and intensity of photosynthesis of cucumber plants injured by *T. cinnabarinus*

Cultivar	LDI ± SE	Photosynthesis (ul. CO_2 x s^{-1} x $m.^{-2}$)	
		Control ± SE	Infested ± SE
Corona	3.5 ± 0.22	16.9 ± 1.82	10.1 ± 0.91
Corona + PGPR	2.9 ± 0.18	17.6 ± 1.84	10.4 ± 1.39
Aramis	2.5 ± 2.50	13.9 ± 1.52	10.1 ± 1.39
Aramis + PGPR	2.3 ± 0.13	13.2 ± 1.96	12.1 ± 1.12

The presence of PGPR in cucumber root system decreased plant injury, caused by mite feeding, by about 18% for cv. Corona and by about 8% for Aramis. The intensity of photosynthesis in infested Corona was strongly inhibited and the presence of bacteria did not significantly changed this situation. Probably the leaves were too seriously injured.
Infested leaves of cv. Aramis showed a lower level of inhibition in photosynthesis, as compared to injured Corona leaves, and the presence of bacteria had a positive effect on this process.

From obtained data it can be concluded that the presence of plant growth promoting rhizobacteria (PGPR) in the soil of cultivated cucumber has an influence on its susceptibility to spider mites. This phenomenon is particularly evident for the cultivar more susceptible to these pests. The tested bacteria can change the suitability of cucumber for spider mites, possibly by induced antibiosis, and has a negative influence on the biology of *T. cinnabarinus*.

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Life history parameters of *Aphidius colemani* (Hym.: Aphidiidae) on sweet pepper in different temperature regimes

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Abstract: The performance of the aphid parasitoid *Aphidius colemani* (Hym.: Aphidiidae) against *Myzus persicae* (Hemiptera: Aphididae) on sweet pepper was evaluated at various constant temperatures. Biological parameters of the parasitoid including adult longevity, mummy production, development time, percent emergence and sex ratio of the progeny were investigated at 20, 25, 28 and 30°C. *A. colemani* reduced *M. persicae* populations at 20 and 25°C, but no clear conclusions could be drawn at 28 and 30°C where the temperature influenced critically the whole tritrophic system.

Keywords: biological control, *Myzus persicae, Aphidius colemani*, aphid parasitoid, and temperature

Introduction

Aphidius colemani, a solitary endoparasitoid of aphids has been used successfully as a biocontrol agent against *Myzus persicae* on a commercial basis. However, some growers have experienced difficulties in controlling *M. persicae* during the summer despite success earlier in the season (GreatRex, pers. comm.). Therefore, further work is required to confirm whether or not *A. colemani* is capable of reducing *M. persicae* populations at high temperatures (i.e. above 20°C).

The objectives of the present study were: a) to assess the longevity of *A. colemani* adults in a greenhouse-like environment at various high temperatures, b) to estimate the fecundity of the mated female parasitoids, and c) to determine the number, sex and development time of progeny produced at temperatures above 20°C.

Material and Methods

Myzus persicae and Aphidius colemani cultures
The aphids were cultured on 10-week old pepper plants (*Capsicum annum* L., variety Bell Boy) maintained in glasshouse conditions where the temperature never dropped below 20°C. Mummies of *Aphis gossyppi* containing the parasitoid were obtained from Novartis (Colchester, Essex, U.K.) and the adults that emerged were cultured on *M. persicae* at 20°C for one generation prior the experiments. The experiment took place in four controlled environment rooms at L:D 16:8h and 70% R.H. where temperatures were held constant at 20, 25, 28 or 30°C.

The longevity of *A. colemani* adults was tested in two ways:(i) they were offered aphids on a sweet pepper plant and (ii) they were fed with honey solution (30% v/v) and water with no aphids being present.

(i) A mixed clonal population of 200 *M. persicae* aphids was placed carefully on one-month-old pepper plants (6-8 leaves), using a fine paintbrush. An insect-proof perforated bag (0.5 x 0.3m) was put over each plant and tied around its base above the soil surface with a

plastic covered wire strap. The plants were kept in controlled environment rooms for 24h prior to the start of the experiment to allow for the free distribution of aphids on the plant, honeydew production and acclimatisation of the plants and aphids to the temperature. A single, newly emerged (0-24h), mated, *A. colemani* female was introduced into each bag. Each female was confined with a male wasp in a glass tube to allow for mating for two hours before the start of the experiment. The parasitoids were not fed prior to the experiment. Ten replicates were used at each of the following temperatures: 20, 25, 28 and 30°C. Records of the longevity of the wasps introduced and the number of mummies produced were made daily. The mummies formed were kept individually in gelatin capsules at the same temperature that they were produced to record the percentage emergence and the sex of the offspring.

(ii) Pairs (male and female) of *A. colemani* were confined in Petri dishes (9 cm diameter). Two cotton balls, one saturated with honey solution (30% v/v) and the other with water were used to provide parasitoids with food. The cotton balls were changed every other day to prevent fungal growth and the Petri dishes were sealed with parafilm to prevent the insects from escaping. Each experiment was carried out at three temperatures: 20, 25 and 28°C and replicated 10 times.

The statistical analysis was carried out using the generalised linear modeling package GLIM v3.77. Statistical significance was tested at the 5% level. Longevity of the plant-based adults of *A. colemani* was analysed by regression analysis, while differences in the longevity of the parasitoids fed on honey solution were tested using ANOVA. Fecundity data were analysed by ANOVA using a Poisson error distribution. Emergence data were analysed by ANOVA using a binomial error distribution and the William's procedure was used to account for overdispersion in the data. Data on development time were tested using the t-test, while data on sex ratio were analysed using the χ^2 test. The Mann-Whitney U test was used to analyse the emergence rates of *A. colemani* males and females.

Results and discussion

Comparison of longevities of *A. colemani* adults (males and females), fed on honey solution and water showed statistical differences at 20, and 25°C ($F=8.03$, $df=59$, $p<0.05$) compared with adults on plants. The number of mummies harvested and the percentage emergence varied according to temperature (Table 1).

Table 1. Longevity (mean days ± s.e.) of *A. colemani* adults when feeding on different feeding regimes, mummies produced and percent emergence of progeny (mean ± s.e.) at four temperatures (n=10, at 20°C n=8).

	honey solution		on plants	No. mummies	% emerg.
	females	males	females	females	both
20°C	2.9 ± 0.4a	2.0 ±0.4a	5.3 ± 0.8d	54.5 ±10.5	76.64 ±3.12
25°C	4.3 ± 0.4b	3.9 ±0.5b	6.0 ± 0.8d	123.5 ±11.28	78.77 ±4.16
28°C	3.3 ± 0.3c	3.0 ±0.4c	3.4 ± 0.8c	8.7 ±2.18	17.10 ±4.6
30°C	-	-	-	0.7 ±0.25	0

Values followed by the same letter at the first three rows are not significant ($p<0.05$).

The parasitoids developed significantly faster (t_{32}=28.1, $p<0.05$) at 25°C compared to 20°C (Table 2). Data at 28°C and 30°C were not included in the analysis due to the low emergence rate (see Table 1). The numbers of male and female progeny produced at 20 and 25°C were 1:1.

Table 2. Number (mean ± s.e.) and development period of *A. colemani* offspring (mean days ± s.e.) produced at 20 and 25°C (n=10, at 20°C n=8).

		oviposition to emergence		mummification to emergence		No of progeny produced		n
20°C	males	13.8	±0.30	6.5	±0.06	22.8	±1.39	213
	females	14.0	±0.30	6.6	±0.08	16.6	±1.44	117
25°C	males	11.6	±0.90	4.8	±0.05	44.0	±1.16	426
	females	11.9	±0.06	4.9	±0.04	54.1	±1.47	534

Males and females have the same life span at the temperatures tested. However, when females were confined with aphids they lived longer at 20 and 25°C than when fed with honey solution. It is possible that honeydew (a natural food of *A. colemani*) contains elements essential for the parasitoid's nutritional requirements. These results are in agreement with Fernández & Nentwig (1997).

Production of mummies reached the highest level at 25°C (123.5 mummies/female) whereas at 20°C 54.4 mummies/female were produced. Hofsvang and Hågvar (1975b) recorded 48 mummies/female at 21°C. At 28°C fecundity was dramatically reduced (9.1 mummies/female), while at 30°C only 0.7 mummies/female were recorded. Also at the highest temperature (30°C) it was observed that aphid mortality was 100% by 3-4 days after the start of the experiment. A number of explanations for reduction in mummy-production at high temperatures can be offered; a) host suitability and acceptance could be affected by high temperatures, b) the parasitoids enter diapause as observed in the field during summer (Starý, 1988) or c) symbionts in the aphid body die (28°C is their upper thermal threshold) (J. Hardie, pers. comm.). At 30°C the death of the symbionts could pose a problem to the digestion procedure of *M. persicae*, which would eventually die.

Percentage emergence was similar at 20 and 25°C but was reduced at the higher temperatures (Table 1). Hofsvang and Hågvar (1975b) also found the percentage emergence of the parasitoid to be 75% and 76% at 24 and 21°C respectively. Although at 28 and 30 °C only a small number of mummies was initially formed (Table 1), adult emergence was recorded at a very low level (17.1 and 0 at 28 and 30°C respectively) as well. Changes in host suitability probably result in decreased adult emergence with increased temperatures.

In most studies, it is usually the number of eggs laid that are counted in estimates of parasitoid fecundity, rather than the number and sex of the offspring as described in this project (Table 2). The latter estimate was considered to be more reliable as the number of eggs or even the number of mummies produced are not equivalent to the number of offspring emerged (Table 2).

A mixed instar aphid population was offered to *A. colemani*, and this may have restricted its maximum potential because aphid parasitoids often prefer to oviposit in young aphid instars (Hågvar and Hofsvang, 1991). In natural conditions though, parasitoids encounter all aphid life stages, so the progeny production in this study provides a more realistic outcome.

Also, *A. colemani* is known to parasitize adult aphids (46.6% of the whole population) at 21°C (Hofsvang and Hågvar, 1975a).

It takes about 14 days from oviposition to adult emergence at 20°C and 12 days at 25°C (Table 2). The same length of time is required for both males and females and they are produced at the same frequency. This agrees with previous findings on the same tritrophic complex (Hofsvang and Hågvar, 1975b).

In the present study it seems that 25°C is the most favourable temperature for the parasitoid's adult longevity, development time of immature stages and progeny production of mated females. However, although the study attempted to simulate realistic situations (e.g. whole plants were used) the temperature was held constant at all times. In commercial glasshouses, the temperature fluctuates, and this may cause different performance features in this tritrophic system. This remains to be investigated.

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Tomate sous abri en France : méthodes et perspectives de lutte contre la pourriture grise due à *Botrytis cinerea*

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Résumé : La pourriture grise due à *Botrytis cinerea* est l'une des maladies aériennes les plus importantes sur tomate, notamment sous abri. Après un rappel des principaux dégâts sur tiges, feuilles et fruits, les facteurs favorables au développement de la maladie, puis les possibilités de lutte telles que les techniques culturales, la gestion du climat et la lutte chimique raisonnée sont développées. La lutte biologique est abordée en tant que perspective prometteuse.

Mots clés : *Botrytis cinerea*, integrated control, control means, tomato, disease, fungus, greenhouse.

Introduction

La pourriture grise due à *Botrytis cinerea* est l'une des maladies aériennes les plus importantes sur tomate sous abri en France car les dégâts ont des répercussions directes sur le rendement et sur la qualité de la récolte.

Depuis 1990, des travaux ont été développés par la Station de Pathologie Végétale de l'INRA d'Avignon afin d'élargir les possibilités de lutte (Nicot et Baille 1996). Plus récemment, des études en stations expérimentales et en serre de production ont aussi été réalisées en collaboration avec différents partenaires, Ctifl, APREL[1], Chambre d'Agriculture des Bouches-du-Rhône...

Les dégâts

Les attaques du champignon sous forme de lésions de tissus ou chancres sont observées sur la tige au niveau des plaies d'effeuillage, d'ébourgeonnage ou de "rafles" de bouquets notamment sous serre verre chauffée. Elles peuvent rapidement tuer les plantes.

Les dégâts se manifestent aussi sur les fruits par des auréoles blanches appelées "taches fantômes" ou par des pourritures. Sous abri froid, des taches nécrotiques prenant un aspect d'anneaux concentriques peuvent se développer sur les feuilles entraînant des dessèchements.

Le champignon responsable de ces dégâts est déjà présent dans les serres sous forme de spores ou de mycelium. Celui-ci se conserve dans le sol, sur les structures des abris ou sur les débris végétaux. Les spores transportées par le vent peuvent aussi pénétrer par les ouvrants.

[1] APREL : Association Provençale de Recherche et Expérimentation Légumière.

Facteurs favorables au développement de la maladie

Les facteurs climatiques, en particulier l'hygrométrie, ont un rôle important sur le développement de la maladie en favorisant la germination des conidies (ou spores), soit directement, soit par l'intermédiaire des exsudats (ou gouttelettes) des plantes.

Une forte densité de plantes ou des plantes vigoureuses peuvent aussi entraîner un microclimat favorable aux attaques sur tiges ou sur feuilles.

Mais ces facteurs ne sont pas les seuls en cause. En effet, *Botrytis cinerea* est un parasite de faiblesse et toutes les opérations qui provoqueront un affaiblissement des plantes pourront favoriser les attaques du champignon : à savoir les à-coups climatiques, de fertilisation... Enfin, *B. cinerea* est un parasite de blessures. Si l'inoculum est présent, les facteurs favorisant les attaques sont en particulier les blessures, les nécroses, les plaies. Les techniques de conduite provoquant des plaies, même superficielles sont des facteurs favorables aux attaques du champignon (ex "couchage" des plants, rassemblement des tiges en cas de contreplantation).

Les possibilités de lutte

Les techniques culturales :

- L'effeuillage : les feuilles inférieures sont retirées tous les 8 à 15 jours ou trois semaines selon l'organisation du travail.

Des travaux à l'INRA effectués de 1991 à 1993 en serres de production et en serres expérimentales ont montré qu'un effeuillage à ras de la tige réduit significativement les attaques (Decognet et al. 1998). De plus, l'effeuillage doit être effectué de préférence le matin, en évitant les jours pluvieux. La coupe des "restes" de rafles de bouquets est aussi préconisée et peut éventuellement être effectuée en même temps que l'effeuillage. L'ébourgeonnage devra être régulier ; en cas de retard de l'opération, il est important de protéger préventivement les plaies comme pour l'effeuillage.

Dans toutes ces manipulations, y compris celle du "couchage" des plantes, il est essentiel d'éviter les blessures de tissus (feuilles, tige...).

Enfin, les plantes mortes dues à des chancres sur tige constituent une source d'inoculum importante et doivent être éliminées rapidement et proprement.

La gestion du climat :

- Des études à la Station de Pathologie de l'INRA Montfavet ont montré en conditions de laboratoire et en serres de production, qu'une plaie (ex. d'effeuillage) reste sensible à la pénétration des spores de *Botrytis* pendant 24 heures. De même, des phénomènes de sudation ont aussi été observés, en particulier lorsque les plantes ne transpirent pas assez (ex. la nuit). Suite à une reprise de la transpiration des plantes, ces gouttelettes peuvent être réabsorbées et ceci favoriserait la pénétration du *Botrytis* dans les tissus des plantes. De plus, le liquide exsudé stimule fortement la germination des spores de *B. cinerea*.
- Les mesures culturales et climatiques auront donc pour but de limiter le "suintement" des plantes, la présence d'eau libre sur les feuilles et de favoriser une cicatrisation rapide des plaies :

– Mise en place de tubes de croissance, de thermosiphons en bas des plantes ou d'un chauffage basse température constitué de "tuyaux" agrothermes installés en palissage le long des plantes pour un assèchement du milieu autour de ces plantes.

– Effeuillage pratiqué le matin, en conditions de serres chauffées.

Lutte chimique raisonnée :

• Pour limiter le risque d'apparition des résistances et améliorer l'efficacité des traitements, il est essentiel d'alterner les familles chimiques de produits.

D'après les études réalisées en 1991, puis en 1994, par l'INRA, en collaboration avec la Chambre d'Agriculture des Bouches-du-Rhône, les CETA[2] et l'APREL, les spores résistantes sont en réalité présentes au niveau d'une région et des échanges de spores entre serres par les ouvrants doivent jouer un rôle non négligeable dans l'évolution de la proportion de ces souches résistantes au cours du temps dans une même serre. Un choix raisonné de produits devrait tenir compte de la situation réelle de la serre, en terme de présence de souches résistantes (Nicot 1997).

• Le plus souvent, les traitements sont effectués préventivement le plus tôt possible après l'effeuillage ou l'ébourgeonnage. En effet des essais mis en place en 1997 au Ctifl, en conditions de pression élevée d'inoculum de *Botrytis* (inoculation avant les traitements) montrent qu'une application d'Euparène juste après effeuillage a permis de réduire significativement le nombre de chancres sur les plaies traitées (32,5% plaies avec chancres) par rapport à une application réalisée 48 heures après (52,9% plaies avec chancres) et par rapport au témoin traité à l'eau (80% plaies avec chancres).

L'effet des "badigeons" sur les plaies avec du Silbos DF, l'Euparène et un traitement à l'argile (argile de potier Fournès) ont été étudiés dans les mêmes conditions expérimentales. Les deux premiers produits ont été significativement meilleurs que le témoin (traité à l'eau) et l'argile. Ce dernier traitement pourrait être intéressant seulement s'il est appliqué en préventif (contrairement aux conditions de l'essai).

Si des symptômes sont observés en culture (ex chancres sur tige), des mesures curatives sont impératives : un curetage et une protection par une pâte fongicide à base de produits de contact ou une pulvérisation sur l'ensemble du feuillage, notamment sous abri froid.

• Les traitements sont effectués soit par pulvérisation localisée sur les plaies d'effeuillage, soit par "badigeons" (ou pâte fongicide permettant de protéger les plaies).Le traitement par pulvérisation doit être réalisé à une pression suffisante et sur toutes les lignes de culture.

Une nouvelle technique est aussi récemment appliquée en serres de production. Elle consiste à utiliser un sécateur pulvérisateur (ex. Felco 19) avec pulvérisation de produit sur la lame du sécateur. D'après les premières observations sur les exploitations concernées, cette technique permet de protéger efficacement les plaies. Même si sa mise en œuvre est plus lourde, les observations ont pu montrer que des traitements complémentaires n'ont pas été nécessaires en cours de culture : on peut s'attendre à un gain de temps global sur l'ensemble de la culture, de plus, les travaux peuvent être réalisés plus régulièrement.

Perspectives de lutte biologique

A l'issue des travaux qui ont débuté à l'INRA en 1992 (Nicot et al. 1996 ; Nicot 1997), plusieurs souches antagonistes ont été sélectionnées pour leur efficacité à protéger les plaies d'effeuillage; leur efficacité a été vérifiée en conditions de serres expérimentales à l'INRA, puis de 1996 à 1998 au Ctifl de Balandran avec inoculation artificielle de la maladie.

Des résultats prometteurs ont été obtenus avec une souche de champignon antagoniste *Fusarium* sp. qui protège efficacement les plaies d'effeuillage contre *Botrytis cinerea,* soit par pulvérisation localisée des plaies d'effeuillage, pulvérisation de tige ou par l'intermédiaire

[2] CETA : Centre d'Etudes des Techniques Agricoles.

d'un sécateur pulvérisateur (Decognet et al 1998). Quelle que soit la technique d'application, sur un total de 11 expériences, le pourcentage moyen de plaies avec chancres est de 57,3% dans les parcelles témoin (eau), 9,7% dans celles avec *Fusarium* sp. et 1% dans celles avec Sumico L.

L'efficacité de *Fusarium* sp doit être approfondie, en particulier sur d'autres cibles potentielles de *Botrytis*, à savoir les fruits et les feuilles. Mais les étapes essentielles restent à franchir : elles concernent la mise au point d'une production de masse du champignon antagoniste, l'optimisation de la formulation sous forme de produit commercial et enfin l'homologation de ce produit.

Conclusion

La pourriture grise due à *Botrytis cinerea* reste une maladie économiquement importante sur tomate sous abri en France. Si les facteurs climatiques ont un rôle important, leur maîtrise n'est pas toujours facile. D'autres conditions sont essentielles à respecter : les mesures prophylactiques pour limiter l'inoculum initial, des opérations culturales de qualité et effectuées régulièrement sans à-coup, dans de bonnes conditions ; enfin, une lutte chimique raisonnée basée sur des interventions essentiellement préventives et parfois curatives, en cas d'attaques observées sur tige, feuilles ou fruits. En terme de perspective, la recherche de matériel végétal résistant est en cours à l'INRA et la recherche de champignons antagonistes a donné des résultats prometteurs. L'avenir de la souche sélectionnée par l'INRA dépendra de l'intérêt que lui portera une firme commerciale pour le développement et l'homologation d'un produit commercialisé en France.

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Pour les résultats d'essais au Ctifl, consulter le site www.ctifl.fr (base de données Oryx).

Tomato crop under greenhouse in France : control means against grey mould caused by *Botrytis cinerea*

Abstract : Grey mould caused by *Botrytis cinerea* is one of the most important foliar disease in tomato crops under greenhouses in France.

After a short description of the main damage on stems, leaves and fruits, the factors favouring the development of the disease and the different control means are described : cultural techniques, climate management, chemical control and biological control as a very promising method.

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Integrated control of the green peach aphid *Myzus persicae* in sweet peppers using the nicotinyl insecticide imidacloprid.

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Abstract: Imidacloprid was studied to investigate whether the compound can be used in IPM programs in glasshouse sweet peppers. The efficacy of drench applications on the green peach aphid *M. persicae* and the side-effects on the predatory bug *Orius laevigatus* was tested. Imidacloprid efficiently controlled the green peach aphid at a dosage which was harmless to the predatory bug.

Key words: Sweet peppers, aphid control, imidacloprid systemic toxicity, *Myzus persicae,* side-effects, *Orius laevigatus,* integrated pest management, biological control.

Introduction

The green peach aphid, *Myzus persicae* (Sulzer)(Hem.: Aphididae), is one of the main pest problems in sweet peppers in Belgium, grown under glass from January until November.
The current control is carried out with *Aphidius* spp., *Aphidoletes aphidimyza* and ladybird beetles (*Harmonia axyridis, Hippodamia* spp.) and if biological control is poor, selective chemical control with pirimicarb is possible, but the product can be toxic to most adult beneficials, especially at repeated sprayings. The aphids can thus be controlled, but the ongoing biological control of other pests can be seriously hampered.
The chloronicotinyl insecticide imidacloprid is a systemic polar compound with good xylem mobility and hence feasable for soil or tank mix application in crops grown in soil or rockwool. The product is highly active against aphids, whiteflies and leafhoppers (Elbert et al., 1991), but does not negatively affect spider mites, predatory mites e.g. *Phytoseiulus* spp., *Amblyseius* spp. and nematodes. After uptake through the root system, the product is spread throughout the plant via the xylem (upward stream), and the compound is also found in the phloem through diffusion (Stein-Dönecke *et al.*, 1992). However, imidacloprid is toxic to bees and bumble bees.
We have studied in glasshouse tests whether imidacloprid can be applied systemically for the control of the green peach aphid in sweet peppers without risk to the biological control of the western flower thrips with the predatory bug *Orius laevigatus* (Fieber) (Hem.: Anthocoridae).

Materials and methods

Test insects

M. persicae aphids were collected in a commercial glasshouse on sweet pepper plants and further reared in the laboratory on sharp pepper plants, at 24-28°C, RH: 75% and L/D: 16/8 in rearing cabinets (50x50x80 cm); continuity of the aphid culture was assured by regular infestations of new pepper plants.

O. laevigatus predatory bugs (80-100) were put in a "drum" cell (diameter: 9 cm; height: 3 cm) containing a small sharp pepper plant, hanging with its roots in water through a small hole in the container. Food (flower pollen and frozen *Ephestia kuehniella* eggs) was given every 2 days; every 2 days plants were replaced by fresh ones. The plants containing *O. laevigatus* eggs were kept at 24-28°C, RH: 75% and L/D: 16/8. Three to 4 days after egg laying, the nymphs emerged. They were used either for experimental purposes, or for continuing the predatory bug culture.

1. Toxicity of imidacloprid to* M. persicae *and to* O. laevigatus *nymphs

Imidacloprid was applied to the stem base of young sweet pepper plants (60-80 cm high), grown in a commercial sweet pepper glasshouse (6,000 m²). The dosages used were: 0.1, 1, 3 mg AI imidacloprid per plant (in a volume of 10 ml) (recommended dose rate: 2.5 mg AI per plant); as toxic reference oxamyl was used, in a dosage of 25 mg AI per plant; untreated plants served as a control. Ten plants per treatment were used. One week after the treatments, per dosage 5 young leaves were removed from the plants and brought to the laboratory placed in "drum" cells, in such a way that the petioles hung in a water reservoir; 15 alate aphids (nymphs and adults) were put on the leaves. To check the toxicity of imidacloprid on the predatory bug nymphs, "drum" cell containers were fastened at the sweet pepper plants; in each cell a young leaf was put. Per dosage 10 containers were used. One week after the drench application, 10 *O. laevigatus* nymphs were put in each container. At the time of adult development, the mortality was determined.

2. Effect of imidacloprid on the egg hatch of* O. laevigatus *and further development of the nymphs.

Imidacloprid was applied to the stembase of sweet pepper plants at dosages of 1 and 2 mg AI per plant (30 ml solution per plant). Four plants per treatment were used. Untreated plants served as a control. Ten days after the treatments, "drum" cell containers were attached to the plants and in each container a sweet pepper leaf was placed. In each container, 5 female and 1 male *O. laevigatus* were put, together with *E. kuehniella* eggs as food. The containers were kept in the crop for 15 days; then the no. of nymphs and adults in the containers was checked.

Results and discussion.

1. Toxicity of imidacloprid to* M. persicae *and to* O. laevigatus *nymphs. Fate of imidacloprid in the sweet pepper leaves.

The mortality of *M. persicae* nymphs and adults, which were exposed during 4 days to imidacloprid treated sweet pepper leaves (one week after treatments) is given in Table 1.

Table 1. Mortality (%) of *M. persicae* nymphs and adults on sweet pepper leaves one week after treatment of the plants with imidacloprid and oxamyl.

Dose (mg AI per plant)	Replicates	Mortality (%) (± s)
0 (untreated)	5	8.5 (± 17.6)
0.1 (imidacloprid)	5	76.7 (± 38.1)
1 (imidacloprid)	5	98.7 (± 3.0)
3 (imidacloprid)	5	100 (-)
25 (oxamyl)	5	13.3 (± 13.3)

Imidacloprid was very toxic to *M. persicae* at a dose of 3 mg AI per plant. The application of 1 mg AI per plant did not eradicate the aphids, but was still very efficient. However, oxamyl, a broadspectrum carbamate insecticide, used as a drench at 25 mg AI per plant was not toxic to the aphid. This indicates how sensitive *M. persicae* is towards imidacloprid. The dose of 3 mg AI per plant corresponds to 75 g AI per ha (with 25,000 plants/ha).

The mortality of *O. laevigatus* nymphs, exposed to imidacloprid treated sweet pepper leaves (one week after treatment) is given in Table 2.

Table 2. Mortality (%) of *O. laevigatus* nymphs on sweet pepper leaves one week after treatment of the plants with imidacloprid and oxamyl.

Dose (mg AI per plant)	Replicates	Mortality (%)(± s)
0 (untreated)	3	0.0 (-)
0.1 (imidacloprid)	3	2.8 (± 4.8)
1 (imidacloprid)	3	3.3 (± 5.8)
3 (imidacloprid)	5	20.5 (± 11.8)
25 (oxamyl)	3	15.6 (± 5.1)

The statistical analysis shows significant differences between the 3 mg AI treatment and the control.
However, 20% mortality is under the threshold level for toxicity in field experiments (25%). The aforementioned dose imidacloprid, which is approximately the one used in practice, did not affect the predatory nymphs. Oxamyl neither did affect the predatory bugs.

2. Effect of imidacloprid on fecundity and fertility of O. laevigatus and further development of the nymphs.

Twenty five days after the imidacloprid treatments, a check was made to find out whether the eclosed nymphs could develop to the adult stage. For technical reasons, it was impossible to separate the adults from the nymphs in the containers, so that cannibalism could occur; but we found predatory bugs of the L4-L5 nymphal and adult stage. Results are given in Table 3.

Table 3. Fecundity, fertility and no. of developed nymphs and adults, 15 days after exposure of adult *O. laevigatus* to imidacloprid treated sweet pepper plants.

Object	Mean no.of deposited eggs per leaf	Mean no. of hatched eggs per leaf	Percent hatching	Mean no. of developed L4-L5 nymphs	Mean no. of developed adults
Control (untreated leaves)	136	130	95.0	7	4
Imidacloprid (1 mg AI per plant)	53	46	87.6	3	7
Imidacloprid (2 mg AI per plant)	93	76	84.6	1	2

This table shows that the fecundity and fertility (egg hatching) is not affected by the imidacloprid dosages, but that the no. of developed nymphs and adults found is abnormally low, due to cannibalism. It is thus not possible to compare the number of developed nymphs and adults between the treatments, but we can conclude that *O. laevigatus* survives imidacloprid treatments of 1 and 2 mg AI per plant, and is able to reproduce.

Conclusions

Imidacloprid meets the requirements needed for pesticides to be used in integrated pest management of the green peach aphid, *M. persicae* in glasshouse sweet peppers. The compound was very toxic to the aphid at 1 mg active ingredient per plant, it was already toxic one week after systemic application and did not adversely affect the predatory bug at this dose. Imidacloprid, applied with the irrigation system, is not toxic to the parasitoids *Aphidius colemani* and the predatory gall midges *Aphidoletes* spp. and *Therodiplosis* spp. (Biobest Brochure, 1998). *A. colemani* wasps are key biological control agents for aphid control; they efficiently control *M. persicae*, but are also able to control the cotton aphid, *Aphis gossypii*, which cannot be controlled with *Aphidius matricariae*. The predatory gall midges *Aphidoletes* and *Therodiplosis* efficiently control resp. aphids and spider mites.

With regard to the principles of integrated pest management, priority must be given to natural limiting factors and economic damage thresholds; according to this principles, imidacloprid can thus best be used in glasshouse sweet peppers, when biological control of the aphids is poor. If possible, imidacloprid should be applied locally whithin the glasshouse to eradicate aphids at high density spots.

Systemically applied imidacloprid, used at the recommended dosage, e.g. 2 mg AI per plant, is of little toxicity to bees, *Apis mellifera* but very toxic to bumble bees, *Bombus terrestris*. Since bumble bees are often used in sweet peppers, the complete crop treatment with the product cannot be done when this biopollinator is used.

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Development of biological control of *Trialeurodes vaporariorum* with *Encarsia formosa* and *Amitus fuscipennis* on greenhouse tomato in Colombia.

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Abstract: Trials were conducted to develop a biological control system for the greenhouse whitefly on tomato in unheated greenhouses at the Bogota plateau. Compared to previous trials made in the Netherlands, a lower longevity, a higher oviposition frequency and a similar fecundity were found for *Trialeurodes vaporariorum* on beef tomato. It is suggested that a difference exists between the Dutch and the Colombian whitefly strain. Biological control of *T. vaporariorum* by *Encarsia formosa* was more efficient in an automated glasshouse than in a plastic greenhouse with a climatic screen. The on average slightly higher average temperatures in the glasshouse compared to the temperature in the plastic house had a higher effect on the calculated life history parameters of *E. formosa* compared to *T. vaporariorum*. At 20 °C the native, parthenogenic parasitoid *Amitus fuscipennis* has a higher intrinsic rate of increase than *E. formosa*, indicating that it could be a good natural enemy of *T. vaporariorum*

Key words: biological control, greenhouse, Colombia, tomato, *Encarsia formosa, Amitus fuscipennis, Trialeurodes vaporariorum*

Introduction

In Colombia, the production area of greenhouse tomatoes is on the increase. The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) is an important pest in this production system. The climate of unheated greenhouses on the Bogota plateau is cool with a mean daily temperature of 15 to 16 °C. In most cases, beef tomatoes are cultivated, and these are better host plants for *T. vaporariorum* than round tomato cultivars. A cold greenhouse climate and a good host plant are two conditions that can impede successful biological control of *T. vaporariorum* by *Encarsia formosa* Gahan. No information exists on the behaviour of the Colombian whitefly strain on beef tomato. Research was therefore started at the Centro de Investigaciones y Asesorías Agroindustriales, located just outside of Bogota, to evaluate if the biological control system of *T. vaporariorum* with *E. formosa* could be adapted to the local greenhouse conditions. Included in the research program is the evaluation of a native parthenogenic parasitoid, *Amitus fuscipennis* (McGown & Nebeker) as a possible alternative for or an addition to the use of *E. formosa.*

Materials and methods

Experiment 1: Longevity and fecundity of the greenhouse whitefly on greenhouse tomato.
In a greenhouse, pairs of recently hatched whiteflies were mounted on tomato leaflets using clip cages. Every two days the clip cages were moved to new leaflets. Mortality was

registered, and fecundity and longevity were calculated. The net reproduction rate (Ro), the mean generation time (T) and the intrinsic rate of increase (r_m) were calculated using the equation of Andrewartha *et al* (1954). The immature survival rate (0.833) and sex ratio (48.3 % females) were taken from van Roermond (1995) and the immature development time was calculated based on the hourly temperature data and the equation of van Roermond (1995).

Experiment 2: Effect of the climate on the control of T. vaporariorum by E. formosa.

The effect of the climate generated by two greenhouses, a plastic greenhouse with a thermal screen and an automated glasshouse, was evaluated. Three *E. formosa* adults per m^2 were introduced in the crop on a weekly basis, starting 4 weeks after transplant, until a total of 66 adults/m^2 was reached. Every week adult whiteflies were counted on the eight upper leaves of the plants of a stratified sample of 10 % of the plants. To determine the percentage of parasitization, the number of parasitized and non-parasitized pupae were counted weekly on one leaf of the plants of a stratified sample of 10 % of the plants. Based on the hourly temperature data, the equations for the egg maturation rate and the development rate of van Roermond *et al.* (1995) and the estimations of r_m of van Lenteren *et al.* (1996), hourly values for those parameters were calculated and averaged for the whole trial period.

Experiment 3: Life history parameters at 20 °C of A. fuscipennis.

In a climatized room, leaflets of tomato plants infested with L1 whitefly larvae were introduced in transparent plastic cylinders and one recently hatched *A. fuscipennis* female was introduced in each cylinder. The females (n=28) were permitted to oviposit and every three days they were moved to new leaflets of new plants until all females had died. When the parasitoids reach the pupal stage, the whitefly pupae turn grey. At that moment the parasitized pupae were removed from the leaves and introduced into a polysaccharide capsule until they hatched. Life history parameters were calculated as in experiment 1.

Results and discussion

Experiment 1

The mean temperature and relative humidity were 16.0 ± 5.1 °C and 81 ± 13.3 % respectively. The mean longevity of the females and males were 36.5 ± 18.2 and 47.2 ± 20.0 days respectively. The total fecundity was 208.5 ±146 eggs per female. The generation time was 69.9 days, the net reproduction rate was 91.2 and the intrinsic rate of increase was 0.0645.

Longevity, fecundity and oviposition frequency change with temperature, tomato cultivar and whitefly strain (van Roermond,1995). At 22.5 °C, van Es (1982) found higher longevities than we found (57.4 and 68.6 days on beef tomato cv. Dombo and Portanto, respectively). In contrast with the longevity, the oviposition frequency was lower in van Es'study: 3.7 and 3.2 eggs per living female per day on cv. Dombo and Portanto respectively, compared to 5.7 in our study. As a result, the total fecundity in both trials was similar: 215 and 219 eggs/female on the cv. Dombo and Portanto respectively, compared to 209 in this trial. The differences can be explained in part by manipulation: van Es transferred the whiteflies every week, while we moved them every two days. Furthermore, the temperatures were different. Van Roermond (1995) found that whitefly longevity reaches its maximum at 16 to 18 °C, and oviposition frequency at 22 °C. The trial of van Es (1982) was done at 22.5 °C., sub-optimal temperature for longevity and optimal temperature for oviposition frequency. The present trial was undertaken at 16 °C, sub-optimal temperature for both parameters. As a result we would expect a higher longevity and a lower oviposition frequency in this trial, the opposite of what was found. It is therefore suggested that a difference exists between the Dutch and the Colombian whitefly strain. The r_m found for *T. vaporariorum* in this experiment (0.0645) is considerably lower than the estimated r_m for *E. formosa* at 16 °C. (0.0974, van Lenteren *et al.*,

1996). So successful biological control will not be limited by the potential population growth of *E. formosa* but on other factors such as searching efficiency. Experiment 2 provides a partial answer to this question.

Experiment 2

The mean temperatures were 15.5 ± 5.4 °C and 16.5 ± 5.1 °C in the plastic greenhouse and the glasshouse, respectively. The mean daily relative humidity was 78 ± 16 % in the glasshouse and 82 ± 15 % in the plastic greenhouse.

The whitefly populations in the glasshouse and the plastic greenhouse were 0.47 and 0.57 whiteflies per plant, respectively, 5 weeks after the transplanting. The population build-up from week 29 to 33 was higher for the glasshouse compared to the plastic greenhouse (Figure 1), which can be explained by the higher temperature in the glasshouse. The lower rate of increase in the plastic greenhouse from week 33 can be explained by the effect of parasitism: the time required for immature development was calculated to be almost seven weeks and the first adults would therefore have emerged in week 32. In the glasshouse the whitefly population started to decrease from week 33, indicating a higher parasitization efficiency of *E. formosa*, as an effect of the higher temperature. However, towards week 45 the population increased in both greenhouses and a chemical treatment was needed. A mere 2.8 % of the plants was sprayed in the glasshouse whereas in the plastic greenhouse, the infestation was so high that all plants required spraying.

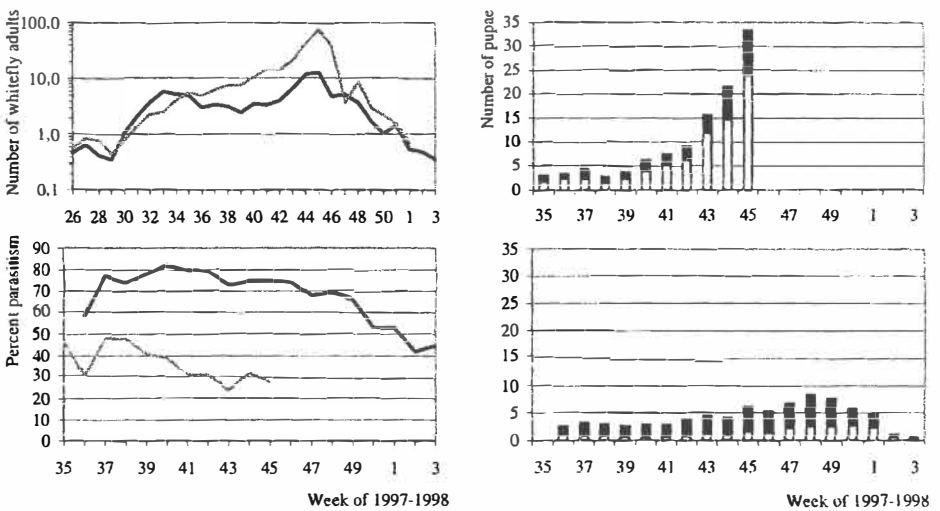

Figure 1. Mean number of whitefly adults on the 8 upper leaves of a plant (upper left) and parasitization percentage (lower left) in the glasshouse (black) and in the plastic greenhouse greenhouse (grey). Mean number of parasitized pupae (black) and non-parasitized pupae (white) per leaf in the plastic greenhouse (upper right) and the glasshouse (lower right)

Parasitism fluctuated between 42 and 82 % in the glasshouse and between 28 and 47 % in the plastic greenhouse. The total number of pupae per sampled leaf increased exponentially in the plastic greenhouse to 34.3 per sampled leaf in week 45 compared to only 6 in the glasshouse. The increase in the plastic greenhouse coincides with the adult whitefly population increase (Figure 1).

The temperature difference between the two greenhouses seems small but created a big difference in the efficiency of *E. formosa*, as the small temperature difference had more effect on *E. formosa* than on *T. vaporariorum*. The calculated egg production of one *E. formosa* adult increased by 25 % and by only 8 % for *T. vaporariorum*. The time required for immature development calculated of *E. formosa* decreased by 9.4 % and only by 7.1 % for *T. vaporariorum*. The calculated r_m of *E. formosa* increased by 19 % and by only 10 % for *T. vaporariorum*..

In conclusion, the higher temperature in the glasshouse had a positive effect on the biological control of the greenhouse whitefly by *E. formosa* when compared to the plastic greenhouse. With a better temperature management, a lower initial whitefly population, and higher *E. formosa* introductions, complete biological control should certainly be possible in the glasshouse and possibly also in the plastic greenhouse.

Experiment 3

Fecundity of *Amitus fuscipennis* was 270 ± 77 eggs per female; mean longevity was 13.6 ± 4.6 days; development time from egg to adult was 30.8 ± 3 days; mortality in the grey stage was 2 %; the net reproduction rate (Ro) was 264; the mean generation time (T) was 34.8 days; and the intrinsic rate of increase (r_m) was 0.160. This r_m is higher than the estimated r_m of *E. formosa* (0.149, van Lenteren *et al*, 1996) at 20 °C. Although the time required for the immature development is higher for *A. fuscipennis* than for *E. formosa*, the mean generation time (T) is shorter because *A. fuscipennis* lays its eggs in a very short period. The fecundity found in the present experiment was considerably higher than the fecundity of 104 eggs per female, observed by Medina *et al.* (1994).

The previous studies have shown that biological control in unheated greenhouses should be possible, but some adaptations to the introduction system as well as to the greenhouse management are needed. Starting with clean plants, introducing more *E. formosa* and a warmer greenhouse climate should improve the biological control of *T. vaporariorum* by *E. formosa*. This is the subject of further greenhouse trials. Although little is known about *A. fuscipennis*, these first results indicate that this parasitoid could be an interesting addition to the list of natural enemies of *T. vaporariorum*. Additional research on the life history parameters and the behaviour of this parasitoid will be undertaken to confirm this.

Ackowledgements

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The Meaning of Registration of Beneficials in Japan

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Abstract: The biological control should be supported by all the parties including basic researchers , field researchers, field advisers, distribution people and consumers. In Japan interests and efforts, among people of plant protection field except most pesticide companies, to incorporate biological control in the conventional plant protection is very noticeable.
Registration requirements have been regarded as typical obstacles for the biological control but once beneficials are registered, the registration system can even start to support the biological system.

Key words: natural enemies, beneficials, registration, biological control

How registration system worked in Japan?

The Japanese registration requirement on beneficial insects has been criticized since it may hinder realization of biological control.
Because it may take at least 3 years to launch a new natural enemy according to the current system. It is necessary to have registration on each crop for each natural enemy.

This requirement means to check each natural enemy's performance on each crop's cultivation condition and on each crop's specific problem such as difficulty to use *Amblyseius cucumeris* on tomato. But in fact it is a big burden to conduct so many trial before commercialization.

On the other hand, the registration system had helped field researchers to officially recommend biological control in the plant protection guideline book of each region. It had eventually helped transfer of knowledge from local field researchers such as extensions, cooperative advisers and regional experiment station people to growers.

Another benefit is for quality and quantity assurance. According to the law which regulates natural enemies, those products which can not satisfy the guaranteed quantity and quality lead to pending of sales or even lead to cancellation of registration. This encourage producers to maintain the quality of products.

Registration requirements

Basically the current system is based on the guideline for chemical pesticides, most of the toxicological studies are exempted from scientific view point. But more biological and ecological data are required. Monitoring study after release is one of important one.

It is more likely if the biology of the insects are well elucidated, they could be registered in Japan sooner or later as long as they do not attack commercial crop. The Japanese plant quarantine law prohibits importation of phytophagus insects as other countries do. An important natural enemy, *Macrolophus*, could not be imported to Japan under this condition. With addition of insects like *Macrolophus*, whitefly control with *Encarsia* will be improved. So far in Japan natural enemies similar to *Macrolophus* are not found yet.

Registration requirements for Beneficials in Japan

The Japanese registration requirements are summarized below.

1. More than 6 valid efficacy data over 2 years at Japanese regional experiment stations
2. Establishment of guaranteed viable quantity control in Japan
3. Impact on other beneficial organism such as silkworm, honeybees and other beneficials
4. Skin sensitization and irritation studies or evidence of no incidence

These are for arthropods and for microbials, the requirements are similar with the one in the U.S.A.

Release timing

One failure may nullify 5 successful controls.

The reasons of failures are mainly from low temperature in the greenhouses. Because the temperature in a typical Japanese tomato greenhouses is usually kept below 10 degree celsius to have a higher sugar content. Therefore the earlier steps to succeed in biological control should be as follows:

A. To recommend to heat up
B. To wait release timing until the greenhouse air temperature goes up above 15 degree.

Because of high oil/gas price in Japan, it is difficult to persuade growers to increase temperature only just for biological control. The fact that a grower who can use heated water from neighboring power plant always succeeds is a clear evidence of this problem. Due to high temperature in summer, transplanting season is around August through September and end of plant is around June-early July. This makes early season release(August-September) rather difficult because the population of pest insects are high in summer. So far the best timing of release is around from March when average temperature goes up above 10 degree and pest insect population is low.

Discussion:

It is not easy to say which is better for biological control in a long run, with registration or without registration. Voluntary quantity and quality control has its limitation as not all the producers are eligible for even a small standard. But most of important products in most developed countries were more or less under strict control. Otherwise the future development of the biological control might be limited.

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Effect of different prey amounts on the population development of the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus* in a single- and in a two-species system on detached rose leaves

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Abstract: We observed the population development of *Phytoseiulus persimilis* A.H. (Acari: Phytoseiidae) and *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) in a single- and two-species system on detached rose leaves during 10 days by offering *Tetranychus urticae* Koch (Acari: Tetranychidae) as amply prey, limited prey and no prey. At amply prey *P. persimilis* reached higher population densities than *N. californicus* in the single-species system, whereas the population densities of both predators were equal in the two-species system. At limited prey *P. persimilis* reached its maximum density at day 4 and then decreased rapidly both in single- and two-species system. In contrast, *N. californicus* built up a stable population in both systems. Without prey, *P. persimilis* escaped from the leaf arenas until day 4 in the single- and two-species system, whereas a small number of *N. californicus* was able to persist until day 10 in both systems.

Key words: biological control, generalist, greenhouse roses, mixed releases, specialist, spider mites

Introduction

The predatory mite *P. persimilis* belongs to the most frequently used biological control agents of *T. urticae* in greenhouse cut roses (Van Lenteren and Woets, 1988). Due to its high dispersal capacity and predatory potential against spider mites *P. persimilis* is able to suppress *T. urticae* populations rapidly (Sabelis and Dicke, 1985). However, the highly specialized predator often overexploits its prey (Laing and Huffaker, 1969), which results in a breakdown of the predator population and in short-term control of the pest. As phytoseiids with a broader range of food are able to persist at low spider mite densities, a combined release of the specialist *P. persimilis* and a generalist feeder could lead to both effective and long-term control of two spotted spider mite in greenhouse cut roses. Preliminary tests indicated that the phytoseiid mite *N. californicus* is an ideal candidate for combined releases with *P. persimilis* (Walzer, 1998). However, combined releases of two predatory mite species may result in direct and indirect competition between the two predators, which could endanger the success of biological control of *T. urticae*. In the present study population development of *P. persimilis* and *N. californicus* on detached rose leaves in a single- and two-species system was compared in each of the systems with regard to the population persistence, when offered ample prey, limited prey and no prey (Walzer, 1998).

Materials and methods

Predator sources and rearing, general methods
P. persimilis originated from a commercial producer (Biohelp, Austria) and was reared in a glasshouse on potted French beans (*Phaseolus vulgaris* L.) infested with *T. urticae* at 22°C, 65% RH and 16 h photoperiod. *N. californicus* was collected from greenhouse roses (*Rosa sp.*) and was kept on artificial arenas with *T. urticae* (Walzer and Schausberger, 1999a). The rose leaves

(cultivar @First Red@) were picked from commercially produced cut roses. Arenas in all experiments consisted of detached rose leaves, which were placed upside down on water saturated cotton wool in petri dishes and kept in environmental chambers at 25 " 1°C, 65 " 5% RH and 16 h photoperiod.

Experimental procedures
Two mated females either of P. persimilis or of *N. californicus* in the single-species systems or one female each of both species in the two-species systems were placed onto the leaf arenas. Every two days the developmental stages were recorded. Each treatment lasted 10 days and was replicated 7 times. The predators were fed amply supply of mixed stages of *T. urticae* in experiment 1 at regular intervals of 2 days. In experiment two 40 eggs of *T. urticae* were placed on each leaf arena once at the beginning of the trial. In experiment 3 the predators were held without prey.

Results

Experiment 1: The population densities of both predator species increased rapidly during the experimental period in the single-species system. Both predators reached their maximum density on day 10 with 28 mobile stages per leaf arena for *P. persimilis* and 20 stages for *N. californicus* (Fig. 1A). In the two-species system the populations of *P. persimilis* and *N. californicus* increased more slowly than in the single-species system and both predators peaked at 10 active stages (Fig. 1B).
Experiment 2: In the single-species system *N. californicus* densities increased until day 8 and peaked at an average density of 9 mobile stages. The maximum mean number of *P. persimilis* per leaf arena was reached on day 4 with 8 mobile stages and decreased rapidly to 2 mobile stages on day 10 (Fig. 1C). In the two-species system *P. persimilis* amounted to the maximum mean density of 4 active stages on day 4 and dropped to 1 mobile stages on day 10. In contrast, the population density of *N. californicus* reached a peak at 5 mobile stages on day 8, which was two times higher than the corresponding mean number of mobile stages of *P. persimilis* (Fig. 1D).
Experiment 3: Both in the single- and in the two-species system only a few females of *P. persimilis* were found on the leaf arenas on day 2. In contrast, a small number of *N. californicus* were able to survive without *T. urticae* as prey until day 10 (Fig. 1E; Fig. 1F).

Discussion

When *T. urticae* was abundant, the specialist *P. persimilis* reached higher population densities than the generalist *N. californicus* in the single-species system due to its high reproduction rate. In contrast, in the two-species system competition between the predators might have led to a slower population growth of both species. At diminishing prey the two predators showed different behaviour. Both in the single- and in the two-species system *P. persimilis* was observed to escape frequently from rose leaves because survival, development and reproduction of this specialist predator depends on the availability of spider mites as prey. In contrast, the generalist *N. californicus* remained on the leaves, since *N. californicus* requires lower amounts of prey for oviposition and survival than *P. persimilis* (Friese and Gilstrap, 1982). In the two-species system interspecific predation was frequently observed and the higher population densities of *N. californicus* indicate, that *P. persimilis* was more affected by predation by *N. californicus* than vice versa. *N. californicus* might benefit from its ability to discriminate between con- and heterospecifics, which is lacking in *P. persimilis* (Walzer and Schausberger, 1999b). Without

prey, scavenging and/or cannibalism could have resulted in surviving of a small number of *N. californicus* in both systems, whereas *P. persimilis* have a pronounced tendency to escape from leaf arenas. In summary, the generalist *N. californicus* seemed to be a better competitor than the specialist *P. persimilis* and might be able to outcompete the latter in times of food scarcity, when dispersal was not allowed. However, in complex ecosystems several other factors (environmental conditions, structure of the microhabite, intra- and interplant dispersal of prey and predators, presence of alternative and supplementary food) can influence the success of biological control of *T. urticae*, so that a specialist and generalist predator may coexist and complement each other under greenhouse or field conditions. Combined releases of phytoseiid species with different predation types resulted in more successful control of spider mites than after the release of a single species, whereas combined releases of two species of the same predator type had about the same effect as either alone (McMurtry and Croft, 1997). Preliminary investigations in commercial cut rose greenhouses indicate advantages of combined releases of *P. persimilis* and *N. californicus* for spider mite control compared to single-species releases. However, more detailed long-term studies on the population dynamics of *P. persimilis* and *N. californicus* both in the laboratory and under greenhouse conditions are needed to verify the prediction that a combined release of *P. persimilis* and *N. californicus* predators ensure both effective and long-term control of *T. urticae* in greenhouse roses.

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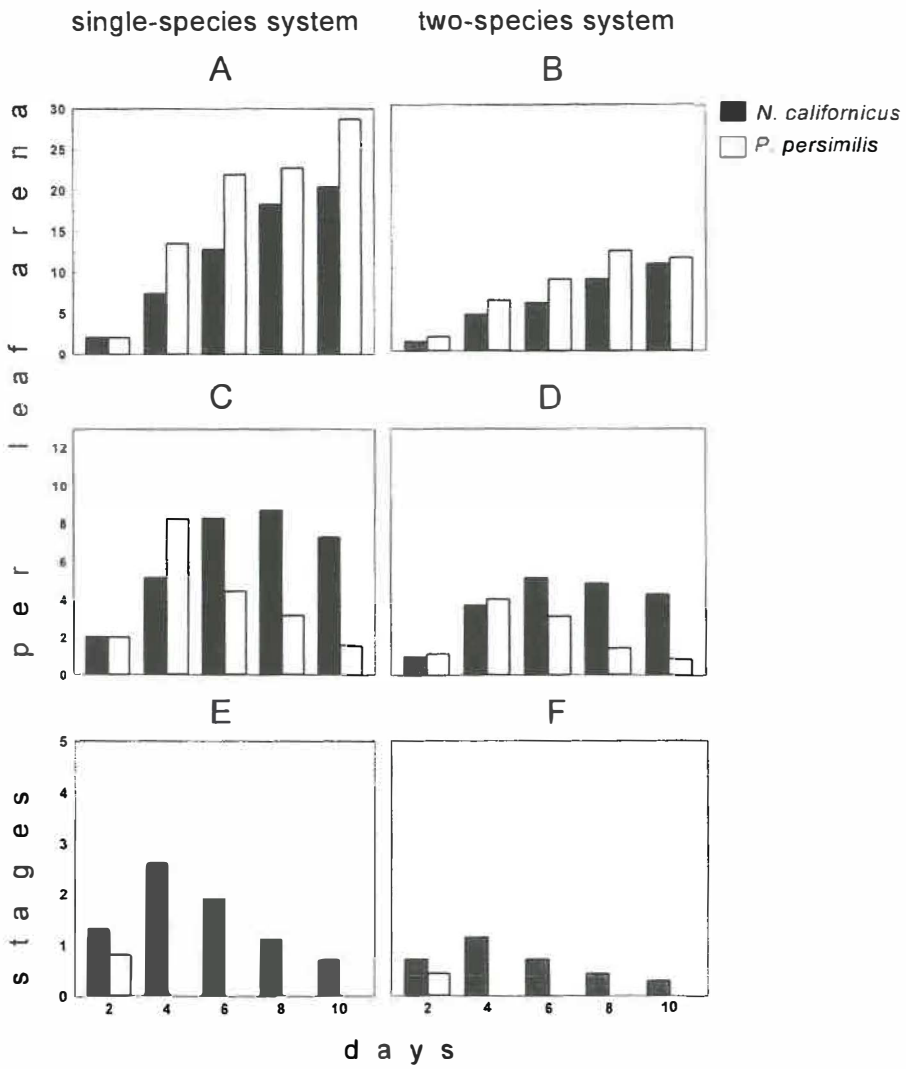

Figure 1: Population development of *P. persimilis* and *N. californicus* over time in a single- and two-species system, when offered amply prey (Fig. 1A,B), limited prey (Fig. 1C,D) and no prey (Fig. 1E,F).

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Evaluating the costs of biological pest control in protected crops

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Abstract: A computer programme has been designed to manage the delivery, distribution and costing of biological control agents on the nursery. The grower initially completes a proforma for each crop, or section of crop, which includes details of plant number, area involved, upto six biocontrol components and their rates of use and prices. The programme calculates the weekly requirements for ordering each biocontrol and accumulates the data for on-going calculations of cost per plant and per square metre. Costs of pesticide treatments and labour can be incorporated in the calculations.The programme is capable of dealing with year-round cropping as well as monocrop situations. The data is provided to growers as a charged service and may be delivered in tabular or graph format.

Introduction

Many horticultural growers are very sophisticated in their use of natural enemies in IPM programmes for protected crops. However, they are less sophisticated in organising their seasonal requirements for their variety of crops or range of greenhouses. In addition, most growers do not know the detailed cost of their IPM programmes. Accurate costings can help in planning , delivering and adjusting IPM programmes. Normally, it is a simple matter to make financial calculations using standard spreadsheets but it is more complicated when year-round cropping programmes are involved. Plant numbers generally increase in steps to peak production and decline later as the programme winds down.

The computer programme described here was initially designed for year-round crops but it is also effective for monocrops. In addition, components of cropping programmes other than natural enemies can also be costed.

Materials and methods

The computer programme was developed through collaboration between a grower (**M.H**), an entomologist consultant (**L.R.W.**) and a computer programmer (**D.J.F.**).

Information for the programme is obtained on a proforma following discussion with the grower who has his own file within the programme. To start the programme, the greenhouse identification and the name of the crop are entered. Crop details of planting date, frequency of planting, the life of the crop, total cropping period (if year-round), area and plant density are then entered. The programme is now ready for details of 'treatments' which can be a list of up to six biological agents or other inputs.

For IPM 'treatments', the size of pack (detailed by number, volume or weight) and its price, as purchased by the grower, are required. These figures vary considerably from grower to grower. The rate of use (whether by plant, area or greenhouse) and the start and finish date and frequency of introductions of 'treatments' are required.

A special sub-section for inputting 'treatment' and application details is available in the programme. Matters are simplified by using a 'drag and drop' technique from prepared lists.

Results of calculations for all the components or for individual components as required, are instantaneously available on the monitor screen. Data can be directly transferred to Excel for further work i.e. preparation of tables or graphs.

Results and discussion

Weekly* Total *data

The example in Table 1 is typical of a year-round ornamentals programme in which each weekly batch of 3135 begonias is set out in 285 sq.m. areas of greenhouse. The programme in this example begins in Week 51 and continues to Week 28 the subsequent year. Each batch takes eight weeks from planting to harvest, hence peak production is reached by Week 6.

Table 1. Weekly costs (£) of the components of an IPM programme in year-round protected begonias.

Week number	***Ambly-seius***	***Aphidius***	***Encarsia***	Total	£ per sq.m.	£ per plant
51	44.5	22.1	0.8	67.3	0.24	0.02
52	88.9	44.2	1.6	134.7	0.47	0.04
1	133.4	66.3	2.4	202.0	0.71	0.06
2	177.8	88.4	3.2	269.4	0.95	0.09
3	222.3	110.5	4.0	336.7	1.18	0.11
4	266.8	132.5	4.7	404.0	1,42	0.13
5	311.2	154.6	5.5	471.4	1.65	0.15
/	**Peak**	**production**	**for**	**sixteen**	**weeks**	**/**
22	0	154.6	5.5	160.2	0.56	0.05
23	0	132.5	4.7	137.3	0.48	0.04
24	0	110.5	4.0	114.4	0.40	0.04
25	0	88.4	3.2	91.5	0.32	0.03
26	0	66.3	2.4	68.6	0.24	0.02
27	0	44.2	1.6	45.8	0.16	0.01
28	0	22.1	0.8	22.9	0.08	0.01
TOTAL	5335.2	4064.6	145.4	9545.1	33.49	3.04

The programme also automatically accumulates data and provides on-going costs per sq.m. and per plant as in the example for a chrysanthemum monocrop in Table 2 below.

Table 2. Total costs of the components of an IPM programme in a protected monocrop of pot chrysanthemums..

Week number	**All bugs**	***Ambly-Seius***	***Aphi-dius***	***Dacnusa/ Diglyphus***	***Phyto-seiulus***	***B.t.***	***Vert/ Mycot***
51	£19	£5.63	£3.44	£5.11	£3.60	£0.23	£0.89
52	£38	£11.25	£6.88	£10.22	£7.19	£0.46	£1.78
1	£57	£16.88	£10.32	£15.33	£10.78	£0.70	£2.68
2	£76	£22.50	£13.76	£20.44	£14.38	£0.93	£3.57
etc	/	/	/	/	/	/	/
8	£198	£56.25	£34.40	£51.10	£35.95	£2.32	£8.92
9	£208	£61.88	£37.84	£56.21	£39.54	£2.55	£9.81
10	£227	£67.50	£41.28	£61.32	£43.14	£2.78	£10.70

Weekly* Individual *component data

Data for each component can be examined separately as shown in Table 3.

Table 3. Weekly accumulated costs per square metre and per plant of three of the components of an IPM programme in a 12 week duration chrysanthemum monocrop.

	Amblyseius	*Aphidius*	*Vert/Myc.*	*Amblyseius*	*Aphidius*	*Vert/Myc.*
Week no.	Per sq.m.	Per sq.m.	Per sq.m.	Per pot	Per pot	Per pot
51	0.9p	0.55p	0.14p	0.028p	0.017p	0.004p
52	1.8p	1.10p	0.29p	0.056p	0.034p	0.009p
1	2.7p	1.65p	0.43p	0.084p	0.052p	0.013p
2	3.6p	2.20p	0.57p	0.112p	0.069p	0.018p
3	4.5p	2.75p	0.71p	0.141p	0.086p	0.022p
4	5.4p	3.30p	0.86p	0.169p	0.103p	0.027p
5	6.3p	3.85p	1.00p	0.197p	0.120p	0.031p
6	7.2p	4.40p	1.14p	0.225p	0.138p	0.036p
7	8.1p	4.95p	1.28p	0.253p	0.155p	0.040p
8	9.0p	5.50p	1.43p	0.281p	0.172p	0.045p
9	9.9p	6.05p	1.57p	0.309p	0.189p	0.049p
10	10.8p	6.60p	1.71p	0.338p	0.206p	0.054p

The costs per square metre and per pot or plant for the combined total of the components of a programme are also available.

The programme has the facility to transfer data for an individual component to an Excel spreadsheet. This enables a complete weekly record for that component to be made for other crops or greenhouses on the nursery. It is then a simple matter to total the weekly requirements for that component, making forecasting and ordering much easier. It is also more convenient for despatching components to individual cropping areas on the site.

Benefits of costings data

A total annual cost of IPM can seem very expensive to many growers, especially if they have not had pest problems due to the success of the programme. These costs are often psychologically more acceptable if they are broken down to cost per unit area or plant. The computer programme is also able to prepare cost profiles for comparative chemical programmes which can be useful for justifying expense on natural enemies, especially when costs of labour are included.

Obviously, the data is useful for preparing and monitoring financial budgets but figures can also be useful for exercising economies in various areas of the IPM programme. Such financial decisions need to be integrated with the technical implications of any adjustments.

The data can be used to plan IPM programmes which is also useful to the supplying biocontrol companies. However, the grower is often in a position to bargain for discounts in these circumstances.

Acknowledgements

We would like to thank the growers who have allowed us to use data from their crops to use as examples in this paper.

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Developing a strategy for the control of *Spodoptera littoralis* with entomopathogenic nematodes in greenhouses

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Abstract: Two species of entomopathogenic nematode were applied as a foliar application to *Spodoptera littoralis* larvae. *Steinernema carpocapsae* was less effective in controlling *S. littoralis* than *S. feltiae*, which reduced larval survival to c10%. Investigations into the movement and survival of *S. feltiae* on capillary matting showed that more nematodes were able to enter larvae of *Galleria mellonella* when the target insect was placed at 5cm compared to 10cm from the nematode source, regardless of the length of time allowed for movement. The type of capillary matting did not significantly affect the overall numbers of nematodes that invaded a host but it did affect the length of time taken to reach their target.

Key Words: *Spodoptera* sp., *Steinernema* spp., foliar application, capillary matting, control

Introduction

Spodoptera littoralis (Boisduval) is a major pest in many regions of the world, with sporadic outbreaks occurring in glasshouses in the UK. It is highly polyphagus was first intercepted in the UK in 1963 following the start of the importation of *Dendranthema* (chrysanthemum) cuttings for all year round cut flower production. Although a number of outbreaks have subsequently occurred (Bartlett & Macdonald, 1993), this pest has been successfully contained and eradicated in the UK by Plant Health action. However, in response to demand from industry for novel biocontrol treatments and to increasing reports of resistance to pesticides and bio-insecticides such as *Bacillus thuringiensis* Berliner, a need to develop alternative strategies was identified. This paper investigates the potential of applying entomopathogenic nematodes (EPNs) as a foliar application and as a drench to capillary matting as part of a control strategy for *S. littoralis*.

Materials and Methods

Susceptibility of S. littoralis *larvae to a foliar application of EPNs*

Larvae of *S. littoralis* were cultured on an artificial diet in propagating trays at 25 ± 3°C with a 12:12 h light:dark regime as described in Williams (1997). A suspension of *S. feltiae* (Nemasys ®) made up to 1000 nematodes/ml with 0.02% of the wetting agent Agral (a. i. alkyl phenol ethylene oxide), was applied to run off at a rate of 900-1200 l/ha (9-12 nematodes/cm^2) using a hand sprayer to 18, two week old broad bean plants (cv. The Sutton). After application, the soil was covered with dry filter paper (Whatman No 1) to minimise the exposure of the larvae to the soil. A similar treatment but without EPNs was applied to a further 18 plants as a control. Twenty, first instar *S. littoralis* larvae were placed on the leaves of each of three of the plants sprayed with *S. feltiae* and on three without nematodes. Each plant was then covered and sealed with a vented plastic container to maintain a high humidity.

The experiment was repeated for the all six larval instars of *S. littoralis*. Plants were maintained at 20 ± 1°C and >85% r.h. under a 12:12 h light:dark regime in which the first 12 h after treatment were in darkness. Larval survival was assessed after 48 h and expressed as a ratio of the control and arcsin transformed before analysis using ANOVA. The experiment was repeated with *S. carpocapsae* (Exhibit SC-WDG®).

Movement and parasitism by* S. feltiae *on capillary matting

Sixty four, 14 cm diameter plastic Petri dishes were lined with Vattex F®, a mixed fibre capillary matting of 4 mm thickness with an absorption rate of approximately 3 litres/m^2. To each dish, 14 ml of water was added to provide moisture for nematode movement. All dishes were left for two hours at 20°C to facilitate the uniform absorption of water by the matting. A 1ml aliquot of a 1000 nematode/ml suspension of *S. feltiae* was then placed at one end of the Petri dish. Late instar *Galleria mellonella* (L.) larvae were sealed in plastic mesh pouches and placed, one per Petri dish, at two distances from the nematodes: 5cm and 10cm. The dishes were maintained at 20 ± 1°C and >90% r.h. until sampled. Larvae were removed from the pouches at 96, 120, 144 and 168 h and assessed for mortality and the presence of nematodes.

Four types capillary matting were also investigated: two Black Lantor®, one mixed fibre Vattex F® and one grey Fibretex SF250® of 6, 2, 4 and 2 mm thickness respectively. Each type of matting was cut into 14 cm diameter circles and inserted inside twelve, 14 cm plastic Petri dishes. In each dish, a late instar *G. mellonella* larva was placed inside a plastic mesh pouch and positioned 10 cm from a 1 ml aliquot of 1000/ml suspension of *S. feltiae*. The Petri dishes were assessed for *G. mellonella* mortality at 48, 72, 96 and 168 h. For each time period investigated there were three replicates.

Results and discussion

Susceptibility of* S. littoralis *larvae to a foliar application of EPNs

Significant mortality of the larvae of *S. littoralis* was recorded after foliar applications of both *S. feltiae* and *S. carpocapsae* ($F=174.63$, d.f. 2,54, $p<0.001$; Fig 1). However, comparative analysis between the two nematode species showed that *S. feltiae* was more effective than *S. carpocapsae* ($F=76.62$, d.f. 1,24, $p<0.001$). Larval susceptibility to *S. carpocapsae*, differed significantly between instars ($F=9.07$, d.f. 5,24, $p<0.001$).

A number of laboratory investigations have been carried out on the susceptibility of different larval instars and species of *Spodoptera* to EPNs (Kaya, 1985; Kondo & Ishibashi, 1985; Fuxa *et al.*, 1988; Glazer, 1992). Contradictory results have been obtained with differences being attributed to the bioassay techniques employed by individual researchers. Accordingly, extrapolation of these data to the field has been difficult and the application of nematodes to whole plants in these investigations aimed to simulate the likely susceptibility in the field by incorporating larval behaviour into the design.

Species, temperature and perhaps strain of nematode are all potentially important in maximising nematode efficacy for *S. littoralis* control. Bohan & Hominick (1995) showed that the average probability of host infection (i.e. transmission coefficient) varied with temperature, the optimum temperature for *S. feltiae* infection of a host being 20°C. For *S. carpocapsae*, the optimum temperature for infectivity has been reported to be 25°C. A factor in the lower mortality of *S. littoralis* recorded after an application of *S. carpocapsae* might, therefore, be the temperature at which efficacy was assessed.

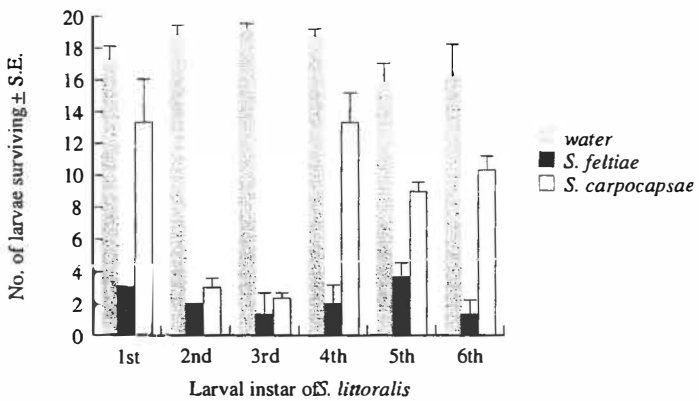

Figure 1. Susceptibility of *S. littoralis* larvae to a foliar application of *S. feltiae* and *S. carpocapsae* on broad bean at 20 ± 1°C and >85% r.h.

Movement and parasitism by* S. feltiae *on capillary matting

Larvae of *S. littoralis* have been observed to drop from plant pots and move on the ground or capillary matting around the pot. By demonstrating that EPNs can survive and move effectively through capillary matting, an additional arena for larval control has been identified. EPNs can successfully travel through capillary matting to infect a susceptible host. The greater the distance between the *G. mellonella* larvae and the point of nematode application, the smaller the number of nematode infested hosts recorded. Significantly more nematodes were recovered from *G. mellonella* placed at 5cm compared to those at 10cm distance ($F=8.04$, d.f. 1,56, $p<0.001$; Table 2). The access time of the host to the nematodes did not affect on the overall host mortality ($F=1.09$, d.f. 3, 56, $p>0.05$).

Table 2. Distance moved by *S. feltiae* after different time periods on Vattex F capillary matting; 8 replicates.

	Mean number of nematodes ± standard error entering late instar *G. mellonella* larvae			
	Exposure Time (hours)			
Distance	96	120	144	168
5cm	9.5 + 5.0	26.6 + 15.3	9.4 + 3.9	9.9 + 4.9
10cm	2.6 + 1.2	2.3 + 1.5	0.4 + 0.4	1.0 + 0.5

Table 3. Ability of *S. feltiae* to move on or through different capillary matting types over time; 3 replicates.

	Mean number of nematodes ± standard error entering late instar *G. mellonella* larvae			
	Exposure Time (hours)			
Matting type	48	72	96	168
Black Lantor (thin)	1± 1	9.7 + 6.0	19 ± 17	48.7 ± 25.2
Black Lantor (thick)	0	20.3 ± 13.2	102.3 ± 2.6	1.3 ± 1.3
Fibretex grey fibre	0	35 ± 35	80 ± 42.2	51 ± 24.8
Vattex mixed fibre	10 ± 6.8	13.7 ± 8.1	18.3 ± 8.1	35.3 ± 12.6

Different types of capillary matting did not significantly affect the number of nematodes that reached their target host after different time periods ($F=0.11$, d.f. 3,41, $p>0.5$; Table 3). There was, however, a significant effect of time on the number of nematodes able to invade a host. Fewer EPNs were able to travel 10cm through the capillary matting after 48 h and reach their target compared to those which were given longer to invade the host. A mortality of 25% was recorded after 48 h compared to 81% after 96 h.

Prepupae, pupae and emerging adults of *Spodoptera* sp. have been shown to be highly susceptible to nematode parasitism after a soil application (Kaya & Grieve, 1982; Williams, 1997). For pot plants, therefore, a drench application over both the pot soil and capillary matting would be likely to enhance the control of the later larval instars. A potential control strategy for *S. littoralis* would include a foliar application of *S. feltiae* at 20°C and >85% r.h to target younger *S. littoralis* larvae with a soil application plus an additional drench on capillary matting for the older instars.

Acknowledgments

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A bioassay technique to determine the functional response of different predators of *Frankliniella occidentalis* in ornamentals.

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Abstract: A bioassay technique to determine the predation of individual thrips predators, at a range of prey densities, on individual leaves or flowers of different ornamental plants is described. Details are given of the functional response of the predatory mite *Amblyseius cucumeris* (Oudemans) to first instar larvae of *Frankliniella occidentalis*(Pergande) on New Guinea impatiens. The importance of a reliable bioassay technique is discussed in relation to formulating a generic model that can be used to develop future biological control strategies in ornamentals.

Keywords: *Frankliniella occidentalis*, thrips predators, functional response, generic model

Introduction

For biological control to be effective against *Frankliniella occidentalis* (western flower thrips) in protected ornamentals, a different approach to that used in edible crops needs to be developed. The main reason for this is that there is a larger diversity of plant species in floriculture crops, with a much lower tolerance to thrips damage. With crops such as peppers, tomatoes, and cucumbers, the foliage can withstand a certain level of thrips before the fruit are damaged. In ornamentals, densities as low as 1-2 thrips per plant can cause visible damage resulting in unmarketable plants. It is difficult to investigate the effectiveness of natural enemies unless unrealistically high thrip densities are used. This highlights the need to formulate a generic model for biological control in ornamentals that can use experimental data to predict the outcome at very low thrips densities.

A modelling approach is being developed at HRI for predicting future biological control strategies against the main pests of ornamentals (the details of this can be found elsewhere in these proceedings). For *F. occidentalis*, the model requires information on the number of thrips consumed by individual predators in 24 hours. This predation rate will be affected by the large diversity in the morphology of ornamental plants. As a way of quantifying this predation, a bioassay technique was developed that could measure the functional response of predators on different plants and at a range of thrips densities. The bioassay was adapted so that predation could be investigated on individual leaves or flowers. The functional response measured on leaves should give an indication of the maximum number of thrips that could be eaten in 24 hours, while that on individual flowers should give a far more realistic predation rate and reflect how the thrips and mites distribute themselves. The aim of this paper is to describe the bioassay techniques and show how they can be used to build up realistic functional response data for use in the model. Information will be presented on the functional relationship between the predatory mite, *Amblyseius cucumeris* and first instar *F. occidentalis* on New Guinea impatiens. These plants were chosen because they have one of the simplest flower and leaf forms of all the ornamental plants and this makes them an ideal model plant.

A. cucumeris was chosen because it is the most widely used biocontrol agent of *F. occidentalis* in the UK.

Materials and methods

Plant and insect rearing

Cultures of *F. occidentalis* were maintained on potted chrysanthemums. To obtain a uniform aged cohort of first instar thrips larvae, female thrips were removed and placed on dwarf French bean pods ('Masterpiece') for 3-4 days. The newly hatched larvae (0-6 hours old), which were white in colour because they had not yet fed, were used in the bioassays. *A. cucumeris* was obtained from Novartis BCM and maintained in culture with the flour mite, *Acarus siro* L. The experiments used healthy adult females taken directly from the cultures to imitate the quality of mites supplied to growers. Cuttings of New Guinea impatiens ('Dark Delias') were bought from Findon's Nursery (Stratford upon Avon) and grown on at HRI. Special care was taken to ensure that the experimental plants were not contaminated with thrips.

Experimental set-up for the leaf bioassay

A single New Guinea impatiens leaf (cut into a 3.5cm diameter disc) was placed on a smaller disc of Oasis ™ (3cm diameter x 0.3cm height) for support. This was floated on the surface of 20 mls of tap water in a pot (5.5cm diameter x 2.5cm height) and held in position by two pins that pierced through each end of the leaf and into two small pieces of Bluetac™ on the base of the pot. This provided a natural water barrier and prevented the leaf from drying out.

First instar thrips were transferred carefully to each leaf using a moistened paint brush at rates of 2, 4, 8, 12, 16, 20 and 30 thrips per leaf. A single adult female predatory mite was then placed in the centre of each leaf disc. Between 15 and 20 replicates were set up for each of the seven thrip densities. The dishes were placed inside transparent plastic boxes (four per box) to reduce desiccation and maintained in an incubator at 25°C ± 1°C (L16: D8). After 24 hours, the number of thrips remaining on the leaf was recorded.

Flower bioassay

A single New Guinea impatiens flower with its stem intact, was placed upright in the centre of a clear glass jar (size 300mls) containing 1cm deep of set agar (Oxoid, technical agar No. 3). First instar thrips were put onto the flower petals at the same densities per flower as used in the leaf bioassays, and one mite was put in the centre of each flower. This procedure was carried out on black surfaces so that the thrips were clearly visible. The jars were covered with cling film, pulled tight to provide a good seal and maintained in an incubator at 25°C ± 1°C (L16: D8). After 24 hours, the flower was removed and placed in a glass Petri dish containing 70% alcohol to prevent the thrips from escaping. The empty jar was also rinsed thoroughly with alcohol to remove any remaining thrips. Using fine tweezers and a dissecting needle the flower was carefully dissected in the alcohol to find and count the remaining thrips and mite. These dissections were time consuming so the flower and thrips were stored in alcohol for assessment later. Nine or 10 replicates were done for each thrips density.

Results and discussion

The functional response data for *A. cucumeris* when predating on larvae of *F. occidentalis* (0-6 hours old) was fitted to the type II functional response model using Rogers equation (Rogers, 1972) and is illustrated in Figure 1.

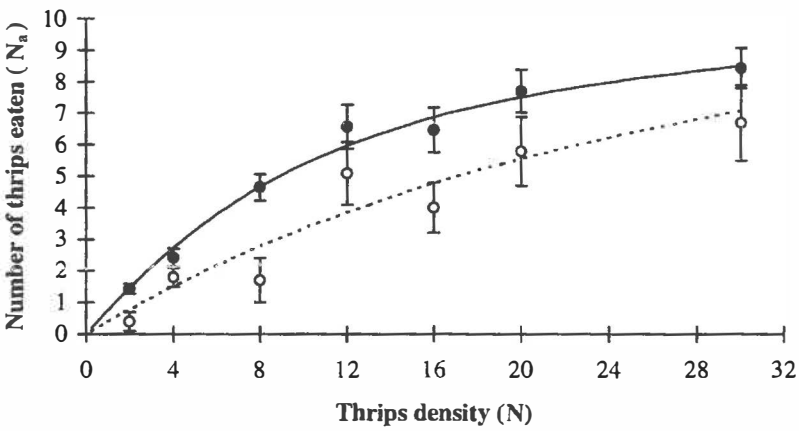

Figure 1. The functional response of *A. cucumeris* to first instar western flower thrips, *F. occidentalis*, on New Guinea impatiens leaves (●, data: —, fitted) and flowers (O, data: - - fitted). Curves are lines of best fit as predicted by Rogers equation (each data point represents the mean ± se).

There are many aspects of the bioassay methodology that need to be considered when interpreting these data. The duration of 24 hours was chosen firstly because it allowed for any changes in the natural feeding and resting stages in the mites' daily cycle and secondly, thrips larvae that are more than one day old, are likely to be unsuitable prey for *A. cucumeris* (Hoeven & Rijn, 1990). With only one surface exposed, the area of the leaf disc is relatively small, making it easy for the mite to find its prey. Care was needed to ensure that the leaf was pinned through the main leaf vein, otherwise the leaf tended to curl and the thrips could hide underneath. Provided the pots were handled carefully, the water barrier was very effective at preventing the thrips from escaping off a flat leaf. It was important to choose the flattest leaves for the bioassay, to reduce this effect. New Guinea impatiens leaves are naturally smooth and free of leaf hairs, a factor that must be considered when assessing the mites activity. The shape of the response curves suggests that 8-9 thrips larvae is the maximum number that can be eaten on New Guinea impatiens leaves in 24 hours. Similar work on sweet pepper leaf discs suggests that a maximum of 10 first instar *F. occidentalis* can be eaten if adults have been starved for 24 hours beforehand (Shipp and Whitfield, 1991).

The mean number of thrips larvae killed was lower on the flowers than on the leaves at each prey density. There was also more variability in the predation data for *A. cucumeris* on flowers. However, this variability is most likely to be a reflection of the structural complexity of the flowers and not due to inaccuracies with the bioassay methodology. Preliminary studies to refine the flower bioassay had overcome many of the practical problems associated with minimising the variability in the results. For example, if ventilated lids were used, the delicate flowers wilted in less than a day. If plastic pots were used, the build up of static electricity made it difficult to transfer the thrips. First instar thrips are difficult to see, but the use of black working surfaces ensured that the thrips could be seen at all times while being handled directly and when the flowers were transferred to the dish of alcohol. Refining the bioassay

technique ensured that all the thrips were recovered from the flowers when controls were set up without mites at each thrips density.

The complexity of flowers probably results in the mites spending more time searching for their prey. In addition, New Guinea impatiens produce a lot of pollen and because this can provide an alternative source of food to both the thrips and the mite, its availability will affect the predation of thrips within the flower. This variability in the thrips' and mites' behaviour is likely to be seen when studying other ornamental flowers because of the wide diversity in flower morphologies. The model is being developed in a stochastic way to take into account much of this inherent variability.

At HRI, the bioassay is being used to quantify the functional response of other thrips predators, such as *Amblyseius degenerans* Berlese and nymphs of *Orius laevigatus* (Feiber), on New Guinea impatiens. The functional response of all 3 predators is also being investigated on the complex leaves and flowers of Chrysanthemums using the same techniques.

All the information gained on the functional response of different thrips predators on various ornamental plants can then be used in the thrips model which, when completed, will be used to predict biological control strategies for ornamental crops in the future.

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Recent advances in the study of biocontrol with indigenous natural enemies in Japan

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Abstract: Some indigenous *Orius* species and eulophid parasitoids have been studied for commercial use in greenhouses in recent years. Although *O. sauteri* has been demonstrated to be effective as a biocontrol agent for thrips and has been registered as a biotic pesticide, *O. strigicollis* has been studied as a more promising species. The parasitoid fauna of *Liriomyza trifolii* was surveyed, and four eulophid species were found to be predominant. One of the species, *Hemiptarsenus varicornis*, which adapts well to the greenhouse environment, seems to be a good candidate as a biocontrol agent for *L. trifolii* in greenhouses.

Key words: indigenous natural enemies, *Thrips palmi*, *Orius* predators, *Liriomyza trifolii*, *Hemiptarsenus varicornis*, parasitoids

Introduction

Both introduced and indigenous natural enemies are used for the biocontrol of greenhouse pests. The advantages of using indigenous natural enemies are: (1) use of natural enemies adapted to the domestic environment (2) avoidance of unexpected effects on domestic ecosystems following the use of imported natural enemies.

The use of polyphagous predators that may become established in Japan demands particular caution. *Orius* species, which are widely used to control thrips pests in Western countries, have not been imported into Japan. Instead, Japanese species have been tested for practical use in biocontrol in greenhouses. In *Liriomyza trifolii* (Burgess) biocontrol trials using imported parasitoid species, it was found that many indigenous parasitoid species sometimes out-competed introduced species and effectively suppressed leaf miners and this finding stimulated study of *L. trifolii* biocontrol by indigenous parasitoid species.

Study of *Orius* predators

Most studies of indigenous *Orius* species have focused on *O. sauteri* (Poppius) because its effectiveness in suppressing *Thrips palmi* Karny has been demonstrated both in the field and in greenhouses (Nagai, 1993; Kawai, 1995; Yano, 1996). Rapid progress has been made in studies on the taxonomy and life history of Japanese indigenous *Orius* species in the past few years.

Taxonomy

Yasunaga (1997) recorded seven Japanese *Orius* species, four of which, *O. sauteri*, *O. minutus* (Linnaeus), *O. strigicollis* (Poppius) and *O. tantillus* (Motschlsky), are considered major natural enemies of thrips in the field. *O. sauteri*, *O. minutus*, and *O. strigicollis* are found on vegetables, such as cucumbers and eggplants, and on fruit trees, herbaceous plants

and flowers. *O. sauteri* and *O. minutus* are distributed almost nationwide, whereas *O. strigicollis* is distributed only in the southwestern part of the four main islands of Japan. *O. nagaii* is the most predominant species in rice fields. *O. tantillus* is distributed only in the southwestern islands of Japan (Ryukyu Islands) and is commonly found on the grasses of the Gramineae. The phylogeny of ten *Orius* species, including the indigenous species in Japan, has been analyzed based on DNA sequence of ITS-1 region of nuclear ribosomal DNA (Honda et al., 1998b).

Life history

The development, survival, oviposition, and reproduction of *O. sauteri* reared on *Thrips palmi* larvae were studied at different temperatures in the laboratory (Nagai & Yano, 1999). The female oviposition rate per day was 3.6 at 25°C, but dropped to 0.3 at 15°C. The intrinsic rate of natural increase per day ($=r_m$) at 15°C, 20°C, 25°C, and 30°C was 0.0135, 0.0763, 0.128 and 0.166, respectively. Honda et al. (1998a) compared the development, reproduction and longevity of *O. minutus* and *O. sauteri* reared on *Ephestia kuehniella* eggs at 25°C. Total fecundity was significantly higher for *O. minutus* (105.36) than *O. sauteri* (68.40). However, the r_m values for *O. minutus* (0.137) and *O. sauteri* (0.132) were similar because of higher mortality of *O. minutus*. The minimum number of *E. kuehniella* eggs required for the development and oviposition of *O. sauteri* was investigated by changing the number of eggs given to *Orius* nymphs and adults (Yano, unpublished). Nymphs and adults of *O. tantillus* collected in Okinawa were reared on *Throphagus putres*centiae (Schrank), *E. kuehniella* eggs, and *T. palmi* larvae. *E. kuehniella* eggs were found to be the most suitable diet for *O. tantillus* (Nagai et al., 1998). The effects of temperature on the development of eggs and nymphs of *O. tantillus* were investigated by rearing individuals with *T .palmi* larvae at different temperatures (Nakashima & Hirose, 1997a). The estimates of lower developmental threshold temperatures for eggs, male nymphs, and female nymphs were 13.7°C, 11.9°C, and 13.4°C, respectively. These estimates were lower than the average winter temperature at night in greenhouses in Japan.

The reproductive diapause of three *Orius* species, *O. sauteri*, *O. minutus* and *O. tantillus* was studied at different daylengths and temperatures. *O. tantillus* does not enter diapause despite exposure to low-temperature and short daylength (Nakashima & Hirose, 1997b).This indicates that *O. tantillus* can continue to reproduce even under short-day and low-temperature conditions.Geographic variations were found in regard to induction of reproductive diapause of *O. sauteri* and *O. minutus* at 22 °C. The critical photoperiods for induction of diapause were 14-14.5L: 9.5-10D for *O. sauteri* and 14.5-15L: 9-9.5D for *O. minutus* in Sapporo (43.0°N)(Ito & Nakata, 1998), and 12-12.5L: 11.5-121D for both species in Kurume (33.2°N)(Kohno, 1997). Temperature affects reproductive diapause induction of *O. sauteri*. All females of *O. sauteri* entered diapause at 22 °C but only 37% initiated oviposition at 26 °C under 10L: 14D (Kohno, 1998). The diapause incidence of *O. strigicollis* is lower than *O. sauteri* at short daylength.

The release strategy of *O. sauteri* to control *T. palmi* was examined in a simulation study. It was found that the predators should be released earlier and at higher density to obtain good results. When the same numbers of predators were released, control on cucumbers was found to be more difficult than on sweet peppers and eggplants (Yano, unpublished).

Prospects for commercial use

O. sauteri was registered as a biotic pesticide in 1998. It can be used to control *T. palmi* and *Frankliniella occidentalis* (Pergande) on sweet peppers. Mass production of *O. sauteri* and *O. strigicollis* has been tried with *E. kuehniella* eggs as their diet. Biocontrol trials with these two *Orius* species on eggplants, sweet peppers, and cucumbers are now being conducted in many agricultural experiment stations. Most of the trials have been yielding satisfactory results. The

major limitation is the insufficient effect in late autumn and winter. Since diapause incidence of *O. strigicollis* is lower and is easier to rear than *O. sauteri,* the former species may be more promising than the latter. Although *O. tantillus* does not enter diapause and can reproduce under short-day and low-temperature conditions, it is not considered as good candidate for a biocontrol agent because of its low reproductive potential.

Study of parasitoids of *L. trifolii*

Parasitoid fauna of L. trifolii

The domestic parasitoid species fauna that attack *L. trifolii* was surveyed in Japan. Twenty-eight species were recorded as parasitoids of *L. trifolii*. An illustrated identification system was developed to identify these parasitoid species of *L. trifolii* (Konishi, 1998). Twenty-one of the species are eulophid species, and the others belong to Braconidae, Eucoilidae, and Pteromalidae. Four eulophid species, *Hemiptarsenus varicornis* (Girault), *Chrysocharis pentheus* (Walker), *Neochrysocharis formosa* (Westwood) and *N. Okazakii* (Kamijo), are the predominant species in Japan.

Prospects for biocontrol with indigenous parasitoids

In Shizuoka, central Japan, all four species are found both in fields and greenhouses as parasitoids of *L. trifolii*. *H. varicornis* was the most important species in greenhouses where synthetic pesticides were applied frequently (Saito et al., 1996). The development period from egg to adult emergence of *H. varicornis* was studied at different temperatures. It was about 9 days at 25°C, and the estimated lower threshold temperature for development was 9°C (Saito et al., 1997). *H. varicornis* is an ectoparasitic parasitoid. Adults kill many larvae of *L. trifolii* not only by parasitization but by host feeding. The size of host larvae affects the manner of attack by *H. varicornis* as well as the size and sex ratio of the adults in the next generation (Yano, unpublished). *N. formosa* is the most important parasitoid of *L. trifolii* in western Japan. Mass production of *L. trifolii* and these parasitoids are being carried out by using potted beans (*Phaseolus vulgaris*). Mass production of these eulophid parasitoids has been started, and biocontrol trials will be conducted soon. *H. varicornis*, which is adaptive to the greenhouse environment in Japan, seems to be a promising species for commercial use.

Conclusions

Several imported natural enemy species and strains, i.e., *Phytoseiulus persimilis*, *Encarsia formosa*, *Aphidius colemani*, *Aphidoletes aphidimyza*, *Amblyseius cucumeris*, *Dacnusa sibirica* and *Diglyphus isaea* have been registered as biotic pesticides in Japan. *O. sauteri* is the only registered indigenous species. Commercialization of indigenous natural enemies will be important from the point of view of environmental safety. The number of indigenous species and strains for biocontrol will be increased and replace with some imported species in future.

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