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Integrated Control in Protected Crops, Temperate Climate

Lutte Intégrée en Cultures Protégées, Climat Tempéré

editor:

Annie Enkegaard

**IOBC wprs Bulletin
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Annie Enkegaard

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The Publication Commission:

Dr. Horst Bathon
Federal Biological Research Center
for Agriculture and Forestry (BBA)
Institute for Biological Control
Heinrichstrasse 243
D-64287 Darmstadt (Germany)
Fax +49-6151-407290
e-mail: h.bathon.biocontrol.bba@t-online.de

Prof. Dr. Luc Tirry
University of Gent
Laboratory of Agrozoology
Department of Crop Protection
Coupure Links 653
B-9000 Gent (Belgium)
Tel. +32 9 2646152, Fax +32 9 2646239
e-mail: luc.tirry@rug.ac.be

Address General Secretariat IOBC/WPRS:

INRA – Centre de Recherches de Dijon
Laboratoire de Recherches sur la Flore Pathogène dans le Sol
17, Rue Sully – BV 1540
F-21034 Dijon Cedex
France

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Preface

This bulletin contains the proceedings of the joint meeting between the “Greenhouse, Nursery, & Ornamental Landscape IPM Working Group” (IOBC/NRS) and WG “Integrated Control in Protected Crops, Temperate Climate” (IOBC/WPRS – the 11th full meeting).

The bulletin has kept its volume compared to our last meeting in Brest, 1999 which I find impressive – 74 contributions on all kinds of aspects of biological and integrated pest management in protected crops in Europe and North America. I thank all contributors for your efforts trying to comply with Authors Instructions. Only few contributions had to be returned for shortening. All the same, a number of articles violated the instructions by e.g. setting aside page format. I have corrected this without altering the text in most cases. However, in a few instances slight changes or rephrasing of the text has been necessary to keep the paper within 4 pages.

A few contributors have complained about the limitation of 4 pages for each contribution – which has been the tradition of our WPRS WG for several years and which here has been strictly enforced, except for a few invited speakers. Of course 4 pages is not much, but on the other hand meeting this limitation can become an art in itself. More importantly, the purpose of our bulletin is to present the latest developments within specific topics to increase impetus for discussions at the meeting and to facilitate pinpointing of possible collaborators among contributing colleagues. Finally, I must point out that no limits on page numbers or a limit of e.g. 8 pages per contribution would cause our bulletin to swell out of all proportions (making it expensive too). Imagine having to browse a bulletin of about 600 pages before the meeting!

The local arrangements for the “Victoria 2002” meeting was organised by David Gillespie, Bob Costello, Brian Spencer, Peter Isaacson, Jim Matteoni, Barb Peterson and Anna Luczynski, who have done a tremendous job to ensure us a scientifically and socially inspiring stay in British Columbia. On behalf of the organisers and the participants I thank David and his team for their great efforts. At the same time I extend my thanks to Kevin Heinz and Les Shipp, convenors of the NRS WG, for their efforts as co-organisers of the Victoria meeting.

Finally, I am thankful for the assistance rendered by Sonja Graugaard, Danish Institute of Agricultural Sciences, Department of Crop Protection, in adjusting the manuscripts and compiling this bulletin.

Annie Enkegaard, Convenor WPRS WG
8th February 2002



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Prospects for the insect parasitic nematode *Thripinema nicklewoodi* (Siddiqi) against Western flower thrips, *Frankliniella occidentalis* (Pergande) in ornamentals

Steven Arthurs, Kevin M. Heinz

Biological Control Laboratory, Department of Entomology, Texas A&M University, College Station, Texas 77843-2475, USA, E-mail: KMHeinz@neo.tamu.edu

Abstract: There is an urgent need for effective biological control agents for western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) infesting greenhouse ornamentals. We are evaluating potential introduction strategies for the thrips parasitic nematode, *Thripinema nicklewoodi* Siddiqi, which although not lethal causes sterility of female WFT. Laboratory studies show that the nematode can infect and reproduce in WFT over the range of temperatures recorded in a Texas (U.S.A.) commercial greenhouse during thrips outbreak periods. Moreover, greenhouse studies using potted chrysanthemums demonstrate that it may establish within WFT populations following low level inoculation. However, relatively poor transmission and slow speed of kill prevented it from being effective over a single crop cycle. *T. nicklewoodi* may have value in a longer term thrips management strategy and/or in combination with other biological control agents.

Key words: *Thripinema nicklewoodi*, Western flower thrips, nematodes, biological control

Introduction

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), has become a serious and widespread pest of over 200 species of vegetables and ornamentals due to direct feeding damage, disease vectoring and control costs (Lewis, 1997). The routine use of agrochemical insecticides as a management strategy against WFT has come under increasing scrutiny due to emerging problems of direct and cross resistance to commonly used insecticides and the need to integrate biological control tactics for concurrent pest problems (Parrella & Murphy, 1996; van Lenteren & Loomans, 1998).

However, while predacious phytoseiid mites and anthocorid bugs have successfully controlled outbreaks of WFT in protected vegetable crops, attempts at biological control has failed to provide acceptable protection for ornamental plants such as chrysanthemums (Hessien & Parrella, 1990; Parrella & Murphy, 1996) and marigolds (Smitley, 1992). On such host plants, WFT typically feed within open flowers and buds, areas that are generally impenetrable for insecticides and most natural enemies.

Entomopathogens formulated as biopesticides are attractive thrips biological control agents due to their small size, ability to be vectored and transmitted in cryptic areas and if necessary applied as an insecticide compatible spray. No effective protozoan or bacterial pathogens are known and thrips viral pathogens cannot be manipulated as inoculative biological control agents (Brownbridge, 1995). The use of entomopathogenic fungi appears to hold more promise, and several strains have shown short term potential against WFT in laboratory trials (Helyer *et al.*, 1995; Parrella & Murphy, 1996; Vestergaard *et al.*, 1995).

Unique amongst thrips natural enemies, the allantonematid nematode, *Thripinema nicklewoodi* Siddiqi, naturally and obligatorily parasitizes *F. occidentalis* residing within the

flower buds and foliar terminals, causing sterility of females without killing them. In a year long survey of field- and greenhouse-grown carnations, chrysanthemums and roses in California, *T. nicklewoodi* was the numerically dominant natural enemy associated with *F. occidentalis* inhabiting flower buds; representing 88.3% of all natural enemies recovered (Heinz *et al.*, 1996). This has led to speculation on the value of introductions of such nematodes to biological control, as *T. nicklewoodi* will target the pest population in its key habitat. By contrast, entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* only have a high attack rate against thrips pupal stages in the soil (Ebssa *et al.*, 2001; Helyer *et al.*, 1995).

We report on studies evaluating the ability of *T. nicklewoodi* to infect WFT and develop under typical greenhouse temperatures during WFT infestation periods and on the nematode's ability to suppress WFT on potted chrysanthemums using an inoculative release of nematode infective WFT.

Material and methods

T. nicklewoodi colony

A culture of *T. nicklewoodi* has been maintained for more than 2 years using a simple *in vivo* method. Nematode transmission between WFT actively releasing nematodes and susceptible immature WFT stages occurs in 1.5 ml Eppendorf vials maintained at 100% humidity with 2×8 mm bean leaf discs. Following a 48 hour infection period, exposed thrips are reared on bean leaves in polypropylene containers (15 × 15 × 5 cm) for 12 days at 25°C to allow *T. nicklewoodi* to develop.

Laboratory temperature bioassay

To assess the ability of *T. nicklewoodi* to thrive in typical temperatures found within commercial greenhouses, we exposed infection arenas and rearing boxes (6 replicates per treatment) separately to a range of constant and fluctuating temperatures in the laboratory and measured the proportional infection and development of second generation *T. nicklewoodi*.

Greenhouse studies

The ability of a *T. nicklewoodi* inoculum to suppress WFT was evaluated on infested potted chrysanthemums (*Dendranthema grandiflora* cv. Golden Polaris). WFT populations on pots with a low inoculum of nematode-infected WFT (introduced approximately 8 weeks post transplanting) were compared to pots receiving no inoculum. Each plant was infested with 38 WFT larvae of various instars and 4 mated adult females. In treated lines, 2 of the adults were infected with and actively releasing second generation *T. nicklewoodi* (~5% inoculation). There were 4 plants per replicate and 6 replicates. Replicate plants were placed in ventilated plastic buckets (30 diam. × 40 cm) with clear plastic lids and maintained in a greenhouse. Plants were destructively harvested after 25 days when in full bloom (approximately 1½ thrips generations) to assess thrips numbers and rates of *T. nicklewoodi* infection in adult female thrips. We repeated the study at a higher starting thrips density and infection rate (mean 170 thrips per plant / ~11% inoculation).

Results

Laboratory temperature bioassay

Immature stages of WFT were readily infected (>60%) at 15-25°C, with an optimum at ~20°C and an upper limit ~30°C. Following infection, second generation *T. nicklewoodi* developed

over a similar temperature range, although with an upper limit close to 35°C. Climate data we recorded over 2 years in a commercial greenhouse (Brenham, Texas) suggests temperatures do not generally fall below 15°C or exceed 30°C in early March through mid June, when WFT are most abundant. While this suggests that upper daytime temperatures may restrict infection (but not development), exposure to daily fluctuating temperature in the laboratory suggests that *T. nicklewoodi* may readily infect WFT during periodically cooler (nighttime) temperatures.

Greenhouse studies

The impact of *T. nicklewoodi* inoculations on WFT densities in potted chrysanthemums is shown in Fig. 1. In neither case did *T. nicklewoodi* affect final WFT densities compared with untreated controls. However, in both cases, *T. nicklewoodi* established and increased 2-3 fold during the ~2 nematode generations represented.

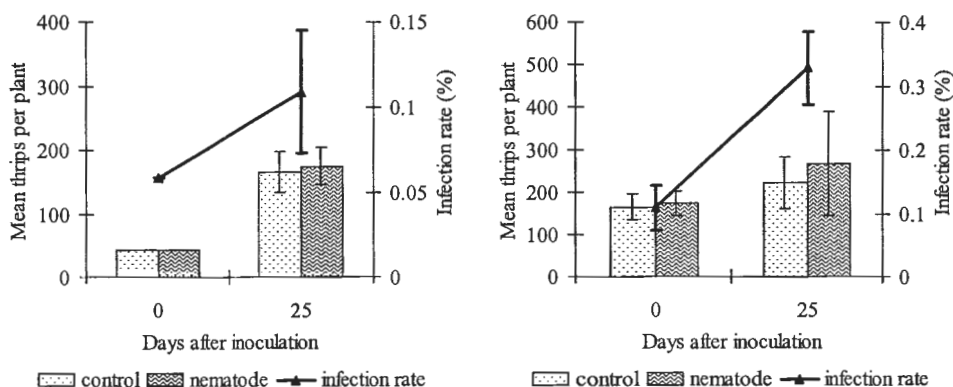


Figure 1. Mean thrips densities and nematode infection rates (in adult female thrips) (\pm SEM) on greenhouse chrysanthemums before and 25 days after low level inoculation with the thrips parasitic nematode *T. nicklewoodi*.

Discussion

Currently lack of mass rearing techniques limit the potential of inundative release of *T. nicklewoodi*. However, unlike the application of rhabditid nematodes, which are typically short lived in foliar environments and have not been shown to be effective against thrips, our studies show *T. nicklewoodi* may establish in WFT infested greenhouse ornamentals (at least under the host densities used) following low level inoculation. Moreover, the laboratory assays indicated the nematode would persist within the temperatures we recorded in Texas commercial greenhouses throughout the thrips infestation period.

However, our greenhouse trials were unable to demonstrate a reduction in thrips densities within a single chrysanthemum crop following low level inoculation of the nematode at the first open bud stage. Thus *T. nicklewoodi* is unlikely to offer a short-term control solution, at least following relatively low inoculation rates. The limited effect of *T. nicklewoodi* may be explained by the relatively poor transmission success and slow speed of kill (infected thrips live about the same time as healthy ones suggesting a delayed effect). This latter point is

backed up by the different adult: larval ratio between treated and control thrips lines at the end of the second run of greenhouse study. The ratio was 3.5 fold higher in the treated lines suggesting nematode infection caused a relative accumulation of adults over immature stages. This suggests a reduction in thrips population in a single crop may occur at higher nematode inoculation densities rates than we tried, or over a longer time period when thrips are moving between batches of crops/refugia. In a laboratory study, (Mason & Heinz, 1999) detected no significant reduction of WFT numbers on rooted bean plants in small rearing boxes following low level inoculation of *T. nicklewoodi* until the fifth thrips generation, when the experiment was terminated. We plan to investigate the potential of early season introductions of *T. nicklewoodi*, alone and in combination with other control tactics such as using predatory bugs, in a longer term thrips management strategy.

Acknowledgements

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The costs of biological pest control in protected tomato crops

Andrzej Bednarek¹, Wojciech Goszczyński²

¹Warsaw Agricultural University (SGGW), Department of Animals Environment Biology, Nowoursynowska Str. 166, 02-789 Warsaw, Poland, E-mail: abednarek@eranet.pl; ²Catholic University in Lublin (KUL), Department of Plant Protection, Al. Raclawickie 14, 20-950 Lublin, Poland

Abstract: Five commercial greenhouses near Warsaw were examined for the evaluation of costs of full biological control of tomato culture pests. The total area of greenhouse blocks ranged from 9000 to 20000 m². Growers used inert substrate, drip irrigation system, climate regulation and natural pollination system. Natural enemies and the knowledge of usage were supplied by the Dutch Company Koppert B.V. All growers were able to reduce whiteflies, leaf miners, spider mites and aphids with the use of beneficial insects only. No zoocides were applied during the vegetation period. The total costs of biological pest control differed in relation to specific farms and ranged from 0.74 to 1.08 PLN/m². In 1998 it was equal to the price of 0.5 kg tomatoes or 1% turnover.

Key words: tomato crops in greenhouses, biological pest control, natural enemies, cost of plant protection, whiteflies, mites, leaf miners

Introduction

In Poland, protected tomato crops on inert substrates, pollinated by bumblebees and largely bio-protected have become a standard (Bednarek *et al.*, 1996). At present, tomato crops comprise over 80% of all the crops cultivated under covers. The average yield is 35 kg, and sometimes it can even reach 51 kg/m². Natural enemies are applied almost exclusively in greenhouses of 360 ha area, which comprises over 45% of the highly modernised greenhouse tomato crops. It is estimated that in northern Europe natural enemies are applied on tomato crops surface area of 1410 ha (O'Neill, 1990). Our study aimed at assessing the possibilities to eliminate zoocides in greenhouse tomato crops, and apply biological control instead. It is essential here to assess the costs of biological control and their share in total production costs. In Polish realms, in which the price of the offered products is still low, the costs of their production are decisive when it comes to the choice of crop technology.

Material and methods

The research was carried out in family greenhouses in the region of Karczew village, near Warsaw in 1999. The farms there have specialized in tomato crops (mainly the Dutch variety Cunero) in soil-less technology (mineral wool mats cultivation). The observations were carried out in farms, of which the total surface area ranged from 8000 to 20000 m². Prior to plants introduction to a final destination spot, the construction of the greenhouses were disinfected with sodium hypochlorite. Before the mats were provided, the greenhouses were laid out with white garden foil in order to isolate the plants from the soil. Seedlings were produced by growers in rock wool blocks. The cultivation was carried out since the beginning of February until the end of October. The plants were pollinated by bumblebees (*Bombus*

terrestris). 20 yellow sticky traps per 1 ha were distributed in order to carry out a scouting of pest population. The occurrence of pests was observed weekly.

The growers applied natural enemies supplied by the Dutch supplier Koppert BV. In the control of whitefly (*Trialeurodes vaporariorum*), *Macrolophus caliginosus*, a predatory heteropteron and 2 parasitoid species, *Encarsia formosa* and *Eretmocerus californicus*, were applied. In the early period of cultivation (until the appearance of the first winged whiteflies), sterile eggs of the butterfly *Ephestia kuehniella* were applied to feed *M. caliginosus*. In order to fight leaf miners (*Liriomyza* sp.), two predatory hymenopterons, *Dacnusa sibirica* and *Diglyphus isaea*, were introduced simultaneously. Spider mites (*Tetranychus urticae* and *T. cinnebarinus*) were controlled by introducing predatory mites *Phytoseiulus persimilis*, a variety selected on tomato, and a predatory hymenopteron, *Feltiella acarisuga*. Dipterous predator *Aphidoletes aphidimyza* and hymenopterous parasitoid *Aphidius ervi* were introduced for aphid control. In the greenhouse neither insecticides nor acaricides were applied. Fungicides were applied sporadically, only in particular cases in late Autumn.

The assessment of economical results of biological control was based on the summing up of purchase costs of the introduced natural enemies, as well as on the application of sticky traps according to current prices. Labour costs related to the display of entomophags and crops monitoring were not taken into account. Moreover, the costs of natural pollination were not taken into consideration.

Results and discussion

Whiteflies were found to occur in all the farms, although its intensity varied in particular farms. It occurred regularly in previous years. Therefore, in the strategy of pest control preventive procedures took place. These were based on the introduction of heteropterous predator, *M. caliginosus* to the surface of crops before the whitefly appeared (table 1). In the first month, on average 0.6 individual per m² were displayed. Since leaf miners and spider mites, which are an alternative food for this predacious insect, did not occur in that season, there arose a need to introduce the eggs of butterfly *E. kuehniella*. Only in farms no. 1 and 5 *E. formosa* was applied (0.75 individuals/m²), after first individuals of whitefly were observed on the sticky traps. In the spring and summer when the greenhouse temperature reached over 25°C, *E. californicus*, was applied in addition to two of the farms. *E. californicus* is characteristic for resistance to high temperature. On average, 0.72 individuals/m² of *M. caliginosus* and 7.18 individuals/m² of *E. formosa* and *E. californicus* were introduced altogether during the entire season.

Leaf miners were found to occur in all the greenhouses. Their numbers, however, were relatively low because of an early application of *M. caliginosus*. *D. sibirica* (75%) and *D. isaea* (25%) were introduced in order to limit the pest population numbers. In one farm only was there a need to provide extra *D. isaea*. It has a capacity to inhibit the growth of leaf miners larvae. On average 0.54 individuals/m² of *D. sibirica* and *D. isaea* were introduced altogether. Spider mites occurred only in farm no. 2, where *P. persimilis* and *F. acarisuga* were applied in spider mites spots. On average 0.54 individuals/m² were applied. Moreover, *Aulacorthum solani* - the glasshouse and potato aphid, appeared in farms no. 3 and 5. Then, *A. ervi* and *A. aphidimyza* (0.27 ind./m²) were applied.

An analysis of pests occurrence in the investigated farms shows that *T. vaporariorum* was a dominating species there. Other species were not significant, except for leaf miners in two farms. It corresponds to the situation in other Northerneuropean countries, where these two species are considered to belong to the group of the so-called key pests in the greenhouse tomato crops (Onillon, 1990; van Lenteren *et al.*, 1992). When comparing the amount of *M.*

caliginosus that was applied in the farms with the European data, one may observe that it is smaller in Poland. Gabarra & Besri (1999) have observed that in Northeuropean conditions 4 individuals/m² of this heteropteron is displayed 2-3 times. Also the amount of *E. formosa* that was displayed in the farms was smaller. According to these authors, in Europe one applies 2-28 individuals/m², within at least 4 introductions.

Table 1. Introduction of natural enemies and the cost of biological pest control in protected tomato crops in five greenhouse farms in central Poland in 1999.

Natural enemies	Farm No. /area (m ²)	1/20000	2/10000	3/9000	4/8000	5/20000
	Total cost (EURO)	4,212.78	2,215.64	2,711.94	1,758.61	4,578.05
<i>Macrolophus caliginosus</i>	First introduction	28.04	18.03	11.03	27.05	11.03
	# of introductions	2	3	1	1	4
	Avg. ind./m ²	0.60	0.60	0.55	0.50	0.81
<i>Encarsia formosa</i>	First introduction	29.04	27.05	10.06	17.06	22.04
	# of introductions	8	5	4	9	6
<i>Eretmocerus californicus</i>	Avg. ind./m ²	6.46	9.30	6.60	10.36	3.04
<i>Dacnusa sibirica</i>	First introduction	15.07	17.06	11.03	1.07	11.03
	# of introductions	3	2	1	2	2
<i>Diglyphus isaea</i>	Avg. ind./m ²	0.38	0.25	0.5	0.63	0.65
<i>Phytoseiulus persimilis</i>	First introduction			17.06		
	# of introductions			2		
	Avg. ind./m ²			2.6		
<i>Feltiella acarisuga</i>	First introduction			10.06		
	# of introductions			1		
	Avg. ind./m ²			0.06		
<i>Aphidoletes aphidimyza</i>	First introduction			10.06	10.06	
	# of introductions			2	1	
<i>Aphidius ervi</i>	Avg. ind./m ²			1.02	0.31	

ind.=individuals

On the basis of current prices of natural enemies offered by the ROL-EKO Company, a distributor of the Koppert products in Poland, the costs of predators, parasites and sticky traps introduction were calculated (table 1). The costs varied from 0.21 to 0.30 EURO/m². Then the costs of the applied biocontrol agents were confronted with the median value. With the average yield of 35 kg/m², and mid-1999 tomato price of 0.97 EURO/kg, the costs of natural enemies and traps purchase was less than 1% of the obtained income which equals the price of 0.3 kg tomatoes. Similar results were obtained by Nawrocka *et al.* (1996) in case of the integrated tomato crop protection. In this case the plant protection costs were equivalent to the income obtained from the sale of 0.5 kg tomatoes. The authors observed that as insecticides were applied, the costs were comparable with 0.5 or 0.7 kg tomatoes, respectively, depending on the technique of spraying. The studies carried out in eight greenhouses of the total area of 600-110000 m² showed the costs of the applied entomophags which were 0.19-0.28 EURO/m² (Bruzda, 1998). In the Netherlands, van Lenteren (1990) compared the costs of whitefly control with the use of zoocides and *E. formosa*. The results obtained pointed out to the fact that the biological method of this pest control was 50% cheaper in comparison with the chemical method. Nevertheless, after the costs of risks connected with the use of insecticides (phytotoxic effects, delayed yield) were calculated, the costs of chemical control may increase, reaching up to 0.88 EURO/m² (Nawrocka *et al.*, 1996). Certainly, in the biological control method such risks do not take place.

The presented results provided above by other authors point out that the costs of biological pest control in tomato crops under covers are comparable or less than the chemical protection method.

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Choice of predatory mites for biological control of ground-dwelling stages of western flower thrips within a 'push-pull' strategy on pot chrysanthemum

Jude Bennison, Kerry Maulden, Heather Maher

ADAS Boxworth, Boxworth, Cambridge, CB3 8NN, UK, E-mail: Jude.Bennison@adas.co.uk

Abstract: Laboratory bioassays with *Stratiolaelaps (Hypoaspis) miles* and *Gaeolaelaps (Hypoaspis) aculeifer* showed that both species had similar daily predation rates on western flower thrips second instar larvae and pupae. A glasshouse experiment with caged pot chrysanthemums indicated that both predators multiplied at similar rates and gave similar reductions in thrips numbers over a 5-week period. Results to date suggest that both predators should have similar potential against ground-dwelling stages of thrips in a 'push-pull' biological control strategy being developed for chrysanthemum.

Key words: western flower thrips, *Frankliniella occidentalis*, *Orius laevigatus*, *Stratiolaelaps (Hypoaspis) miles*, *Gaeolaelaps (Hypoaspis) aculeifer*, pot chrysanthemum, biological control

Introduction

Reliable biological control methods for western flower thrips (WFT), *Frankliniella occidentalis* still need to be developed for chrysanthemums. Many growers rely on regular use of dichlorvos for control of WFT, but this pesticide is likely to be withdrawn. There are no alternative effective insecticides and pressures are increasing to minimize pesticide inputs.

A 'push-pull' strategy is being developed, whereby semiochemicals are used to repel (push) WFT from the crop and attract (pull) them to 'lure' chrysanthemum cultivars where predators can be released or attracted for cost-effective control of all thrips life stages (Bennison *et al.*, 2001). Pot chrysanthemums are being used as a model crop and flowering cv. 'Swingtime' was selected as a suitable 'lure' plant. *Orius laevigatus* are being used in the strategy, as both adults and nymphs were shown to be good predators of both WFT adults and larvae on 'Swingtime' buds and flowers. A biological control agent against ground-dwelling thrips stages would be a useful component in the strategy, as 97% second instar WFT larvae (L2's) were shown to fall from 'Swingtime' plants to pupate in the compost or substrate.

Stratiolaelaps (Hypoaspis) miles and *Gaeolaelaps (Hypoaspis) aculeifer* are being used increasingly within IPM on various protected crops, not only against sciarid flies, but also for supplementing other beneficials against WFT. However, there is little data on their comparative efficacy against WFT. On cucumber, both *G. aculeifer* and *S. miles* have contributed to WFT control (Gillespie & Quiring, 1990; Wilde, 2001). On Saintpaulia, *G. aculeifer* reduced WFT increase but *S. miles* did not (Glockeman, 1992). Laboratory studies indicated that *G. aculeifer* has greater potential against WFT than *S. miles* (Altena *et al.*, 1997). On pot roses, *S. miles* reduced numbers of WFT adults (Linnamäki *et al.*, 1998) but *G. aculeifer* was considered ineffective on cut roses grown in rockwool or peat (Vänninen & Linnamäki, 2001). The work described in this paper aimed to compare the predation and multiplication rates of *S. miles* and *G. aculeifer* on WFT on pot chrysanthemums, in order to select which species to use in the development of the push-pull strategy.

Materials and methods

***S. miles* and *G. aculeifer* predation on WFT larvae and pupae - laboratory bioassays**

Five WFT late L2's or pupae were offered to a single, unstarved adult female predator of each species on either 1 cm-depth damp compost or a 6 cm-diameter disc of capillary matting, placed in a tightly-fitting, ventilated plastic Petri dish. Pre-pupae were not included as this stage lasts for only two days at 21°C and predation rates were assumed to be similar to those on L2's or pupae. The dishes (6 cm diameter, 3 cm depth) had a 1cm layer of set plaster of Paris and charcoal in the base, which was dampened with water. Ten replicate dishes were used for each predator species, thrips life stage, substrate and controls without predators. The dishes were incubated for 24 hrs at 21°C, 11:13 hrs L:D, consistent with chrysanthemum production conditions. After 24 hrs, the numbers of predators and live or dead thrips per dish were recorded. Predated pupae appeared as flattened, shrivelled empty skins and sometimes the only recognisable larval remains were the head and antennae.

Population increase of S. miles and G. aculeifer and WFT control on pot chrysanthemum - cage experiments

Twenty WFT females were released to individual pot chrysanthemum cv. 'Swingtime' plants at the opening bud stage in 12 mesh cages (0.5 x 0.5m) in a glasshouse. Four replicate cages were used for each of three treatments: untreated, *S. miles* and *G. aculeifer*, in a randomised block design. Predators were released at 50 per plant i.e. 15 females, 9 males, 24 nymphs and 2 eggs, consistent with the mean life stage ratios present in the fresh commercial packs of the two species. The predators were released to the compost 12 days after WFT release, when sticky traps under plants in an extra cage showed that L2's had started to drop for pupation.

All plants had been grown in a thrips-free glasshouse and had been treated only with *Aphidius colemani* as a precaution against aphids. The plants were eight weeks old when placed in the cages, thus invertebrates such as sciarid fly larvae and springtails were available as alternative prey for the predatory mites, as on a commercial nursery. Following predator release, the plants were left in the cages for a further five weeks, at min. night temperature 18°C, venting at 21°C, 11:13 hrs L:D. After five weeks, total numbers of WFT, *S. miles* or *G. aculeifer* per cage were assessed. Each plant was cut off at compost level and tapped vigorously over a large white plastic tray and numbers of WFT adults and larvae falling onto the tray were recorded. *S. miles*, *G. aculeifer* and WFT in the compost were extracted using Tullgren funnels for one week. Any predatory mites or WFT remaining in the cages were trapped on two blue sticky traps per cage over a further two weeks.

Results and discussion

Predation rate bioassays

The data were analysed using a Generalised Linear Model (GLIM) in Genstat, treating the data as binomial. Predation rates on L2's on capillary matting were not analysed as the L2's tended to burrow into the matting fabric, making it difficult to judge predation. The mean predation rates of each predatory mite on WFT L2's and pupae were statistically similar over 24 hrs and are given in table 1. Numbers of pupae predated by *S. miles* are similar to those reported by Brødsgaard *et al.* (1996), who recorded mean 24 hr predation rates of 1.2 and 1.7 when offered 10 WFT pupae as a choice of prey with mushroom sciarid larvae, *Lycoriella solani* or entomopathogenic nematodes, *Steinernema feltiae* respectively. However, the results differ from those given by Altena *et al.* (1997), who reported that daily predation rates on WFT pupae were significantly higher for *G. aculeifer* (2.6) than for *S. miles* (1.8). These

results may have varied from those in this paper, due to slightly different experimental conditions used (Mulder, personal communication).

Table 1. Numbers of WFT pupae and L2's predated by single female *S. miles* and *G. aculeifer* on compost or capillary matting over 24 hrs.

Predator species	Substrate	Mean no. WFT pupae predated \pm S.E.	Mean no. WFT L2's predated \pm S.E.
<i>S. miles</i>	Compost	1.5 \pm 0.7	1.7 \pm 0.3
	Capillary matting	0.9 \pm 0.6	-
<i>G. aculeifer</i>	Compost	0.7 \pm 0.5	1.3 \pm 0.3
	Capillary matting	1.3 \pm 0.6	-

Cage experiment

The data were subjected to log transformation and analysis of variance (ANOVA). The mean numbers of *S. miles*, *G. aculeifer* or WFT per cage are given in table 2. As final numbers of WFT were unusually low in one control cage, this block was omitted from the analysis, giving only three replicate cages per treatment. Population increases of *S. miles* and *G. aculeifer* were statistically similar after 5 wks, i.e. 5.2 and 4.9 x as many respectively as those released.

Table 2. Mean numbers of *S. miles*, *G. aculeifer* and WFT adults and L2's 5 wks after predator release. (Log-transformed means given in brackets below back-transformed means).

Predatory mite sp.	Mean no. predatory mites (log-transformed S.E.D. = 0.19)	Mean no. WFT adults (log-transformed S.E.D. = 0.10)	Mean no. WFT larvae (log-transformed S.E.D. = 0.21)
Untreated control	0	745.6 (2.87)	272.7 (2.44)
<i>S. miles</i>	257.5 (2.41)	307.0 * (2.49)	32.7 * (1.53)
<i>G. aculeifer</i>	246.4 (2.39)	250.6 * (2.40)	48.3 * (1.69)

* significantly different from the control, P<0.05

The analysis indicated that numbers of WFT adults and larvae were significantly lower in cages treated with either *S. miles* or *G. aculeifer* than in the control cages, with no significant differences between the two predators. Mean numbers of WFT adults were 59% and 66% lower in cages treated with *S. miles* and *G. aculeifer* respectively, than in the control cages. These values are similar to the approximately 50% control of WFT adults reported by Vänninen & Linnamäki (2001) in pot roses treated with 50 *S. miles* per pot. However, the same authors reported that *G. aculeifer*, released to maintain densities of at least 500 per m² did not contribute to WFT control on cut roses grown in rockwool bags or peat beds.

The data indicates that either *S. miles* or *G. aculeifer* may contribute equally to WFT control on pot chrysanthemums when used to supplement other natural enemies. It is possible

that other species of predatory mites may have more potential against WFT pupae in the future (Vänninen & Walter, 2001). *S. miles* was selected for further research in this project and is currently being used in a repeated cage experiment with additional replicates over a longer time period. The role of both *O. laevigatus* and *S. miles* in controlling WFT in the push-pull strategy will be evaluated in glasshouse experiments during the rest of the project.

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IPM on protected hardy ornamental nursery stock in the UK

Jude Bennison¹, Roger Umpelby², John Buxton²

¹*ADAS Boxworth, Boxworth, Cambridge, CB3 8NN, UK, E-mail: Jude.Bennison@adas.co.uk;*

²*ADAS Rosemaund, Preston Wynne, Hereford, HR1 3PG, UK*

Abstract: The use of biological control agents within IPM on protected hardy ornamental nursery stock in the UK is increasing. Examples of IPM methods for the major pests are given, and likely future developments are discussed.

Key words: pests, biological control, integrated control, IPM, hardy ornamental nursery stock

Introduction

Hardy ornamental nursery stock (HNS) is a valuable horticultural commodity in the UK, currently grown on 8,688 ha (DEFRA, 2001). Over the past ten years, the proportion of this area grown in containers under protection has increased, and IPM is being used on a greater number of these crops, both in glasshouses and polythene tunnels. Growers adopting IPM are now using more comprehensive biological control programmes. The main drivers for this increased uptake have been severe pesticide resistance in some of the major pests, withdrawal of pesticides, demand for quality plants grown with sustainable, environmentally-responsible methods, and concern for the staff working environment. The successful adoption of IPM on HNS has been encouraged and made possible by training and advice given by IPM consultants including ADAS and the biological control suppliers.

IPM programmes for HNS

The HNS market demands high quality, pest-free plants, therefore IPM strategies aim for pest 'overkill', rather than a balance between pests and natural enemies as on edible crops. IPM programmes must be planned and managed carefully, and this requires an extensive knowledge of which pests attack each of the huge range of plant species and varieties grown, and of how each beneficial performs on each plant at all production stages. Conditions are not always ideal for some biological control agents, e.g. liner and container units are given only frost protection, thus diurnal temperatures can fluctuate widely. Research is beginning to address gaps in knowledge to improve biological control methods on protected HNS. Meanwhile, growers and advisers are continually gaining practical experience and using this to further develop the use of biological control within IPM.

Control of the major pests within IPM

A summary of currently used IPM methods for the major pests of protected HNS, and the main problem areas are given below.

Aphids

Aphids are one of the main pests and are best controlled in the early stages of infestation by inoculative releases of parasitoids, supplemented with predators, mainly *Aphidoletes aphidimyza*, if necessary. The melon and cotton aphid, *Aphis gossypii* is the main species. This aphid is resistant to many pesticides and has a wide host range, *Euonymus* and *Hebe* are examples of highly susceptible plants. The potato aphid, *Macrosiphum euphorbiae* is also common with a wide host range, *Cistus* and *Photinia* are particularly susceptible. *Aphidius colemani* and *Aphidius ervi* can give good control of *A. gossypii* and *M. euphorbiae* respectively if releases are managed well, and the use of 'banker plants' has improved control on some nurseries. Biological control of some of the more host-specific aphids can be a problem and reliable methods need to be developed. IPM-compatible aphicides e.g. imidacloprid, pirimicarb or pymetrozine are used for control when necessary.

Mites

Two-spotted spider mite, *Tetranychus urticae* is the main mite pest species and is a major HNS pest. Careful management of *Phytoseiulus persimilis* releases can give effective control on many HNS hosts. However, control on highly susceptible plants e.g. *Buddleja* and *Sambucus* can be problematical, particularly during hot, dry periods which are unfavourable for *P. persimilis*. The predatory midge, *Feltiella acarisuga* can be used to supplement control, particularly in heavy infestations. *Amblyseius californicus* is now re-licensed for release under protection in the UK, and although slower to establish than *P. persimilis*, it is more tolerant of hot, dry conditions and has been shown to have good potential on HNS (Buxton, 1999). The citrus red mite, *Panonychus citri* is becoming an increasing problem within IPM e.g. on *Elaeagnus* and *Skimmia*. *P. persimilis* seems to be ineffective, but other predatory mite species could have potential. Other mites such as eriophyids and tarsonemids can cause occasional problems but are usually controlled by routine use of *Amblyseius cucumeris* against thrips. IPM-compatible acaricides such as abamectin, fenbutatin oxide or tebufenpyrad can be used if necessary.

Thrips

Western flower thrips, *Frankliniella occidentalis* has become an increasing pest of HNS, due to severe pesticide resistance and increased plant traffic. Growers who had serious problems with chemical control of the pest, now achieve good biological control with routine releases of *A. cucumeris*. Use of *Hypoaspis* spp. against sciarid and shore flies is also thought to contribute to control of thrips ground-dwelling stages. On some nurseries, maintaining biological control on flowering plants e.g. *Clematis* and *Rosa* can be a problem, and research is needed on supplementing control with other potential natural enemies such as *Orius* spp. or entomopathogenic nematodes.

Whiteflies

The glasshouse whitefly, *Trialeurodes vaporariorum* is the most common species, e.g. on *Fuchsia* and *Lavatera*. *Encarsia formosa* can give successful control when temperatures are suitable, but problems with establishment can occur early in the season in structures given only frost protection, leading to problems later on. *E. formosa* does not always work well on aromatic or hairy-leaved plants such as *Salvia* and *Lavendula*. *Verticillium lecanii* can give useful control of whiteflies during propagation when relative humidities and temperatures are suitable. Control of *T. vaporariorum* with compatible pesticides is difficult, due to widespread resistance, including to buprofezin, with some cross-resistance to teflubenzuron (Gorman *et*

al, 2000). Where necessary, imidacloprid can be used, or regular 'soft' pesticides such as fatty acids.

Caterpillars

Caterpillars are an increasing problem within IPM, e.g. on *Choisya* and *Skimmia*. The carnation tortrix, *Cacoecimorpha pronubana* is thought to be the main species but the light brown apple moth, *Epiphyas postvittana*, is becoming increasingly prevalent in S. England and Wales (Umpelby, unpublished data). Correct identification is essential to ensure that appropriate pheromone traps are used for monitoring moth immigration to time control treatments. Caterpillars are difficult to control with *Bacillus thuringiensis* or the IPM-compatible pesticides diflubenzuron or teflubenzuron, particularly tortricid species which are protected within webbed leaves. Research is needed on improved biological control methods, as growers are currently having to resort to broad-spectrum pesticides which disrupt IPM.

Vine weevil

Vine weevil, *Otiorhynchus sulcatus*, is a widespread, major pest of many HNS species and most growers apply routine control measures to prevent larval infestations. Although *Heterorhabditis* or *Steinernema* spp. are used on some HNS nurseries, due to temperature limitations there are difficulties with optimal timing of application, and many growers use routine compost incorporation of imidacloprid or chlorpyrifos for long-term control. Although the side-effects of both these pesticides in the compost have not been fully tested against biological control agents, in practice, both seem to be largely compatible with IPM.

Sciarid flies

Sciarid flies, *Bradysia* spp. can damage young plants during propagation, particularly under adverse growing conditions. *Hypoaspis* spp. are now widely and successfully used against the pest, and *Steinernema feltiae* will also give good control. Shore flies, *Scatella* spp. are not HNS pests but are sometimes confused with sciarid flies and can cause concern if present in large numbers. Avoiding over-watering and good nursery hygiene procedures can reduce problems with both species.

Slugs and snails

Most growers apply molluscicide pellets regularly against slugs and snails, but control is usually inadequate and growers would prefer to use non-chemical control methods. In a current research project funded by HDC and DEFRA, aiming to develop integrated control methods for slugs and snails on HNS, the main slug species was identified as *Deroceras panormitanum* and the semi-aquatic snail *Oxyloma pfeifferi* is also widespread. The parasitic nematodes *Phasmarhabditis hermaphrodita* are being tested against both species. Laboratory studies to date have been shown them to be very effective against *O. pfeifferi* (Bennison & Maher, unpublished data). Potential repellents against slugs and snails have also been identified in the project (Schüder, unpublished data).

Other pests

'Minor pests' e.g. capsids, leafhoppers, scale insects and suckers can become significant pests on certain crops when IPM is adopted and broad-spectrum pesticides are no longer used. Research is needed to develop reliable biological control methods for these pests. However, natural beneficials can sometimes give control within IPM and provide potential new commercial control agents, e.g. the leafhopper egg parasitoid *Anagrus atomus* was developed commercially after giving natural control of the glasshouse leafhopper, *Hauptidia maroccana*

on tomatoes (Wardlow, 1990). The chrysanthemum ('sage') leafhopper, *Eupteryx melissae* is now becoming a serious pest within IPM e.g. on *Salvia* and *Lavendula*. Although *A. atomus* is reported not to attack *E. melissae*, (Cooper, 1993), naturally-occurring strains of the parasitoid were found on this pest on several herb nurseries during a survey funded by the HDC (Bennison, 2001).

Future development of IPM on HNS

The uptake of IPM is likely to increase on protected HNS. All growers who have adopted IPM are realising the benefits of improved pest control, higher quality plants, reduced time inputs and improved working environment. Research is needed to improve biological control of 'difficult' pests or on problem crops, and to employ potential new or unexploited natural enemies. Integrated control methods for diseases need further development and adoption within an Integrated Crop Management (ICM) approach. Growers are now wishing to expand the use of IPM outdoors and this is now a major challenge for researchers. MAFF-funded research confirmed a wide range of naturally-occurring beneficial species on outdoor HNS nurseries (Buxton, 1997). Many growers are trying to use mainly selective pesticides outdoors, to encourage natural enemies and increase biodiversity, and they would be keen to adopt any new IPM methods developed. Continued exchange of ideas and results, and knowledge transfer and advice to growers will continue to be essential for further IPM development and successful uptake.

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Results of quality control tests with *Phytoseiulus persimilis*, *Neoseiulus cucumeris* and *Orius laevigatus* in Austria

Sylvia Blümel, Hermann Hausdorf

BFL, Institute of Phytomedicine, Spargelfeldstr. 191, A-1226 Wien, Austria, E-mail: sylvia.bluemel@relay.bfl.at

Abstract: Batches of mass-reared phytoseiids (*Phytoseiulus persimilis*, *Neoseiulus cucumeris*) and *Orius* species from different commercial producers were controlled for their quality according to standard test guidelines. In 2 out of 22 controlled batches all quality requirements were fulfilled completely, whereas in the remaining batches at least one quality criterion was not met. *P. persimilis* offered on leaf substrate showed especially a non-compliance with regard to an acceptable predator-prey ratio. In *N. cucumeris* batches the required minimum number of individuals was missed considerably. The main quality problem with *Orius* batches was the short longevity of females and in this connection the low cumulative reproduction per female. Reasons for the observed quality problems are discussed.

Key words: beneficial arthropods, mass-rearing, quality control, Phytoseiidae, Anthocoridae

Introduction

For the successful implementation of biological and integrated pest control measures into horticultural production a high quality of mass reared beneficial arthropods, which are released for biocontrol, belongs to one of the key factors. During 1998 and 2001 the quality of several batches of commercially produced predatory mites *Phytoseiulus persimilis* A.H., *Neoseiulus cucumeris* (Oudem.) (Acarina: Phytoseiidae) and of the flower bugs *Orius laevigatus*, *O. majusculus* (Heteroptera: Anthocoridae), which were distributed to the horticultural growers in Austria was checked at the BFL. Standardised quality test guidelines, which were developed by the Global IOBC WG: "Arthropod Mass-Rearing and Quality Control" were applied during this study.

Materials and methods

Test organisms

Four batches of *P. persimilis* offered on detached bean leaves (*Phaseolus vulgaris* as substrate) were quality tested in 1998 according to the guideline proposed by Steinberg & Dale (1998) for *P. persimilis* in carrier material. Five batches of the predatory mite *N. cucumeris* in carrier material (wheat bran) from 1999 and six batches from 2000 were quality checked according to van Schelt & Stepper (1998). Four batches of either *O. majusculus* or *O. laevigatus* from 2000 and 2001 were quality investigated according to the method described by Tommasini & Bolckmans (1998).

Quality criteria

Quality criteria tested included:

- the compliance of the number of living individuals on the batch label and actually present in the batch
P. persimilis: minimum batch size: 500 mobile individuals (assessment of number of *P. vulgaris* leaves per batch and of number all live and dead developmental stages of *T. urticae* and *P. persimilis* on 20 randomly selected leaves)
N. cucumeris: minimum batch size: 25000 mobile individuals
Orius sp.: minimum batch size: 500 mobile individuals
- sex-ratio
P. persimilis > 70% females (n=100)
N. cucumeris > 50% females (n=100)
Orius sp. > 45% females (n=100)
- longevity
P. persimilis: at minimum 5 days for 80% of females, minimum sample 30 females (out of technical reasons 4 days were observed, n: 24 females)
- cumulative reproduction per female
P. persimilis: within 5 days after first egg-laying ≥ 10 eggs/female (n: 24 females) (out of technical reasons 4 days were observed, ≥ 8 eggs/female (n: 24 females)
N. cucumeris: > 7 eggs/female within 7 days from the 2nd test day on (n: 30 females)
Orius sp.: ≥ 30 eggs/female within 14 days (n: 30 pairs)

Standard test conditions

Temperature: $25 \pm 1^\circ\text{C}$ ($22 \pm 1^\circ\text{C}$ for *N. cucumeris*), r.h. $65 \pm 5\%$, photoperiod 16h L: 8h D.

Results and discussion

Phytoseiulus persimilis

In all four controlled batches the number of predatory mites actually present exceeded the amount stated on the label. The surplus of mobile stages ranged between 1.2% and 195.6% and increased up to 285.6% if the eggs were included (table 1). The exceeding of the amount of phytoseiids actually present in the batch compared to the label statement might on the one hand turn out as a long term economic loss for the producer and on the other hand could lead to problems for the grower, because biocontrol success might be related to label statement amounts rather than to the actually introduced number of macrobials. Therefore, if *P. persimilis* from another producer with better compliance of phytoseiid amounts present in the batches and their label statements were introduced, the actually released numbers would be lower than with the leaf substrate described above, which might lead to biocontrol problems.

The percentage of females from the number of living mobile phytoseiids ranged between 54% and 66%, however in comparison to the total number of mobile stages (dead + living) in the batches this portion reached only 40%, thus missing the required 70%. Between 85% and 95% of the females lived up to 5 days at minimum, thus the required quality limit for this criterion was met. The mean cumulated reproduction per female (n = 24) within 4 days ranged between 7.7 (95% CI: 5.5 – 9.8) and 9.3 (95% CI: 7.2 – 12.1) and was neither between nor within the batches significantly different (ANOVA-SPSS, Bonferroni-Test, $\alpha \leq 0,05$). One out of four tested batches did not match the quality criteria with regard to both the percentage of females and the reproduction per female.

Table 1. Quality test results for *P. persimilis* on leaf substrate.

Batch date	leaves/ batch	n <i>PP</i> mobile	n <i>PPs</i> eggs	% <i>PP</i> * mobile	% <i>PP</i> * total	% <i>PP</i> dead	% live F	% F > 5d longevity
06.04.98	46	1012 ¹	307	101.2	131.9	15.2	54	85
15.06.98	69	1478 ²	450	295.6	385.6	13.4	66	95
13.07.98	93	980 ²	587	196.0	313.4	1.7	56	85
16.08.98	91	1298 ²	763	259.6	412.2	0.2	65	95

*% of label statement, *PP*: *Phytoseiulus persimilis*, F: females, ¹label statement: 1000 *PP*,
²label statement: 500 *PP*

The predator: prey ratio for mobile stages was in 1 out of 3 batches unacceptably high (>1:10) (table 2), which could result in additional infestation with *T. urticae*, rather in controlling the pest.

Table 2. Predator: prey ratio in quality control samples of *P. persimilis*.

Batch date	n <i>TU</i> mobile	n <i>PP</i> mobile	<i>PP</i> : <i>TU</i> ratio mobile	n <i>TU</i> total	n <i>PP</i> total	<i>PP</i> : <i>TU</i> ratio total
15.06.98	3180	275	1 : 11.6	10738	376	1 : 28.6
13.07.98	597	184	1 : 3.2	3066	304	1 : 10.1
16.08.98	1907	248	1 : 7.7	4442	334	1 : 13.3

TU: *Tetranychus urticae*, *PP*: *Phytoseiulus persimilis*

Neoseiulus cucumeris

Only in 1 out of 11 batches the minimum required number of phytoseiids as stated on the batch label was reached. The other batches contained up to 89% less *N. cucumeris* than stated on the batch label (table 3). The minimum percentage of females per batch was exceeded by 12% to 29% in all batches. The required minimum mean cumulated number of eggs per female was reached in all batches.

Orius majusculus and Orius laevigatus

In 3 out of 6 batches the minimum required number of individuals present was exceeded, whereas the other 3 batches contained 7% – 11% less individuals in total than stated on the batch label (table 4). The percentage of living individuals did not reach 50% in 3 of the tested batches, respectively ranged between 68% and 84% of the label statement in the other batches. The minimum percentage of living females per batch was not reached in any of the checked batches. In four out of six batches the mean cumulated reproduction per female could not be assessed, as none of the females survived the whole trial period and thus had to be excluded from the evaluation according to the guideline.

Producers and/or distributors of mass reared arthropods were informed about the quality control results of their batches and possible reasons affecting the quality during production were identified. Especially for *P. persimilis* the non-compliance with acceptable predator-prey ratios can be explained with the rearing method on plants and the related mode of counting and harvesting of the phytoseiids. However, it has to be assumed that the product quality is additionally strongly influenced by postproduction factors, such as transport conditions and

duration (lack of food, climatic changes) and the conditions and duration of intermediate storage. Such factors have presumably contributed to the quality deficiencies of *N. cucumeris* and *Orius* batches. Although producers cannot take responsibility for circumstances influencing the product quality after production, it should be considered whether and how the product quality could be maintained to a certain extent until release. The presented results indicate the importance of regular quality control checks of mass reared beneficial arthropods in order to guarantee the efficacy of these biocontrol agents in pest control.

Table 3. Quality test results for *Neoseiulus cucumeris* 1999-2000.

Batch Date	n NC total	n NC larvae	% label* NC	% F	Eggs/F (CI 95%)
31.05.1999	2850	665	11.4	78.3	7.8-11.2
08.06.1999	16240	2400	64.9	72.3	9.9-12.9
16.06.1999	12934	1450	51.7	75.3	6.3-10.5
22.06.1999	26304	8232	105.2	63.2	6.2-10.0
06.07.1999	8260	700	33.0	74.1	8.9-11.5
30.06.2000	14246	102	56.9	79.1	11.1-16.8
14.09.2000	19454	3104	77.8	75.1	9.2-12.9
06.07.2000	18557	168	74.2	69.9	8.9-13.5
14.09.2000	7512	67	30.1	77.5	8.9-12.5
14.07.2000	11088	242	44.4	77.7	4.3-9.8
18.08.2000	4834	198	19.3	61.9	10.7-15.3

* in % of label statement, NC: *Neoseiulus cucumeris*, F: females

Table 4. Quality test results for *Orius* spp. in 2000-2001.

	Batch Date	n OR total	% OR* total	n OR total alive	% OR* living	% F living	Eggs/Female
<i>O.m.</i>	28.09.2000	443	89%	117	23%	8.5	/
	06.10.2000	623	125%	207	41%	92	/
	17.10.2000	591	118%	126	25%	87	/
	25.10.2000	684	137%	342	68%	68	43.8-85.9
	12.02.2001	464	93%	418	84%	64	43.5-70.5
	16.02.2001	467	93%	413	83%	58	/

* in % of label statement, OR: *Orius laevigatus*, *O.m.*: *Orius majusculus*, F: females

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Successive release of *Neoseiulus californicus* McGregor and *Phytoseiulus persimilis* A.H. (Acari, Phytoseiidae) for sustainable biological control of spider mites in greenhouse cut roses – Interim results of a two years study in a commercial nursery

Sylvia Blümel¹, Andreas Walzer², H. Hausdorf¹

¹BFL, Institute of Phytomedicine, Spargelfeldstr. 191, A-1226 Vienna, Austria, E-mail: sylvia.bluemel@relay.bfl.at; ²Institute of Zoology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Abstract: Successive introductions of the phytoseiid species *Neoseiulus californicus* and *Phytoseiulus persimilis* were evaluated as release strategy for both rapid and long-term suppression of *T. urticae* populations in greenhouse cut roses with regard to an economically feasible production. Spider mite control could only be achieved in combination with repeated acaricide treatments at mid of July, when the predator/pest ratio had reached >1 : 10. The percentage of infested rose shoots was variety dependent and declined to zero at the beginning of August for the rest of the growing season. As a main reason for the failure of the biological spider mite control in the first half of the growing season, the quality of the released predatory mites (*P. persimilis*) is discussed.

Key words: greenhouse roses, biological control, integrated control, *Tetranychus urticae*, *Phytoseiulus persimilis*, *Neoseiulus californicus*

Introduction

The combined release of predatory mite species with different life-styles has been proposed as biological control strategy for *Tetranychus urticae* Koch (Acari: Tetranychidae) with regard to the efficiency and the sustainability of the control success (McMurtry & Croft, 1997; Schausberger & Walzer, 2001). In former trials the simultaneous release of *P. persimilis* and *N. californicus* successfully controlled naturally occurring *T. urticae* infestations on greenhouse cut roses under integrated pest management conditions, but at the expense of high introduction rates of both beneficials (Blümel & Walzer, 2002). *P. persimilis* as a diet-specialist which depends on spider mites for its population establishment, is able to suppress spider mite populations rapidly, but often guarantees only short-term control when released alone. In contrast the diet-generalist *N. californicus* is less voracious and prolific than *P. persimilis*, but can utilise various food sources, which allow a persistence of the population at low or diminishing spider mite densities (McMurtry & Croft, 1997). In the present study successive releases of both *N. californicus* and *P. persimilis* should therefore be tested as alternative release strategy, starting with preventive introductions of the prey generalist at the start of the growing season in order to suppress or to delay the population growth of *T. urticae* and proceeding with releases of *P. persimilis* into high density infestation patches.

Materials and methods

The two seasons trial was started in March 2001 in two 900 m² greenhouses of a commercial nursery planted with four (greenhouse 1) respectively three different rose varieties (greenhouse 2). In each greenhouse *N. californicus* on vermiculite carrier substrate was released at the beginning of March before any visible spider mite infestation. *P. persimilis* on leaf substrate colonised with *T. urticae*, was distributed on rose plants with high infestation patches of spider mites since the end of March.

The control success was evaluated on the one hand by counting all rose shoots with buds ready for harvest, which were undamaged and those with visible sucking damage by *T. urticae* and/or the presence of spider mite developmental stages every second week in order to determine the percentage of infested rose shoots per greenhouse. On the other hand 180 single leaves per greenhouse were randomly collected from the lower leaf canopy and the number of spider mite and predatory mite developmental stages per leaf was assessed with the binocular at fortnight intervals.

Results

Despite the release of sufficient amounts of both phytoseiid mite species according to practical experience (22.2 *N. californicus*/m² and 49.4 *P. persimilis*/m² in greenhouse 1; 17.8 *N. californicus*/m² and 41.1 *P. persimilis* /m² in greenhouse 2) spider mite control was only achieved with the support of repeated acaricide treatment until mid of July. In mid July the predator/pest ratio reached >1:10 in both greenhouses, which was sufficient to suppress spider mite populations for the rest of growing season. The mean number of *P. persimilis* developmental stages per leaf in greenhouse 1 was higher than that in greenhouse 2, and vice versa for *N. californicus*. This might be due to the high infestation level with *T. urticae* in greenhouse 2 at the mid of April, which could have favoured the population development of the prey specialist more than that of the prey generalist. *N. californicus* was the dominant predator in each greenhouse, although *P. persimilis* was released in higher numbers than *N. californicus* (Fig. 1). Spider mite infestation frequency was rose variety dependent. In greenhouse 1 the percentage of infested shoots of the varieties "Akito" and "Isis", which had been newly planted, increased constantly from the start of April until a peak at the beginning of June (90.2% "Akito"; 98% "Isis"). In contrast the portion of infested rose shoots never exceeded 37% in the variety "Trixx". In the variety "First Red" two infestation peaks occurred, one at the end of April (66.7%) and the other at the end of June (46.9%). In greenhouse 2 the increase of the percentage of infested rose shoots was delayed four to six weeks compared to greenhouse 1, but also showed two infestation peaks (end of May: 61.3%; beginning of August: 32.7%). The variety "Trixx" showed an infestation peak at the start of May (67.8%) but remained at a low infestation level for the rest of the season (< 24%). The variety "Prophyta" peaked at 38.7%. The percentage of infested rose shoots declined to zero at mid of august until the end of the season.

Discussion

The failure of spider mite control by the predatory mites in the first half of the growing season is probably due to the inefficient performance of *P. persimilis*, which did neither build up high population densities rapidly as known from experience, nor reduced the pest density sufficiently. The main reason turned out to be the poor quality of *P. persimilis* released on bean leaves, which concerned both the low number of living predators, and at the same time

the high number of living *T. urticae* on the leaf substrate, which led to an additional release of the pest. An unacceptable predator/prey ratio of *P. persimilis* and *T. urticae* on *Phaseolus* leaf substrate was described as one of the main factors for the non-compliance of test batches with product quality standards (Blümel & Hausdorf, 2002). Another reason for the rapid build-up

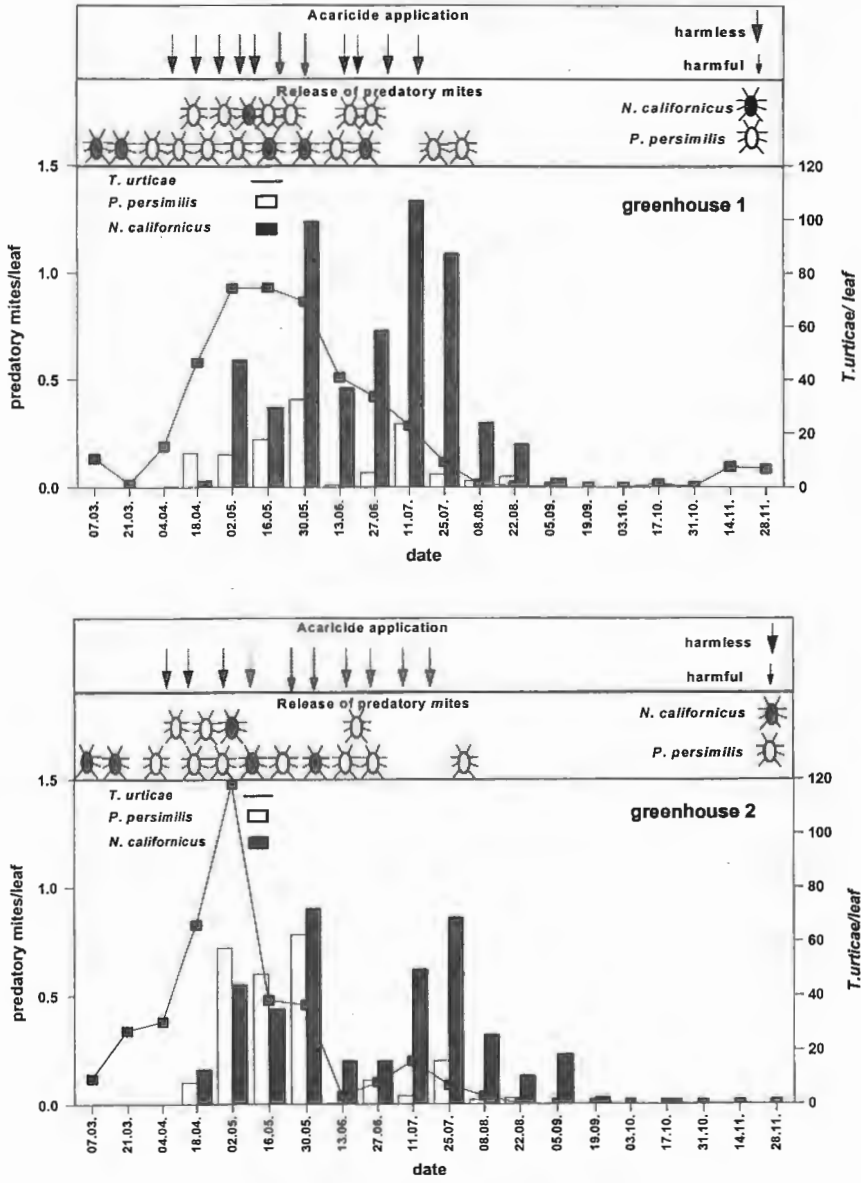


Figure 1. Population development of predatory mites and spider mites in both greenhouses during the growing season.

of the spider mite population could be the intensive pruning of the roses at the beginning of the growing season after *N. californicus* had been released already two times and could thus have reduced the number of *N. californicus* present on the plants and delayed its population development. To counteract this disadvantage, *N. californicus* was released again in May and June. Additionally disadvantageous for the biological control agents was the two times application of acaricides, which are known to have moderately harmful effects on *P. persimilis* and might have led to a reduction of the population. Finally, in times of food scarcity asymmetric intraguild predation of *N. californicus* on *P. persimilis* may have reduced the population of the latter (Schausberger & Walzer, 2001; Walzer *et al.*, 2002). For the next season changes of the trial conditions with regard to the timing and establishment of early released *N. californicus* and especially the predatory mite quality (*P. persimilis*) should improve the long-term suppression of *T. urticae* populations. Notwithstanding of the unfavourable results of the first part of the experiment a final evaluation of the long-term release strategy will be possible only after the second trial season.

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Biological pest control in eggplants in the Netherlands

K.J.F. Bolckmans, A.N.M. Tetteroo

Koppert Biological Systems, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands, E-mail: Kbolckmans@koppert.nl

Abstract: About 75 hectares of eggplants are currently grown in glasshouses in the Netherlands. Eggplants are an excellent host plant for many arthropod pests. Therefore biological pest control in eggplants has long been very challenging. More than 90% of the Dutch eggplant growers are currently successfully using biological pest control for control of thrips, whiteflies, aphids, spider mites, leafminers and caterpillars. An overview is given of the current status of biological pest control in eggplants in the Netherlands.

Introduction

In 2001 about 75 hectares of eggplants are grown in glasshouses in the Netherlands. Eggplants are planted in November and December at a density of 1.6 plants/m². The crop is finished one year later at the end of October. In the Netherlands eggplants yield on average about 45 to 55 kilograms per m². Prior to 1990 biological control in eggplants seemed impossible. Eggplants are an excellent host plant for many arthropod pests. The main bottleneck was biological control of western flower thrips (*Frankliniella occidentalis*). Each year thrips caused growers giving up on biological control in March when the pest pressure started to increase. The main pests in eggplants are tobacco aphid and green peach aphid (*Myzus persicae nicotianae*, *Myzus persicae*), foxglove aphid (*Aulacorthum solani*), potato aphid (*Macrosiphum euphorbiae*), onion thrips (*Thrips tabaci*), western flower thrips (*Frankliniella occidentalis*), two-spotted spider mite (*Tetranychus urticae*), leafminers (*Liriomyza bryoniae*, *L. trifolii*), greenhouse whitefly (*Trialeurodes vaporariorum*) and since a few years occasionally *Bemisia tabaci*. Additionally, also caterpillars (*Chrysodeixis chalcites*, *Laconobia oleracea* and *Mamestra brassicae*) can cause severe damage in eggplants.

The first real successes with biological control in eggplants were booked after 1990. In 1995 about one third of the eggplant growers was using biological pest control successfully. In 1997 the auctions decided to offer a surplus of 0.02 Dfl. per kilogram for eggplants grown with biological control. This meant an extra income of 1 Dfl. per m² per year for the growers, which are a member of the auction. From then on more than 90% of the eggplant growers started using biological control. Biological pest control in eggplants is still considered as the most challenging of all greenhouse vegetables in the Netherlands. In this publication we give an overview of the current status of biological pest control in eggplants in the Netherlands.

Thrips

Western flower thrips can successfully be controlled with a combination of the predatory mite *Amblyseius cucumeris* and the minute pirate bug *Orius laevigatus*. In the past growers would fumigate the crop with dichloorvos shortly after planting. Now that dichloorvos is discontinued in the Netherlands most growers spray the crop with abamectine after planting.

Large scale practical experience has shown that, especially during winter, treatments with abamectine can interfere with the initial successful establishment of beneficials in greenhouse vegetables. If *A. cucumeris* is introduced immediately after planting, treatment with chemicals to “desinfect” the crop is not necessary. After planting *A. cucumeris* can be introduced by dosing 5 millilitres (about 50 predatory mites) of *A. cucumeris* - *Tyrophagus putrescentiae* - bran - mixture in a small heap on each plant pot. The bran mite *T. putrescentiae* which feeds on the bran in the mixture provides *A. cucumeris* with a substrate to develop a population which can then migrate into the crop. Since a few years however, eggplants are more and more grafted on a tomato rootstock. Because of the glandular hairs on the tomato rootstock the *A. cucumeris* mites have great difficulty migrating into the crop.

Three to four weeks after planting, when the plants start to flower and to touch each other, 4,000 sachets (1 on every fourth plant) of *A. cucumeris* are introduced. These “breeder sachets” will produce about 1,000 predators per week for a period of 6 to 8 weeks. Furthermore *Orius laevigatus* is introduced in the beginning of March at a rate of minimum 1 *O. laevigatus* per m². They build up a large population in the crop and have a major contribution to thrips control. There are no selective chemicals available to correct thrips populations without interfering with the established natural enemies in the crop.

Also *Echinothrips americanus* is observed more and more as a pest in eggplants in the Netherlands. Special care has to be taken not to carry over an *E. americanus* infestation from one cropping season to the next by secure sanitation measures between cropping seasons. Experience has learned that *Macrolophus caliginosus* and *O. laevigatus* both contribute to the control of *E. americanus*. Many growers combine weekly scouting and spot treatments with abamectine to control *E. americanus* in their crop.

Whiteflies

Whitefly populations (*Trialeurodes vaporariorum*) can develop very quickly in eggplants due to their very high fecundity and quick development on this host plant and because of the rather high growing temperatures (night: minimum 18°C, day: minimum 20°C). Therefore it is very important to have a good control of whiteflies from the beginning otherwise whitefly control often remains a concern for the rest of the growing season. Starting immediately after planting, *Encarsia formosa* is weekly released preventively. In case the crop has been sprayed with abamectine a waiting period of 3 weeks after the last treatment is needed before starting to release whitefly parasites. When whiteflies are detected, high numbers of *Eretmocerus eremicus* are released in hot spots (inundative releases, hostfeeding) and the weekly rates of *E. formosa* are increased. Once 80% parasitism by *E. formosa* is observed, further releases of *E. formosa* are stopped (seasonal inoculative releases). On average about 12 *E. formosa* and about 10 *E. eremicus* will be introduced in total per m² per season.

Because of the relatively high populations of *Macrolophus caliginosus* towards summer and because of the use of *E. eremicus*, *Bemisia tabaci* rarely becomes a problem, although it is a rather new pest for eggplants in the Netherlands.

Also the predatory mirid bug *Macrolophus caliginosus* is used successfully in eggplants. Especially the high growing temperatures assure their quick establishment. *M. caliginosus* is introduced at about 1 per m² in the beginning of the cropping season. *M. caliginosus* is also thought of contributing to control of thrips in eggplants. Because of the high relative humidity at which eggplants are grown, flowers of eggplants can be infected by the bacteria *Erwinia carotovora*. *M. caliginosus*, *O. laevigatus* and bumblebees which are used for pollination are thought to increase the spread of this disease.

In case of outbreaks of whiteflies, population corrections can be done by spraying Mycotal-Addit (*Verticillium lecanii*) or pyriproxifen.

Aphids

Until April mostly foxglove aphid (*Aulacorthum solani*) is observed. This aphid can cause light purple spots on the fruits, deformation of the leaves and bushy growth at high populations. Preventive and curative introductions of the parasites *Aphidius ervi*, *Aphelinus abdominalis* and the predatory gall midge *Aphidoletes aphidimyza* are used rather successfully to control this aphid species. After April also the potato aphid *Macrosiphum euphorbiae* can be observed and easily controlled with *A. ervi* and *A. aphidimyza*. At this moment foxglove aphid is probably the most challenging pest to control in eggplants because of the immediate influence on fruit quality at low aphid densities.

Tobacco aphids (*Myzus persicae nicotianae*) and green peach aphids (*Myzus persicae*) are controlled with *Aphidius colemani*. Bankerplants are not used very much yet in eggplants. The hoverfly *Episyrphus balteatus* has been tried without success in eggplants. Probably the larvae are hampered by the hairy leaves.

Usually pirimicarb is used to correct local aphid outbreaks. Because of the very important contribution of *Orius laevigatus* to thrips control and *Macrolophus caliginosus* to whitefly control, it is not recommended to use the systemic insecticide imidacloprid for aphid control throughout the greenhouse because it will badly affect the population of these predatory bugs which also feed on plant juices. Only localised applications ("spot treatments") of systemic insecticides can be combined with predatory bugs.

Two-spotted spider mites

A common problem in eggplants is that outbreaks of spider mites are often detected too late by the grower. Very positive results have been obtained with "pest-in-first releases" whereby spider mites are purposely released in the crop together with the predatory mite *Phytoseiulus persimilis*. With this method it has been possible to establish an active population of *P. persimilis* in the crop before spider mites, which come out of hibernation, start to infest the crop.

In case of an infestation with spider mites, *P. persimilis* is introduced throughout the greenhouse at a rate of 3 to 4 predators per m² with extra predators in hot spots. Spider mites can often build up high populations around the edges of the greenhouse close to the heating pipes where temperatures are higher and the relative humidity lower. *P. persimilis* is not able to control spider mites successfully under these conditions. Instead *Amblyseius californicus* is used at a rate of 5 to 10 predators/m².

Larvae of the naturally occurring predatory gall midge *Feltiella acarisuga* are frequently observed in spider mite colonies. Excellent results have been obtained over the past few years by releasing 250 *F. acarisuga* during 3 to 5 weeks into hot spots of spider mites. Another technique, which is successfully used to control two-spotted spider mite outbreaks in eggplants, is by releasing nymphs of *M. caliginosus* into hot spots. Fenbutatin oxide is used in case of chemical corrections.

Leafminers

Leafminers (*Liriomyza bryoniae*, *L. trifolii*) are effectively controlled with the parasitoid *Diglyphus isaea*. *D. isaea* is introduced 1 to 2 times at a rate of 0.25/m² once sufficient leafminer activity is observed in the crop to allow for successful establishment of the parasite. Exact timing of the releases is very critical. There are no registered possibilities for chemical corrections, which are compatible with biological control.

Caterpillars

Caterpillars can cause very big damage in eggplants. The most common species is *Chrysodeixis chalcites* but also *Laconobia oleraceae* and *Mamestra brassicae* are frequently observed. In glasshouses where *Macrolophus caliginosus* is well established, problems with caterpillars are often much smaller because *M. caliginosus* will feed on moth eggs and small caterpillars. Very good results have been obtained by weekly releases of the pentatomid bug *Podisus maculiventris* in hot spots. At the moment the high production cost of *P. maculiventris* is prohibitive for its large-scale use. Mostly, *Bacillus thuringiensis* is used to control caterpillars. Insect growth regulators of the class of the benzoyl ureas cannot be used because of their incompatibility with the predatory bugs *O. laevigatus* and *M. caliginosus*.

Others

When the greenhouse is located in the vicinity of a potato field also infestations of colorado potato beetle (*Leptinotarsa decemlineata*) can occur. The larvae can very successfully be controlled by releasing *Podisus maculiventris*. Preparates based on *Bacillus thuringiensis tenebrionis* work excellent against colorado potato beetle larvae in eggplants but are not registered in the Netherlands. Alternatively localised systemic treatments with imidacloprid can be used.

In southern France and in Spain eggplants are also often infested by the green vegetable stink bug *Nezara viridula* which can severely upset a biological control program. In the Netherlands this pest has not been a problem in eggplants yet.

Conclusion

Biological pest control in eggplants is now very feasible in the Netherlands. Conditional to success are a thorough monitoring and scouting program to detect pest outbreaks in an early stage combined with weekly preventive releases of beneficials. Biological pest control in eggplants costs between 0.60 and 1.30 Dfl. per m² (2000 – 2001) with an average of 1 Dfl/m²/season depending on pest pressure and on the experience of the grower and his biocontrol advisor.

Biological control of soil-dwelling life stages of Western Flower Thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) by entomopathogenic nematodes and *Hypoaspis* spp. (Acari: Laelapidae)

Christian Borgemeister¹, Lemma Ebssa¹, Dammini Premachandra¹, Oliver Berndt¹, Ralf-Udo Ehlers², Hans-Michael Poehling¹

¹Institute of Plant Diseases and Plant Protection, Hannover University, Herrenhäuser Str. 2, 30419 Hannover, Germany, E-mail: borgemeister@ipp.uni-hannover.de; ²Institute for Phytopathology, Department for Biotechnology and Biological Control, Christian-Albrechts-University Kiel, Klausdorfer Str. 28-36, 24223 Raisdorf, Germany

Abstract: Western Flower Thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is worldwide one of the most important pests on vegetables and ornamental crops under greenhouse and field conditions. Conventional chemical and biological control tactics, targeting only at the foliar-feeding stages, usually provide no satisfactory control levels. Thus we investigated the potential of entomopathogenic nematodes (EPNs) and soil inhabiting predacious mites (*Hypoaspis* spp.) against soil-dwelling life stages of WFT. Our results indicate that all soil-dwelling life stages of WFT are susceptible to the tested EPN strains/species. Virulent strains, applied at a dose rate of 400 infective juveniles cm⁻² resulted in 80 and 40–60% WFT mortality under laboratory and microcosm conditions, respectively. Releases of *H. aculeifer* (Canestrini) at 2,800 mites m⁻² reduced WFT population by 78%. Combined applications of EPNs and *H. aculeifer* significantly lowered the number of emerging WFT adults compared to the untreated control as well as to individual releases of EPNs and predacious mites. These findings may open up a new venue for biological control of WFT.

Key words: Western Flower Thrips, *Frankliniella occidentalis*, entomopathogenic nematodes, *Hypoaspis* spp., biological control

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important cosmopolitan pest of vegetables and ornamentals, in greenhouses and in the field. WFT lay eggs in the plant tissue. Both first and second instar larvae feed on the plant. However, the majority of the late second instar larvae leaves the host plant and descends into the soil where they develop into non-feeding prepupae and pupae, while a smaller part of the larval population remains on the plant and completes its development in buds, flowers and leaf axils. The emerging adults leave the soil or other cryptic habitats and commence feeding on the host plants. WFT spends more than one-third of its developmental time in the soil. Thus, the conventional chemical and biological control strategies, targeting the foliar-feeding stages of WFT, can not provide sufficient control of WFT, especially in high value crops like ornamentals.

Entomopathogenic nematodes (EPNs) (Nematoda: Rhabditida) of the Steinernematidae and Heterorhabditidae families and soil-inhabiting predaceous mites of the

genus *Hypoaspis* (Acari: Laelapidae) are of great potential in controlling soil-dwelling pest insects (Boemare *et al.*, 1996; Wright & Chambers, 1994). We thus tested the efficacy of EPNs and *Hypoaspis* spp. against soil-dwelling developmental stages of WFT.

Materials and methods

Efficacy of EPNs against WFT

The efficacy of 12 EPN strains was tested in the lab and under microcosm conditions against all soil-dwelling developmental stages of WFT. In the laboratory studies, an assay arena was filled with a commercial growing substrate (Fruhstorfer Erde). Thereafter, at least ten individual WFT of one developmental stage were transferred to the arena. Finally, one of the EPN strains was pipetted to the top of the soil. Three to four days later (depending on the tested thrips developmental stages), the number of emerging adults was recorded on daily bases. The efficacy of a given EPN strain was determined by comparing the number of emerged adults from the EPN applied treatment to that of a water-treated control. In follow-up experiments, the most virulent EPN strains were further tested at different dose rates of the EPNs (100, 200, 300, 400, 800, or 1,000 infective juveniles (IJs) cm⁻²). In microcosm experiments, the efficient strains were subsequently tested under more practical conditions where adult WFT were released on seedlings of potted green bean (*Phaseolus vulgaris* L.). EPNs were applied to the soil at a time when the thrips F1 had commenced descending from the plants in order to pupate in the soil. Efficacy of EPNs was again assessed by comparing emergence rate of adult thrips (F1) in the treatments to the water-treated control.

Efficacy of Hypoaspis spp. against WFT

Predaceous mites (*Hypoaspis miles* [Berlese] and *H. aculeifer* [Canestrini]) and synchronized first instar WFT larvae were introduced to potted green bean seedlings. Adult mites were released on the soil surface once the thrips F1 had commenced leaving the plants to pupate in the soil. To study the density-dependent effects of the mites, two thrips densities (by introducing 10, or 50 WFT larvae per pot) were established and three predator densities (5, 10, or 20 adult *H. miles* or *H. aculeifer* per pot) were released. In the control no mites were released. Efficacy of *Hypoaspis* spp. was calculated by comparing the number of emerged WFT adults from the mite-treated and untreated pots.

Compatibility of EPNs and H. aculeifer for control of WFT

Initially WFT adults were released to caged green bean seedlings in microcosms (for details see previous paragraph). Ten adult mites (1,400 mites m⁻²) were released on the soil surface once the thrips F1 started leaving the plants for pupation in the soil. Two days later *Heterorhabditis bacteriophora* (Poinar) HK3 and *Steinernema feltiae* (Filipjev) hybrid (the commercial formulation Nemaplus®) at 400 IJs cm⁻² were applied to the soil. The combined or single effects of EPNs and *H. aculeifer* were determined by comparing the numbers of emerged WFT adults in the treatments and in the control.

Results and discussion

Efficacy of EPNs against WFT

All soil-dwelling developmental stages of WFT were susceptible to the tested *Steinernema* and *Heterorhabditis* strains. However, strains significantly differed in their virulence (Ebssa *et al.*, 2001a). At a dose rate of 400 IJs cm⁻² EPNs caused 80% and 40–60% thrips mortality under laboratory and microcosm conditions, respectively (Ebssa *et al.*, 2001a).

For the majority of the tested EPN strains/species increasing dose rates up to 400 IJs cm⁻² significantly increased thrips mortality in the laboratory experiments and yielded mortality > 80%. Concentrations > 400 IJs cm⁻² did not significantly increase mortality in WFTn (Ebssa *et al.*, 2001a; Premachandra, 2001). Yet in the microcosm experiments, increasing the dose rate from 400 to 1,000 IJs cm⁻² significantly increased WFT mortality from 40 to 60% in certain EPN strains (Ebssa *et al.*, 2001b). Chyzik *et al.* (1996) also recorded high mortality of WFT prepupae and pupae at a dose rate of 400 IJs cm⁻². However, fungus gnats can be efficiently controlled by EPNs at comparatively lower concentrations (Harris *et al.*, 1995).

Efficacy of *Hypoaspis* spp. against WFT

Releases of *H. miles* and *H. aculeifer* significantly reduced the number of emerging WFT adults. Data from laboratory life-table experiments show that *H. aculeifer* is a more voracious than *H. miles* (O. Berndt, unpublished data). In microcosm experiments increasing the density of both *H. miles* and *H. aculeifer* from 5 to 20 (equivalent to 700 to 2,800 mites/m²) significantly increased WFT mortality from 51 to 78% (O. Berndt, unpublished data).

Table 1. Effect of two EPN strains and *H. aculeifer* on emergence of adult WFT (Control = water application only; H = *Hypoaspis aculeifer* only; HK3 = *Heterorhabditis bacteriophora*, strain HK3 only; SFN = *Steinernema feltiae*, commercial formulation Nemaplus® only; H-HK3 = combined applications of *H. bacteriophora* strain HK3 and *H. aculeifer*; H-SFN = combined applications of Nemaplus® and *H. aculeifer*). Means followed by the same letter are not significantly different at *P* < 0.05 (LSD multiple range test; after square root transformation).

Treatments	Adult WFT emerging (mean ± SE)
Control	34.7 ± 4.86a
H	18.8 ± 1.50b
HK3	13.6 ± 1.75bc
SFN	19.0 ± 5.00b
H-HK3	6.4 ± 1.66d
H-SFN	10.0 ± 2.55cd

Compatibility of EPNs and *H. aculeifer* for WFT control

Single releases of 10 *H. aculeifer* alone already reduced WFT populations by 46% (Premachandra, 2001). Application of the two EPN strains at a dose rate of 400 IJs cm⁻² caused similar reductions of WFT density (table 1). However, combined releases of EPNs and *H. aculeifer* yielded a significantly higher control level than individual application of both biocontrol agents.

These results indicate a new venue for biological control of WFT through combined releases of two biocontrol agents targeting at the soil-dwelling life stages of thrips. Possibly such a combined approach, together with releases of predators against soil-dwelling thrips life stages, will assure sufficient control levels even in high value crops such as ornamentals.

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Higher-order predators in greenhouse systems

Jacques Brodeur¹, Conrad Cloutier², Dave Gillespie³

¹*Département de phytologie, Université Laval, Québec, Canada, G1K 7P4, E-mail: Jacques.Brodeur@plg.ulaval.ca;* ²*Département de biologie, Université Laval, Québec, Canada, G1K 7P4;* ³*Pacific Agri-Food Research Centre, Agriculture & Agri-Food Canada, P.O. Box 1000, Agassiz, B.C., Canada, V0M 1A0*

Abstract: A growing number of biological control programs tend to combine two or more species of natural enemies to reduce populations of arthropod pest species. Recent evidence from field situations indicates that higher-order predators may disrupt biological control by interfering with other beneficial organisms, mainly through intraguild predation. In greenhouse crops, higher-order predators are usually released as a second line of defense, when pest populations have reached high densities. We argue that interference by higher-order predators is less likely to be important in greenhouses than in other agroecosystems. The negative effects of intraguild predation would be reduced by the low probability of establishment of higher-order predators and the periodic releases of high numbers of more specialized biocontrol agents.

Key words: greenhouse crops, biological control, intraguild predation, generalist predators

Introduction

Intraguild interactions have become a hot topic in community ecology and biological control. There is increasing evidence that higher-order predators (i.e. generalist predators of herbivores that also attack other predators) may have a significant effect in driving fluctuations in populations of natural enemies (Rosenheim, 1998). Understanding competitive outcomes and mortality patterns among natural enemies are therefore meaningful for implementing effective pest control strategies. For instance, a better choice of combinations of beneficial organisms would lead to a reduction in the ratios of biocontrol agents to pest organisms that are released in greenhouses, thereby less money to spend for growers and better incentive to biological control (Brodeur & Rosenheim, 2000).

In this paper, we first describe some unique ecological characteristics of greenhouse systems. Next, we introduce the most common groups of higher-order predators used in greenhouse crops and examine their role in biological control. Finally, we describe some of the interactions that may occur between higher-order predators and other beneficial organisms and discuss the consequences on biological control. This paper is intended to stimulate curiosity on the role of higher-order predators in greenhouses rather than satisfy it. Nowadays, patterns are emerging but do not rely on strong experimental investigations.

The greenhouse environment

Greenhouse systems are unique. Several ecological characteristics make them completely different from other natural or managed ecosystems. This creates a specific environment that not only determines the nature of pest infestation but also shapes the role of biocontrol agents.

•In greenhouses, climatic conditions are constant and provide a favorable environment for the survival and reproduction of arthropods once they have invaded a crop. In greenhouses, there is no wind, no rain, no frost, no drought.

•Greenhouse crops are transient. They last from a few weeks to a few months. They are also of high commercial value which favors the implementation of biological control.

•Greenhouses are isolated units, especially during the cold season in temperate regions. They are characterized by very low rates of emigration and immigration by both herbivores and natural enemies. As opposed to field systems, greenhouse crops poorly benefit from natural control.

•In greenhouses, food webs are totally artificial. It is basically the growers that create and determine the nature of the food webs in their crops. In general, arthropod communities share the following characteristics:

- *Low number of pest species.* In vegetable and ornamental crops, we observe very few species of herbivores, likely 4 to 6 different species per crop.
- *Low number of predator species.* For example, a maximum of 23 species of predators, belonging to 6 families of insects and to 2 families of mites, can possibly be observed on greenhouse tomatoes if a grower introduces all species of predators that are currently commercially available in Canada. A few species of spiders that naturally occur in greenhouses, and a number of incidental natural enemy species that invade from outside the greenhouse should be added to this number.
- *Low level of interactions between ground and foliage arthropods.* Several greenhouse crops are grown on artificial media which means that organisms from the soil are excluded from the food web.
- *Low level of co-evolution between interacting species.* Greenhouse communities are made up with microbials, parasitoids, and predators coming from different sources and released together within an alien environment. Except for specialist species, biocontrol agents have not co-evolved with targeted pest species.
- *Unstable trophic interactions.* Because the complexity of food webs is reduced in greenhouse crops, trophic and guild interactions have a lower propensity for stability. Greenhouse systems rarely, if ever, reach equilibrium dynamics.

The identity and role of higher-order predators

Because greenhouse crops are short-lived and fail to provide a stable environment for the establishment of natural enemies, they are best suited for multiple releases of biocontrol agents (Parrella *et al.*, 1999). In vegetable crops, growers usually proceed with periodic inoculative releases of more or less specialized biocontrol agents throughout the growing season. These natural enemies are efficient in reducing pest populations and constitute a 'first line of defence' against pest species. They usually have a short generation time and a good rate of establishment in the crop. Furthermore, they are not expensive to produce commercially. Whitefly parasitoids, aphid parasitoids, and phytoseiid mites are typical candidates of the first line of defence.

Occasionally, when pest densities reach high levels and infestations become out of control, growers tend to introduce higher-order predators in their crops. These predators constitute a 'second-line of defence'. They are often released in combination with the usual cocktail of more specialized natural enemies from the first line of defence.

Higher-order predators used in greenhouses share the following attributes. They are generalist feeders. They not only attack different types of herbivores but they can also feed on each other (intraguild predation) and exploit prey at different trophic levels. Some are

omnivorous and can derive some to considerable nutrition from plant sources. These predators are robust and voracious animals. They have a long generation time and a low rate of establishment, if any, in the crop. Finally, in contrast to biocontrol agents from the first line of defence, most higher-order predators are expensive.

There is a limited number of species of higher-order predators that are available from commercial suppliers (table 1). They belong to 8 genera from 3 families of insects. Species from the genera *Macrolophus* and *Dicyphus* are omnivorous and have the capacity to feed on both animal prey and plant material.

Table 1. Higher-order predators in greenhouse systems.

Order	Family	Genus
Neuroptera	Chrysopidae	<i>Chrysoperla</i>
		<i>Chrysopa</i>
Heteroptera	Anthocoridae	<i>Orius</i>
	Pentatomidae	<i>Podisus</i>
	Miridae	<i>Macrolophus</i> <i>Dicyphus</i>
Coleoptera	Coccinellidae	<i>Hippodamia</i>
		<i>Harmonia</i>

Do generalist predators disrupt biological control in greenhouses?

In field and greenhouse systems, the success of release programs depends upon a number of factors: nature of the crop plant, nature of the pest, density of pest organisms, quality and density of biocontrol agents, climate, and interactions between biocontrol agents. The latter has recently received a great deal of attention. Experimental evidence from laboratory and field situations now indicates that biological control can be disrupted by direct and indirect interactions such as competition, apparent competition, intraguild predation, and behavioral interference between natural enemies.

Do generalist predators disrupt biological control implementation in greenhouses? Very few studies have examined the interactions among biocontrol agents that are commonly used in greenhouse systems. Two conclusive ones come from the work of Cloutier & Johnson (1993) and Janssen *et al.* (1998) who studied the relationships among arthropods on greenhouse cucumbers. They have carefully described the nature of trophic and guild associations that may occur between two pest species, the western flower thrips *Frankliniella occidentalis* and the two-spotted spider mite *Tetranychus urticae*, and their biocontrol agents. Of interest, they showed that thrips predators may interact not only with each other but also with a predator of the two-spotted spider mite. The anthocorid bug *Orius* sp., a generalist predator used to control thrips population, has the capacity to prey upon two phytoseiid mites, *Amblyseius cucumeris* and *A. degenerans*, which are also released against thrips on cucumbers. It was further shown that *Phytoseiulus persimilis*, a predatory mite specific to *T. urticae*, may suffer high level of predation from *Orius*. These results nicely indicate that higher-order predators release to control a pest species may also interfere with biocontrol agents of a second pest species.

However, to our knowledge, all the published studies that measured interference between generalist predators and other biocontrol agents of greenhouse pests were conducted in small

arenas and during a short period of time. These studies are determinant to identify potential interactions between natural enemies, but they are not reliable to predict the prevalence and frequency of such interactions, as well as their impact on biological control in a greenhouse, at the crop level.

We believe that *interference by higher-order predators is less important in greenhouses than in annual or perennial agroecosystems*. In greenhouses, higher-order predators are released in crops as a corrective measure mainly during severe pest infestation. Although they are likely to interfere with other biocontrol agents, intraguild predation probably does not have significant consequences on biological control for two reasons. First, higher-order predators tend to disappear from the system once pest populations have been reduced. They appear to have a low capacity to survive, reproduce and build up populations when manoeuvring in unstable, poorly diversified food webs. However, such may not be the case for omnivorous species, which can sustain populations to some degree on plant food sources. Second, biological control is continuously restored by periodic inoculative releases of high numbers of specialized natural enemies. Therefore, the negative effects of intraguild predation on biocontrol agents from the first line of defence are likely to be compensated, except if higher-order predators have the capacity to increase in numbers using the introduced biocontrol agents as a food source.

Conclusion

At this point, the discussion on the role of higher-order predators in biological control in greenhouse remains preliminary, indeed speculative. Models developed by ecologists studying community structure, population dynamics, and their applications to biological control poorly apply to greenhouses because of their inherent ecological characteristics (see above). As a consequence, studies on higher-order predators in greenhouses can not rely on a strong theoretical framework. Experimental work conducted at the greenhouse scale and for extended periods of time are necessary to provide a reliable portrait of the role of higher-order predators in biological control.

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The potential of *Atheta coriaria* Kraatz (Coleoptera: Staphylinidae), as a biological control agent for use in greenhouse crops

V.A. Carney¹, J.C. Diamond², G.D. Murphy³, D. Marshall⁴

¹Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, AB, T1J 4B1, Canada, E-mail: carneyv@em.agr.ca; ²Niagara Peninsula Conservation Authority, 250 Thorold Rd. West, Welland, ON Canada, L3C 3W2; ³Ontario Ministry of Agriculture, Food and Rural Affairs, 4890 Victoria Ave N., Vineland Station, ON L0R 2E0; ⁴Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 4902 Victoria Avenue North, Vineland Station, ON, Canada L0R 2E0

Abstract: An efficient, cost-effective protocol for rearing the staphylinid *Atheta coriaria* Kraatz on an artificial diet was developed. Laboratory trials showed *A. coriaria* to be highly predatory against fungus gnats, shoreflies and western flower thrips. A naturally occurring population of *A. coriaria* in a commercial greenhouse demonstrated its potential for long-term establishment and survival.

Key words: biological control, *Atheta coriaria*, rearing, predation, monitoring, greenhouses

Introduction

As part of a research program on fungus gnats and shore flies at AAFC, Vineland, a soil-dwelling staphylinid, *Atheta coriaria* Kraatz was observed feeding on laboratory cultures of fungus gnats. Since various pests of greenhouse crops (e.g. fungus gnats, shoreflies, thrips) have life stages living in the growing medium of the crop, it was felt that they may be vulnerable to predation by this beetle. The predatory nature of *A. coriaria* has been documented (Miller, 1981; Miller & Williams, 1983), but not against greenhouse pests.

Before investigating the biological control potential of these beetles, an efficient culturing method was needed to produce sufficient beetles for experimental needs. Laboratory populations of the beetle were reared successfully on fungus gnats; however, the high prey consumption rate resulted in cultures that were inefficient and highly labour intensive. This paper describes preliminary studies into: a rearing protocol using artificial diet; the predatory capacity of the beetle; and monitoring of *A. coriaria*

Materials and methods

Insect rearing

Individual colonies of *A. coriaria* were reared in 4-l plastic containers containing 2 l of moistened, expanded coconut fibre. A tight fitting lid with a 10 cm diameter screened hole was used for ventilation. Rearing colonies were maintained in a research greenhouse with a mean temperature of 25°C (±3°C), RH of 60%, and seasonal photoperiod.

A number of potential artificial diets (including raw and cooked ground beef, cat food and dog food) were evaluated. All were found to sustain *A. coriaria* populations similarly; however, the product found easiest to use was a pelletized commercial trout food (Martin #3 sinking trout food) product from Martin Mills Inc., Elmira, Ontario, Canada. The pellets were

ground to a coarse powder and combined with the coconut fibre in a ratio of 1.25 g to 1 l, respectively. Twice weekly, this diet was mixed thoroughly into each culture.

Biology

Short-term reproductive potential of the beetle was assessed by introducing fifteen adult females and 5 males of indeterminate ages into each of ten rearing containers. Populations were assessed after 23 days to eliminate the possibility that any of the parent generation were included in the F1 count (preliminary investigations indicated that adult beetle longevity did not exceed 21 days under the conditions used in this trial). Larvae, pupae and adults were collected by passing the rearing substrate through a 20 mesh per inch screen. Complete life table studies were beyond the scope of the project.

Predation

Trials were carried out in petri dishes lined with moistened filter paper, held at 25°C, 16:8 photoperiod and 60% R.H. Beetles were starved for a 6-hour period and predation measured after 24 hours. Predatory capacity of the beetle was assessed against fungus gnats, *Bradysia impatiens* Johannsen (eggs, larvae, pupae), shoreflies, *Scatella stagnalis* Fallen (eggs, larvae, pupae) and thrips, *Frankliniella occidentalis* Pergande (late 2nd instar larvae and pupae).

Monitoring

Preliminary studies into monitoring techniques showed that sticky cards resulted in inconsistent, low numbers of adults, with poor correlation to actual population size. Trap pots (filled with coconut fibre and baited with artificial diet) were also evaluated. Trap pots were effective, but extraction methods to quantify numbers were labour-intensive and inefficient. The trap pot method was used for research trials but was not considered feasible for large scale regular monitoring.

In May 2001, a grower of commercial miniature roses reported a high *A. coriaria* population. The roses were grown in a peat mix in 5 cm pots. Adults and larvae could be seen on the growing medium and the bench surface. The number of beetles on the growing medium surface was monitored weekly by visual observations. Four pots were monitored per bench (800 pots per bench). There were approximately 200 benches in the compartment. Thrips and fungus gnat populations on sticky cards were also monitored.

Results and discussion

Insect rearing

Laboratory cultures have been maintained exclusively on the above diet for more than 2½ years, with no visible reduction in colony vigour (apart from instances of contamination described below). Adults from the culture were sexed on three occasions: Sep. 2000 (n=108), Nov. 2000 (n=164) and Mar. 2001 (n=133). The mean percentage of females was 58%, consistent with a population (n=266) collected from a commercial greenhouse (61% female).

Two problems relating to the general culturing of this beetle are worth noting. Firstly, fungal contamination of the culture with *Actinomyces elegans* (Eidam) Benjamin & Hesseltine, presented a serious problem on occasions. It was found that beetle cultures could be sustained and the fungus managed by maintaining a substrate moisture level of 27% (by weight), incorporating the diet well into the substrate and good ventilation.

Secondly, cultures were sporadically contaminated by phoretic deutonymphs of a mite species resembling *Rhizoglyphus* sp., specimens of which have been submitted to Agriculture and Agri-Food Canada, Ottawa, Canada for identification. *Rhizoglyphus* sp. deutonymphs

have been documented to be carried by various insects (Diaz *et al.*, 2000). The mite can have a severe impact on *A. coriaria*, restricting mobility, feeding and mating.

The rearing protocol and diet presented here are efficient and economical for research purposes. However, if production is to be elevated to levels required by commercial producers, optimal environmental conditions must be determined and regulated.

Biology

Assessments of F1 populations 23 days after set up, showed that 29% of beetles were adults and 56% were 3rd instar larvae. Therefore, the egg-adult development time is approximately 3 weeks; however, there is an obvious need for more detailed life table studies on this insect. First-generation population increases of the beetle varied considerably, with a mean of 15.6 (± 6.4) progeny per female parent. The influence of temperature and food availability on the development of *A. coriaria* has been explored (Miller, 1981; Miller & Williams, 1983), however, little is known about other rearing parameters.

Predation

Table 1 shows the predation potential of *A. coriaria*. With increasing prey density presented, the beetles killed more insects than they actually consumed. Miller & Williams (1983) documented several other prey species for this insect; however, there is considerable scope for further work on its host range, and other pest species that may potentially be controlled.

Table 1. Predation of *A. coriaria* against fungus gnats, shoreflies and thrips (maximum number of insects eaten in 24 hours).

Rove beetle life stage	Fungus gnats					Shoreflies				Thrips	
	Egg	1 st	2 nd	3-4 th	Pupa	Egg	1 st	3 rd	Pupa	2 nd	Pupa
1 st Instar	24	30	21	-	-	nt	nt	nt	nt	nt	nt
2 nd Instar	100	30	30	8	-	nt	nt	nt	nt	nt	nt
3 rd Instar	100	100	10	6	1						
Adult	154	150	46	10	4	134	33	6	1	95	78

nt- not tested

Monitoring

Monitoring data from a commercial miniature rose crop (Fig. 1) demonstrates that *A. coriaria* can establish and survive under commercial greenhouse conditions, even when prey (fungus gnats and thrips) is reduced. There was no obvious reason for the sudden decline of the three species in late July, although high temperatures (between 35°C and 40°C) during that period may have been a cause.

It is interesting to speculate on an apparent correlation between fungus gnats and rove beetles in the latter half of the year, however further work is needed to confirm the relationship. If a relationship does exist, then population curves should be fairly synchronous. Numbers of adult fungus gnats on sticky cards are a function of the size of larval populations about two weeks previously. When fungus gnat larval populations are high, the increased food supply for the beetle would likely result in a subsequent population increase coinciding with that of the adult fungus gnats. This is supported by the fact that population peaks for the beetle are comprised primarily of larvae (88% larvae on Sep 25; 81 % larvae on Oct 31).

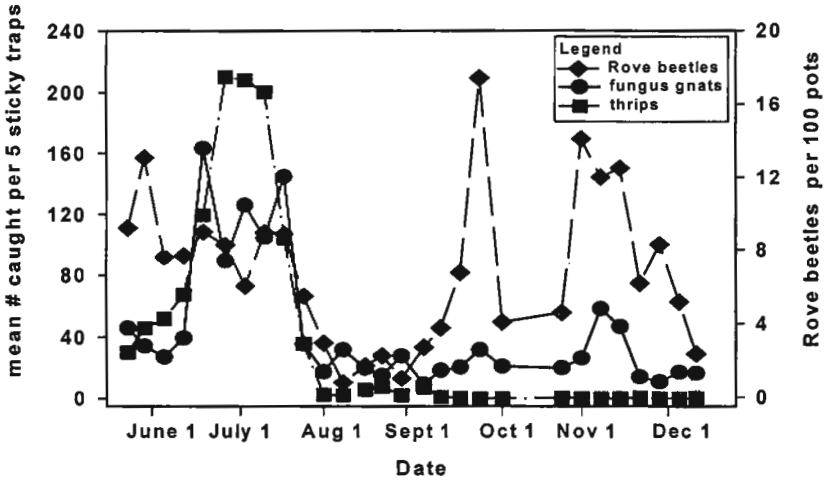


Figure 1. Weekly population counts for *A. coriaria*, western flower thrips and fungus gnats in a commercial miniature rose crop.

Conclusion

A. coriaria shows excellent potential for biological control of soil-dwelling insect pests. It is a voracious generalist predator that survives well in a variety of growing media including peat, coconut fibre and rockwool. Its soil habitat should confer some protection from pesticides applied to crop foliage, although compatibility trials with commonly used pesticides should be a priority. Continued research is needed on the beetle's biology, and interaction with other natural enemies such as *Hypoaspis* sp. Mass-rearing studies and field trials on introduction rates are needed and will most likely be undertaken by commercial producers, but they are essential for a better understanding of the potential value of this predator to the greenhouse industry.

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Distribution, thresholds, and biological control of the twospotted spider mite (Acari: Tetranychidae) on bent cane cut roses in California

Christine Casey, Michael Parrella

Department of Entomology, University of California, One Shields Avenue, Davis, CA 95616 USA, E-mail: cacasey@ucdavis.edu

Abstract: The goal of this project was to develop an IPM program for the twospotted spider mite on greenhouse grown bent cane cut roses. This work was part of a larger project to develop a model IPM program for floriculture crops that incorporated the use of fixed precision sampling, action thresholds, and biological control. Specifically, we used Taylor's power law to quantify twospotted spider mite spatial distribution in order to develop a sampling plan. We also examined the relationship between spider mite density and photosynthesis in the rose plant to develop an action threshold for this pest. Finally, we looked at a technique to enhance movement of the mite predator, *Phytoseiulus persimilis*, in this crop.

Twospotted spider mite spatial distribution was shown to be highly aggregated on bent cane roses under both chemical and biological control. Using the Taylor's power law coefficients we determined that a sample of 38 plants per 929 m² could predict mite density with a precision of 0.25. The relationship between spider mite density and rose plant photosynthesis suggested that our nominal action threshold of 5 mobile stages/leaf/week was too low. We also demonstrated that the use of interplant bridges facilitated predatory mite movement between rose plants in the bent cane system. Implementation of the IPM program developed from this study resulted in key pest densities and control costs that were comparable to or less than those obtained under a conventional control program.

Key words: sampling, thresholds, photosynthesis, biological control, roses, *Tetranychus urticae*, *Phytoseiulus persimilis*

Introduction

Rose production is the strongest component of the \$500 million cut flower industry in California. In 1999, California growers produced 71% of the US crop with a wholesale value of \$54 million (USDA, 2000). The key pests of this crop (*Tetranychus urticae* [twospotted spider mite]; *Frankliniella occidentalis* [western flower thrips]; and *Sphaerotheca pannosa rosae* [rose powdery mildew]) are among the most difficult pests to control in the greenhouse. This, coupled with the high quality standards for this crop, has traditionally meant that it is subject to 40 to 50 pesticide applications per year in California. The need for frequent pesticide applications has resulted in the development of pesticide resistance and has interfered with attempts to use biological control.

Recent regulatory changes in the US have favored the development of reduced risk pesticides that tend to be more specific. These products often target a single pest or even a single pest life stage, and are often more compatible with natural enemies than traditional materials. In addition, the bent cane production method, which spatially separates the flowers and lower canopy thereby creating a refuge for predatory mites, has been widely adopted.

These changes have led many rose growers to reconsider IPM methods for pest management. The objective of this study was to develop an effective IPM program for twospotted spider mites on cut roses that emphasized the use of sampling, thresholds, and biological control.

Materials and methods

T. urticae spatial distribution

To describe *T. urticae* spatial distribution we sampled two greenhouses where mite chemical control was used and one greenhouse where mite biological control with the predator, *Phytoseiulus persimilis*, was used. Twenty randomly selected plants (variety 'Kardinal') were sampled weekly over a period of 18 months. Leaves were taken from the first, third, fifth, seventh, and ninth vertical leaf positions above and below the bend to ensure that all vertical strata of the plant were sampled. Mites were counted by leaflet as eggs, immature, females, and males using a 40x dissecting microscope. Taylor's power law (Taylor, 1961) was used to quantify spatial distribution.

Evaluation of the nominal action threshold

The nominal action threshold used by rose growers was 5 mobile *T. urticae*/leaf/week. To evaluate the appropriateness of this threshold, we examined the relationship between *T. urticae* density and photosynthesis using both bent (third leaf below the bend, variety 'Kardinal') and upright shoots (third leaf above the bend, variety 'Orlando'). Mites generally occur on foliage below the harvest point and thus it is appropriate to base a threshold on physiological, rather than aesthetic, effects. Twospotted spider mites were obtained from a laboratory colony raised on miniature rose. Mite density was measured as mite-days, where mite-day = [number of mites x number of days]/leaf area. There were five replications of four target mite-day levels on the bent shoots (0, 5, 20, and 50) and six replications of three target mite-day levels on the upright shoots (0, 10, and 50). Mites were transferred to the sample leaf, on which the petiole was banded with Tanglefoot® to prevent dispersal. The control was an adjacent leaf on the same stem that was free of mites. Mites were removed after 6 days and photosynthesis was measured with a Licor LI6400 photosynthesis system (Licor, Lincoln, NE, USA). Regression analysis was used to examine the relationship between mite density and photosynthesis.

Enhancing *Phytoseiulus persimilis* movement

Phytoseiulus persimilis efficacy in the bent cane system is sometimes reduced by gaps in the lower canopy that limit interplant dispersal. We evaluated the use of interplant bridges of 2.5 cm wide orange plastic flagging tape to overcome this limitation. Each replicate consisted of five 5-liter square pots, each containing one rose plant (variety 'Orlando'). The pots were placed so that the sides were touching, but the foliage was not. The center plant in each group of five was kept free of mites, while *T. urticae* were established on the four remaining plants one week prior to initiation of the experiment. The two treatments (bridges present or absent) were replicated five times. Bridges connected the five plants of each replicate through the lower canopy. *Phytoseiulus persimilis* were obtained from a commercial insectary and released 24 h after arrival. Four hundred predators were released onto the center plant of each replicate. The number of predators on each plant was counted 24 h after release using a two-minute timed search. The effect of each treatment on the number of predators recovered was analyzed with analysis of variance.

Results and discussion

T. urticae spatial distribution

The Taylor's power law analysis indicated that *T. urticae* has a highly aggregated spatial distribution under both chemical ($b = 2.25$; $P = 0.0001$) and biological ($b = 2.64$; $P = 0.0001$) control. Calculation of relative net precision (Ruesink, 1980) suggested that the first leaf above the bend was an appropriate sample unit. The Taylor's coefficients were used in the formula of Wilson *et al.* (1983) to calculate that 38 samples of this leaf per 929 m² are required to estimate *T. urticae* density at the desired precision of 0.25.

Evaluation of the nominal action threshold

There was a weak relationship between mite density and photosynthesis in the lower canopy ($R^2 = 0.33$, $P = 0.03$), while the relationship between these factors was strong in the upper canopy ($R^2 = 0.87$, $P = 0.0001$). In addition, Jiao & Grodzinski (1998) have shown that only photosynthate produced in the flower shoot is translocated to the developing flower. Thus we used only the data from the upper shoot to evaluate the nominal action threshold of 5 mobile *T. urticae* per leaf per week. We used the equation of the regression line relating mite density to photosynthesis in the upper shoot to make the evaluation. By substituting the mite day value that corresponded to our nominal action threshold (5 *T. urticae*/leaf/week = 0.58 mite-days based on an average leaf area of 60cm²) we could determine the effect of mites at this density on photosynthesis. This analysis is as follows:

Photosynthesis as a percent of control = $101.8 - 1.1x$

$x = 0.58$ mite-days

Change in photosynthesis as a percentage of the control = 0.6 percent

Thus the current action threshold corresponds to a drop in photosynthesis of less than 1 percent per infested leaf, which suggests that the nominal action threshold should be raised.

Enhancing *Phytoseiulus persimilis* movement

Significantly more mites were recovered from plants that were connected by bridges than from those that were not (mean bridges present = 21.0 ± 3.91 ; mean bridges absent = 9.4 ± 2.45 ; $p = 0.02$), suggesting that bridges may be used to facilitate predator movement in the greenhouse. This expands the results of laboratory studies (Takafuji, 1977) that have demonstrated enhanced *P. persimilis* movement with interplant bridges. Ongoing experiments will examine the longer term effects of this technique on twospotted spider mite densities in a commercial setting.

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Demonstration and implementation of a reduced risk pest management strategy in fresh cut roses

Christine Casey, Michael Parrella

*Department of Entomology, University of California, One Shields Avenue, Davis, CA 95616
USA, E-mail: cacasey@ucdavis.edu*

Abstract: Our goal was to develop and implement a pest management program using reduced risk pesticides and biological control agents to manage the key pests of cut roses. This program represents the largest effort to implement an IPM program on floriculture crops in the United States. Eight growers spanning the major rose production areas of California participated. Data were collected at each location from an IPM and a conventional practice greenhouse. We used a comprehensive sampling plan that provided information on the density of insects, mites, and diseases. Based on thresholds developed for each of the pests, we took no action; applied a reduced risk pesticide; or released biological control agents.

Twospotted spider mite and western flower thrips densities were the same or significantly lower in the IPM compared to the conventional practice greenhouses. Biological control of mites was successful at all locations. Pesticide use was generally lower in the IPM greenhouses. Efforts to reduce prophylactic applications of fungicides using a model to predict powdery mildew incidence need further study. Control costs were comparable under the two programs.

Key words: sampling, thresholds, biological control, roses

Introduction

The frequency of pesticide sprays typical in a rose range has impeded the implementation of IPM procedures, particularly the use of biological controls. Pesticides targeting hard-to-kill floriculture pests frequently kill natural enemies, which favors continued reliance on conventional pesticides while discouraging the adoption of biological control.

The goal of this project was to develop and implement a pest management program emphasizing reduced risk pesticides and biological control agents to manage the major pests of California fresh cut roses. We proposed a reduced-risk pest management program for fresh cut roses that is comprised of three integrated components: 1) monitoring procedures for key pests; 2) application of economic and action thresholds to guide pest control decisions; and 3) reduction and substitution of conventional pesticides with reduced risk materials and biological control. An economic analysis comparing the success and cost of our reduced risk strategy to conventional grower programs was done at the completion of the project.

Materials and methods

Eight growers spanning the major rose production areas of California participated in the program. Data were collected weekly at each grower location from an IPM and a conventional

practice greenhouse using a comprehensive sampling plan that provided information on the density of insects, mites, and diseases. Specifically, fixed precision sampling plans were developed for twospotted spider mites and western flower thrips. This represents the first use of such plans in a floriculture IPM program. Based on thresholds developed for each of the pests, no action was taken; an application of a reduced-risk pesticide was made; or biological control agents were released.

Twospotted spider mites

We developed a fixed precision binomial sampling plan based on a tally threshold of 5 mobile spider mites per leaf. We have determined that the first leaf above the bend on 38 randomly selected plants per 929 m² of greenhouse area needs to be sampled to predict spider mite density with a precision of 0.25.

These samples were also used to determine co-occurrence of twospotted spider mites with predator mites, *Phytoseiulus persimilis* (Persimilis). In the IPM houses, mites were controlled with abamectin, pyridaben, azadiractin, insecticidal soap, or bifenazate until fewer than 25 percent of the sampled plants had more than 5 mobile stages on the sampled leaf. At that point Persimilis releases based on co-occurrence began. Co-occurrence greater than 80 percent meant no release; co-occurrence between 50 and 80 percent meant spot releases (5 predators per infested plant); co-occurrence less than 50 percent meant broadcast releases (1 to 5 predators per plant). All predator releases were made to leaves just below those on which mites were present.

Western flower thrips

The sampling plan for WFT used yellow sticky traps. Three 10 by 15cm yellow sticky traps were placed per 929 m². This sampling program allowed us to predict the number of thrips per flower (25/card = 1/flower; 50/card = 2/flower). The traps are placed at flower height and are evenly distributed in the house. The lower threshold of 25 thrips/trap/week is used in more susceptible varieties, while the higher threshold of 50 thrips/trap/week is used in less susceptible varieties. There is currently no cost effective biological control agent for WFT in cut roses. Our IPM program was based on directed sprays of spinosad or azadiractin when the threshold was reached as well as regular removal of open flowers from the lower canopy. Pesticides for thrips were directed to the terminal shoots and developing buds.

Powdery mildew

Each IPM house contained an Adcon (<http://www.adcon.com>; Klostemeuburg, Austria) weather station that collected data on temperature, leaf wetness, and relative humidity. A predictive model for rose powdery mildew based on these environmental conditions was evaluated. Powdery mildew is primarily affected by temperature, with infection occurring between 18 and 27°C. Pressure is cumulative, so several days of favorable conditions are needed to create high disease pressure, while several days of unfavorable conditions will create low disease pressure. As the data was collected a measure of disease pressure (ranked on a scale of 0 to 100) was generated from the model. The model is not fully functional but it already provides a tool to better predict the need for mildew control, enabling growers to move away from calendar sprays or routine sulfur volatilization, which may have chronic sub-lethal effects on the Persimilis mite predator. Our IPM program used potassium bicarbonate, piperalin, trifloxystrobin, and azadiractin. The eradicant (piperalin) was used at higher mildew pressure, while the other materials were emphasized under lower pressure.

Secondary pests

Plant inspections for whiteflies, aphids, mealybugs, *Botrytis*, downy mildew, and rust were done as part of the plant inspections for spider mites. Yellow sticky traps were also used to monitor whiteflies and winged aphids. We emphasized the use of materials that were compatible with the *Persimilis* predator for control of these pests.

Results and discussion

Twospotted Spider Mites

There were significantly more plants with no mites ($p = 0.0001$) and significantly fewer plants with mites ($p = 0.0001$) under IPM. The cost of IPM was initially higher than the cost of conventional control, although control costs became comparable after 4 to 8 weeks of IPM. Predatory mites were successfully used in each of the IPM greenhouses and almost eliminated the need for miticide applications in those houses.

Western Flower Thrips

There were significantly fewer WFT caught in the IPM houses than in the conventional houses across all nurseries ($p = 0.0001$). The largest differences in thrips levels between the two treatments occurred during the summer months when WFT pressure is generally highest. The monitoring program, removal of open flowers, and the use of reduced risk pesticides worked very effectively in the IPM greenhouses.

Rose Powdery Mildew

Despite the fact that the index remained near 100 at most sites for the majority of the study period, disease was not always observed. We suspect that other factors such as relative humidity play a role in disease development. Further study is needed to add refinements such as this to the model.

Secondary pests

Effective IPM implementation was hindered at two sites by the citrus mealybug, *Planococcus citri*. This pest is generally not a problem for rose growers until IPM is implemented, when the cessation of broad spectrum pesticide applications can allow this pest to develop. Unfortunately effective natural enemies of the mealybug are not currently available, and the most effective mealybug pesticides are detrimental to spider mite predators. We are working with the natural enemy suppliers to try and change this situation. In the interim, we will continue to evaluate reduced risk pesticides for efficacy against the citrus mealybug.

Overall, we are satisfied that the rose IPM program was successful. This program represents the largest effort to demonstrate and implement an IPM strategy on floriculture crops in the United States. We have shown that high quality roses can be produced with substantially fewer pesticides and with the incorporation of biological control into mainstream floriculture.

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Status of biological and integrated control in greenhouse vegetables in Spain: Successes and challenges

Cristina Castañé

Departament de Protecció Vegetal, IRTA-Centre de Cabrils, E-08348 Cabrils, Barcelona, Spain, E-mail: Cristina.Castane@IRTA.ES

Abstract: Spain is one of the main vegetable producers in Europe. Due to the mild weather greenhouses are unheated semi-open structures in which pest populations reach high levels. Pest origin is mainly from outdoors but there is also a rich fauna of natural enemies that colonize the greenhouses. This is an important consideration for developing effective biological control programs in this region. Nowadays, peppers are the main success in biological control, in spite of the Tomato Spotted Wilt Virus problem transmitted by the western flower thrips. For tomatoes, biological control is only applied in regions free of inoculums of the Tomato Yellow Leaf Curl Virus, transmitted by *Bemisia tabaci*.

Key words: tomato, peppers, thrips, whiteflies, natural enemies

Introduction

Spain is one of the main vegetable producers in the European Union, both in protected and in open fields, the majority of production being exported to other European countries. The vegetable growing region is located along the Mediterranean coast in the mainland, in Mallorca and in the Canary Islands. The greenhouse surface area dedicated to vegetable growing is about 70,335 ha of which 33,560 ha are concentrated in the Almería region (southeast Spain). The main horticultural crops in terms of economic importance are tomato, with 13,222 ha, and peppers, with 9,712 ha, (MAPA, 1999).

Characteristics of the Spanish agro ecosystem in greenhouses

The climate is mild for most of the year with hot summers, and the outdoor environment is a major source of pests and natural enemies. Crops encounter high pest populations, especially in summer and autumn, which move from crop to crop and survive the mild winter. There are also abundant populations of native natural enemies that hibernate in open field crops, weeds and wild flora. Greenhouses are unheated semi-open plastic structures that allow the exchange of pests and natural enemies both inside and outside the greenhouses, leading to a continuous exchange of pest populations between old and young crops, between fields and greenhouses and among greenhouse crops for most of the year. In general, protected crops are mixed with open field crops, except for the Almería region where there are only greenhouses.

Factors promoting the use of biological control

Consumer demand for vegetables with low insecticide residues is one of the reasons forcing administrators to increase the amount of farmland adopting Integrated Production by subsidizing it. Also, the disappearance of several insecticides from the market is encouraging

European governments to stimulate and support the research, extension and technology transfer of non-chemical methods for controlling insect pest and diseases.

Companies that produce natural enemies are also promoting biological control through their technical staff, who show growers the effectiveness of their products and compete with the staff of chemical companies. Finally, biological control is self-promoted since natural enemies are more efficient at controlling some pests than conventional insecticides.

Biological control successes

Biological control successes in our region have occurred when native natural enemies were involved and augmentation-conservation techniques were applied. Native natural enemies are better adapted to local conditions and the agro-ecosystems resulting from conservation strategies are more stable than those produced with exotic beneficials. This is the case with the leafminer parasitoid *Diglyphus isaea*, and the polyphagous predators *Orius laevigatus* and *Macrolophus caliginosus*. The release of these beneficials is performed by synchronizing them with the pest cycle in the crop during periods in which they are not abundant outdoors; however, late in the season their natural populations colonize the greenhouses and are the ones controlling the pest.

When exotic natural enemies were released early in the crop season, in many cases they were replaced late in the season by native species that entered the greenhouses. There are several examples: when *Encarsia formosa* was introduced in the North-eastern tomato greenhouses for the control of the greenhouse whitefly *Trialeurodes vaporariorum*, abundant populations of *M. caliginosus* were commonly found at the end of the crop that fed on the whitefly and on the parasitoid (Castañé *et al.*, 2000a). Also, in some cases, large populations of *Encarsia pergandiella* were found, an exotic species that has been established outdoors in the Mediterranean region. *E. pergandiella* interferes with *E. formosa*, which is also exotic (Gabarra *et al.*, 1999). In the South-eastern greenhouses, when the exotic species *Eretmocerus eremicus* is introduced to control *Bemisia tabaci* in pepper crops, the main species found at the end of the season is the native *E. mundus* (Federico García, personal communication). Abundant natural populations of the aphid predator *Aphidoletes aphidimyza* were found in greenhouse tomatoes, that accounted for the control of *Macrosiphum euphorbiae* (Alomar *et al.*, 1997).

1. Biological Control in greenhouse tomatoes

In the North-eastern region, the main pest of the crop is *T. vaporariorum*. Although *B. tabaci* is present at the end of the summer in regions where tomatoes coexist with ornamentals, it is not widely distributed in the region. Tomato Yellow Leaf Curl Virus (TYLCV) was detected in 2000 but it did not spread in 2001. For greenhouse whitefly control *M. caliginosus* is introduced and/or conserved, and the same for leafminer (*Liriomyza* spp.) control with *D. isaea*, which is mainly conserved and only released if populations are low. Lepidoptera and aphids are controlled with *Bacillus thuringiensis* and pirimicarb, and acaricides in the spots are used for controlling tomato russet mite, *Aculops lycopersici*. This program was applied during 2001 in 52 ha (J. Arifio, M. Martí & M. Pagès, personal communication).

In the South-eastern region the devastating problem with TYLCV transmitted by *B. tabaci* is the main cause of yield losses. This situation has greatly reduced the application of biological control, which is only used in the few spots where the virus is not present. Whiteflies

are controlled with *M. caliginosus* if it is a summer crop and with *E. eremicus* and *E. mundus* if it is a year-round crop (J. van der Blom, personal communication).

2. Biological control in greenhouse peppers

Biological control was widely applied in this crop in 1,000 ha in Murcia and Almería during 2001. The main pest problem is *Frankliniella occidentalis* and the virus that it transmits, the Two Spotted Wilt Virus (TSWV). Resistant varieties to the virus are now commercially available and were used in 20% of the greenhouses during 2001. At the start of the crop, in February, one release of *Amblyseius cucumeris* is introduced to slow down thrips population development while temperatures rise. Afterwards, thrips control is performed with *O. laevigatus* that is released as soon as flowers are present in the plant and temperatures have increased. As the crop season progresses, so does the thrips populations, which continuously enters the greenhouse. The following invasions of thrips during the crop cycle are controlled by *O. laevigatus* and these crops have fewer virus problems than conventional chemical greenhouses (Sánchez *et al.*, 2000; Lacasa & Sánchez, 2002). Biological control has been adopted by 55% of pepper growers in Murcia, which correspond to 900 ha of greenhouses (van der Blom, 2002). *O. laevigatus* is widely distributed in the Spanish Mediterranean coast and is the most common *Orius* species found in crop and non-crop hosts (Riudavets & Castañé, 1994). Other pests of the crop are aphids (*Myzus persicae*, *Aphis gossypii*, *Aulocarthum solani*), spider mites (*Tetranychus urticae*) and *B. tabaci*. For controlling aphids *Aphidius colemani* and *Aphelinus abdominalis* are released; for spider mite control *Amblyseius californicus* or *Phytoseiulus persimilis* (both spontaneous) are released, according to the natural enemy species that is predominant in the greenhouse region; *E. eremicus* (exotic but commercial) is released for *B. tabaci* control, although at the end of the crop what is found in the crop is *E. mundus*. Several natural enemies are now being tested for *Spodoptera exigua* control, a growing problem in this crop when biological control is applied.

Factors limiting the application of biological control

The horticulture is very intensive and dynamic in the South-eastern region, and the type of crops grown and the crop cycles change very rapidly according to market demands. Changes in crop cycles lead to different pest problems, and new problems sometimes arrive more quickly than solutions to previous situations. As an example, the tomatoes grown at present (they used to be cultivated only during summer but are now grown all year round) have increased the whitefly-transmitted virus problem since there is no crop free period from which to start a clean plantation. Some natural enemies are effective in summer and are less efficient in autumn-winter, when other natural enemies have to be released. Therefore, for the same crop and the same pest problem different strategies have to be designed depending on the crop cycle.

Temperatures inside the greenhouses are low during winter because they are not heated. Installation of introduced natural enemies is difficult because their development is generally slower than that of the pest.

Some pests became important for crops that have not been affected previously, as is the case of *S. exigua*, which now is reaching a pest status in biological control greenhouse peppers, for which no effective solution has been found yet. Pest problems and potential solutions of a crop are in constant evolution and demand continuous revision by experts in the strategies for their control.

Some crops are very sensitive to certain pests and their damage threshold is too low. This is the case of Dutch type cucumbers, which are very sensitive to western flower thrips damage (Castañé *et al.*, 2000b)

A major increase in insect transmitted viruses has been detected. If the crop cycle starts when the vector and inoculums are high, growers have dramatic yield losses. This is the case with the tomato crop in south-east Spain, where it is planted at the end of summer. Although insecticides are no more efficient than natural enemies for avoiding virus problems, in general growers are not willing to use biological control in this situation. Only virus-resistant varieties of tomatoes will grow satisfactorily. Meanwhile, the practice is to net the ventilation openings and prevent the entrance of whiteflies (Berlinger *et al.*, 1991). On the other hand, the pepper crop cycle in Murcia starts in winter, when the virus vector population is low. This has led to the massive adoption of biological control by growers due to its greater efficiency in comparison to insecticides.

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Preliminary study on the effect of nitrogen fertilization on cotton aphid, *Aphis gossypii*

Amanda Chau¹, Kevin M. Heinz¹, Fred T. Davies, Jr.²

¹Biological Control Laboratory, Department of Entomology, Texas A&M University, College Station, TX 77843-2475, E-mail: achau@tamu.edu; ²Department of Horticulture, Texas A&M University, College Station, TX 77843-2133

Abstract: The effect of nitrogen fertilization on the abundance of cotton aphid, *Aphis gossypii* Glover, was studied on chrysanthemum, *Dendranthema grandiflora* Tzvelev var. "Charm". We tested five fertilization treatments that consisted of 20%, 50%, 75%, 100% and 130% of the recommended nitrogen level. We transferred five apterous aphids to each pot at the beginning of the experiment and counted aphids weekly for 3 weeks. There were no significant effects of nitrogen on aphid abundance possibly due to delay reaction of the host plants to changes in nitrogen levels.

Key words: cotton aphid, *Aphis gossypii*, nitrogen fertilization, chrysanthemum, *Dendranthema grandiflora*, aphid abundance

Introduction

The cotton aphid, *A. gossypii*, is an important pest of greenhouse ornamental crops such as chrysanthemum, *D. grandiflora*. This aphid is highly polyphagous and has a worldwide distribution (Blackman & Eastop, 2000). Dietary nitrogen is one of the most important factors that influence the development and performance of herbivorous insects like aphids (Dixon, 1970; Mattson, 1980; Douglas, 1993). Nitrogen fertilizers are widely used in greenhouse ornamental production and are an important source of nitrogen for both the plants and their pests. Previous studies on the effects of nitrogen fertilization on aphids focused mainly on body size or weight, fecundity, intrinsic rate of increase and survival of individual aphids in a single generation (van Emden, 1966, Petit *et al.*, 1994; Bethke *et al.*, 1998; Nevo & Coll, 2001). The effect of changes in host plant quality may be obscured by the nutritional quality of the host plants that the parent generation fed on. Like other aphids, cotton aphids are parthenogenetic and viviparous. The nutritional quality of the host plant that the mother was feeding on has a direct impact on her developing embryos (Nevo & Coll, 2001). As a result, we investigated the effect of nitrogen fertilization on cotton aphid abundance across a number of generations on chrysanthemum.

Material and methods

Two hundred rooted cuttings of chrysanthemum, *D. grandiflora* var. "Charm" were transplanted into 50 6-inch standard pots (15.5 cm in dia., 14.5 cm in depth). We used Sunshine Mix#1 (Sun Gro Horticulture Inc., Washington) for potting media and evenly spaced four cuttings within each pot. The plants were kept in 3 growth chambers at 20°C night/24°C day, 75% RH and under 16L: 8D photoperiod. All plants were initially fertilized with 375 ppm N (Peters Professional Peat-lite special 15-16-17, Scotts-Sierra Horticultural Products Company, Marysville, OH, USA). The recommended range of nitrogen for potted chrysanthemum is 350

to 400 ppm N for pulse feeding (Scotts-Sierra Horticultural Products Company). Plants were fertilized twice a week and watered as needed between fertilizations. When there was 2 to 2.5cm of new growth on the plants, each plant was soft pinched to approximately 7 laterals. Ten pots were randomly selected for each treatment. For the five fertilization treatments, we made up solutions with Peters 15-16-17 to the following concentrations: 75 (20%), 188 (50%), 281 (75%), 375 (100%) and 488 (130%) ppm N. A fixed amount of fertilizer (200 ml) was applied to each pot twice a week regardless of the treatment. When there was 2 to 2.5 cm of new growth on the plants, the photoperiod of the growth chambers was shortened to 11L:13D to initiate flower primordia. Seven days after switching to “short day” condition, we applied plant growth regulator (B-Nine WSG, Uniroyal Chemical Company Inc., Middlebury, CT, USA) to all pots at the concentration of 3,500 ppm to reduce internode elongation. Five apterous early adults of *A. gossypii* were transferred to each pot the day after B-9 application. The aphids were placed on the apical region of the plants. The plants were approximately 5 weeks old at the time of aphid transfer. Cotton aphids used in this experiment were from a colony established with individuals collected from naturally infested chrysanthemums (var. “Miramar”) in the greenhouse and maintained in the laboratory at $26\pm 1^{\circ}\text{C}$, $46 \pm 5\%$ RH and 11L:13D photoperiod on chrysanthemums (var. “Charm”). The number of aphids on each pot was counted weekly until apterous aphids or alates started to leave the pots. The data were analyzed using two-factor, repeated-measures ANOVA tests (SPSS, SPSS Inc., Chicago, IL, USA) with chamber and treatment (nitrogen concentration) as main effects. The Greenhouse-Geisser adjustment was used to correct for sphericity. The data were transformed to their natural logarithms to meet the requirements necessary for the ANOVA models.

Results and discussion

Our results showed that concentrations of nitrogen in the fertilizer had no effect on the number of aphids within the 21 days trial period (Fig. 1, table 1). However, there were growth chamber effects on aphid abundance (table 1). The mean number of aphids was significantly higher in one chamber when compared to the other two. Nevertheless, our analysis showed that there was no interaction between chamber and treatment effect (table 1). One pot was excluded in our analysis because no aphids were found on it after the initial transfer.

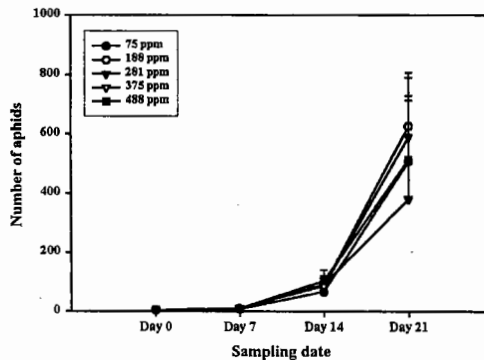


Figure 1. Mean number (+ SE) of cotton aphids, *Aphis gossypii* in treatments with different nitrogen concentrations: 75, 188, 281, 375 and 488 ppm N (n= 10, except for 281ppm N, n=9).

Table 1. Repeated measures two-way ANOVA for effects of treatment (nitrogen concentration) and chamber (growth chamber) on the abundance of cotton aphid, *Aphis gossypii*. The Greenhouse-Geisser (G-G) adjusted test *P* values were also given.

Source	<i>df</i>	<i>F</i>	<i>P</i>	G-G
Treatment (T)	4	0.073	0.990	
Chamber (C)	2	16.630	<0.0001	
T x C	8	0.755	0.644	
Error	34			
Sampling date (D)	3	240.109	<0.0001	<0.0001
D x T	12	0.415	0.955	0.928
D x C	6	19.528	<0.0001	<0.0001
D x T x C	24	0.841	0.678	0.651
Error	102			

Previous studies on the effect of nitrogen fertilization on aphids have shown mixed results. A number of studies on cotton aphid, *Aphis gossypii*, in cotton have shown that the fecundity of the aphids was positively affected by nitrogen fertilization (Rosenheim *et al.*, 1994; Nevo & Coll, 2001). van Emden (1966) showed that the cabbage aphid, *Brevicoryne brassicae* (L.) and the green peach aphid, *Myzus persicae* (Sulz.) on Brussel sprout also responded positively to increase in nitrogen fertilization. However, other studies have found that nitrogen fertilization had no effects on either aphid fecundity or number. Archer *et al.* (1995) showed that the number of Russian wheat aphid, *Diuraphis oxia* (Mordvilko) on field-grown wheat was influenced by irrigation but not nitrogen fertilization. Bethke *et al.* (1998) found that the fecundity of Melon aphid, *Aphis gossypii*, was affected by cultivars of chrysanthemums but not irrigation or fertilizer treatments. Our failure to detect differences in aphid abundance between the nitrogen treatments might have been due to a delay reaction of the host plant to changes in nitrogen levels. The plants were fertilized with the same level of nitrogen initially until "soft pinch". The residual nitrogen might have influenced the effects of subsequent nitrogen treatment. To determine the effects of nitrogen fertilization on aphids, we need to also examine the effects of nitrogen fertilization on the host plant. Schuch *et al.* (1998) showed that leaf and stem dry weight, plant height and leaf nitrogen were significantly affected by fertilizer and irrigation treatment. van Emden & Bashford (1969) and van Emden (1966) also found that the soluble nitrogen concentration within plants was affected by nitrogen fertilization and thereby influenced the fecundity of the aphids.

Reduction of fertilizer usage has been an important goal for the ornamental industry. Environmental concerns related to high level of nitrogen in run off have prompted changes in nitrogen usage for a number of crops such as roses, poinsettias and hydroponically grown chrysanthemum (Kageyama *et al.*, 1991; Cabrera *et al.*, 1993; Rose & White, 1994). The use of nitrogen fertilizer might alter the susceptibility of plants to pest insects and their damage. Understanding the influence of nitrogen fertilizer on the host plant and the pest insects would help us to improve the crop quality and minimize damage or loss due to pest outbreaks.

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Intraspecific interactions among the predators *Orius majusculus* and *Aphidoletes aphidimyza*

Rikke Kirkeløkke Christensen^{1,2}, Annie Enkegaard², Henrik F. Brødsgaard²

¹Copenhagen University, Department of Population Biology, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark, E-mail: Annie.Enkegaard@agrsci.dk; ²Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark

Abstract: Predation by the generalist predator *Orius majusculus* on the widely used aphid predator, *Aphidoletes aphidimyza* was studied in laboratory experiments. In 24-hour tests in small arenas, mortality of *A. aphidimyza* eggs and larvae were measured when exposed to *O. majusculus*. In addition it was examined if the introduction of aphids, *Aphis gossypii*, as alternative prey would affect the degree of predation. *O. majusculus* consumed gallmidge eggs in large numbers, independent of the presence of aphid prey. Larvae of *A. aphidimyza* was killed in substantial amounts when presented to *O. majusculus* as the only prey species, but the predation was significantly reduced in the presence of *A. gossypii*.

Key words: *Aphidoletes aphidimyza*, *Orius majusculus*, intraguild predation, biological control, glasshouse crops

Introduction

The pest complex in ornamental greenhouses is often large. Biological control programs in these crops therefore involve the use of a series of beneficials. For control programs to be effective the beneficial species used must be able to co-exist without deleterious effects on each others control efficiency. For evaluation of compatibility of control agents, food specificity is an important characteristic. A polyphagous beneficial that shows no particular preference for the target pest may prey indiscriminately on all species encountered, pest or beneficial. Anthocorids in the genus *Orius* (Hemiptera: Anthocoridae) normally feeds on trips, aphids, mites, and other soft bodied arthropods (Hodgson & Aveling, 1988). *Orius majusculus* (Reuter) is marketed as a biological control agent of thrips e.g. *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), but is generally known as a polyphagous predator (Zwahlen *et al.*, 2000).

The present study was designed to assess the predation risk of *O. majusculus* on the commonly used aphid predator *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). The gallmidge *A. aphidimyza* is predatory in the larval stage and the larvae feed exclusively on aphids. Generalist predators such as *O. majusculus* could affect the control efficiency of *A. aphidimyza*. Predation was examined when *O. majusculus* had access to *A. aphidimyza* as the only prey, and it was also determined if the availability of alternate prey affected the tendency of *O. majusculus* to kill *A. aphidimyza*. The predation risk was examined for both eggs and larvae of *A. aphidimyza*.

Material and methods

Prey and predators

All experimental animals were reared separately on chrysanthemum (*Dendranthema grandiflorum*, cv. Purple Cindy) in greenhouses in mesh covered cages (61 x 66 x 75 cm) at 22°C, L:D 16:8 h, 70% RH. Both predator species were fed on *Aphis gossypii* (Glover) (Hemiptera: Aphididae). Each generation of *A. aphidimyza* was synchronised by removing all adults after a 24-hour egg laying period so that all eggs or larvae in a colony were of similar known age.

Experimental design

The predation of *O. majusculus* adult females on *A. aphidimyza* eggs and larvae were examined using the following combinations. In some combinations *A. gossypii* was used as alternative prey.

1. 250 *A. aphidimyza* eggs, 30 4th instar *A. gossypii* nymphs and one *O. majusculus*
2. 250 *A. aphidimyza* eggs and one *O. majusculus*
3. 30 4th instar *A. gossypii* nymphs and one *O. majusculus*
4. 250 *A. aphidimyza* eggs and 30 4th instar *A. gossypii* nymphs
5. 250 *A. aphidimyza* eggs
6. 30 *A. gossypii*
7. 25 *A. aphidimyza* larvae, 500-600 *A. gossypii* of mixed instars and one *O. majusculus*
8. 25 *A. aphidimyza* larvae and one *O. majusculus*
9. 25 *A. aphidimyza* larvae and 500-600 *A. gossypii* of mixed instars
10. 25 *A. aphidimyza* larvae

where combination 4-6 and 9-10 served as controls.

Petri dishes (9 cm Ø), each containing a chrysanthemum leaf (~15 cm²), were used as experimental arenas. All experiments were carried out in climate cabinets at 25°C, RH 65-75%, L:D 18:6 for a 24 hour period. Gallmidge eggs were removed from leaves from the rearing with a fine brush and placed randomly on the underside of the experimental leaf that was placed in the arena with the underside facing up. To ensure that no eggs would hatch during the experimental period, eggs were collected immediately after the egg laying period. In the relevant combinations fourth instar *A. gossypii* nymphs were placed on the leaf and allowed one hour to settle. Likewise gallmidge larvae, 72 hours of age, were placed on an experimental leaf that was either clean or infested with 500-600 *A. gossypii* of mixed instars. This high number of aphids ensured that excess amounts were present throughout the experimental period. One female *O. majusculus*, obtained randomly from the rearing, was then transferred to the leaves and the arena was sealed with parafilm. At the end of the experiments the number of eggs left uneaten and the number of live, dead, and escaped aphids were recorded in combination 1-5. In combinations where predation on gallmidge larvae was examined the data recorded were live, dead and escaped *A. aphidimyza* larvae. The number of replicates varied between 10 and 24.

Statistical analysis

Results were analysed by logistic regression analysis in the procedure PROC GENMOD in SAS (SAS Institute Inc., 1989). In this analysis the predation by *O. majusculus* in the various treatments was analysed using proportions of total number of prey killed (number killed

/number offered). Before analysis corrections were made for the mortality in the relevant control combinations.

Results

The results of the experiments demonstrated that *O. majusculus* will prey upon both eggs and larvae of *A. aphidimyza* and that the predator has a voracious appetite especially for gallmidge eggs (table 1). Even in the presence of aphid prey *O. majusculus* maintained the high predation rate, and the consumption of eggs was not significantly reduced ($F = 1.80$; $df = 1$; $P = 0.19$). However, a significant reduction in the mortality inflicted on the gallmidge larvae was seen when aphids were available as an alternative food source ($F = 57.10$; $df = 1$; $P = 0.001$).

Regarding the predation on aphids by *O. majusculus*, experiments revealed that the number *A. gossypii* killed was not significantly reduced in the combinations where gallmidge eggs and aphids were presented simultaneously ($F = 2.99$; $df = 1$; $P = 0.0925$).

Table 1. Predation, expressed as numbers eaten in 24 hours, by *O. majusculus* on *A. aphidimyza* eggs and larvae, and on *A. gossypii* nymphs. Values in square brackets are 95% confidence limits. Values in round brackets are the number of replicates. Values in the same row are significantly different if followed by different letters. ($P \leq 0.05$).

Type of prey	Numbers of prey killed	
	Aphids absent	Aphids present
<i>A. aphidimyza</i> eggs	205.1 [178.3; 222.5] (16) a	182.1 [152.2; 203.4] (16) a
<i>A. aphidimyza</i> larvae	16.3 [14.6; 17.9] (24) b	6.8 [5.2; 8.7] (20) a
<i>A. gossypii</i>	Eggs absent	Eggs present
	20.2 [17.9; 19.7] (22) a	17.1 [14.4; 19.7] (20) a

Results from control combinations 9 and 10 showed that the mortality of *A. aphidimyza* larvae during the 24-hour period was relatively low. 5.2 % of the larvae died when there was no access to aphids and 1.2 % died when aphids were present in the arena. These results were significantly different ($F = 5.65$; $df = 1$; $P = 0.0246$).

Discussion

The results of the present study confirm the generalist predation character of *O. majusculus* and include the gallmidge *A. aphidimyza* in its range of prey. In all combinations of aphids and gallmidges, *O. majusculus* inflicted high levels of mortality on both gallmidge eggs and larvae. There was no significant reduction in the amount of eggs eaten when *O. majusculus* had access to both gallmidge eggs and aphids simultaneously. However, in addition to the large amount of eggs eaten the predator also killed a substantial number of aphids. *A. aphidimyza* larvae were readily accepted for consumption by *O. majusculus* when there was no other food source available. When *A. gossypii* was presented simultaneously it resulted in a reduction in the larval killing rate, but larvae were still included in the choice of prey. The high aphid density in this part of the experiment may have created a dilution effect that can have increased the survival of the gallmidge larvae.

The present results indicate that the polyphagous character of *O. majusculus* could in fact present a problem in a biocontrol situation requiring the use of both *A. aphidimyza* and *O. majusculus*. A normal greenhouse with full size plants may not, however, be directly comparable to the two-dimensional set-up used in these experiments. A greenhouse environment presents the insects with the ability to seek out preferred microhabitats and gives prey species a better chance to escape when attacked. *O. majusculus*, however, is a very mobile predator that primarily search for prey along the veins of the leaves (Hodgson & Aveling, 1988). *A. aphidimyza* females prefer to lay their eggs on the under side of the leaf where aphid density is high (Markkula *et al.*, 1979) leaving both eggs and larvae vulnerable to predation by *O. majusculus*. The low pest tolerances in ornamental crops create control situations of low pest densities and relatively high natural enemy densities leading to prey scarcity and frequent encounters among predators.

Experiments in glasshouse cabinets have revealed that using a generalist predator like *O. majusculus* for biological control will affect other biological control systems. Beneficials can serve as alternative prey for polyphagous predators when the target prey is scarce, thereby enabling survival of the predator in the culture. However, this predation may result in a poorer control of the pest targeted by the beneficials that served as alternative prey (Brødsgaard & Enkegaard, 1997). Other experiments have indicated that some generalist predators are able to co-exist with other control agents without negative effects on the control efficiency. For example small cage experiments showed that *Orius tristicolor* (White) preyed on the predatory mite *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae), but it was concluded that the two species would be compatible in a greenhouse environment if trips were present (Gillespie & Quiring, 1992). These results illustrate the importance of analysing separately the processes of predation when regarding a polyphagous predator for biological control. Prey preference, habitat preference and search strategies should be carefully considered.

In the case of *O. majusculus* and *A. aphidimyza* the present results have shown a substantial potential of *O. majusculus* to affect negatively on a population of *A. aphidimyza*, even when alternative prey is easily available. Such knowledge should be taken into account in the development of IPM programs.

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The interplay between biological characteristics and interactions among predatory mites in biocontrol on protected crops

Michael de Courcy Williams, Lidija Kravar-Garde

Department of Entomological Sciences, Horticulture Research International, Wellesbourne, Warwicks CV35 9EF, UK, E-mail: Michael.DeCourcyWilliams@hri.ac.uk

Abstract: The survival of the immature stages of four species of phytoseiid mites (*Phytoseiulus persimilis*, *Iphiseius degenerans*, *Neoseiulus californicus* and *N. cucumeris*) was high and the adult life span (after the first egg was laid) varied between 19-58 days between species. Survival of food deprivation differed substantially between species and was doubled when water was available. There was little indication of negative intra- or inter-specific interactions between the immatures of the four species when food was available. Under food deprivation the survival of immatures of the specialist predator *P. persimilis* was reduced by the generalist predators *N. californicus* and *I. degenerans*.

Key words: phytoseiid mites, life span, survival, interactions, intraguild predation

Introduction

Releases of multiple natural enemies, including both specialists and generalists, are a feature of biological control of pests of many crops. The likelihood and extent of interactions between the different natural enemies has become an important issue, particularly with the low pest levels required by growers of protected crops. An understanding of the possible interactions between phytoseiid mites, such as intraguild predation and cannibalism, has revealed important biological processes (Shausberger & Croft, 1999, 2000) that are relevant to pest control systems. A knowledge of how these mites interact and survive periods with little or no food is of direct help to growers in identifying the best combinations of predators and how frequently they should be introduced for biological pest control. This work examines the interactions (under different conditions of food availability) between immatures of four mite species (*Phytoseiulus persimilis*, *Iphiseius degenerans*, *Neoseiulus californicus* and *N. cucumeris*), which are used for the control of thrips and spider mites in protected crops.

Material and methods

Mite rearing

Stock mites were obtained from commercial suppliers (Koppert BV and Syngenta Bioline Ltd) and kept as laboratory cultures (Ramakers & van Lieburg, 1988; Overmeer, 1985). Food was supplied as spider mites (*Tetranychus urticae*) for *P. persimilis* and *N. californicus*, pollen (*Ricinus communis*) for *I. degenerans* and bran mite (*Tyrophagus* sp.) for *N. cucumeris*. All cultures and experiments were kept in illuminated incubators at 20°C, 16L:8D.

Adult life span

Immature mites were collected and reared on platforms (200 mm x 150 mm) and classed as adults when the first eggs were produced. Adult females were kept individually in small black

plastic mesh cages (40 mm square x 3 mm deep with a 16 mm diameter circular hole), covered on one surface by a nylon gauze screen (65 μ aperture) and on the other with a clear perspex cover. Mites were either provided with or deprived of food (*T. urticae*) as two treatments. One set of the food-deprived mites was provided with water and a second set was deprived of water. Water was supplied using a wick (40 mm x 90 mm) of filter paper (Whatmans No.1) with a circular hole (16 mm diameter) that was held between the cover and the body of the cage. The mites were checked daily and provided with food as necessary.

Survival of immature stages

Eggs were harvested and placed individually in mesh cages and provided with excess food just prior to egg hatch. The survival of the immatures was monitored daily until adulthood. A second treatment monitored survival of the immatures without food but with water.

Intra- and Inter-specific interactions between immature stages

To test for intra-specific predation even aged cohorts of 10 eggs of each of the four species were kept in mesh cages and the survival of the developing immatures was recorded on a daily basis. To test for inter-specific predation even aged cohorts of pairs of species (5 eggs each) were allowed to develop and interact in the mesh cages. Survival was recorded for two treatments, one with excess food and a second with no food but provided with water.

Results and discussion

The mean life span of adult female mites (after the first egg was laid) differed between species over the range of 19 to 58 days (table 1). With a mean of 58 days the life span of adult female *N. californicus* was longer ($P < 0.001$) than the other species. No difference was observed between the life spans of adult female *N. cucumeris* (25 days) and *I. degenerans* (28 days), which were longer ($P < 0.05$) than the life span of adult female *P. persimilis* (19 days).

Table 1. Mean (\pm SE) life span in days for adult female mites (after first egg laid) at 20°C under different regimes of food and water provision.

Mite species	With food	(n)	No food	
			Without water 70% rh (n = 10)	With water (n = 11)
<i>P. persimilis</i>	18.6 \pm 4.80	(21)	2.5 \pm 0.37	6.1 \pm 0.56
<i>I. degenerans</i>	27.8 \pm 4.84	(24)	2.8 \pm 0.20	4.0 \pm 2.27
<i>N. cucumeris</i>	25.2 \pm 9.01	(21)	3.9 \pm 0.35	10.0 \pm 1.65
<i>N. californicus</i>	58.4 \pm 13.49	(18)	3.3 \pm 0.15	17.9 \pm 0.92

In the absence of food and water adult female mites survived for a maximum of between 2-4 days and both *N. cucumeris* and *N. californicus* survived longer than the other species (table 1). Survival time was at least doubled when water was available to the mites deprived of food (table 1). For *P. persimilis* and *I. degenerans* life span increased to 6 and 4 days respectively when water but no food was available (table 1). When *N. californicus* had no food but had access to water, its life span of 18 days was comparable to that of *P. persimilis* (19 days) when the latter had access to a continuous food supply (table 1).

The development time of immature stages was shortest for *P. persimilis* (5.2 days) but there were no differences in development time for *I. degenerans* (6.6 days), *N. cucumeris* (6.4

days) and *N. californicus* (6.2 days) (table 2). When kept individually with excess food, the survival of immature stages was 100% after 7 days ($n = 24-33$ for individuals tested). Without food but with water the survival of the immature stages was high at day 4 (80-100%), except for *I. degenerans* (55%). At day 7 survival of immatures was low (0-60%), except for *N. californicus*, which still showed survival of 92% (table 2).

Table 2. Development time (with food) and survival (water and no food) of the immatures of four mite species when kept individually.

Mite species	Food		Water only			
	n	Days (Mean \pm SE)	n	Days (Mean \pm SE)	Percent	
					Day 4	Day 7
<i>P. persimilis</i>	10	5.2 \pm 0.20	33	5.1 \pm 0.22	79%	6%
<i>I. degenerans</i>	9	6.6 \pm 0.18	44	3.6 \pm 0.09	55%	0
<i>N. cucumeris</i>	25	6.4 \pm 0.10	29	6.8 \pm 0.22	100%	59%
<i>N. californicus</i>	34	6.2 \pm 0.13	38	9.8 \pm 0.39	97%	92%

When immatures of the same species were allowed to interact with each other, survival was high with food available and there was little evidence of negative interactions (table 3). Furthermore, there was little indication of intra-specific predation when no food but only water was available as survival of the immature stages was high with the exception of *I. degenerans* and *P. persimilis* after 4 days (table 3). However, survival of most species without food but provided with water fell off by day 7, except for *N. californicus*, which by contrast showed 90% survival (table 3).

Table 3. The percent survival when 10 individual immatures of the same species were confined together and provided with food or with only water for each of the four mite species.

Mite species	Day 4		Day 7	
	Food	Water	Food	Water
<i>P. persimilis</i>	98%	84%	97%	21%
<i>I. degenerans</i>	73%	74%	60%	14%
<i>N. cucumeris</i>	87%	94%	87%	72%
<i>N. californicus</i>	90%	96%	87%	90%

When immatures of pairs of different species were allowed to interact with each other, survival was high with food available for all combinations of species at day 4 (89-98%) and at day 7 (80-98%). In the absence of food but with water available the survival of *P. persimilis* at day 4 was reduced by the presence of *I. degenerans* and *N. californicus* (from 91% to 28% and 62% respectively, Fig. 1b). After 7 days of interaction, without food but with water available, the survival of most species was low (4-40%) except for *N. californicus* and *N. cucumeris*, which had survival rates of 98% and 75% respectively when interacting with *P. persimilis*.

There are substantial differences in the biological characteristics of phytoseiid mites in terms of their life span, survival capabilities and the degree of interaction between species.

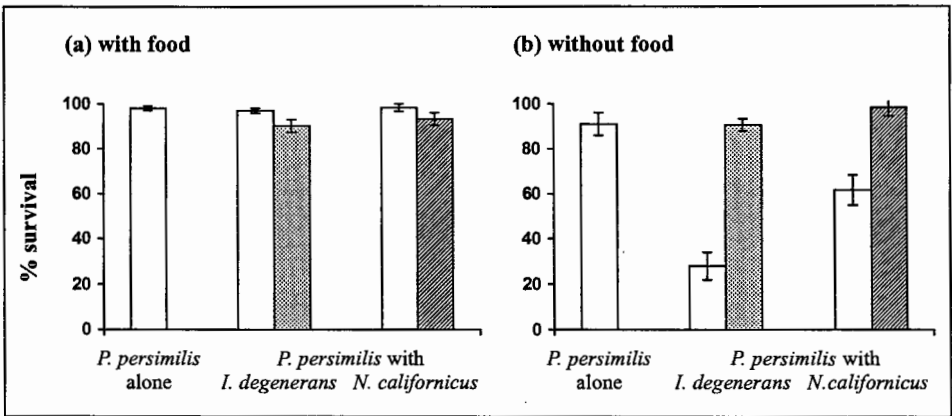


Figure 1. Mean percent survival to four days of immatures of *P. persimilis* alone and when interacting with *I. degenerans* and *N. californicus*. Error bars show standard errors.

This work supports previous conclusions that generalist phytoseiids are more likely than specialists to be intraguild predators (McMurty & Croft, 1997; Shuasberger & Croft, 2000) and that this tendency is evident in the immature stages. This can also be true for long lived species, such as *N. californicus*. It appears that this interaction is likely to be weak when food is abundant. However, under conditions of food deprivation the impact of possible negative interactions need to be evaluated in the context of the life span and survival capabilities of both the intraguild predator and intraguild prey.

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“Resistance” towards biological control

Annie Enkegaard, Henrik F. Brødsgaard

Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark, E-mail: Annie.Enkegaard@agrsci.dk

Abstract: Failure in biological control of the shallot aphid, *Myzus ascalonicus*, a close relative of *M. persicae*, with the parasitoids *Aphidius colemani* and *A. ervi* in a Danish commercial Bellflower culture led to investigations into the interactions between the aphids and the parasitoids. Laboratory experiments revealed that *A. colemani*, *A. ervi* and *Aphelinus abdominalis* either did not parasitise or had an extremely low degree of parasitisation of *M. ascalonicus*. This resulted from behavioural defence mechanisms that caused *M. ascalonicus* to quickly drop from the plants or walk away from the parasitoid in response to the first examining touch combined with apparent emission of alarm pheromones to alert the aphid colony. The parasitoids lost interest in the aphids when they began walking. This, combined with the fact that a large proportion of dropping aphids probably survived by merely landing on lower plant parts of the very compact Bellflower plants, meant that *A. colemani* and *A. ervi* neither directly nor indirectly exerted any effect on the *M. ascalonicus* population – creating, in effect, a situation of resistance to parasitoid biocontrol.

Key words: *Myzus ascalonicus*, *Myzus persicae*, *Aphidius colemani*, *Aphidius ervi*, *Aphelinus abdominalis*, parasitisation, biological control, glasshouse pests

Introduction

In 2000, a Danish producer of Bellflower (*Campanula haylodgensis* Hort.) reported having problems with biological control of aphids with the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) applied in the culture via a banker plant system. Likewise the use of banker plants with *A. ervi* Haliday failed to control the aphids. The aphid species was initially believed to be either *Myzus persicae* (Sulzer) or *M. nicotiana* Blackman (Homoptera: Aphididae) but was later identified as the shallot aphid, *M. ascalonicus* Doncaster, an aphid commonly found on various weeds and occasionally being a pest problem in e.g. shallots and strawberries (Heie, 1994). The aphids presumably arrived in the glasshouse via infested plants that had spend part of their growing cycle outdoor.

M. ascalonicus is very closely related to *M. persicae* and the failure of the *Aphidius*-parasitoids to exert control was baffling. As a consequence a search for an explanation was initiated. After evaluation of the situation at the producer’s site side-effects of insecticides or growth retarders, or poor quality of the parasitoids could be ruled out as causes of the biocontrol failure. Instead it became clear that the cause had to lie in the interaction between the parasitoids and aphids themselves. To elucidate the possible mechanism behind what, in effect, was resistance in *M. ascalonicus* to biocontrol with *Aphidius* spp. the following experiments were undertaken.

Material and methods

Insects

M. ascalonicus was collected in a commercial greenhouse and reared on *C. haylodgensis*, cv. Elisabeth Oliver, in a greenhouse in mesh covered cages (61 x 66 x 75 cm) at 22°C, L:D 16:8 h, 70% r.h.. *A. colemani*, *A. ervi* and *Aphelinus abdominalis* Dalm. (Hymenoptera: Aphelinidae) were obtained from a supplier of beneficials, J.A. Consult, Denmark.

Parasitisation

A. colemani, *A. ervi* or *A. abdominalis* females (max. 1 days old) were exposed individually to a pepper leaf, the petiole wrapped in water satiated cotton wool, with 20 2nd instar *M. ascalonicus* placed in a Petri dish (9 cm Ø). The aphids were allowed 1-2 hours to settle after transfer from the rearing. Parasitisation took place in climate cabinets at 25°C, RH 65-75%, L:D 16:8 for either 5 (*A. colemani*, *A. ervi*) or 24 (*A. abdominalis*) hours with 10 replicates for each parasitoid species. After 2-2½ week the dishes were examined and the number of parasitised and unparasitised aphids recorded

Aphid behaviour

The response of *M. ascalonicus* to the presence of parasitoids was examined by placing 30 aphids, divided in approx. equal proportions of small, medium and large stages, on a Bellflower leaf placed in a Petri dish (9 cm Ø). The petiole of the leaf was placed in a small glass tube. The tube was held in position by modelling clay to prevent the leaf from contact with the Petri dish lid and sides, hereby allowing the aphids to drop from the leaf as a defensive response. The aphids were given 1-2 hours to settle on the leaf after transfer from the rearing. Subsequently, 3 female *A. colemani*, max. 1 day old, were added to the Petri dish and the set-up was monitored for 10 minutes. Monitoring took place in the laboratory at 20-22°C. The total time spent by each parasitoid on the leaf during the 10 minutes was recorded and summed to a total visit time for the 3 females. After removal of the parasitoids the number of aphids on the leaf and in the dish were recorded. The response of *M. persicae* to the presence of *A. colemani* was recorded in the same way to serve as control. 14 and 16 replicates were made for *M. ascalonicus* and *M. persicae*, respectively.

Aphid and parasitoid behaviour

A more detailed examination of the interactions between aphids and parasitoids was made by placing 15 aphids of mixed stages on a Bellflower leaf in a similar set-up as described above. One female parasitoid (*A. colemani* or *A. abdominalis*), max. 1 day old, was placed in each Petri dish and the behaviour of aphids and parasitoids were observed for ½-1 hour under stereomicroscope. 5 and 3 individuals were observed for *A. colemani* and *A. abdominalis*, respectively.

Results and discussion

Parasitisation

Neither *A. ervi* nor *A. abdominalis* parasitised the shallot aphids at all as evidenced by the recovery of no mummified aphids among the 200 aphids exposed to the two parasitoid species. The parasitisation exerted by *A. colemani* was likewise virtually non-existing since only 1 out of the 200 offered aphids mummified. These results are, in the extreme, different from the degree of parasitisation exerted by *A. colemani* on *M. persicae* in similar set-ups

under similar conditions where an average parasitisation percentages of 51% was observed (Enkegaard & Brødsgaard, unpubl. data).

Aphid behaviour

The results of the experiment demonstrated that *M. ascalonicus* had a distinctly different response to the presence of parasitoids than the closely related *M. persicae*. Thus, 54.4% [95% confidence limits: 40.9; 67.4%] of *M. ascalonicus* had abandoned the Bellflower leaf after 10 minutes in the presence of *A. colemani* whereas only 15.8% [8.6; 27.4%] of *M. persicae* responded in the same way. These percentages were significant different ($P=0.002$; PROC GENMOD (SAS Institute Inc., 1989)). There were no differences in the reaction of the different stages of aphids in their response to the presence of parasitoids, for neither *M. ascalonicus* nor *M. persicae* ($P>0.49$, PROC GENMOD (SAS Institute Inc., 1989)).

There was a significant difference ($P=0.0001$, PROC GLM (SAS Institute Inc., 1989)) in the total parasitoid visiting time in set-ups with *M. ascalonicus* (total visit time (\pm s.e.) 5.5 ± 1.55 min) and *M. persicae* (total visit time (\pm s.e.) 16.69 ± 1.49 min). This presumably reflected a reduced parasitoid interest in remaining long on leaves with decreased density of *M. ascalonicus*. There was no correlation between visiting time and the percentage of aphids abandoning the leaf during the experiment for either of the aphid species ($P>0.14$, PROC REG; (SAS Institute Inc., 1989)).

Aphid and parasitoid behaviour

The 5 *A. colemani* females had a total of 50 encounters with *M. ascalonicus*. 76.0% (s.e. $\pm 6.0\%$) of these encounters resulted in the aphids either dropping from the leaf ($40.0\pm 6.9\%$) or abandoning feeding and starting to walk away from the parasitoid ($36.0\pm 6.8\%$). None of the encounters resulted in parasitisation or parasitisation attempts. A similar pattern was seen for *A. abdominalis* where a total of 37 encounters were observed. $89.2\pm 5.1\%$ of the encounters lead to defensive reactions, either dropping from the leaf ($43.2\pm 8.1\%$) or walking away from the parasitoid ($45.9\pm 8.2\%$). Only 1 aphid was stung by the parasitoids. No observations were made for *A. ervi* but *M. ascalonicus*' reaction towards this species is likely to be the same, as judged from the lack of parasitisation.

The results have shown that the behaviour of *M. ascalonicus* is clearly very different from the behaviour of closely related species of *Myzus*. They are more easily disturbed and are quick to either drop from the leaf or walk away from the parasitoid in response to the first examining touch. This "nervousness" clearly affects the parasitoids that lose interest when the aphids start to wander off. This loss of interest may reflect parasitoid adaptation to avoid oviposition in aphids that are likely to drop from the plant hereby posing a mortality risk to the parasitoid offspring (Chau & Mackauer, 1997). Emission of alarm pheromones apparently took places in our experiments – when the first aphids started to walk or drop off the plant several of the other aphids halted feeding and started to walk away. Secretion of alarm pheromones is often associated with defensive reactions in aphids (Nault *et al.*, 1973). The defence behaviour described here for *M. ascalonicus* is a common phenomena in many aphids species but defence behaviour towards parasitoids never seems to prevent successful parasitisation (Chau & Mackauer, 1997; de Farias & Hopper, 1999; Kairo & Murphy, 1999) as completely as in the present case.

The failure of biocontrol of *M. ascalonicus* with *Aphidius*-parasitoids at the Danish Bellflower producer is explained by the obtained results. The defence reactions displayed by *M. ascalonicus* preclude parasitisation almost completely. The aphids that drop from the plants may die if they land on the growth media or end up outside the pots (Dill *et al.*, 1990).

However, Bellflower plants are very compact and a large proportion of the dropping aphids is likely just to land on lower parts of the plants. Thus, they will, together with the aphids that wander off in response to parasitisation attempts, constitute a considerable population of surviving aphids – creating, in effect, a situation of resistance to parasitoid biocontrol. It remains to be seen if the defence behaviour of *M. ascalonicus* is a general species characteristic or whether a selection took place at the particular Bellflower producer. Others (Andrade & Roitberg, 1995) have demonstrated selection for defensive behaviour in aphids. In the present case selection might have been a result of a combination of poor nutritional quality of the crop and high survival rate for dropping aphids.

What happened at the producer's site?

Additional investigations revealed that *M. ascalonicus* did not show defence reactions towards the gallmidge, *Aphidoletes aphidimyza*, which ate shallot aphids in amounts similar to *M. persicae* (Enkegaard & Brødsgaard, unpubl. data). A biocontrol programme based on this beneficial might therefore prove efficient – except for the fact that Bellflowers are cultured under short-day conditions to induce flowering. Thus, the use of *A. aphidimyza* would only be possible in part of the cultural cycle. At present no aphid biocontrol is implemented at the Bellflower producer.

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New pests in Ontario greenhouse vegetables

G. Ferguson¹, Les Shipp²

¹*Ontario Ministry of Agriculture, Food, and Rural Affairs, Greenhouse & Processing Crops Research Centre, Harrow, ON, N0P 2G0, Canada, E-mail: gillian.ferguson@omafra.gov.on.ca;* ²*Agriculture and Agri-Food Canada, Greenhouse & Processing Crops Research Centre, Harrow, ON, N0P 2G0, Canada.*

Abstract: The increasing occurrence of new pests in Ontario is an emerging issue with the industry. The constant need to develop new control programs that are compatible with existing IPM programs has been increasingly difficult. Between 1991 and 2001, eight new pests have been observed in greenhouse vegetable crops in Ontario. Such a situation calls for increased vigilance over the movement of goods and people into, and out of Ontario. It also requires that we give thought to potential new pests and the measures necessary for combating such pests.

Key words: new pests, Ontario, greenhouse vegetables

Introduction

Since 1991, eight new pests (arthropods and diseases) have been observed in greenhouse vegetables in Ontario. This total was divided equally between arthropod pests and disease agents. Six of these were in tomato, and one each in cucumber and pepper. The incidence of an average of one new pest per year within recent years has challenged extension specialists and researchers in offering solutions for their management. Because strategies for combating new pests are often not in place, and because their development is not an instant process, the occurrence of these pests has been disruptive to pest management programs. This paper provides a brief description of the pests that have occurred in Ontario greenhouse vegetables during the last 10 years.

New arthropod pests

Tomato Pinworm (*Keiferia lycopersicella*) (*Lepidoptera: Gelechiidae*)

The tomato pinworm (TPW) is primarily a pest of field tomatoes of tropical and subtropical regions (Lin & Trumble, 1983). The first record of TPW in field and greenhouse tomatoes in Ontario was in 1946 (Garland, 1989) but that infestation did not survive. Two subsequent isolated infestations in 1970 and 1975 in British Columbia also did not survive (Howard *et al.*, 1994). However, since its observation in 1991 in southwestern Ontario (Wang *et al.*, 1997), this pest has persisted in greenhouse tomato until the present time in this province. Economic damage due to this pest has been substantial with yield losses of up to 3 kg per plant. Such losses occurred because the tomato pinworm attacks both fruits and foliage, and its feeding habit of tunneling within plant tissues provides it with protection from most biological and chemical controls. Following research on mating disruption, this technique has been integrated with UV light traps, biological and cultural controls to minimize the level of damage that can be caused by this pest.

Echinothrips americanus (*Thysanoptera: Thripidae*)

Echinothrips americanus, which has a range from southern Quebec to Florida and west to central Iowa (Stannard, 1968), was first observed in Ontario in greenhouse peppers in 1999. During the same year, this thrips species was also found in two ornamental operations. However, the first incidence of *E. americanus* in Canada in a greenhouse vegetable crop was in British Columbia in 1994 (Opit *et al.*, 1997). In BC, this thrips species has attacked greenhouse cucumbers and peppers. Damage by *E. americanus* can be severe and the release of natural enemies available in this province for other thrips pests has resulted in limited success.

Potato psyllids (*Paratrioza cockerelli*) (*Homoptera: Psyllidae*)

The first reports of potato psyllids were out of Colorado in 1909 (Al-Jabr, 1999), and it is likely that its occurrence in more northerly regions, including the western provinces of Canada, originates from populations migrating from the southern states and Mexico. Potato psyllids were first observed in greenhouse tomatoes in Ontario in 1998, and subsequently in 2001. To date, potato psyllids have occurred in only a few tomato greenhouses but its incidence has certainly been disruptive to biological control programs.

Tomato bug (*Engytatus modestus*) (*Heteroptera: Miridae*)

The tomato bug ranges from the southern United States through Mexico to South America, occurs in the West Indies, and is considered “adventive in Hawaii, where it was detected in 1924” (Wheeler, 2000). This bug was first observed in southern Ontario in tomato greenhouses during late spring to summer of 2001. Damage by this pest directly affected yield due to its inclination to feed on developing shoots and inflorescences. Feeding can result in girdling, breakage of shoots and entire inflorescences, and flower abortion. By early fall, the incidence of the tomato bug had spread to about 36 operations. Given the lack of available controls for this pest and the severity of its feeding damage, there is concern that this pest may persist and seriously upset ongoing biological control programs for other pests.

New disease agents

Tomato powdery mildew (*Oidium sp.*)

Tomato powdery mildew was first observed in tomato greenhouses in Ontario in 1995, one year after its first incidence in Canada was reported (Belanger, 1994). Prior to its occurrence in Canada, tomato powdery mildew was already known to occur in Dutch greenhouse so its appearance in Ontario was not altogether unexpected. Frequent travel by personnel between Europe and Canada, and importation of product from the Netherlands could have facilitated spread. This disease, if left unchecked, causes severe chlorosis and leaf necrosis. Fortunately, growers are able to successfully manage this disease by integrating manipulation of the environment with few applications of fungicides that are relatively compatible with biological control agents.

***Fusarium crown and stem rot in cucumber* (*Fusarium oxysporum f.sp. radicum-cucumerinum*)**

This disease was first described from Greece in 1996 (Vakalounakis, 1996) and also occurs in the Netherlands. It was first observed in Canada in British Columbia in 1994 and then in Ontario around 1998. Since that time, the incidence of this disease in Ontario has spread to a few operations and plant loss attributed to this disease has been as much as 35%. Fortunately, the host range of this disease appears to be restricted to cucurbits. Controls for this potentially

devastating disease in Canada are restricted to manipulation of the environment, use of sanitation techniques, and one bioagent, *Streptomyces griseoviridis* (Mycostop™).

Pepper powdery mildew (Leveillula taurica)

Powdery mildew in peppers was first observed in greenhouse peppers in Ontario in 1999 (Cerkauskas *et al.*, 1999). Of the greenhouse vegetable crops, pepper, tomato, and eggplant tend to be favoured by this mildew. Cucumbers can also be infected but reports indicate, not as readily.

Generally, crops become more susceptible to this mildew as they mature and older leaves are the first to show symptoms. Initially, the characteristic white powdery symptoms are somewhat difficult to detect in peppers because while the upper leaf surfaces often appear green, the undersides of leaves can have considerable white powdery-like fungal growth. Later on however, yellow spots become visible on the upper leaf surfaces. Conditions that favour development of this disease differ with the host plant. *Leveillula taurica* is reported to infect peppers under very humid conditions, whereas in most other crops, as in tomatoes, infection takes place more readily under dry conditions. To date, growers have been able to successfully manage this disease without excessive disruption of other control programs.

Pepino Mosaic Virus (PepMV)

PepMV was originally reported from Peru in 1980, and then from The Netherlands in 1999 where it occurred in greenhouse tomato. This virus was subsequently first observed in greenhouse tomato in Ontario during 2000 (French *et al.*, 2001). However, the relationship among isolates from Europe, Peru, and Ontario is not clear because of differences in reaction in plant inoculation tests. PepMV is a very contagious disease that is easily spread mechanically and the virus is thought to remain viable in dry plant material for as long as three months. Long distance spread of this virus could have been facilitated by several means including contaminated fruits and seed material, and possibly as a contaminant on clothing and footwear of travelers. The ease of spread of this disease led to its carryover into 2001 when a significant number of operations became infected. This virus has caused much concern among growers, not only because of potential yield losses, but also because of the restrictions that the presence of such an easily spread disease imposes on movement of personnel and equipment within a crop. One impact of such restrictions has been the reduction of crop scouting for pests and diseases.

Conclusions

The rapidity with which new pests have occurred in greenhouse vegetables in Ontario has caused much concern among growers, extension specialists and researchers alike. Such concern is primarily due to the lack of available or effective controls for these pests and the disruption that they cause in crop management and biological control programs. To stem the incidence of further new pests, a collaborative and an integrated approach is necessary. Growers and managers of packing houses need to continuously enforce implementation of sanitation measures within and between operations. Government agencies need to be more vigilant regarding import of plant material. Finally, researchers and extension specialists need to anticipate not just the occurrence of potential new pests, but also measures required to address their possible introduction into the province.

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Biological and integrated control in vegetables in British Columbia: The challenge of success

David R. Gillespie

Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, PO Box 1000,
 Agassiz, British Columbia, Canada, E-mail: gillespie@em.agr.ca

Abstract: The production of greenhouse vegetable crops in British Columbia has grown in the past two decades, and relies heavily on natural enemies for control of pest insects and mites. This growth, increased sophistication of the industry, and increased complexity of the IPM system, constrains the application of natural enemies for biological control of pests and predisposes the IPM system to failure. These constraints must be addressed if biocontrol-based IPM in greenhouses is to continue.

Key words: pests, natural enemies, integrated control

Introduction

In this article, I will review in a very general way the development of biological control applications in the greenhouse industry in British Columbia (BC). The objective is not to provide a sophisticated and exhaustive statistical treatment. Identification of the major trends provides a framework from which to identify and discuss challenges arising in the application of biological control in greenhouse vegetable crops both locally and globally. These challenges arise from growth in the greenhouse industry, increase in the diversity of herbivores attacking the crops, and success of biological control approaches in greenhouses.

Evolution of biocontrol in BC greenhouses

In 1982, biological control in BC had just begun to be used widely in commercial greenhouse vegetable crops. One small company sold *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae) and *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) for release against two pests, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), respectively. These were used on approximately 30 ha of cucumber and tomato crops, with an average size of less than 0.5 ha (BCMAF, 1985). These replaced insecticide and miticide applications. Greenhouses were family owned and operated. In BC, one federal entomologist, one provincial entomologist, one provincial extension specialist, and the staff of the biocontrol company, Applied Bionomics, were the technical support available to the industry. Costs of research and development were borne by federal and provincial funding sources. There was no monitoring of pest or biological control numbers. Master of Pest Management graduates from Simon Fraser University were only beginning to study greenhouse-related problems in their thesis work. The top tomato variety carried no resistance to diseases and was grown on trellises that were 2 to 2.5 m high. Flowers were pollinated by hand and yields of tomatoes were in the order of 10 kg/m² (BCMAF, 1985).

In 2002, just 20 years later, five biological control companies are selling products in BC. There are four key crops – beefsteak tomato, cluster tomato, sweet bell pepper and cucumber.

There are about 28 pests and 29 natural enemies are routinely applied for biological control against 19 of these. There are over 200 ha of greenhouse vegetable production. The top beefsteak tomato varieties have resistance to a range of diseases, and yields average 65 kg/m² (P. Isaacson, BC Greenhouse Growers, pers. comm.). Plants are grown on trellises over 4 m high. Bumble bees are used universally for pollination in tomato crops. Greenhouse areas average in excess of 3 ha, and many are owned by investors and operated by employee growers. The BC Greenhouse Vegetable Research Association manages grower levies that contribute to the costs of R&D. The equivalent of one and one-half federal entomologists, a provincial entomologist and a provincial industry specialist constitute the public technical support. Advisory and technical support for IPM and biological control comes mainly from private consultants, technical reps from biocontrol companies, IPM employees from individual greenhouse operations and educators. All pests, including plant diseases, are viewed as potential biological control targets. Simon Fraser University, Kwantlen College and University College of the Fraser Valley provide IPM education for the industry at various academic and technical levels.

In the first comprehensive treatment of the subject in many years Murphy *et al.* (2002) described the extent of use of biological controls in Canada. In BC all greenhouse vegetable growers surveyed use biological control. Across Canada, depending on the crop and the pest, from 47% to 80% of greenhouse vegetable growers were satisfied with their biological controls (Murphy *et al.*, 2002). This success has been accompanied by an increase in the scientific and technical support available to growers and greenhouse owners. In parallel with the increase in use of biological controls, the industry has expanded by about 10% per year since 1982, and yields have simultaneously increased by about 10% per year.

Reasons for adoption of biological control

Biological controls were adopted in greenhouse crops out of desperation. In the 1970's, widespread pesticide resistance among twospotted spider mites and greenhouse whiteflies forced growers to apply biological control agents. Each new biological control agent has arisen from similar circumstances. Growers have experienced disastrous crop losses either because of resistance of new pests to available pesticides or because pesticides kill natural enemies and release other pests from suppression. Two early successes with biological control, and one early disaster with pesticide contamination in greenhouse cucumbers (The Vancouver Sun, June 3, June 5 and June 6, 1985) produced a very early "incentive" for biological control.

Good communications also contributed to the successful uptake of biological control. Until very recently, the greenhouse vegetable industry in BC was a unified producer group represented by a single organization based on a cooperative model, and researchers and extension advisors could respond to the priorities identified by growers. The BC Greenhouse Vegetable Research Association has been a crucial link, providing funding to promote the industry's priorities, coordinating grower study groups and disseminating new information. A twice-yearly greenhouse IPM meeting and an annual IPM conference for growers provide opportunities for exchange of information among researchers, consultants and growers.

The challenge of success

Aside from the contingencies of the world marketplace, the application of biological control in greenhouses is presently hampered by several specific problems. One new pest arises per year, based on an increase from 2 to 28 pests over 20 years in BC. Some of these are exotic

pests arriving either from other greenhouse-producing regions, or from warm-temperate or subtropical agricultural regions. Others are simply local horticultural pests that have "discovered" the greenhouse system. Pesticides applied against these new pests can cause outbreaks of other pests because of insecticide-induced loss of natural enemies. Efficacy of formerly successful biological control agents is apparently declining, judging from anecdotal reports from growers and IPM consultants. This has led to concerns about the quality of biological control products, and to substantial increases in the release rates for some. These problems leading to increased dissatisfaction with biological control. Governments are responding to these concerns, and to the increase in sales of natural enemies with legislation to register and regulate biological control agents. The addition of new natural enemies and the loss of confidence and efficacy of old biological control agents are leading to increased costs for biological control. This further erodes the competitiveness of biological control relative to strictly pesticide-based approaches, and threatens to put the industry back onto the pesticide treadmill, where new pesticides are continuously needed to replace those lost to pest resistance. Conversely pesticide resistance and customer demand for pesticide-free product preclude the industry returning to a pesticide-only model of pest management. Is the trend to increased use of biological controls sustainable? Is it possible under the constraints of the modern greenhouse production system to manage plant pests with natural enemies alone?

Many of these proximate problems originate from the success of the greenhouse industry. Greenhouse vegetable production area and yield per unit area have increased over the past 2 decades. It is a highly technological industry delivering large quantities of high quality produce to an increasingly broad market area and could be described as industrial-scale horticulture. Industrial-scale agriculture works in opposition to the operation of "natural" systems (Pollan, 2001). Application of natural enemies for pest control and bees for pollination suggests that greenhouse vegetable production requires at least some of a contrasting philosophy.

The success of the greenhouse industry in the past two decades has contributed to a trend to larger greenhouses, with crops grown on higher trellises. In large greenhouses, herbivore and natural enemy distributions show a distinct patchiness. Movements of organisms are not bounded by short rows and glass walls as in smaller houses, and opportunities for herbivores to escape into enemy-free space are increased. Ecologists describe this patchiness as "metapopulation ecology" (Rhodes *et al.*, 1996). Immigration and emigration are as important as natality and mortality in determining population size and growth in patches (Hanski, 1996). Pest management models in greenhouses tend to focus on reproduction and predation, and ignore immigration and emigration. Metapopulation ecology may help describe how interactions between natural enemies and pests change with increasing greenhouse size.

In the vertical dimension, tomato and pepper crops resemble forests, with distinct canopies and understories, more than they do herbaceous crops. The gap between floor and canopy in tomato crops has changed from zero to 2 m or more in 20 years, changing both the biotic and abiotic environment. Notwithstanding the impacts of defoliation for plant management in tomato crops, this vertical structure could modify the outcome of natural enemy-pest interactions by changing movement and reproduction of natural enemies and pests.

The range of pest species on any one of the greenhouse vegetable crops has increased. This increase in herbivore diversity is inevitable. Global traffic in horticultural goods provides a mechanism for dispersal of pests, global warming opens up new habitats for pest species, and the increased size of greenhouse crops in landscapes makes them more apparent to herbivores. The preferred method of managing new pests in the past two decades has been to develop new natural enemies for biological control. This has led to a greater diversity of

biological control products. As the range of biological controls increases, the frequency with which growers resort to pesticide use will also increase. This is because each biological control agent has a finite probability of failing to control its target pest. As the number of natural enemy/pest interactions increases, the probability of at least one of these failing, often from human error, also increases, and eventually approaches certainty. The important point is that the more complex the system, the more likely it is that some component will fail, or, conversely, the more expensive it is to prevent failure. This is different from ecological theory that asserts that stability increases with increasing diversity (Schowalter, 1996). The optimum number of natural enemies to be introduced against any pest is an ongoing debate in classical biological control (e.g. Myers *et al.*, 1989). The optimum structure for natural enemy communities is a debate equally important in greenhouse systems.

Biological control is the fundamental approach to pest management in greenhouse vegetable crops in BC, as it is elsewhere. The continued production of greenhouse vegetable crops without pesticides will require new approaches, as also noted by van Lenteren (2000). The increasingly complex nature of an IPM system that is based on biological controls generates increased risk of failures and increased costs and threatens the continued acceptance of this approach by the industry. Increasing the use of natural enemies in greenhouse vegetable crops is not as simple as adding another species of natural enemy or replacing old species with "new and improved" species. Managing natural enemies in an environment where greenhouse size and the complexity of herbivore and natural enemy communities are constraints will require substantial changes in the philosophy underlying IPM in greenhouse vegetable production.

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Mass rearing of *Aphidoletes aphidimyza* Rondani for control of aphids

Hyun Gwan Goh

Division of Research Coordination, Rural Development Administration (RDA), 441-707, Suwon, Korea, E-mail: hggoh@rda.go.kr

Abstract: Mass rearing of predatory gall midge *Aphidoletes aphidimyza* Rondani was studied. All of the three foods must be grown or reared: plant-aphid-gallmidge. The mass rearing was studied in three steps: 1) growing pea and cucumber plants, 2) rearing of aphids, and 3) rearing of *A. aphidimyza*. The pea and *Megoura crassicauda* were used for *A. aphidimyza* larva. The cucumber and *Aphis gossypii* served as a stimulus of oviposition of the *A. aphidimyza* adult.

Key words: mass rearing, *Aphidoletes aphidimyza*, *Megoura crassicauda*, *Aphis gossypii*

Introduction

Aphids have great numbers of natural enemies in the field conditions. Aphid has been controlled largely by the use of broad-spectrum insecticides which can inhibit application of biological control agents against other insect pests.

The predatory gallmidge *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) has been used for biological control in many countries because of their effectiveness against aphids in greenhouses (Adams & Prokopy, 1980). The aphidophagous species of Cecidomyiidae is widely distributed over the world and highly effective against aphids, particularly in glasshouse (Meadow *et al.*, 1985). It is broadly oligophagous and it is known to attack more than about 80 aphid species (Yukawa *et al.*, 1998). It is effective at low aphid population density in greenhouse and it reduces their prey not so rapidly. It lays eggs directly in aphid colonies. It was employed in the biological control by the inoculative method (Havelka & Zemek, 1988).

The researches on mass rearing of *A. aphidimyza* are just beginning in Korea. Selection of aphid species as food for the gallmidge is prerequisite to mass rearing. In this paper current research activities on selection of aphid species and mass rearing technique of *A. aphidimyza* are reported.

Materials and methods

The *Acyrtosiphon pisum* was collected from alfalfa (*Medicago sativa* L.) growing in the field near Suwon. *Megoura crassicauda* was collected from *Vicia amurensis* in Kwangreung. Both aphids were reared on pea plants (*Pisum sativum* L.) for about 3-4 generations under laboratory conditions. For experiment, adults of both aphids were transferred to pea leaves, and allowed to lay eggs for six hours. Fecundity of both aphids was observed. Also, fecundity of *A. gossypii* was observed on cucumber leaves. Test aphids were randomly sampled from the population of newborn nymphs, and transferred to each plant. The plants were incubated in a climatic room at the condition of 22°C and 25±1°C, 60±10% RH, and a L18:D6 photoperiod. Ten individuals of each aphid species were examined for their fecundity.

Results and discussion

Selection of aphid for mass rearing

A. aphidimyza is broadly oligophagous, and it is known to attack many aphid species. Selection of aphid species as food for the gallmidge is very important for mass rearing. The aphid should be able to produce a large biomass within a short period of time. In addition, preference of the gallmidge to host and egg-laying female is another factor to be considered on selection of the aphid species. *Megoura crassicauda* and *Acyrtosiphon pisum* were chosen as candidates to food sources of *A. aphidimyza* larvae, *A. gossypii* as a stimulus for oviposition, and pea and cucumber as a host plant for the aphids.

The number of progeny was highest in *A. gossypii*, followed by *M. crassicauda* and *A. pisum* (table 1). Body size of the *M. crassicauda* was much bigger than that of *A. gossypii*. This result indicated that the *M. crassicauda* is a suitable food source of the *A. aphidimyza* larvae, and *A. gossypii* can serve as a stimulus of oviposition of the *A. aphidimyza* adult.

Table 1. Comparison of reproduction among 3 different species during 7 days.

Species	No. Larvae produced / female	
	Mean	Range
<i>Aphis gossypii</i>	78.5 ± 56.3	24 – 194
<i>Megoura crassicauda</i>	44.9 ± 21.6	19 – 81
<i>Acyrtosiphon pisum</i>	37.8 ± 6.1	27 – 42

Laboratory mass rearing

Up to date artificial medium for rearing of the gallmidge larvae has not been developed. As a result, all of the three food sources, plant-aphid-gallmidge, must be grown or reared as follows.

Growing pea and cucumber plants

The pea plants were grown on the medium of pine tree sawdust in 8 cm high pots (φ10 cm). Dry sawdust filled the pot, and 9 healthy beans were placed on it. The beans were covered with another layer of sawdust and watered. The pot was placed in the greenhouse. The cucumber plants were grown in the pot filled with soil. Two seeds per pot were buried. After germination, the pot was transferred to the greenhouse.

Rearing of aphids

Ten pots of pea plants were placed in an acrylic cage (50x30x30 cm). Leaves of the pea plant were infested with *M. crassicauda* when they were about 5 cm in height. When the pea plant was covered with large colonies of *M. crassicauda*, two pots were placed beside two healthy plants in a new cage. The aphids moved to healthy, young plants within approximately two days. *M. crassicauda* in rest of pots was used as a food source for the larvae of *A. aphidimyza*. The aphid multiplied very well, increasing its density, and were available about 10 days after infestation.

For rearing of *A. gossypii*, 9 pots of the cucumber plant were placed in an acrylic cage (40x40x50 cm). Leaves of the plant were infested with *A. gossypii* when they had produced about 4 leaves. When the cucumber plants were covered with large colonies of *A. gossypii*, two pots were transferred to a new cage containing fresh cucumber plants. *A. gossypii* in the

other pots was used as a stimulus of oviposition as well as a food source of the *A. aphidimyza* larvae.

Rearing of *A. aphidimyza*

All developmental stages were markedly hygrophilic. The optimal relative humidity was 90-100%; optimal temperature was 25°C. Non-diapause larval development required an 18-hour photophase. The insectarium housing adult *A. aphidimyza* was shaded during daylight.

A two-storied shelf in the insectarium was suitable for rearing of *A. aphidimyza*. It was made of a stainless steel. Oviposition cage was placed on the shelf. Cocoons of *A. aphidimyza* were transferred into the oviposition cage, and incubated until emergence. As soon as adults emerged, 5% sucrose or honey solution was supplied to them. A small volume of cotton was soaked in the solution and placed on the bottom of oviposition cage (70x50x50 cm). One cage contained about 100-200 individuals of both sexes.

Cucumber plants infested with 60-100 *A. gossypii* per leaf were placed in the oviposition cage for stimulating oviposition of *A. aphidimyza*. The aphid-infested cucumber plants were replaced every other day. The cucumber leaves with cecidomyiid eggs were cut off and placed over 1-2 cm layer of fine sands in a plastic rearing container. Sands were cleaned with the boiled water prior to use. The rearing container was covered with a nylon-netted lid. When eggs in the container hatched, *M. crassicauda* were supplied daily until cocoons appeared. Cocoons were harvested by sieving sands. The cocoons were placed into a container, and used in greenhouses or for reproduction. The amount of daily production of the cocoons was dependent on the rearing unit. Emergence began at 25±1°C one week later.

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Developments in IPM for protected cropping in Australia

Stephen Goodwin, Marilyn Steiner

National Centre for Greenhouse Horticulture, Horticultural Research and Advisory Station,
Locked Bag 26, Gosford, New South Wales 2250, Australia, E-mail:
stephen.goodwin@agric.nsw.gov.au

Abstract: The Australian protected cropping industry is receiving support for improvements to pest and disease management through a number of initiatives. These include the provision of two new Centres for R&D dedicated to the industry, the development of publications to assist producers to adopt IPM strategies and as a resource for a new training course designed specifically for them, and a program of research to continue the development of native natural enemies as biocontrol agents. The last mentioned started with the commercial release of the indigenous phytoseiid mite, *Typhlodromips montdorensis* (Schicha) for use against western flower thrips, *Frankliniella occidentalis* (Pergande) and other key thrips species such as onion thrips, *Thrips tabaci* Lindeman and plague thrips, *Thrips imaginis* Bagnall. The research program also includes the evaluation of new biorational products involving azadirachtin (AzaMax™) and the beneficial fungi, *Beauveria bassiana*, *Metarrhizium anisopliae* and *Verticillium lecanii*, and a range of chemistry new to the Australian horticultural industry.

Key words: IPM, protected cropping, Australia

Introduction

As in many other countries, in Australia the protected cropping industry comprises both floricultural and vegetable crops, although the nursery industry has only 5% of production area under cover and doesn't consider itself a significant player in this industry *per se*. The floriculture industry is evenly distributed, while greenhouse vegetables are mainly produced in New South Wales (NSW), Victoria and South Australia (SA), with some in Tasmania, Queensland and Western Australia. In the cutflower and vegetable industries, where monocultures create more significant pest problems, approaches to pest and disease management continue to rely on chemicals in most areas. This is mainly due to a poor appreciation of more sophisticated approaches to pest management by NSW and SA growers, who in the main, are from a non-English speaking and non-horticultural background. In Victoria, there is a more progressive approach to pest and disease management, due mainly to their interest in, and support for, consultant advice on IPM.

Despite this generally pessimistic picture, a number of developments aimed at educating and training growers in IPM, and in providing opportunities for the development of alternative pest and disease management strategies to chemicals, provides optimism for a more sustainable industry than currently exists. Aside from actual research and development, the establishment of two new Centres dedicated to greenhouse vegetable production R&D in the past 12 months has provided a fillip to this industry. In NSW, NSW Agriculture has established the National Centre for Greenhouse Horticulture at Gosford to facilitate research into, and industry demonstration of, new technology including IPM under Australian conditions. In SA, a consortium including the SA Government has established a Greenhouse

Modernisation Project, which aims to demonstrate new technology including biocontrol and other IPM strategies to its industry, while operating as a commercial venture.

We report here on progress in IPM and biocontrol in our programs to date.

Education and training in IPM

Extension publications

In a recently completed project, industry workshops conducted in three States identified the need for education and training materials on IPM as a high priority. As a result, two comprehensive IPM technical manuals and field identification guides have been published for both the ornamental and greenhouse vegetable industries. The ornamentals set was released 12 months ago and already it is into its second edition, while the vegetables set was recently released. These are entitled “Integrated Pest Management in Ornamentals/Greenhouse Vegetables: Information Guide” and “Pests, Diseases, Disorders and Beneficials in Ornamentals/Greenhouse Vegetables: Field Identification Guide”.

Both documents for the respective crops are cross-referenced and are aimed at providing in-the-crop assistance in identification and more detailed information on the target organisms, plus practical advice on how to practice IPM, guidance on pest-risk minimisation actions for the property, information on the availability of biocontrol agents, plus who supplies them and how to use them. The manual also contains a comprehensive directory with contact details on where to obtain monitoring tools, analytical and diagnostic services and IPM consultants.

Training

The next step was to develop an accredited training course in IPM that could be delivered nationally by technically qualified trainers. Called IPM OnFarm, this course targets the processes essential to successful IPM, providing practical training in identifying key pest, disease and biocontrol organisms, correctly using sticky traps, crop inspection techniques and in designing and implementing an IPM program. It uses the above publications as resource material, obliging participants to familiarise themselves with the contents.

The competency-based training course is the necessary first step towards lifting the awareness of growers in IPM and to gaining more widespread industry adoption amongst those groups where greater than normal challenges in changing practices exist.

Progress in research and development

Biocontrol

The introduction of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) into Australia in 1994 resulted in the recent development of the indigenous predators, *Typhlodromips montdorensis* (Schicha), *Typhlodromalus* sp. (Phytoseiidae) and *Stratiolaelaps scimitus?* (*Hypoaspis miles*) (Laelapidae). At the Horticultural Research and Advisory Station, Gosford, current research conducted in commercially sized greenhouse crops with environmental controls, has convincingly demonstrated the ability of *T. montdorensis* to manage thrips (WFT, onion thrips, *Thrips tabaci* Lindeman) populations in winter and spring/summer Lebanese cucumber crops and in gerberas (Steiner & Goodwin, 2002, in this Bulletin) and WFT and plague thrips, *Thrips imaginis* Bagnall, in commercial hydroponic strawberry crops (Steiner, 2002, in this Bulletin). These new thrips predators are an important addition to the small number of commercially-produced biocontrol agents in this country. *Typhlodromips montdorensis* is also now commercially available overseas through Syngenta Bioline as Amblyline-M™ in some countries, with permits being sought in Canada and the UK.

In a continuation of this work to identify native natural enemies for development as biocontrol agents (BCAs), current project investigations are targeting some key areas. These include the identification of native phytoseiid mite species for development as BCAs against tomato russet mite, *Aculops lycopersici* (Masse). Currently eight species are being evaluated for their ability to develop and reproduce on a russet mite diet, determine their russet mite consumption potential, determine their survivorship on tomato plants, and with selected species, their performance as a BCA in a greenhouse crop environment. Other current targets in this biocontrol project include the comparison of a new braconid parasitoid with *Aphidius colemani* Viereck against green peach aphid, *Myzus persicae* (Sulzer) and cotton aphid, *Aphis gossypii* Glover, plus the evaluation of a promising lacewing species against the same hosts. To complement this work a program of laboratory testing of the effects of some new chemistry against *Encarsia formosa* Gahan and *T. montdorensis* is also being conducted.

Biorational chemicals

Since 2000, the current research program has also been engaged in a series of greenhouse trials to evaluate the effectiveness of a neem product (AzaMax™, also known overseas as NeemaZal™ TS), against a range of key pest species in greenhouse ornamental, vegetable and strawberry crops. Phytotoxicity trials were also conducted. Crops included tomatoes, cucumbers, capsicums, strawberries, gerbera and roses.

AzaMax™ demonstrated suitability for registration and inclusion in IPM programs against greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), two-spotted spider mite, *Tetranychus urticae* Koch, green peach aphid and WFT, but not tomato russet mite, which gave erratic and unacceptable control despite repeated trials. Natural establishment of *T. montdorensis*, *E. formosa* and *A. colemani* was observed on plants treated with AzaMax™ during these trials.

Successful IPM continues to be thwarted by the absence of a reliable biorational chemical for caterpillar control. Bacterial and viral pesticides cannot be relied upon to protect high value crops grown in greenhouses. At the Station, a planting of Gerbera cv Mammut is being used to trial a range of new chemistry, mainly IGRs against two economic caterpillar pests, cluster caterpillar, *Spodoptera litura* (Fab.) and lightbrown apple moth, *Epiphyas postvittana* (Walker). In another area of research, chemical treatments to inhibit the transfer of tomato spotted wilt virus in capsicums, are being trialled.

Microbial pesticides

Currently Australian growers do not have access to any commercially produced fungal pesticides. Arising from the thrips research workshop held in Ontario, June 2000, an international collaborative group was formed comprising Dr. Michael Brownbridge, UVM, USA; Dr. Ken Fry, ARC, Canada; Rob Jacobson, HRI, UK and ourselves, to combine resources to screen a wide range of isolates in the laboratory using comparable methodology. In October-November 2001, we collaborated with Brownbridge at the Station to initiate a joint research program. Isolates of *Beauveria bassiana*, *Metarrhizium anisopliae* and *Verticillium lecanii* obtained from the USA, Canada, and two locations in Australia are being tested against WFT, greenhouse whitefly, green peach aphid and cotton aphid. The immediate aims of the research are to screen isolates and to undertake dose-response bioassays with the most promising of these. This will be followed by a program of crop trials to evaluate additives for commercial formulation and of laboratory tests of selected isolates against some beneficial organisms.

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Physical methods for the control of *Bemisia tabaci* and its impact on TYLCV infection in greenhouse tomato in Morocco

A. Hanafi¹, B. Murphy², I. Alaoui¹, R. Bouharroud¹

¹Integrated Production and Protection Unit, Complexe Horticole, Institut Agronomique et Vétérinaire Hassan II, BP. 18/S, Agadir, Morocco. E-mail: hanafi@marocnet.net.ma; ²CAL AGRI PRODUCTS LLC, 10720 McCune Ave. Los Angeles, California 90034, USA

Abstract: The development of IPM tactics in greenhouse crops in Morocco has evolved quickly over the last ten years showing impressive reductions in pesticide use while improving crop quality and yield. Unfortunately, the introduction of Tomato Yellow Leaf Curl Virus (TYLCV) and its vector, *Bemisia tabaci* has significantly increased the need for pesticide applications and impacted the ability of many growers to grow disease free crops. In response growers have been adapting IPM tactics focused primarily on prevention of disease transmission. A number of tactics have been implemented with some degree of success such as resistant cultivars, rouging diseased plants, and alternate planting dates. For managing the vector, *B. tabaci*, farmers have adopted improved pest monitoring and control guidelines, mass trapping, exclusion nets, and pesticide rotation to preserve effective pesticides. However, the two tactics most relied on by farmers are chemical controls and exclusion nets to reduce disease transmission by *B. tabaci*. Nowadays, over 98% of greenhouses use insect nets of various mesh gauge sizes to exclude *B. tabaci* from greenhouses. Considering the time and expense devoted to exclusion nets, evaluations were begun to determine the benefits of using insect nets within the overall IPM program. Factors such as the effect of mesh size on disease incidence and *B. tabaci*, impact on biological control, disease management and crop yields are included. The first objective of the program compared the efficacy of two mesh gauge sizes most commonly used by farmers, the 10x14 and the 10x20 gauge mesh. Preliminary field comparisons suggest there may be significant differences in efficacy between the most common mesh gauges and their value to pest management.

Key words: Tomato Yellow Leaf Curl Virus, exclusion nets, physical control, *Bemisia tabaci*

Introduction

TYLCV was introduced to Morocco from neighbouring Spain most probably through infected plantlets that were imported from Almeria between 1996 and 1997. Since then it has spread to the major tomato production areas of Morocco and represents a major challenge for the production of field and greenhouse tomato. During the 2001 season, the area planted with tomato saw a 28% reduction over the previous year. Field grown tomato has suffered the most damage causing many farmers to prematurely destroy their crop.

Widespread adoption of Integrated Pest Management programs (IPM) began in Morocco 10 years ago and the required number of insecticide sprays was reduced to 3 or less for the entire crop cycle in many tomato greenhouses. Unfortunately, the introduction of TYLCV caused farmers to react by relying on chemical controls against the vector, *B. tabaci*. As a consequence, the number of insecticide applications in tomato increased from 10 to 20-fold within a few seasons and *B. tabaci* rapidly developed resistance to a number of insecticides. Faced with increased restrictions on pesticide residues from importing countries (EU primarily) on the one hand, and pressure to use insecticides to control disease transmission on the other, farmers began to adopt a number of IPM tactics to help manage the crisis. These

tactics included practices such as the use of resistant tomato cultivars, early rouging of infected plants, pest-monitoring using yellow sticky cards and mechanical controls such as mass trapping and insect exclusion nets. No doubt the use of all these components has been essential in the management of *B. tabaci* and TYLCV. However, exclusion nets, by far have been used as the first lines of defence against TYLCV transmission. The primary purpose of the exclusion nets is to prevent migratory *B. tabaci* from vectoring TYLCV to a new crop rather than controlling insect densities. The use of insect nets has already cost the greenhouse industry in Morocco over 14 millions US dollars, yet little is known of the effectiveness of the various mesh sizes employed, the impact on natural enemy populations, and foliar and soil disease management in tomato greenhouses.

In response, a program was begun to evaluate the costs and benefits associated insect exclusion nets in greenhouse tomato. The first objective evaluated the relative effectiveness of different size mesh gauge on excluding *B. tabaci* and reducing TYLCV incidence for two of the most commonly used mesh gauges. Ongoing research includes the effect of insect screens on foliar disease management programs as well as their impact on natural enemy migration into greenhouses.

Materials and methods

The experiments were performed at farm level, in two hectare units of tomato grown under a 5 m high canary type wooden greenhouse located in the Souss Valley in South Morocco. Both greenhouses were equipped with a double SAS at their entrance and relied on natural ventilation. They were equipped with insect nets on all opening (30% of greenhouse covered area). The side wall and roof ventilation and the rest of the area of the greenhouse was covered with ordinary plastic of 200 microns. In site 1 the greenhouse was equipped with an insect net of a gauge mesh of 10x14 whereas in site 2 the insect net was of a gauge mesh of 10x20. The two greenhouses were planted early august 2000 with the cultivar *Daniella* planted at a density of 2 plants/m². The tomato crops were irrigated by drip irrigation and received the same cultural practices as any commercial greenhouse production. Insecticides were used as needed by the farmers.

Flight and TYLCV phenology

The indoor as well as outdoor populations of whiteflies were monitored in the two sites using 2 pairs of yellow sticky cards (dimension 10 x 20 cm) on each direction of the greenhouse (total of 8 per ha) and 2 pairs at the middle of each quarter ha inside the greenhouse (total of 8 per ha). Sticky cards were suspended at a height of 2 m outside. Inside the greenhouse traps height was adjusted to crop height to be near the upper part of the tomato plants. All traps were monitored and changed weekly.

The plants in each greenhouse were monitored weekly for symptoms of TYLCV and their numbers were registered. These plants were systematically rouged by the farmers beginning since planting until the end of October. After November, farmers stopped rouging infected plants because of the yield they carry.

Results

Average captures of adults whiteflies in sticky cards placed inside the two greenhouses equipped with insect screens of 10x14 and 10x20 gauge mesh are reported in Fig. 1. This figure shows three periods with different trends of captures of whiteflies in the two greenhouses. Between November 2000 and January 2001, whiteflies captures in sticky cards

were consistently higher in the greenhouse equipped with the screen 10x14 than in the greenhouse equipped with the screen 10x20. Many of the whiteflies invading the greenhouse in fall to early winter originate from open field tomato crops, which have generally high incidence of TYLCV. From an epidemiological point of view these early flight of *B. tabaci* are the most critical as they coincide with the young stage of tomato plants in the greenhouse and when they are most sensitive to TYLCV infection. Between February and end of March 2001 captures were consistently low in the two greenhouses. After April 2001 when whiteflies captures were the most intense especially in the greenhouse equipped with the 10x14 screens. Whereas cold winter temperatures (9-18°C) affected negatively whiteflies development inside the greenhouse, the prevailing warmer temperatures (>25°C) during the spring created favourable conditions for whitefly outbreaks indoor as well as outdoor.

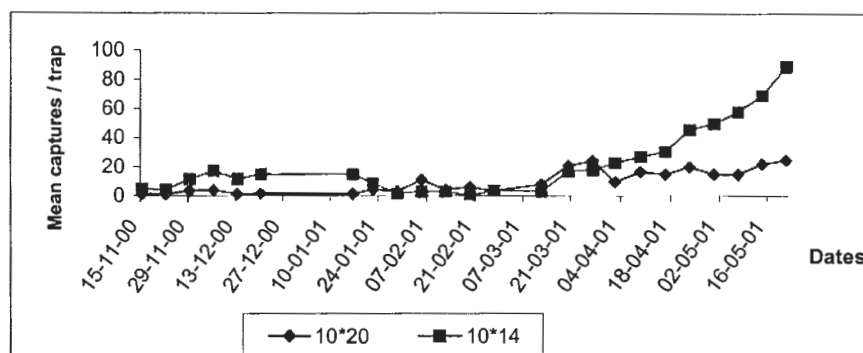


Figure 1. Evolution of mean captures of *Bemisia tabaci* on yellow sticky cards inside tomato greenhouses equipped with 10x14 and 10x20 mesh screens, in the Souss Valley in Morocco 2001.

Table 1. Cumulative captures of whiteflies on sticky cards placed inside and outside greenhouses equipped with insect nets 10x14 and 10x20 and TYLCV incidence in tomato, in the Souss Valley, Morocco 2002-2001.

Period of captures	Mean cumulative captures of adult whiteflies per sticky card			
	Insect screen 10x14		Insect screen 10x20	
	Indoor	Outdoor	Indoor	Outdoor
Early season	63.5	156.8	12.3	133.0
Season long	508.8	831.4	234.3	1737.9
Final TYLCV incidence	7.48% (Total of 1495)		0.04% (Total of 7)	

Outdoor captures indicates that whitefly pressure early in the season was comparable between the two sites but was significantly higher over all the season in the greenhouse equipped with screens 10x20 (table 1).

Early season as well as season long, outdoor traps captured significantly higher number of whiteflies than indoor traps indicating that both types of screens exerted some exclusion of *B. tabaci*. In the greenhouse equipped with the 10x20 screens indoor traps captured 10.8 and 7.4 fold less whiteflies than outdoor traps early season and season long, respectively. Whereas

in the greenhouse equipped with the screen 10x14, whiteflies exclusion was less efficient and indoor traps captured only 2.5 and 1.6 fold less whiteflies than outdoor traps, early season and season long, respectively.

The relation between indoor and outdoor captures was later analyzed and correlation between indoor and outdoor captures was significant only in the case of the screen 10x14 ($R=0.94$). This confirmed the superiority of the screen 10x20 in excluding adult whiteflies from the greenhouse.

Final TYLCV incidence was significantly higher (187 fold) in the greenhouse equipped with the screen 10x14 (7.48%) than in the greenhouse equipped with the screen 10x20 (0.04%).

Discussion

The results of this study demonstrated clearly that the screens 10x14 are neither efficient in excluding *B. tabaci* nor in preventing TYLCV and this despite the heavy reliance on insecticides. Over all season a total of 27 and 13 insecticides applications were used against *B. tabaci* in the greenhouse equipped with the screen 10x14 and 10x20, respectively. The superiority of the screen 10x20 was clearly demonstrated in this study whether in terms of whitefly captures and final TYLCV incidence or in terms of insecticide reduction (48%). However, their effectiveness could even be improved if the screens are fixed neatly to greenhouse structure in order to avoid the smallest hole possible. We recognize that this is not easy to achieve in the case of the wooden canary type greenhouse. It is equally important that greenhouses are equipped with insect screens on all ventilation openings (roof and side walls) long before planting.

Airflow resistance, primarily a function of mesh size varies among screen types (variable thread diameters or thickness). Consequently, nets with high airflow resistance, often costly in terms of requiring greater screening area to maintain adequate natural ventilation, are the best way to exclude *B. tabaci* with a minimum effect on greenhouse environment and consequently on crop health. However, the overriding concern should be that of overall efficacy. The unfortunate fact is that fine mesh screens like the 10x20 when coupled with dusty conditions that prevails in the South could have a negative impact on greenhouse climate.

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Evaluating a new non-toxic pesticide for integrated control of *Bemisia tabaci* in protected agriculture in Morocco

A. Hanafi¹, R. Bouharroud¹, B. Murphy²

¹Department of Plant Protection, IAV Hassan II, Complexe Horticole d'Agadir, BP.18/S, Agadir, Morocco, E-mail: hanafi@marocnet.net.ma; ²CAL AGRI PRODUCTS, 10720 McCune Avenue Los Angeles, California, USA

Abstract: The introduction of TYLCV and its insect vector *Bemisia tabaci* have exacerbated crop losses, disrupted IPM programs and have seen the loss of pesticides to insect resistance from overuse. In the Mediterranean and North African regions this pest complex has single-handedly challenged the ability of the region to produce high quality and quantity of horticultural products for the export market. To address this problem, investigations were begun to modify current IPP practices to include more effective management tactics for TYLCV and *B. tabaci*. One focus of this research was the evaluation of alternative pesticides for control of *B. tabaci* populations that would not disrupt current IPM programs. Here we present preliminary results evaluating the efficacy, field performance and user recommendations for one alternative pesticide, AGRI-50 (CAL AGRI PRODUCTS, Los Angeles, California, USA). Evaluations were conducted in the laboratory to ascertain efficacy at all life stages of *B. tabaci*. Further studies were conducted in commercial tomato greenhouses in Morocco to determine optimum application rate, spray volume and compatibility with commercial cultural practices. Laboratory trials have shown AGRI-50 to be effective against the adult and all nymph stages of *B. tabaci*, particularly, the tenacious pupa stage at concentrations from 2500 to 5000 ppm. A single application was found capable of nearly complete control of late *B. tabaci* pupae and prevented emergence of the newly developed adults. In commercial tomato greenhouse trials AGRI-50 was found to achieve economic control equal to or greater than many conventional pesticides. Furthermore, it was found that this level of control could be achieved using standard application equipment and spray volumes. Research examining compatibility of AGRI-50 with current IPM practices have shown that bumble bee pollinators are unaffected by Agri-50 applications and evidence has been accumulated showing relatively low impact on important natural enemies such as *Eretmocerus* sp. and *Diglyphus* sp. parasitoids.

Key words: IPM, *Bemisia tabaci*, AGRI-50, low risk insecticides, greenhouse

Introduction

Development of IPM tactics for protected agriculture in Morocco has been progressing rapidly during the last 15 years. During its implementation, the number of pesticide applications for tomato production had been reduced to as little as 3 for the whole crop cycle. However, with the introduction of TYLCV the number of insecticide applications for control of *B. tabaci* increased to as many as 30 to 60 per crop cycle negatively affecting IPM in greenhouse crops. The necessity for frequent pesticide sprays has precluded the possibility of biological control, disrupted bumble bee pollination, resulted in the loss of pesticides due to resistance and greatly increased the cost of pest control and crop production to growers.

A number of tactics have been implemented to address this issue, such as improved pest monitoring and control guidelines, insect screens, mass trapping, altering planting dates, resistant cultivars and early removal of infected plants. However, these tactics alone have not been sufficient to manage the pest and the disease. Current research has now been focusing on

the evaluation of low to non-toxic alternative pesticides that can be integrated with minimal disruption to existing tactics. These pesticides generally tend to be more selective against the target pest and often minimally disruptive to other IPM and production practices. In protected tomato production, the need for frequent applications of standard pesticides have virtually precluded the use of predators and parasites for *B. tabaci* control. Further, these materials often impact bumblebee pollinators required for maximizing tomato yields and suppress natural enemies such as *Diglyphus* sp. that often reach sufficient numbers to maintain leaf miners below economically significant densities.

Here we present results evaluating the efficacy, field performance and user recommendations for an alternative pesticide product, AGRI-50 (CAL AGRI PRODUCTS LLC, Los Angeles, California, USA) within greenhouse IPM programs. AGRI-50 is classified as a non-toxic foliar pesticide that uses a proprietary formulation of potassium phosphate as the active ingredient. Although it has efficacy against a number of foliar pests and pathogens, our research focused on evaluations for *B. tabaci* control and its compatibility with current tomato greenhouse IPM tactics.

Material and methods

Whole plant bioassays

Whole potted lantana (9 plants) and tobacco (9 plants) were caged with 300 to 500 adults *B. tabaci* released per plant. Nymphs were allowed to develop to the appropriate stage for evaluation. All adults were removed and AGRI-50 was applied (doses of 1:200 and 1:400) using a hand held atomizer to individual leaves of the potted plants. Control leaves were treated with water.

Petri dish bioassays

Infested leaves with *B. tabaci* were collected in an untreated tomato greenhouse. In this particular greenhouse whitefly populations were allowed to develop without any insecticide applications in order to evaluate these cultivars for TYLCV tolerance. The nymphs on leaves were observed under microscopes to select individuals with higher number of pupa stages and leaf-discs of 5cm diameter were cut and dipped in two concentrations of AGRI-50 solution. Control discs were dipped in a distilled water solution only. All leaf discs were placed in petri dishes on an agar media (15 g/l) prepared previously. Two doses of AGRI-50 were tested at 1:200 and 1:400 dilutions (5,000 and 2,500 ppm, respectively) and there were 10 petri dish replicates per treatment. Evaluations were conducted the day of treatment (pre-count) and subsequently at 4, 8 and 12 days after treatment.

Field trials

The trials were conducted at a commercial greenhouse (5,000 m²) located in the Souss Valley in southern Morocco. Two trials were conducted in this greenhouse during the fall of 2001 (October-November). The first trial tested and verified the optimal concentration of AGRI-50 for pest suppression and the second field trial tested the optimal spray volume.

Experimental design

The same experimental design was used in each of the two field trials. In this design the greenhouse was divided into 3 plots. Each plot (0.15 ha) consisted of 6 lines (100 m long) planted with pepper at a distance of 30 cm within rows and 1.2 m between rows.

Coverage trial

Treatment 1: Positive control using the neocotinoid Thiamitoxam (Actara of Syngenta) at a dose of 20 g/hl, 2,000 l/ha (SV). Treatment 2: AGRI-50 (dose of 1:200) using standard spray volume of 2,000 l/ha (SV). Treatment 3: AGRI-50 (dose of 1:200) using double spray volume of 4,000 l/ha (DV)

Concentration trial

Treatment 1: Positive control using the IGR Buprofezine (Applaud of Aventis) at a dose of 100 ml/hl. Treatment 2: AGRI-50 (dose of 1:300) using standard spray volume of 2,000 l/ha. Treatment 3: AGRI-50 (dose of 1:150) using standard spray volume of 2,000 l/ha

Leaf sampling

Leaf samples were collected prior to insecticide application and then at 4, 10 and 14 days after treatment applications. For each replicate, 4 pepper plants were randomly selected, flagged, and systematically sampled during the trials. For each plant sampled two leaves from the upper and two leaves from the lower portion of the plant canopy were collected and transferred to the laboratory for microscopic examination.

Results and discussion

Preliminary evaluations of AGRI-50 conducted on whole plants in the laboratory at IAV Hassan II demonstrated that AGRI-50 was efficacious against nymphs and pupa of *B. tabaci* with percent mortality varying between 86.7 and 96.9%. Of particular note was the very effective mortality achieved against pupal stage in the petri dish trial where only 3.7% and 6.6% of pupa completed development to adults in the 1:200 and 1:400 concentrations, respectively. No significant difference was detected between lower and higher doses of AGRI-50.

The first commercial scale field trial confirmed our laboratory results demonstrating AGRI-50 had achieved economic control of *B. tabaci* (greater than 80% mortality) at both concentrations tested (Fig. 1). The neocotinoid, Thiamitoxam, performed poorly in this trial with mortality rates below 50%, most probably due a high degree of *B. tabaci* resistance to the Neocotinoids that include Thiamitoxam and Imidachlopride (unpublished data). In previous studies, Thiamitoxam has been shown, and is commonly known, to severely impact bumblebees in tomato greenhouses, which requires the colonies to be removed before application. Comparable trials using AGRI-50 have shown no adverse effects to bumblebees or fruit set after repeated applications.

The second commercial scale trial confirmed the ability of AGRI-50 to economically control *B. tabaci* populations relative to standard pesticides. AGRI-50 achieved mortality rates comparable to or greater than Buprofezine (Applaud). In addition, AGRI-50 caused significant mortality to adult *B. tabaci* that was easily observed on foliage after applications. In contrast to the previous trial, no significant resistance of *B. tabaci* was indicated for Buprofezine. Guidelines have strongly recommended that Buprofezine should not be used more than 3 times per crop cycle to minimize the risk of *B. tabaci* resistance. Overall both products performed well against *B. tabaci* nymphs achieving economic control (greater than 80%) by the end of the trial (Fig. 2). However, AGRI-50 showed superior control of pupa relative to Buprofezine. The second trial also confirmed that neither doubling the concentration of AGRI-50 nor doubling the quantity of spray solution significantly increased *B. tabaci* mortality.

No impact was observed on bumblebee when colonies remained in the greenhouse during the application time indicating its safety for pollinators. Sampling of leaf miners larvae also showed that AGRI-50 had no detectable impact on *Diglyphus* sp. populations. Although preliminary, these studies suggest a degree of compatibility with other practices that make the product a potential candidate for tomato greenhouse IPM programs.

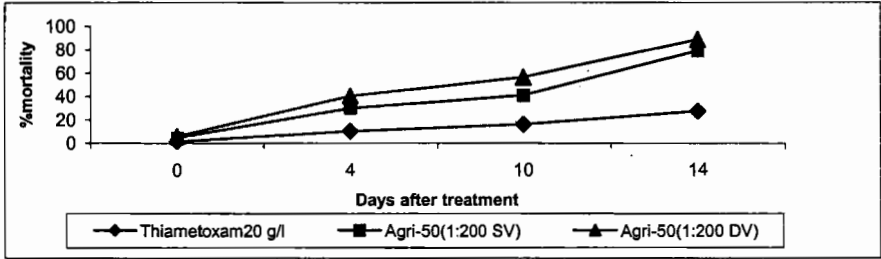


Figure 1. Percent *B. tabaci* nymph and pupa mortality after Agri-50 application (1:200) in a standard (2,000 l/ha) and double (4,000 l/ha) volume of water in pepper greenhouse in the Souss valley of Morocco, fall 2001.

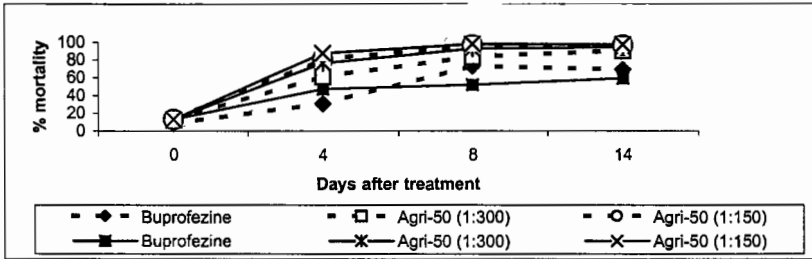


Figure 2. Percent *B. tabaci* nymph (-----) and pupa (____) mortality using Agri-50 at simple dose (1:300) and double dose (1:150) in a standard volume of water (2,000 l/ha) as compared to Buprofezine, applied in pepper greenhouse in the Souss valley of Morocco, fall 2001.

Results thus far have shown Agri-50 suppresses *B. tabaci* numbers over a period of time comparable to many pesticides currently used for *B. tabaci* control. While no single strategy is likely to control TYLCV and its vector adequately, our results suggest AGRI-50 could be harmoniously used with other IPM practices in greenhouse grown tomato. As research on management tactics for TYLCV and *B. tabaci* continues, IPM compatible pesticides such as AGRI-50 coupled with other tactics (insect nets, mass trapping, plant rouging) may form the backbone of future management strategies for *B. tabaci* and TYLCV in Morocco.

Biological control of cabbage root fly using entomopathogenic nematodes in glasshouse experiments

Andrew J. Hart, Deena M. Willmott

Department of Entomological Sciences, Horticultural Research International, Wellesbourne, Warwick CV35 9EF, United Kingdom, E-mail: Andrew.Hart@hri.ac.uk

Abstract: A number of different species and isolates of entomopathogenic nematodes were used against cabbage root fly, *Delia radicum*, infesting potted cauliflower under glasshouse conditions. *Steinernema affine* was found to be the most effective isolate tested. This isolate was then compared with a commercial product (*S. feltiae*, marketed as 'Nemasys'®) at a range of doses. *Steinernema affine* gave a significantly higher level of control compared to the commercial strain.

Key words: entomopathogenic nematodes, *Steinernema* spp., biological control, *Delia radicum*

Introduction

Entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) are important natural enemies of soil dwelling insects, and have great potential as biological control agents in glasshouse and field systems. Two families of nematodes, Steinernematidae and Heterorhabditidae, which are mutualistically associated with pathogenic bacteria, are widely employed in biocontrol programmes. Entomopathogenic nematodes have a range of advantages including: host seeking capabilities; high pathogenicity for insects, yet safe for vertebrates; ease of mass rearing, storage and application; exemption from government regulation (Gaugler & Kaya, 1990). Numerous sampling studies have been carried out across a range of habitats in an attempt to identify the most effective nematodes for biological control (e.g. Chandler *et al.*, 1997, Hominick *et al.*, 1995; Stuart & Gaugler, 1994). Once isolated, such nematode samples can be assessed for potential use as biocontrol agents in laboratory bioassays, controlled glasshouse experiments and field trials.

Cabbage root fly *Delia radicum* (Diptera, Anthomyiidae) is a serious pest of brassica crops in the field with the larvae causing damage to root systems of the plants, and therefore applications of entomopathogenic nematodes may be able control the soil dwelling stages. An extensive soil sampling survey was carried out in the UK, which identified a number of novel isolates of entomopathogenic nematodes (Gywnn & Richardson, 1996). In this preliminary study a selection of nematode isolates from this sample library were tested against cabbage root fly in potted cauliflower under controlled glasshouse conditions.

Material and methods

Ten nematode isolates were selected from the culture collection maintained at Horticultural Research International and mass produced *in vitro* using the methods described by Bedding (1981). Cabbage root fly were reared on swede in sand filled pots, with pupae being picked out and allowed to develop to adults in jars. Eggs were collected from ovipositing flies and used to inoculate young potted cauliflower plants held in a cool glasshouse maintained at 16°C ± 2°C with a 16L:8D light/dark cycle. Nematodes were applied to the soil at a dose of

1.6×10^4 juveniles per pot 7 days after inoculation of the cauliflower with cabbage root fly eggs. Approximately one month later, cabbage root fly larvae (alive or dead) were recovered from the soil in the pots and counted.

Using the findings from the first experiments, the most pathogenic nematode, *Steinernema affine*, was taken forward for further assessment. It was compared with a commercially available nematode *S. feltiae* A1 (isolate UK76, marketed by Microbio as Nemasys®) in a multiple dose response experiment using four different doses (8.0×10^3 , 1.6×10^4 , 3.2×10^4 and 6.4×10^4 infective juveniles per pot).

Results and discussion

Steinernema affine was found to provide the best control of cabbage root fly larvae out of the ten selected isolates. This was selected for use in the second experiment and was found to give greater control of cabbage root fly compared to a commercial strain of *S. feltiae*. Both nematode species showed a dose response (Fig.1).

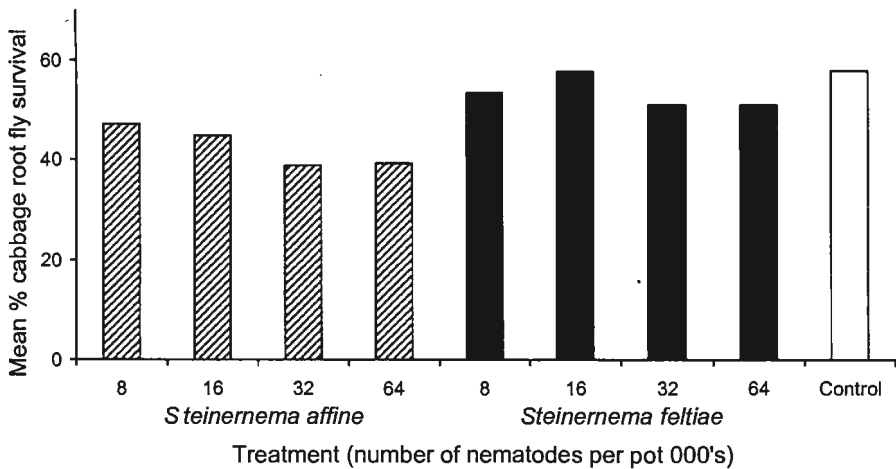


Figure 1. Effect of treatment using *Steinernema affine* and *Steinernema feltiae* at different doses on mean percentage survival of cabbage root fly in potted cauliflowers maintained at 16°C, 16L:8D.

Studies such as the one described here allow the identification of new nematode isolates, which can be used in biological control programmes. *Steinernema affine* may have potential against a range of glasshouse pests and may prove to be more effective than current commercial products. Further experiments, using similar methods outlined here, need to be carried out using *S. affine* against other pest insects, in conjunction with laboratory bioassays.

Acknowledgements

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Development of an integrated control strategy for leafminers in leafy salads with potential for extrapolation to other cropping systems

Justine Head, Lisa F. Palmer, Keith F.A. Walters

Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK, E-mail: j.head@csl.gov.uk

Abstract: Leafminers are serious pests of many cropping systems throughout the world. With increased resistance to pesticides new approaches are required to obtain high levels of control. The efficacy and compatibility of chemical insecticides and foliar applications of the entomopathogenic nematode, *Steinernema feltiae*, were evaluated for the control of *Liriomyza huidobrensis*. In the laboratory, pesticides routinely used against leafminers resulted in less than 30% larval mortality, whereas 65% mortality followed treatment with abamectin. In contrast, up to 97% larval mortality was obtained by one application of *S. feltiae* made to an infested Chinese brassica crop as part of an integrated program. Information obtained in this study has potential for use in other cropping systems.

Key words: *Liriomyza huidobrensis*, entomopathogenic nematodes, insecticides, integrated pest control

Introduction

The South American leafminer, *Liriomyza huidobrensis*, is a major polyphagous pest, which causes considerable damage to a wide variety of edible and ornamental crops. Since its introduction to the UK in 1989 outbreaks have occurred annually in protected crops and statutory measures are taken to prevent movement of the pest to other commercial premises.

Few pesticides are available for the control of *L. huidobrensis* in the UK, especially on edible crops, and those pesticides which are available vary in their effectiveness. A non-chemical control method utilising the entomopathogenic nematode, *Steinernema feltiae*, as a foliar treatment against larval instars has been developed (Williams & Walters, 1994; Williams & Walters, 2000). To facilitate the inclusion of this agent into an integrated pest management (IPM) program for *L. huidobrensis* on leafy salads it was necessary to determine its compatibility with other available control techniques.

This paper describes components of an IPM approach for use on lettuce and Chinese brassica crops, including results from a glasshouse trial, which has potential for extrapolation to other cropping systems.

Material and methods

Pesticide and nematode compatibility

The effect of cypermethrin residue on foliar applications of *S. feltiae* was investigated using well established techniques (Head *et al.*, 2000). The *L. huidobrensis* culture used was known to display high levels of resistance to chemical insecticides routinely used for leafminer control. In the first experiment direct exposure of *S. feltiae* to pesticide was investigated by suspending nematodes in either cypermethrin (as Toppel 10) at the field rate for lettuce (65 ml in 100 l) or in water, for 24 hours in constant darkness. Sand tube bioassays were conducted with both washed and unwashed nematodes to assess infectivity (Fan & Hominick, 1991).

The second experiment investigated the effect of nematode exposure to pesticide residues. A spray of either cypermethrin or water was applied to leafminer infested petunia plants, followed 24 hours later by an application of either a suspension of 10,000 *S. feltiae*/ml (as Nemasys®, MicroBio UK Ltd.) with 0.02% v/v of a non-ionic wetting agent Agral (a.i. alkyl phenol ethylene oxide), or a water control. *L. huidobrensis* survival was assessed after 96 hours.

Pesticide Efficacy

To determine whether the insecticides used against leafminers on leafy salad crops were effective against an outbreak population of *L. huidobrensis*, tomato plants were exposed to 50 adult leafminers for 48 hours to allow egg laying. The adults were removed and the plants incubated until late second instars were present (Head *et al.*, 200-). Excised tomato leaves infested with larvae were dipped into an insecticide solution diluted to the field rate for protected lettuce. Leaves were dried, placed onto filter paper in petri dishes and incubated for 72 hours at $20 \pm 1^\circ\text{C}$, 16:8 hours light:dark and 65% relative humidity (r.h.) before larval survival was recorded. This procedure was repeated using four registered insecticides and with abamectin (which is not approved in the UK for use on leafy salad crops).

Glasshouse trial

A Chinese brassica production glasshouse of approximately 1940 m², divided into 7 bays, was selected to test components of the IPM approach. An outbreak of *L. huidobrensis* had been present on this site for several months. Between crops the glasshouse had been treated with nicotine shreds to kill any *L. huidobrensis* adults as they emerged from pupae. Yellow sticky curtain traps were erected throughout the glasshouse and individual yellow sticky cards were used for monitoring. When the crop was at the second true leaf stage, baseline samples were taken to determine which pest larval instars were present. Glasshouse temperatures and *L. huidobrensis* larval development data (Head *et al.*, 200-) were then utilised to estimate when 2nd and 3rd instar larvae (the stages most susceptible to *S. feltiae*) predominated in the populations. Suspensions of *S. feltiae* infective juveniles (IJs) containing 0.02% Agral, were prepared and applied at one of three rates at optimal times; 2 bays received an application of either 0.36 or 0.54 million IJs m⁻², and 3 bays received a lower rate of 0.18 million m⁻². During spraying the glasshouse was at the ambient temperature of $10.5 \pm 1^\circ\text{C}$, before being raised to $15 \pm 1^\circ\text{C}$ for a period of eight hours post treatment.

Plants were sampled randomly from the glasshouse before spraying, with further samples collected from each bay 24 hours post-treatment (approximately 20 plants per bay). Plants were incubated to enable all larvae present at the time of nematode application to pupate. Larval mortality was expressed as the number of pupae per treated plant as a percentage of those which emerged from untreated plants.

Results and discussion

Pesticide and nematode compatibility

Direct exposure of *S. feltiae* to cypermethrin rendered the nematodes ineffective when mixed with pesticide, with zero nematode infectivity recorded in the bioassay compared to means of 34 and 42% in the unwashed and washed control groups (unwashed: $F = 18.65$, $df\ 1, 18$, $P < 0.01$; washed: $F = 97.41$, $df\ 1, 18$, $P < 0.01$). However *S. feltiae* infectivity was not reduced when used in the presence of a cypermethrin residue resulting from an application 24 hours earlier (treatments 'pesticide & *S. feltiae*' vs. 'water & *S. feltiae*': $F = 1.4$, $df\ 1, 6$, $p > 0.05$). These results complement a previous study which showed that the residues of 5 other

pesticides caused no significant reduction in nematode efficacy against leafminer larvae infesting lettuce (Head *et al.*, 2000).

Pesticide efficacy

Abamectin and dimethoate were the most effective chemicals with 65 and 30% mortality of *L. huidobrensis* larvae respectively recorded following application (Fig. 1). The other insecticides resulted in low mortalities that were not significantly greater than the control treatment of water ($F = 1.32$, $df\ 3,8$, $P > 0.05$). Cypermethrin, deltamethrin and nicotine had been used regularly against this leafminer population, thus the pest may have developed the ability to tolerate these chemicals. Although the efficacy of pesticides tested against this outbreak population were poor and therefore not included in the IPM programme, the potential for the sequential use of some foliar applied chemical insecticides and *S. feltiae* is clear, where effective chemicals are available. Sequential treatments offer a greater flexibility in timing applications of the different control agents, many of which are known to cause differential mortality to the various life stages of this pest (Williams & Walters, 1994).

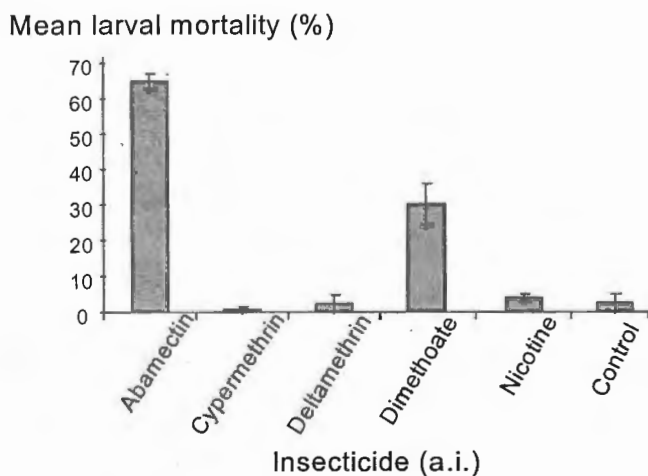


Figure 1. The efficacy of selected insecticides available in the UK against larvae from a population of *L. huidobrensis* infesting Chinese brassica.

Glasshouse trial

The glasshouse trial of components of the IPM strategy yielded promising results. Although nematodes were applied whilst temperatures were at the lower end of the range for *S. feltiae* activity, over 80% larval mortality was obtained by the lowest rate of 0.18 million IJs m^{-2} and the two higher rates yielded between 94 and 97% mortality (Fig. 2). This level of control is similar to the mortalities recorded following *S. feltiae* applications to lettuce in commercial glasshouses (Williams & Walters, 2000).

Targeting of a particular life stage with the most appropriate control measure (Solomon & Morgan, 1994) remains a viable option which relies on the availability of appropriate pest development data. Other biological control agents for leafminers could also be included for

use on integrated programmes where the cropping cycle is longer and the crop is of a higher value.

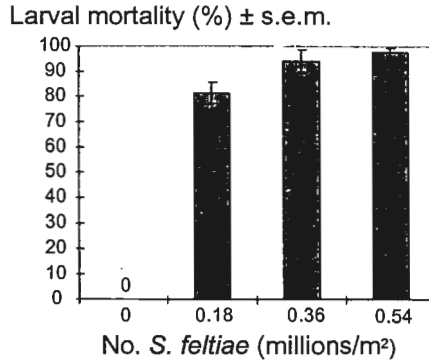


Figure 2. *Liriomyza huidobrensis* larval mortality following application of *Steinernema feltiae* to Chinese brassica in a commercial glasshouse.

Acknowledgements

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Abamectin plus pymetrozine; an extremely useful addition to the IPM armoury

Neil Helyer

Fargro Ltd. Toddington Lane, Littlehampton, West Sussex, BN17 7PP, UK, E-mail: ipm-fargro@btinternet.com

Abstract: Abamectin is active against spider mites and, at a higher rate, leaf miners and thrips, whereas the selective pesticide pymetrozine has activity against many sap sucking insects such as aphids, mealybug and whitefly but minimal side effects on most beneficials. The two products can be safely mixed together for spray application against a wide range of pest organisms including as above plus leaf hopper, psyllids and scale insects. Trials indicate good crop safety when applied to poinsettia for control of autumn migrating whitefly and thrips, whilst enabling growers to maintain a background biological control programme.

Key words: abamectin, pymetrozine, integrated control, sucking pests, phytotoxicity, poinsettia, whitefly

Introduction

Abamectin is a translaminar acaricide with good insecticidal activity against several pests, it can be integrated with biological control agents with reasonable success particularly during summer months when its persistence is shortest. Pymetrozine is from a new group of insecticides that are systemic and have excellent activity against many sucking pests while showing extreme safety to beneficials (Kayser *et al.*, 1994). Several growers in the UK have been tank mixing the two active ingredients together to produce a broader spectrum IPM compatible spray for pest hot spot treatments and end of season clean-up sprays. Trials were done to determine any possible phytotoxic reaction and spray deposit to poinsettia plants when treated in full colour.

Materials and methods

Representative cultivars of poinsettia including Cortez White, Cortez Red, Spotlight Red, Marble Star and Purple Rain were treated with one of four treatments; abamectin, pymetrozine, water control and abamectin plus pymetrozine. The pesticide treatments were done at the highest UK approved rates of application: 5 mls of abamectin (1.8% w/v) in 10 litres, and 8g pymetrozine (50% w/w WDG) in 10 litres. Spraying was by hand held pressure spray at 3 bar applying approximately 2,000 l ha. as a high volume wet spray to incipient run off. Two applications were made at just 4 days apart to simulate a worse case scenario, the second spray also had 5.0 ml per 10 l. of the wetting agent PBI Spreader (nonylphenol ethylene oxide condensate) added to all the treatments. Weather conditions during both treatment periods were bright autumn sunshine with no crop shading in use over the plants. The plants were maintained in a heated glasshouse along with the main crop for sale later that autumn. Observations were made 4, 7 and 14 days after treatments.

Results and discussion

No discernible phytotoxic reaction was visible to any of the treated plants, the white bracts of Cortez White showed no deposit or loss in colour and the cyathia continued to develop quite normally. The purple and red cultivars similarly showed no phytotoxic problems on either bract or cyathia development. However, a noticeable spray deposit was left from the pymetrozine applications on both leaf and bract. The addition of the wetting agent to the second spray helped to reduce the deposit and should be considered for use with applications of higher rate pymetrozine.

Migrations of flying insect pests such as whitefly frequently occur in the autumn months particularly just before salad crops are terminated. The reasons for the pest populations are several; reduced biological control inputs in the few weeks preceding crop termination, reduced efficacy of biocontrol agents during the autumn months and a general desire to reduce pesticide applications to edible crops. It is quite common to apply abamectin to control spidermites in early autumn before they enter diapause however this treatment can interfere with biological control agents for several days after spraying. When applied during summer months the side effects are reduced and the biological agents can bounce back to full controlling numbers quite quickly, as autumn progresses the effects on natural enemies becomes more pronounced. The standard treatment for migrating adult insects on a densely leafed crop such as poinsettia has been fumigant smokes such as nicotine shreds or propoxur. All of which drastically interfere with biological control agents, the treatment discussed above offers an IPM compatible alternative which can be used as a corrective treatment.

Acknowledgements

I thank Peter Hill, Hill Brothers Ltd. Chichester, West Sussex, UK for providing the plants and facilities.

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Development and life-span of *Macrolophus pygmaeus* Rambur at different temperatures and influence of host plants and prey

Martin Hommes, Stephanie ter Horst

BBA, Institute for Plant Protection in Horticulture, Messeweg 11/12, D-38104 Braunschweig, Germany, E-mail: M.Hommes@BBA.de

Abstract: In Germany, releases of *Macrolophus* bugs to control whiteflies in protected tomato crops have developed into a standard procedure. Currently two different species, the Mediterranean *M. caliginosus* Wagner and the endemic *M. pygmaeus* Rambur are offered on the market. To find out which species should be preferred for biological pest control some investigations on the biology of the endemic species were conducted. The life-span of *M. pygmaeus* adults was very long, on average from 24 days at 25°C to 140 days at 15°C for females and from 41 to 192 days for males, respectively. The mean development time for all larvae stages together took 15.5 days at 25°C to 37.5 days at 15°C. The investigations on host plants and prey showed that if enough prey food was offered, the influence of the host plant was negligible. Whereas without sufficient food supply the host plant has a great effect on the development and mortality of larvae stages.

Key words: biology, life-span, development time, predatory bug, *Macrolophus pygmaeus*

Introduction

In recent years the releases of *Macrolophus* bugs in tomato crops cultivated in greenhouses to control whiteflies has established in Germany. In general, predatory bugs were released as a precautionary measure 1 to 2 times shortly after the cultivation of the crop had started. The great advantage of these beneficials is that they are able to live and multiply on the crop without any prey, sucking from the tomato plants only. Currently the Mediterranean species *M. caliginosus* and the endemic species *M. pygmaeus* are offered on the German market. Basic data on the biology of *M. caliginosus* were published by Fauvel *et al.* (1987) and of *M. pygmaeus* by Perdikis & Lykouressis (1997, 1999, 2000).

To find out which species should be preferred in our country for biological pest control and also how our local strain will react, some fundamental research on the biology of the local *M. pygmaeus* strain was done.

Material and methods

Insect rearing

The strain of *M. pygmaeus* originated from Flora Hangelsberg, a natural enemy supplier located near Berlin. Rearing took place in insect cages of Macrolon plastics (50x50x60 cm) at a temperature of 20°C and a photoperiod of 16(L):8(D) h. As host plants 3 *Nicotiana tabacum* L. cv. Samsun plants were placed in a cage, and twice a week eggs of *Sitotroga cerealella* Olivier were stewed on the leaves as an additional food source.

Experiments

Investigations on development time for the different larvae instars and the life-span of adults were conducted in petri dishes with a diameter of 9 cm. The bottom of the dishes was covered

with filter paper and a young tobacco leaf placed in a 2 ml reaction vial filled with water was offered as host plant. For water and humidity supply a moistened dental cotton roll was provided. As additional food a few *Sitotroga* eggs were spread on the leaf surface. Filter paper and leaves were changed once a week or more often when necessary. The experiments on the influence of host plants and prey were done in a similar way in 13 cm petri dishes at a constant temperature of 20°C. The egg development was observed on leafless plant sticks, which were placed in a rearing cage for 24 hours. Each treatment was replicated 20 times.

Results and discussion

Influence of temperature on life-span of M. pygmaeus adults

Adults of *M. pygmaeus* lived relatively long compared to other beneficials used for biological pest control. The life-span of the adults observed in the experiments lasted on average from 24 days at 25°C to 140 days at 15°C for females and from 41 to 192 days for males respectively (table 1). It was conspicuous that at every tested temperature males lived distinctly longer than females. In comparison to the data published by Fauvel *et al.* (1987) for *M. caliginosus*, *M. pygmaeus* females had a 26% longer life-span at 15°C, a similar one at 20°C and a 40% shorter one at 25°C. Similar results were obtained for males.

Table 1. Influence of temperature on life-span (in days) of adults of *Macrolophus pygmaeus*.

°C	15		20		25	
	F	M	F	M	F	M
min	33	133	51	75	16	20
max	193	237	103	149	41	58
ξ	139.6	191.9	89.0	104.8	23.8	41.0
σ	± 46.2	± 37.5	± 14.8	± 22.2	± 9.6	± 14.0

ξ = Mean in days and ± standard deviation (σ), F = Female, M = Male

Influence of temperature on development time and mortality of egg and larvae stages

As expected, temperature had also a major influence on the development of the egg stage and the different larvae instars (table 2). The development time for the egg stage decreased rapidly from 29.3 days at 15°C to 10.7 days at 30°C and for the larvae instars in total from 37.5 days at 15°C to 15.5 days at 30°C. At a constant temperature of 32.5°C and above, none of the larvae reached the adult stage. This data indicates that larvae of the local *M. pygmaeus* strain are very sensitive to temperatures above 30°C. The same results were stated by Fauvel *et al.* (1987) for *M. caliginosus*. The development times for eggs were the same at 25 and 30°C for both species but at 15°C *M. pygmaeus* developed 7.5 days faster than *M. caliginosus*. At 20°C the difference was only one day in favour of the endemic *M. pygmaeus* species.

Similar results to the data from Fauvel *et al.* (1987) were reported by Perdakis & Lykouressis (1999) regarding their *M. pygmaeus* strain. Astonishing is also the tolerance of the Greek strain to temperatures above 30°C. A complete development, even if it was a little bit delayed, could be observed at a constant temperature of 35°C. This points towards a different local strain which has adapted to the Mediterranean climatic conditions.

Table 2. Influence of temperature on development time and mortality (M) of egg and larvae stages (L1-L5) of *Macrolophus pygmaeus* on tobacco.

t [°C]	n	mean development time [d ± σ] ¹							M [%]
		egg	L1	L2	L3	L4	L5	total L1-5	
15	20	29.3 ± 0.6 ^{a2}	8.7 ± 1.2	7.4 ± 1.1	5.3 ± 1.4	6.7 ± 0.9	9.4 ± 0.7	37.5 ^{a2}	20
20	20	17.2 ± 0.8 ^b	6.3 ± 1.1	4.1 ± 0.4	3.6 ± 0.6	3.8 ± 0.6	7.2 ± 0.4	25.0 ^b	5
25	20	11.8 ± 0.5 ^c	5.3 ± 0.0	3.1 ± 0.4	2.6 ± 0.4	2.7 ± 0.2	4.5 ± 0.2	18.2 ^c	15
30	20	10.7 ± 0.6 ^d	3.9 ± 0.5	2.3 ± 0.4	2.2 ± 0.4	2.9 ± 0.3	4.3 ± 0.2	15.5 ^d	25
32.5	20	- ³	- ⁴	-	-	-	-	-	100

¹ Days (d) ± standard deviation (σ)

² Means followed by different letters are significantly different at P = 0.05 (Tukey-Test)

³ not investigated

⁴ 100% mortality of the larvae

Influence of host plants on development time and mortality of larvae stages of M. pygmaeus

Investigations on the influence of host plant and food showed that if enough food was offered the influence of the host plant is more or less negligible (table 3). Whereas without additional food supply the host plant has a great influence on the development time and mortality of the larvae stage. The fastest development without additional food could be observed on eggplant cv. Mastoma with a prolonged development period of 49%, followed by tobacco with 60% and by tomato cv. Sparta with 74%, respectively. In sweet pepper cv. Mazurka a complete development could not be achieved without additional food. These results contradict the data from Perdakis & Lykouressis (1999) who only found slight differences in the development rate of eggplant and pepper. This is even more surprising because more or less the same methods were used and the data received for the development time with additional food was very similar. Reasons for the observed differences could be the above mentioned development of a local biotype or a great influence of the tested host plant variety.

Table 3. Influence of different host plants with and without prey on development time and mortality (M) of larvae stages (L1-L5) of *Macrolophus pygmaeus* at 20°C.

host plant	prey	mean development time [d ± σ] ¹						M [%]
		L1	L2	L3	L4	L5	total	
tobacco	-	10.1 ± 2.5	7.4 ± 1.6	5.1 ± 1.0	7.4 ± 1.1	9.8 ± 1.7	39.8	45
pepper	-	-	-	-	-	-	-	100
eggplant	-	6.7 ± 1.0	4.8 ± 0.6	5.8 ± 1.2	6.9 ± 1.2	10.7 ± 1.1	34.8	40
tomato	-	7.8 ± 1.5	9.1 ± 1.4	7.2 ± 1.5	8.5 ± 2.1	10.3 ± 0.8	43.0	80
tobacco	S.c. ³	6.3 ± 1.3	4.1 ± 1.1	3.6 ± 0.7	3.8 ± 0.9	7.2 ± 0.9	24.9a ²	5
pepper	S.c.	5.9 ± 1.4	4.4 ± 0.6	4.4 ± 0.8	3.8 ± 0.5	4.5 ± 1.3	22.8a	10
eggplant	S.c.	6.1 ± 1.2	4.2 ± 0.8	4.2 ± 0.9	3.9 ± 0.9	5.0 ± 0.7	23.4a	15
tomato	S.c.	6.6 ± 1.1	4.6 ± 0.4	4.0 ± 0.6	4.6 ± 0.6	5.0 ± 0.4	24.7a	5

¹ Days and (d) ± standard deviation (σ)

² Means followed by different letters are significantly different at P = 0.05 (Tukey-Test)

³ S.c. = eggs of *Sitotroga cerealella*

Influence of natural prey on development time and mortality of larvae stages of *M. pygmaeus*

Eggplant leaves infested with *Aphis gossypii* Glover represented a good diet for this predatory bug (table 4). The developing period was only slightly (7%) prolonged compared to the standard system 'leaves with *Sitotroga* eggs' (table 3). On tobacco leaves infested with white flies (*Trialeurodes vaporariorum* Westwood), *M. pygmaeus* larvae could also prosper very well but the development time was prolonged by 12% in comparison to the standard system. These results do not differ too much from the data given by Perdikis & Lykouressis (1999). The development time they worked out at 20°C for the system 'eggplant infested with *Myzus persicae*' was 22 days compared to 25 days in our investigations with *A. gossypii*.

Table 4. Influence of natural prey on development time and mortality (M) of larvae stages (L1-L5) of *Macrolophus pygmaeus* at 20°C.

host plant	prey	n	mean development time [d ± σ] ¹					Total	M [%]
			L1	L2	L3	L4	L5		
tobacco	T.v.	20	8.4 ± 1.4	4.9 ± 0.8	3.5 ± 0.5	4.0 ± 0.6	7.1 ± 0.6	27.9 ²	15
eggplant	A.g.	20	5.6 ± 1.1	4.2 ± 0.9	4.3 ± 1.0	4.8 ± 0.6	6.3 ± 1.3	25.1 ^b	5

¹ Days and (d) ± standard deviation (σ)

² Means followed by different letters are significantly different at P = 0.05 (Tukey-Test)

³ T.v. = *Trialeurodes vaporariorum*; A.g. = *Aphis gossypii*

The experiments on *M. pygmaeus* have shown that for a successful biological pest control applying *Macrolophus* bugs it is important to know which species and biotypes are offered on the market. Starting out from the results we recommend using the German *M. pygmaeus* strain for crop cultivation at temperatures around 20°C. For temperatures around 25°C or higher *M. caliginosus* or an adapted Mediterranean strain of *M. pygmaeus* should be preferred. If no prey is present in the crop it is also recommended to promote the predatory bug population by additional food supply.

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Predation and oviposition rate of the predatory bug *Orius laevigatus* in the presence of alternative food

Jan Hulshof, Marika Linnamäki

MTT, Agrifood Research Finland, Plant Production, R-building, FIN-31600 Jokioinen, Finland, E-mail: jan.hulshof@mtt.fi

Abstract: The effect of the presence of alternative food (*Ephestia kuehniella* eggs and pine pollen) on the predation and fecundity rate of *Orius laevigatus* was studied. The presence of pine pollen enhanced the predation rate of 3rd instar nymphs and adult bugs on thrips larvae (*Frankliniella occidentalis*), whereas the predation rate of the 4th instar nymphs on thrips larvae and that of adult female bugs on adult thrips was not affected. Both nymphal stages killed, irrespective of the presence of alternative food, only thrips larvae, but not adults, when both stages were offered simultaneously. Even in the absence of thrips as prey, both pine pollen and *E. kuehniella* eggs supported the bugs' fecundity. In preliminary greenhouse tests, the alternative food did not enhance the bugs' persistence in the cucumber crop, probably due to cannibalism. Further experiments should therefore consider not only the alternative food, but also the assumed cannibalistic behavior of the bugs.

Key words: *Orius laevigatus*, *Frankliniella occidentalis*, alternative food, pollen, *Ephestia kuehniella*

Introduction

Biological control of the Western Flower Thrips (*Frankliniella occidentalis*) seems more successful in sweet pepper than in cucumber. This might be due to the difference in establishment and persistence of the predators in these two crops. In cucumber, populations of *Neoseiulus cucumeris* or *Orius laevigatus* invariably declined after predator release, necessitating repeated releases to achieve sufficient control, whereas in sweet pepper *O. insidiosus* population remained constant even in the absence of thrips prey (van Rijn & Sabelis, 1990; Chambers *et al.*, 1993; van den Meiracker, 1999). The predators might use sweet pepper pollen as alternative food, whereas modern cucumber varieties lack pollen (van den Meiracker, 1999). This suggests that supplying alternative food might facilitate the preventive introduction of predators. In cucumber, populations of *Amblyseius degenerans* and *A. limonicus* increased faster, and thrips populations remained smaller in the presence of additional pollen than in its absence (van Rijn *et al.*, 1999). Here we report on the effect of alternative food on the predation and fecundity rate of *O. laevigatus*, which was studied during the evaluation of the practical feasibility of this method for *O. laevigatus*.

Material and methods

Predation rate of *Orius* nymphs. The predation rate of 3rd and 4th instar *O. laevigatus* nymphs on *F. occidentalis* larvae and adults was determined in the presence and absence of additional food: pine (*Pinus sylvestris*) pollen or *Ephestia kuehniella* eggs. Ten thrips larvae and 3 adults were confined on pieces of cucumber leaf (cv. 'Jessica') in mini-chambers (Hulshof & Vänninen, 1999). After 24 hours one nymph (N3 or N4) was added. The number of thrips (alive and dead) was recorded upon adding the nymph and after 2, 4 and 6 h. The

number of thrips was kept constant by adding the same number of thrips that were killed. N = 25 and 20 for the 3rd and 4th instar, respectively.

Predation rate of adult females. The predation rate of adult *O. laevigatus* females on *F. occidentalis* larvae and adults was determined for the same treatments. A cucumber leaf was placed in a glass petridish (ø: 19 cm). The petiole of the leaf was covered with wet cotton to avoid the leaf from wilting. The leaf was infested with 20 thrips larvae and 10 adults 24 hours prior the start of the experiment. At the start of the experiment 1 female bug, which was conditioned for 24 h at the receiving treatment, was released on the leaf. The number of thrips (alive and dead) was recorded upon adding the female and after 24 h. N = 15 per treatment.

Fecundity rate. The fecundity and survival rate of 1 week old adult *O. laevigatus* females was determined in mini-chambers for 5 subsequent days by counting the eggs with a stereomicroscope (Hulshof & Vänninen, 1999). This was done for 6 treatments: cucumber leaf, leaf + pine pollen, leaf + *E. kuehniella* eggs, leaf + thrips mix (10 larvae + 3 adults), leaf + thrips mix + pine pollen; and leaf + thrips mix + *E. kuehniella* eggs. N = 15 per treatment.

Statistical analyses. Non-parametric tests and mixed models for repeated measurements on the same individual were used for the predation rate and fecundity rate data, resp. (SAS Institute Inc., 2000).

Results and discussion

Predation rate

The presence of pine pollen enhanced the predation rate of 3rd instar nymphs compared to that of the other treatments during the 2nd and 3rd two hour interval. For all three treatments the predation rate of the 4th instar nymphs was higher than that of the 3rd instar nymphs. The presence of pine pollen did not have an effect on the predation rate of the 4th instar nymphs (Fig. 1). Tommasini & Nicoli (1994) found that the predation rate of 4th instar nymphs on thrips adults was even lower than that of 3rd instar nymphs. Morphological changes during the 4th instar stage might explain that these nymphs are less interested in food.

Irrespective of the treatment, both nymphal stages killed only thrips larvae, but not adults, when both stages were offered simultaneously, whereas in a non-choice situation they are able to kill adult thrips (Tommasini & Nicoli, 1993).

There is a tendency of the predation rate of adult female bugs on thrips larvae being affected by the presence of alternative food (Kruskal-Wallis test $\chi^2=5.48$, $df=2$, $p=0.06$). In the presence of pine pollen, the predation rate on larvae was significantly higher than in its absence (KW, $\chi^2=5.71$, $df=1$, $p=0.02$), whereas predation rate was not affected by the presence of *E. kuehniella* eggs (KW, $\chi^2=1.17$, $df=1$, $p=0.28$). The predation rate of adult female bugs on adult thrips was not affected by the presence of alternative food (KW, $\chi^2=2.57$, $df=2$, $p=0.28$) (Fig. 2).

The predation rate of *O. laevigatus* on *F. occidentalis* also increased in the presence of chrysanthemum pollen (Bennison, pers. comm.). The reduced awareness of thrips larvae to their environment might explain why they suffer more from predation by *O. laevigatus* in the presence of pollen.

Fecundity rate

E. kuehniella eggs and pine pollen supported the fecundity of the bugs even in the absence of thrips as prey. The fecundity of bugs that were offered both thrips and *E. kuehniella* eggs did not differ significantly from that of the bugs offered only thrips ($t=0.93$, $df=77$, $p=0.36$). The fecundity of the bugs offered both pine pollen and thrips was significantly lower than that of those offered only thrips ($t=-2.71$, $df=77$, $p=0.008$) (Fig. 3).

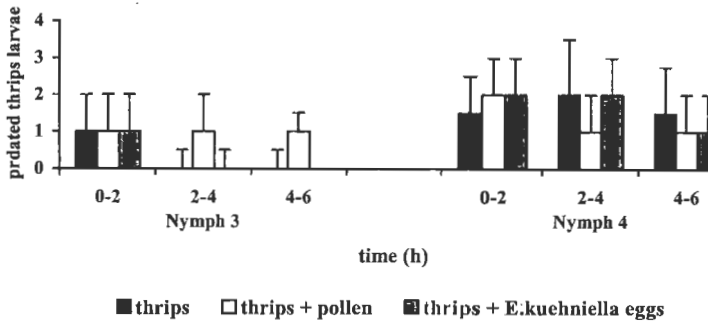


Figure 1. Median predation rate of the 3rd and 4th nymphal stage of *O. laevigatus* on *F. occidentalis* larvae in the presence and absence of alternative food (*E. kuehniella* eggs or pine pollen) during 3 subsequent periods of 2 hours. Error bars: quartile deviation (25%-75% confidence interval/2).

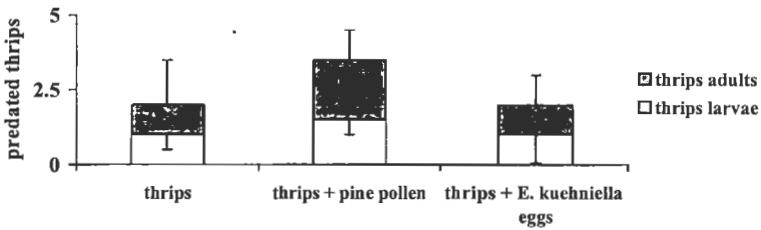


Figure 2. Median predation rate of *O. laevigatus* adult females on *F. occidentalis* larvae and adults in the presence and absence of alternative food (*E. kuehniella* eggs or pine pollen) during 24h. Error bars: quartile deviation, up: thrips adults; down: thrips larvae.

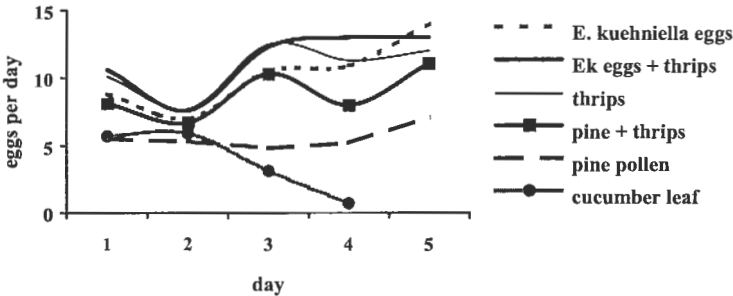


Figure 3. Fecundity rate of *O. laevigatus* supplied with different food sources during five subsequent days.

This is surprising, because the dish experiment had shown that bugs actually can kill more thrips in the presence of pine pollen than without it (cf. Fig. 2). Behavioral observations

should reveal whether the reduced fecundity is due to a change in the way the bugs are feeding on insect prey in the presence of pine pollen. Do they really consume the prey, or does the increased predation rate limit the time available for egg laying, or does it resemble a trade off between fecundity and longevity? This reduced fecundity in the presence of pine pollen does probably not hamper the biological control of thrips, because the increased predation rate should compensate for it. The fecundity rate of bugs engaged on the cucumber leaf decreased after 2 days (Fig. 3). Also the survival rate of these bugs was affected, all bugs were dead at day 5, whereas the survival rate of the bugs receiving the other treatments was still in the range of 80 and 90%.

It was concluded that pine pollen and *E. kuehniella* eggs fulfilled the requirements of alternative food under laboratory conditions: they supported the bugs' fecundity even in the absence of thrips as prey, and they had either a positive or no effect on the predation rate of *O. laevigatus*. In preliminary greenhouse tests the bugs were eventually able to control the thrips, irrespective of the presence or absence of alternative food. However, the presence of pine pollen or *E. kuehniella* eggs did not enhance the persistence of the bugs in the cucumber crop (Hulshof, unpubl.). This might be due to the cannibalistic behavior of the bugs. In sweet pepper, the flowers might not only offer alternative food, but also serve as a shelter place, reducing the cannibalistic behavior of the bugs. Therefore further experiments in cucumber should not only focus on alternative food, but also on ways to minimize the assumed cannibalistic behavior of the bugs (e.g. the way food is offered: dusted or concentrated; or offering refugee places for the bugs).

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***Lygus rugulipennis* Poppius (Het. Miridae): Options for integrated control in glasshouse-grown cucumbers**

R.J. Jacobson

Stockbridge Technology Centre Ltd, Cawood, N. Yorkshire, YO8 3TZ, UK, E-mail: robjacobson.stc@farmline.com

Abstract: Damaging infestations of *Lygus rugulipennis* Poppius are becoming more common in glasshouse-grown cucumbers in the U.K. and IPM compatible control measures are urgently required. The entomopathogenic fungus, *Beauveria bassiana* [Balsumo] Vuillemin, and the antifeedant chemical, pymetrozine, have been shown to reduce *L. rugulipennis* numbers and damage respectively when applied as high volume sprays or low volume mists. A combination of both control measures may provide the ultimate solution to this problem.

Key words: *Lygus rugulipennis*, cucumbers, *Beauveria bassiana*, pymetrozine, IPM

Introduction

Surveys of growers using IPM have confirmed that infestations of *Lygus rugulipennis* Poppius are becoming more common and more damaging in protected salad crops in the U.K. (Jacobson, 1999). Cucumbers are most seriously affected with distorted foliage, dead growing points and malformed fruit causing estimated financial losses of up to £2 per m². As there are no IPM compatible control measures for *L. rugulipennis*, growers have been forced to apply broad spectrum insecticides that disrupt biological control of other pests and result in secondary pest outbreaks. An IPM compatible control measure is urgently required.

The entomopathogenic fungus, *Beauveria bassiana* [Balsumo] Vuillemin (Naturalis-L) was evaluated against *L. rugulipennis* on cucumber plants in experimental glasshouses in 1998 (Jacobson, 1999). A single application of a high volume spray (HV) or a low volume mist (LV) provided similar results with numbers of adult *L. rugulipennis* being reduced by 60% compared to untreated controls. However, the mean relative humidity (RH) in the glasshouse was high (90% in the crop canopy), which favoured the entomopathogen. The present study (experiment 1) was done under more challenging conditions in 1999.

The antifeedant chemical, pymetrozine (Chess), is specific to some plant-sucking Hemiptera, and should be compatible with natural enemies. Preliminary laboratory studies showed that the chemical did not kill adult *L. rugulipennis* directly but the damage to growing points of treated plants was less severe than untreated controls (Jacobson, unpublished data). This was further investigated in the present study (experiment 2).

Materials and methods

Experiment 1

Twenty four cucumber (cv. Enigma) plants were planted on 5 August 1999 in each of five similar experimental glasshouses. The plants were grown hydroponically in peat bags with excess feed solution running to waste and were trained by the cordon-V system. The glasshouses were artificially infested with adult female *L. rugulipennis*. Due to the pest's mobility, the following treatments were each confined to a separate glasshouse:

1. Untreated control.
2. *B. bassiana* (Naturalis-L) applied as HV spray at 400 ml product / 100 l water.
3. *B. bassiana* (BotaniGard WP) applied HV at 125 g BotaniGard / 100 l water.
4. *B. bassiana* (Naturalis-L) applied as LV mist at 14.4 ml product / 280 ml water / 109 m³.
5. *B. bassiana* (BotaniGard WP) applied LV at 4.5 g product / 280 ml water / 109 m³.

Treatments 2-5 were all applied three times; *i.e.* on 8, 9 and 10 September when the plants were about 1.8 m tall. The HV sprays were applied to maximum leaf retention using a fully calibrated Oxford Precision Sprayer at a rate equivalent to 2025 litres per hectare. The LV mist treatments were applied using a fully calibrated Turbair Scamp 240 at a rate equivalent to 70 litres per hectare.

Samples were taken from each spray mixture and the numbers of viable spores (expressed as colony forming units [CFU]) determined by culturing on growth media. The numbers of *L. rugulipennis* per plant were assessed in each glasshouse before spray application and 7 days after application of treatments. Data from each assessment date were analysed by analysis of variance and differences compared using LSD. Some caution is required when interpreting the results from experiments such as this, where it is impractical to fully replicate treatments. There was no true replication and within treatment variation was used as a measure for experimental variation. Dead insects found during the post-treatment assessment were placed individually on moist filter paper in a Petri dish and incubated without light at 23°C. Fungal growth on the cadavers was subsequently sub-cultured on growth media, incubated until sporulation occurred and identified. The humidity in the glasshouses was recorded throughout the experiment.

Experiment 2

The experimental design and method was similar to experiment 1 with the following differences. The cucumbers were planted on 30 August 2000. One of the following treatments was applied in each glasshouse:

1. Untreated control
2. Single pymetrozine HV application at 40 g product / 100 l water.
3. Three pymetrozine HV applications at 40 g product / 100 l water.
4. Single pymetrozine LV application at 40 g product / 1,000 m².
5. Three pymetrozine LV applications at 40 g product / 1,000 m².

Pymetrozine was applied in treatments 2 to 5 on 5 September. The additional applications to treatments 3 and 5 were done on 12 and 19 September. HV sprays were applied at volumes equivalent to 720, 930 and 1,125 litres per hectare on 5, 12 and 19 September respectively. The volumes used increased because the plants were growing and retained more spray. The LV mist treatments were applied at volume equivalent to 16.6 litres per hectare.

Assessments were done in all treatments immediately before each of the three applications of pymetrozine, and 7 days after the final application. On each occasion, the youngest 10 cm of growth of every plant was examined and *L. rugulipennis* damage recorded using the following index:

- 0 No visible damage.
- 1 <2% of foliage damaged (*i.e.* "pin-prick" damage, tearing and/or distortion).
- 2 2-10% of foliage damaged.
- 3 10-40% of foliage damaged.
- 4 40-100% of foliage damaged.
- 5 Growing point killed.

Analysis of variance was done on log transformed data (with the term 0.375 added to all data due to the large number of zeros) and the differences were compared using LSD. As with experiment 1, some caution is required when interpreting the results due to the use of within treatment variation as a measure of experimental variation.

Results and discussion

Experiment 1

The approximate numbers of CFU's of *B. bassiana* per ml in the spray mixtures and the mean numbers of adult *L. rugulipennis* per plant in each treatment, on each assessment date, are shown in table 1.

The data from the post-application assessment showed no significant difference between the numbers of *L. rugulipennis* on plants in the four *B. bassiana* treatments but all had significantly fewer *L. rugulipennis* than the untreated control ($P < 0.05$). The presence of *B. bassiana* was confirmed on dead *L. rugulipennis* that were collected on 17 September.

The overall mean number of adult *L. rugulipennis* in the *B. bassiana* treatments was approximately 78% lower than the untreated control, compared to approximately 60% lower than the untreated control in the previous experiment (Jacobson, 1999). The additional control can be attributed to the programme of three sprays compared to the previous application of a single spray. These levels of control were broadly consistent with that recorded when *B. bassiana* was applied against *Lygus lineolaris* on cotton in the USA and against *Lygus hesperus* in laboratory studies at the University of Idaho (Brown, pers. com.).

The mean relative humidity during this experimental period was approximately 70% in the crop canopy compared to 90% in the previous experiment. This suggests that infection of *L. rugulipennis* by *B. bassiana* is not dependant on high RH in the aerial environment.

Table 1. Experiment 1. Mean numbers of CFU's of *B. bassiana* in spray mixtures and mean numbers of adult *L. rugulipennis* per plant before and after application of *B. bassiana*.

Treatments	Range of CFU's per ml in the three spray mixtures	Mean number of <i>L. rugulipennis</i> adults per plant:	
		Before application of <i>B. bassiana</i>	7 days after application of <i>B. bassiana</i>
1. Untreated control	-	1.75	1.5
2. Naturalis-L - HV	0.9 - 1.3 x 10 ⁵	1.92	0.58
3. BotaniGard WP - HV	0.6 - 0.9 x 10 ⁷	1.75	0.33
4. Naturalis-L - LV	1.2 - 1.6 x 10 ⁶	1.92	0.25
5. BotaniGard WP - LV	0.6 - 0.9 x 10 ⁸	2.08	0.17
LSD (df = 15)	-	0.97	0.48

Experiment 2

The mean damage indices for each treatment at each assessment date are shown in table 2. On the first post-treatment assessment date, the mean damage index in the untreated control was 1.08, while there was no damage recorded in any of the pymetrozine treatments. On the second post-treatment assessment, there was more ($P < 0.05$) damage in the untreated control

(mean index 0.88) than the 3 x LV and 3 x LV pymetrozine treatments (mean indices 0 and 0.04 respectively). Neither the 1 x LV nor 1 x HV treatments was significantly different to the untreated control at that time. The situation was similar on the third and fourth post-treatment assessments, with mean damage indices in the untreated controls, 3 x LV and 3 x HV treatments being 1.42, 0 and 0.04 respectively on the third assessment and 1.71, 0 and 0.25 respectively on the fourth assessment.

The trends throughout the experiment can be clearly seen. Both LV and HV applications of pymetrozine prevented damage during the week following the first application of the anti-feedant chemical. Where there were no further applications of pymetrozine, the damage increased steadily throughout the remainder of the experiment. However, weekly applications of pymetrozine restricted damage to commercially acceptable levels.

Table 2. Experiment 2. Mean damage index (log transformed) in all treatments before the first application of pymetrozine and at weekly intervals over the following four weeks.

Assessment (weeks post- treatment)	Mean damage index (log transformed) on five assessment dates in treatments (#):					LSD [df=12]
	1. (Control)	2. (1 x HV)	3. (3 x HV)	4. (1 x LV)	5. (3 x LV)*	
0	0	0	0	0	0	-
1	1.08 (-0.22)	0 (-0.98)	0 (-0.98)	0 (-0.98)	0	- (0.48)
2	0.88 (-0.48)	0.29 (-0.76)	0.04 (-0.93)	0.17 (-0.76)	0	- (0.41)
3	1.42 (-0.04)	0.54 (-0.47)	0.04 (-0.93)	0.58 (-0.50)	0	- (0.74)
4	1.71 (0.18)	0.96 (-0.18)	0.25 (-0.77)	1.71 (0.20)	0	- (0.93)

* excluded from analysis because all figures were zero.

for comparisons between Treatments 1, 2, 3 and 4 within assessment dates use the LSD [df] shown in table, and between assessment dates use LSD = (0.30) [df = 48].

Overall conclusions

Both *B. bassiana* and pymetrozine reduce damage by *L. rugulipennis* when applied as HV sprays or LV mists. The results indicate that pymetrozine would have to be applied at weekly intervals to provide continuous protection throughout the main *L. rugulipennis* invasion period. LV applications require minimal labour and would therefore allow the cost-effective application of such a series of treatments. The ultimate solution to this problem may be a combination of both control measures, with pymetrozine used to limit crop damage while *B. bassiana* reduces the number of *L. rugulipennis*.

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Interactions between the two polyphagous predators *Orius majusculus* and *Macrolophus caliginosus*

Lene Jakobsen^{1,2}, Annie Enkegaard², Henrik F. Brødsgaard²

¹Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark, E-mail: Annie.Enkegaard@agrsci.dk. ²Department of Crop Protection, Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark

Abstract: The mutual predation between the two polyphagous predators *Orius majusculus* and *Macrolophus caliginosus* was examined in laboratory experiments in the presence and absence of *Frankliniella occidentalis*. Predation occurred but was unidirectional since neither nymphs nor adults of *M. caliginosus* preyed upon *O. majusculus*. Adults of *O. majusculus* preyed upon *M. caliginosus* in absence and in some circumstances also in presence of *F. occidentalis*. *O. majusculus* nymphs did not prey upon *M. caliginosus* either adults or nymphs. The predation rate of *O. majusculus* on *F. occidentalis* was unaffected by the presence of *M. caliginosus*. This suggests that the presence of *M. caliginosus* in a culture will not hamper the biological control of *F. occidentalis*.

Key words: biological control, interactions, *Macrolophus caliginosus*, *Orius majusculus*, *Frankliniella occidentalis*

Introduction

Often greenhouse crops, especially ornamentals, suffer from attack from several different pests necessitating the use of an equal or higher number of beneficial species for their control (Enkegaard *et al.*, 2000). The simultaneous use of several beneficial species raises questions not only about the influence of beneficials on the pests but also about the mutual interactions between the beneficials.

The two predatory bugs *Orius majusculus* (Reuter) (Heteroptera: Anthocoridae) and *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) are used commercially for biological control of thrips and whiteflies, respectively, however being polyphagous they prey on several other small insects as well (Alverado *et al.*, 1997; Tedeschi *et al.*, 1999). A polyphagous trait is favourable e.g. when target pest populations are low, although it could also become a problem when several beneficials are used simultaneous. The present experiments were undertaken to examine if *O. majusculus* and *M. caliginosus* interact by attacking each other in order to clarify the possible implications for a simultaneous use of the two predators.

Materials and methods

Insects

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) was reared on *Phaseolus vulgaris* (L.) var. Montana in net covered cages (85x75x68 cm) in a greenhouse at 22°C, 70% r.h., and 16:8 L:D. *M. caliginosus* and *O. majusculus* were delivered by EWH BioProduction, Denmark.

General conditions

All experiments were conducted in climate cabinets at 25°C, 67 ± 10% RH and L:D 18:6. Chrysanthemum, *Dendranthema grandiflorum* (L.) cv. Purple Cindy was used as model plant. The predators used in the experiments were taken directly from the batches delivered from the producer. The stages of predators used were adult females or 3rd instar. *F. occidentalis* were taken from the rearing and only adults were used. Prior to experimentation the predators were starved for a 24 h period by placing them in pairs in Petri dishes with wet filterpaper. Starving took place under the same conditions as the experiments.

Experiments

Three sets of experiments were conducted to examine the mutual predation between *O. majusculus* and *M. caliginosus* in 1) absence and 2) presence of prey; and 3) to examine the influence of the presence of *M. caliginosus* on *O. majusculus*' predation on thrips.

In the first set of experiments four combinations of predator stages were used: a) adult *O. majusculus* and adult *M. caliginosus*; b) adult *O. majusculus* and 3rd instar *M. caliginosus*; c) adult *M. caliginosus* and 3rd instar *O. majusculus*; and d) 3rd instar *O. majusculus* and 3rd instar *M. caliginosus*. 3rd instars of the two predators are of approximately similar size. In the second set of experiments combinations a), b) and d) were used. In the third set of experiments only combination b) was used.

The experimental set-up consisted of a small water filled, lidded plastic cup (h 4 cm, Ø 3.5 cm) in which a Chrysanthemum cutting with 4-5 leaves was positioned through a hole in the lid. Cotton was used to seal the space between the petiole and the lid to prevent access to the interior of the cup. The cup was placed in a plastic cylinder (h 9 cm, Ø 8 cm) sealed with a lid and further secured by wrapping of parafilm. The predators – and in 2) and 3) also thrips – were added to the set-up. The following number of individuals were used: 1) 10 *O. majusculus* and 10 *M. caliginosus*; 2) 10 *O. majusculus*, 10 *M. caliginosus*, and surplus (130-160) thrips; 3) 1 *O. majusculus*, 3 *M. caliginosus* and 35 thrips. In experiment 3) only *O. majusculus* was starved. After 24 h the number of living and dead insects were counted. In all three experimental set-ups controls were set up separately.

Data analysis

Data were analysed using the statistical method Proc Genmod in SAS (SAS Institute Inc., 1989).

Results

The results of the experiment revealed that predation occurred between *O. majusculus* and *M. caliginosus*. The predation was, however, unidirectional – in none of the combinations did *M. caliginosus* prey upon *O. majusculus* ($P > 0.324$).

Adult *O. majusculus* preyed on both adults and nymphs of *M. caliginosus* when no alternative prey was present (table 1). The predation was independent of the stage of *M. caliginosus* ($P = 0.693$), the individual predation rate being about 0.3 *M. caliginosus*, adults or nymphs (table 1). When alternative prey in the form of *F. occidentalis* was present, adult *O. majusculus* maintained their predation rate on *M. caliginosus* nymphs ($P = 0.976$) unchanged. Their predation rate on adult *M. caliginosus* was, however, significantly reduced ($P < 0.001$) to a level not significantly different from the corresponding control (table 1).

Contrary to the adults, nymphs of *O. majusculus* did not prey upon either adults or nymphs of *M. caliginosus*, even in the absence of alternative prey (table 1).

In spite of the fact that adult *O. majusculus* predated on nymphs of *M. caliginosus* in the presence of *F. occidentalis*, the predation rate of *O. majusculus* on *F. occidentalis* was not significantly ($P=0.456$) influenced by the presence of nymphs of *M. caliginosus* (table 2).

Finally, the experiments revealed that nymphs of *M. caliginosus* did not predate upon *F. occidentalis* in the present set-up ($P=0.278$).

Table 1. The average total mortality [95% confidence limits] inflicted by 10 *O. majusculus* on *M. caliginosus* expressed as numbers of dead *M. caliginosus* in the absence and presence of alternative prey (*F. occidentalis*) in the four experimental combinations. n is number of replicates. P gives the level of significance for the difference between the mortality in the experimental combinations and the corresponding control without *O. majusculus*. Figures in the same row are significant different when followed by different letters ($P < 0.05$).

Combinations of species and stages	Absence of <i>F. occidentalis</i>		Presence of <i>F. occidentalis</i>	
	# of dead <i>M. caliginosus</i>	P	# of dead <i>M. caliginosus</i>	P
<i>O. majusculus</i> adults } <i>M. caliginosus</i> adults }	3.5a [2.40; 4.78] (6)	0.0055	0.44b [0.18; 1.08] (9)	0.0958
<i>O. majusculus</i> adults } <i>M. caliginosus</i> nymphs }	3.17a [2.48; 3.95] (6)	<0.0001	3.18a [2.71; 3.70] (11)	<0.0001
<i>O. majusculus</i> nymphs } <i>M. caliginosus</i> nymphs }	1.11a [0.48; 2.49] (7)	0.318	0.8a [0.32; 1.87] (10)	0.256
<i>O. majusculus</i> nymphs } <i>M. caliginosus</i> adults }	0.67 [0.25; 1.68] (9)	0.882	-	

Table 2. The average mortality [95% confidence limits] inflicted by individual *O. majusculus* on *F. occidentalis* expressed as numbers of dead *F. occidentalis* in the absence and presence of alternative prey (*M. caliginosus*). n is number of replicates. P gives the level of significance for the difference between the experimental combinations and the corresponding control without *O. majusculus*. Figures in the same row are significant different when followed by different letters ($P < 0.05$).

Combinations of species and stages	Absence of <i>M. caliginosus</i>		Presence of <i>M. caliginosus</i>	
	# of dead <i>F. occidentalis</i>	P	# of dead <i>F. occidentalis</i>	P
<i>O. majusculus</i> adult } <i>F. occidentalis</i> adult }	13.58a [12.00; 5.24] (12)	<0.0001	14.38a [13.11; 15.69] (19)	<0.0001

Discussion

The present results have shown that the interactions between *O. majusculus* and *M. caliginosus* are unidirectional and dependent upon the stage of predator and prey in the interaction, as well as upon the presence of alternative prey. The predator in the interaction was adult *O. majusculus* and the prey was nymphs and - in the absence of alternative prey - adult *M. caliginosus*. *O. majusculus* nymphs did not engage in interactions with *M. caliginosus* in the present study where individuals of the two species were approximately similar size. It is, however, possible that larger nymphs of *O. majusculus* will prey upon

smaller sized nymphs of *M. caliginosus*. The results indicate superiority of *O. majusculus* to *M. caliginosus*, perhaps due to a harder and sturdier body structure or due to behavioural differences. Direct observations revealed that adult *O. majusculus* was the aggressor in meetings between the two species, irrespective of the stage of *M. caliginosus*, with *M. caliginosus* showing only defensive reactions and no sign of attempts of attack (L. Jakobsen, pers. obs.). Experiments under more realistic three-dimensional conditions are needed to elucidate the practical implications of the predation of *O. majusculus* on *M. caliginosus* for the outcome of the biological control *M. caliginosus* is set out to exert.

The results showed that adult *O. majusculus* killed an equal number of *M. caliginosus* adults and nymphs, irrespective the presence of alternative prey. A lower predation of adult *M. caliginosus* and a lower predation of nymphs in the presence of *F. occidentalis* would be expected, but it must be kept in mind that the actual degree of consumption of killed individuals was not examined in the present study. Killed individuals may have been only partially consumed.

The predation rate of *O. majusculus* on *F. occidentalis* was not affected by the presence of *M. caliginosus* indicating that the simultaneous use of the two beneficials will not interfere with *O. majusculus*' control of *F. occidentalis*.

Other experiments have been carried out to highlight the influence of the presence of other beneficials on *O. majusculus*' predation on *F. occidentalis*. Experiments have shown that the efficacy of *O. majusculus* for control of *F. occidentalis* was not decreased by the presence of either the gall midge *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) or the predatory mite *Phytoseiulus persimilis* Athios-Henriot (Acarina: Phytoseiidae). The experimental outcome suggested that *O. majusculus* benefited from the presence of alternative prey sources when the target prey population was low (Brødsgaard & Enkegaard, 1995).

In the present experiment there was no sign of *M. caliginosus* preying on *F. occidentalis*. This is in contrast with reports from others (Montserrat *et al.*, 2000; Riudavets & Castane, 1998) and may be due to differences in the experimental set-up or the stages of *F. occidentalis* used as prey.

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Spinosad: An effective biocide for inclusion in integrated pest management programs for *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on greenhouse cucumbers

Terri Jones¹, Cynthia Scott-Dupree¹, Ron Harris¹, Les Shipp², Brenda Harris³

¹Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1, E-mail: csdupree@evb.uoguelph.ca; ²Agriculture and Agri-Food Canada, Greenhouse Processing Crops Research Center, Harrow, Ontario, N0R 1G0; ³Dow AgroSciences Canada Inc., Calgary, Alberta, T2J 5R7

Abstract: Currently there are no efficacious insecticides available for use against western flower thrips *Frankliniella occidentalis* Pergande, that have minimal impact on biological control agents that are used in integrated pest management programs for greenhouse cucumbers. Our research indicates that the biocide spinosad is effective against thrips and has minimal impact on *Orius insidiosus* (Say).

Key words: spinosad, western flower thrips, integrated pest management, greenhouse, biological control agents

Introduction

Western flower thrips (WFT) is an economic pest of greenhouse crops worldwide. It can cause considerable damage to cucurbits resulting in yield loss and decreased market value (Hao *et al.*, 2001). Insecticides are commonly used to control WFT on greenhouse cucumber (*Cucumis sativus* L.). However, it is developing resistance to several of the insecticides presently in use (Broadbent & Pree, 1997). In addition, some of these insecticides have adverse effects on greenhouse biological control agents (BCA) resulting in subsequent pest outbreaks (Blümel *et al.*, 1999). Therefore, there is a need to develop new insecticides with unique modes of action (MOA) that can be used in integrated pest management (IPM) programs for WFT in greenhouses without fear of negative impact on BCA. Spinosad is a new biocide, which has shown promise against WFT (Eger *et al.*, 1998). It is a naturally produced mixture of *spinosyn* A and *spinosyn* D which are produced by the actinomycete *Saccharopolyspora spinosa* under aerobic fermentation conditions. Spinosad has a unique MOA and low to moderate toxicity to common greenhouse BCA (Miles & Dutton, 2000) suggesting it could be an effective product in IPM programs on greenhouse cucumbers.

Our objectives were to: 1) determine the effectiveness of spinosad by both direct and residual to control for adult WFT on greenhouse cucumber; and 2) determine the impact of spinosad on adult greenhouse BCA.

Materials and methods

Insect Rearing

WFT were obtained from a natural colony at Agriculture and Agri-Food Canada's Greenhouse Processing Crops Research Centre (GPCRC) in Harrow, ON. They were maintained on white and yellow pom chrysanthemums for the residual contact bioassay and on greenhouse cucumber for the direct contact bioassay. WFT were held in a growth chamber for

4 weeks on their respective rearing-host plants at $27 \pm 1^\circ\text{C}$, 75% RH and 16L:8D. *Orius insidiosus* (Say) were obtained from Koppert Canada Ltd. (Scarborough, ON) and maintained on kidney bean (*Phaseolus vulgaris* L.) stems. *Ephesttia kuehniella* Zeller eggs (Beneficial Insectary, Oak Run, CA) were provided to *O. insidiosus* as a food source. *O. insidiosus* were held at $27 \pm 1^\circ\text{C}$, 75% RH and 14L:10D during rearing. *Encarsia formosa* Gahan were obtained from En-Strip® cards (Koppert Canada Ltd.) that were held at 21°C , $45 \pm 5\%$ RH, until eclosion.

Insecticide treatments

Two formulated products were evaluated: 1) spinosad (Conserve® SC Turf and Ornamental Insect Control, 120g a.i./L, Dow AgroSciences Canada Inc., Calgary, AB) at 48 (75% recommended rate =S75%) and 60 (recommended rate =SRR) ppm; and, 2) endosulfan (Thiodan® 50% WP, Hoechst Canada Inc., Regina, SK) at 500 (recommended rate =ERR) and 1500 (3 times recommended rate =E3x) ppm. Distilled water was used as the control treatment.

Direct contact bioassay

Fifteen adult WFT were exposed to 8 ml aliquots of an insecticide solution using a Potter spray tower. Treated thrips were transferred to an excised cucumber leaf, which was lying upside down on a piece of moistened filter paper and cotton in a petri dish. A screened cover was placed over the dish and it was wrapped with Parafilm®. Cages were held at $25 \pm 1^\circ\text{C}$, 75% RH and 12L:12D. This procedure was repeated for *O. insidiosus*.

Residual contact bioassay

Residual contact toxicity was determined using a leaf dip bioassay. Excised cucumber leaves were dipped into one of 4 insecticide treatments for 5 sec and left to dry in a fume hood. Plastic 20 dram vials had 2 holes cut into their sides. One hole had a cork inserted into it and the other had a piece of screening over it. A moistened filter paper disk was placed into the lid of each vial. A leaf was then placed upside down over the lid and snapped into the vial. For WFT and *O. insidiosus* trials, 15 insects were gently blown using a mouth aspirator into the vial. As a food source for the *O. insidiosus*, *E. kuehniella* eggs on a piece of moistened filter paper were suspended between the vial and the cork. Strips of parasitized whitefly were suspended in a similar manner for the *E. formosa* trials and were removed at 24 h. Cages were held at $27 \pm 1^\circ\text{C}$, 75% RH and 16L:8D.

Data collection and analysis

All cages were examined at 24 and 48 h to assess % mortality. Insects, which did not move after being probed and/or did not respond to light, were considered dead. Corrections for natural mortality were made using Abbott's formula (Abbott, 1925). Data were arcsine transformed before being subjected to analysis of variance. Means were separated by least significant difference with actual means shown in all tables. Insecticides were ranked: harmless (<25% mortality), slightly harmful (25-50% mortality), moderately harmful (51-75% mortality), and harmful (>75% mortality) according to Pietrantonio & Benedict (1999).

Results and discussion

At 24 h, spinosad was highly toxic to WFT by both direct (n=100/treatment) and residual contact (n=240/treatment [tables 1 and 2]). The thrips were significantly less susceptible to endosulfan (tables 1 and 2). This low toxicity and the fact there was little or no significant

difference between ERR and E3x, suggest that WFT has developed high levels of resistance to endosulfan.

Table 1. Direct contact toxicity of spinosad and endosulfan to adult western flower thrips and *Orius insidiosus*.

Treatment	Rate (ppm)	Average Percent Mortality \pm SEM			
		Western Flower Thrips		<i>Orius insidiosus</i>	
		24 h	48 h	24 h	48 h
ERR	500	2 \pm 1.1c	6 \pm 2.3c	33 \pm 8.8b	38 \pm 12.7a
E3x	1500	13 \pm 6.2b	14 \pm 3.0b	62 \pm 9.6a	63 \pm 8.9a
S75%	48	100 \pm 0a	100 \pm 0a	33 \pm 6.9b	47 \pm 11.1a
SRR	60	100 \pm 0a	100 \pm 0a	35 \pm 5.9b	54 \pm 3.9a
P \leq 0.05		F=630.6 df=3, 36	F=1959.0 df=3, 36	F=4.0 df=3, 32	F=1.6 df=3, 32

Values in a column followed by the same letter were found not to be significantly different.

ERR = endosulfan at recommended rate; E3x = endosulfan at 3 times recommended rate

S75% = spinosad at 75% recommended rate; SRR = spinosad at recommended rate

Table 2. Residual contact toxicity of spinosad and endosulfan to adult western flower thrips and the greenhouse biological control agents, *Orius insidiosus* and *Encarsia formosa*.

Treatment	Rate (ppm)	Average Percent Mortality \pm SEM					
		Western Flower Thrips		<i>Orius insidiosus</i>		<i>Encarsia formosa</i>	
		24 h	48 h	24 h	48 h	24 h	48 h
ERR	500	2 \pm 0.86b	15 \pm 3.2b	63 \pm 4.3b	93 \pm 2.1a	44 \pm 5.6b	86 \pm 3.7ab
E3x	1500	3 \pm 1.4b	22 \pm 3.7b	82 \pm 3.6a	98 \pm 1.2a	77 \pm 4.8a	97 \pm 2.2a
S75%	48	100 \pm 0a	100 \pm 0a	5 \pm 1.5c	14 \pm 3.9b	51 \pm 5.8b	81 \pm 5.9b
SRR	60	100 \pm 0a	100 \pm 0a	10 \pm 3.6c	23 \pm 5.6b	48 \pm 4.1b	95 \pm 2.0a
P \leq 0.05		F=1778.0 df=3, 96	F=929.1 df=3, 96	F=75.3 df=3, 52	F=153.6 df=3, 52	F=8.0 df=3, 56	F=3.8 df=3, 56

Values in a column followed by the same letter were found not to be significantly different.

ERR = endosulfan at recommended rate; E3x = endosulfan at 3 times recommended rate

S75% = spinosad at 75% recommended rate; SRR = spinosad at recommended rate

As direct contact applications both spinosad and endosulfan were classed as slightly harmful to *O. insidiosus* (n=90/treatment [table 1]). As a residual contact treatment, endosulfan was moderately harmful to harmful to *O. insidiosus* whereas, with only 5 and 10% mortality at S75 and SRR, respectively, after 24 h and 14 and 23% mortality after 48 h, spinosad was classed as harmless. *E. formosa* was more sensitive than *Orius* to both spinosad and endosulfan residues (table 2).

Development of selective pesticides is very important in a successful IPM program to ensure a safe and consumer friendly product, the success of biological control programs, and reduce the selection pressure associated with older chemicals (Blümel *et al.*, 1999). When WFT populations reach the economic threshold, spinosad can be applied at the recommended rate with minimal impact on *O. insidiosus*. Since *O. insidiosus* is more active at high

temperatures and vapor pressure deficit (Zhang & Shipp, 1998), spraying when greenhouse conditions are <25°C and 77 kPa will have the least impact. Since *E. formosa* is relatively inexpensive (3000 wasps for ca. \$23 + tx) and spinosad residues are short lived, Miles & Dutton (2000) suggest *E. formosa* can be (re) introduced safely 1 to 2 wk after application.

Spinosad's unique MOA, high toxicity to thrips, negligible toxicity to *O. insidiosus* and limited residual activity make it an ideal biocide for greenhouse IPM programs.

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The potential of Sterile Insect Technique (SIT) as one of the strategies for control of *Liriomyza trifolii* (Diptera: Agromyzidae) infesting greenhouse crops

Roy Kaspi, Michael Parrella

Department of Entomology, University of California, One Shields Avenue, Davis, CA 95616, USA, E-mail: rkaspi@ucdavis.edu

Abstract: *Liriomyza trifolii* (Burgess) is a serious pest of chrysanthemum and other greenhouse crops around the world. The objectives of this study were to determine the possibility of using the Sterile Insect Technique (SIT) against *L. trifolii* infesting greenhouse chrysanthemum. We found that a high level of sterility was achieved with a dose of 155 Gy for both sexes. The copulatory success, longevity, percent emergence and flight ability of irradiated males were similar to that of unirradiated males. The SIT experiments indicated that the release of sterile *L. trifolii* can significantly reduce the reproductive capacity of the normal leafminer population. Our study indicates that sterilization of *L. trifolii* flies is feasible and that sterile males are of high quality and competitive with normal males. Based on these data, we will continue research on the use of SIT against *L. trifolii* populations in greenhouses. We will also determine the feasibility and efficacy of the combination of the SIT with the augmentative release of the parasitoid *Diglyphus isaea* for *L. trifolii* control.

Key words: *Diglyphus isaea*, *Liriomyza trifolii*, SIT

Introduction

Liriomyza trifolii (Burgess) (Diptera: Agromyzidae) is a serious pest of chrysanthemum and other greenhouse crops around the world. Larvae feed within the leaves of the host plants and create serpentine mines (Parrella, 1987). The Sterile Insect Technique (SIT) has been successfully used against some Diptera species in field situations, and it has been most effective against relatively isolated population (e.g., on islands, etc.; Gilmore, 1989; Knipling, 1998; Krafur, 1998). Therefore, SIT against pest populations in greenhouses has a high potential for success.

Our long-term goals are to develop a novel Integrated Pest Management (IPM) program that relies on alternative methods besides the use of pesticides for *L. trifolii* control in ornamental greenhouses. In particular, our study focuses on the augmentative release of both the larval ectoparasitoid *Diglyphus isaea* and sterile males for *L. trifolii* control in greenhouses.

The objectives of this study were to determine the optimum gamma-irradiation dosage required for the sterilization of *L. trifolii*, to determine the quality (percent emergence, flight ability, longevity, and copulatory success) of irradiated males compared with that of the unirradiated (normal) males, and experimentally determine the feasibility of the SIT as a control method in small cages (one generation tests).

Material and methods

Optimum irradiation dosage required for the sterilization of L. trifolii (sterility tests)

Batches of pupae were irradiated at 70-210 Gy, in the ITEH gamma-irradiation facility, UCD (a Cesium-137 source), when they were between 1 and 2 days prior to adult emergence. Irradiated and unirradiated ('normal') flies were allowed to mate in small containers, then mating pairs were placed into individual cages ('cylindrical screened cage' - 25±0.5 cm high × 15.2 cm diameter) with a chrysanthemum plant as an oviposition host (in a 25°C greenhouse). The number of mines made by the females (during their lifetime) was recorded as a determinate of sterility, using the Kruskal-Wallis test.

Emergence percentage, flight ability & longevity

Two days before emergence, 100 pupae were placed in a petri dish, which was placed at the center of an opaque plastic cup (70 mm high, 140 mm diameter base, 116 mm diameter top) that was coated with talcum powder to prevent the flies from walking out (FAO/IAEA, 1998). The cups were covered with transparent plastic cylinders netted on top (80 mm high × 116 mm diameter) that were coated with transparent sticky glue. After all the emerging flies either flew out of the cups (and trapped by the glue) or died, we counted the adults and the unemerged pupae. Ten replicates of 100 flies from each group were evaluated. In order to compare means, t-tests (with arcsin transformation) were used.

After eclosion, irradiated (155 Gy) and normal males were kept in 1.1-liter plastic containers, at densities of 25 males per container, and had *ad libitum* access to water and honey. Dead males were removed daily until all of the flies died. To evaluate effects of male irradiation on fly longevity, nonparametric survival analysis was used.

Copulatory success

Pupae (irradiated & normal) were marked with fluorescent dye (green and pink) before irradiation (FAO/IAEA 1998). Twenty irradiated males (155 Gy), 20 normal males and 20 normal females were released into a 'mating cage' (cylindrical, 5 gallons, screened cage). Flies that were observed copulating were captured with an aspirator and their identity was determined by using a fluorescent microscope. As an index of fly size, the length of one wing was measured using an ocular micrometer. To investigate the effects of irradiation and size on probability of mating, logistic regression was used.

SIT experiments

One normal male and female were placed in a 'screened cage' (25±0.5 cm high × 15.2 cm diameter), with a chrysanthemum plant. In the 'SIT treated cages', 10 irradiated (155 Gy) males were added. After 20 days the number of mines per female was determined, using the Kruskal-Wallis test.

Results

Sterility tests

We found that high sterility level (<1.9 mine per female; or 98.1%) was achieved with a dose of 155 Gy for males ($\chi^2 = 71.4$, $P < 0.001$; table 1). Moreover, the irradiated females (155 Gy) were 100% sterile (irradiated females: $n = 16$, mean = 0, SE = 0; normal females: $n = 15$, mean = 104.7, SE = 9.8; $\chi^2 = 36.3$, $P < 0.001$).

Table 1. Number of mines per normal females that mated with irradiated or normal males.

Experiment:	Irradiated males			Normal males		
	n	Mean	SE	n	Mean	SE
Male sterility test	68	1.89	0.3	36	101.6	9.0
SIT experiment	18	7.5	3.3	27	48.15	4.4

Emergence percentage, flight ability & longevity

We found that the percent emergence and flight ability of irradiated males were similar to that of normal males (percent emergence: $t = 1.29$, $df = 18$, $P = 0.214$; table 2); (flight ability: t -test with arcsin transformation: $t = 0.01$, $df = 18$, $P = 0.99$; table 2). Moreover, the longevity of irradiated males was similar to that of normal males (nonparametric survival analysis: $\chi^2 = 1.57$, $P = 0.21$; Fig. 1).

Table 2. Effect of fly irradiation on emergence and flight ability percentage.

Experiment:	Irradiated males		Normal males	
	Mean	SE	Mean	SE
Percent emergence	76.1	2.9	81.2	2.7
Flight ability	92.4%	1.5	92.2%	1.7

Copulatory success

The copulatory success of sterile males (mean = 6.9, SE = 0.5) was not significantly different from that of the normal males (mean = 8.5, SE = 0.8) (Logistic regression; model significance: $\chi^2 = 9.4$, $P < 0.001$; irradiation: $\chi^2 = 3.3$, $P = 0.07$). However, larger individuals were more likely to copulate than were smaller ones (size: $\chi^2 = 5.9$, $P = 0.016$).

SIT experiment

The results indicated that the release of sterile *L. trifolii* can significantly reduce the reproductive capacity of the normal leafminer population (Kruskal-Wallis test: $\chi^2 = 21.6$, $P < 0.001$; table 1).

Discussion

The serpentine leafminer *L. trifolii* (Burgess) is a major worldwide economic pest of vegetable and ornamental crops (Parrella, 1987). In order to further reduce the use of pesticides to control this pest, novel approaches incorporating alternative methods of control should be developed. It may include cultural, mechanical, autocidal and biological controls. Our study shows that sterilization of *L. trifolii* flies is feasible and that sterile males are of high quality and competitive with normal males. Based on these data, we will continue research on the use of SIT against *L. trifolii* populations in greenhouses, and determine the feasibility and efficacy of the combination of the SIT with the augmentative release of the parasitoid *Diglyphus isaea* for *L. trifolii* control. We hypothesize that the combination of these two techniques is much more efficient than the use of either alone since they mutually complement each other (Knipling, 1998). If proven successful, this IPM program may be applicable in other greenhouse crops and against other greenhouse pests.

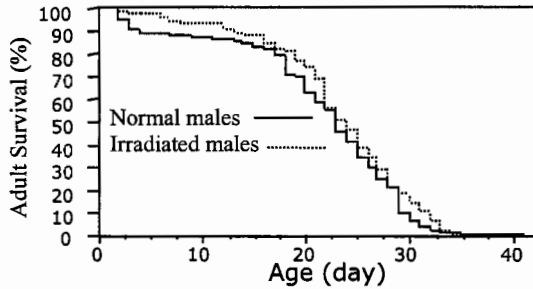


Figure 1. Survival of *L. trifolii* males. Lines show Kaplan-Meier estimates of the probability of male survival. Normal males (n = 128), Irradiated males (n = 122).

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Influence of extracts from sage (*Salvia officinalis* L.) on some biological parameters of *Tetranychus urticae* Koch. feeding on Algerian Ivy (*Hedera helix variegata* L.)

Beata Kawka, Anna Tomczyk

Department of Applied Entomology, Warsaw Agricultural University, Nowoursynowska 166
02-786 Warsaw, Poland, E-mail: Tomczyk@alpha.SGGW.waw.pl

Abstract: Extracts from fresh and dry sage leaves were used to investigate the possibility to apply them for spider mite control on ornamental plants. The tests were conducted on *Hedera helix* leaves. An effect of extracts on fecundity of *T. urticae* females as well as on their longevity was studied. The feeding index for spider mites on *Hedera helix* leaves treated with sage extracts and mortality of all developing stages were also estimated. Significant differences were found between fecundity of females feeding on leaves treated with sage extracts and water. Both extracts made of fresh and dry leaves of *Salvia* reduced by about 35-45% the total number of eggs produced by *T. urticae* females. The female longevity was reduced by about 25%. Activity of females feeding on ivy leaves was strongly affected by sage extracts. The feeding index for mites on leaves previously treated with extracts was 28-35% as compared to control leaves (treated with water). The mortality of preimaginal stages of *T. urticae* was highest on leaves previously treated with extract made of dry *Salvia* leaves, however, in the case of direct use of extracts on mites fresh leaves were more effective.

Key words: sage extracts, *Tetranychus urticae*, biology parameters, *Hedera helix variegata*

Introduction

In recent years botanical insecticides have been tested for insect and mite control. It is known that secondary plant metabolites which are present in the tissues of many herbs can affect the development of different pest populations. The most commonly used in insect and mite control are pesticides based on extracts of the neem tree (*Azadirachta indica* A. Juss). Neem extracts affect pest biology. Thus neem treatments affected moulting processes as well as fecundity in some homopterans (Schmutterer, 1990; Saxena, 1995). Neem extracts had also an influence on *T. urticae*. Fecundity of females was restricted, mortality of larvae and nymphs, as a result of egg treatment, was observed and a repellent effect was recorded (Schauer & Schmutterer, 1980). Increased mortality of adult was also noticed (Mansour & Ascher, 1995). Experiments conducted on some ornamental plants with use of neem-based Bionim showed a decrease in populations of *Frankliniella occidentalis* Pergande, *Myzus persicae* Sulzer and *Tetranychus urticae* Koch. (Gripwall, 1999). Sage extracts are known to be effective in aphid control (Achremowicz & Ciez, 1988).

Our previous experiments showed that extracts prepared from *Achillea millefolium* L. and *Taraxacum officinale* L. can reduce mite fecundity and decrease their population on cucumber (Tomczyk & Szymanska, 1995).

Material and methods

Experiments were conducted under laboratory conditions on *Hedera helix* cv. Glorie de Morengo. The spider mites also originated from that plant. Influence of extracts on spider

mites was tested on whole leaves which were placed in Munger cages and then in the chambers SANYO with temperature 23°C and air humidity 60%. The light period was 16 h and dark 8 h. The control leaves were treated with water.

Extract preparation

Small pieces of fresh or dry leaves of *Salvia* were extracted for 24 h at room temperature with 96% ethanol. Extracts were filtrated and alcohol was removed by evaporation. The pellet was dissolved in water to obtain a 1% solution. For most of tests the leaves of *Hedera* were dipped for 5 seconds in extract, dried and used for spider mites.

Influence of extracts on fecundity and longevity of females

Fertilised females of the spider mites were placed individually on ivy leaves previously treated with *Salvia* extracts or water. Forty females were used in every experimental combination. Eggs laid by females were counted every day and removed, throughout the female life. After every 4 - 5 days females were transferred to a new leaf. Total number of eggs and longevity for every female was noticed.

Estimation of feeding index

The feeding index was based on amount of faeces produced by a female during its whole life and estimated according to Hazan *et al.* (1975) and Gerson & Aronowitz (1980). The feeding index was checked on leaves treated with *Salvia* extracts or water. Forty females were used in every combination as in the experiment for fecundity estimation.

Effect of extracts on mortality of spider mites during development

On every leaf of ivy one female of *T. urticae* was placed. After 24 h the females and eggs were removed with the exception of 1 egg. 250 - 280 eggs were examined. During the next days the development of spider mites from eggs was observed and numbers of non-developing eggs and dead larvae and nymphs were noticed.

Direct effect of extracts on spider mites mortality

In this experiment spider mites were placed on ivy leaves before extract application. Twenty specimens of different mobile stages of spider mites (larvae, nymphs, females) were placed on separate ivy leaves and sprayed with extracts or water. After 24 h the killed mites were counted. The experiment was made in 30 replicates for every combination.

Results and discussion

Influence of extracts on fecundity, longevity and feeding activity of females

The data are presented in table 1.

Table 1. Female fecundity, longevity and feeding index.

Treatment	Total fecundity (no of eggs) ± SE	Longevity (days) ± SE	Feeding index ± SE
Water	81.5 ± 5.80	24.2 ± 0.70	84.7 ± 4.95
Sage fresh	53.7 ± 3.59	18.1 ± 0.57	57.5 ± 2.96
Sage dry	42.9 ± 4.65	18.5 ± 0.93	56.5 ± 1.43

Both studied biological parameters were affected by sage extracts made of fresh as well as of dry plant material. *T. urticae* females placed on ivy leaves previously treated with extracts prepared from fresh leaves of sage laid about 35% less eggs as compared to the leaves treated with water. On leaves treated with dry material the inhibition of fecundity was even higher, 45%. The longevity of females was shorter after application of both tested extracts by about 25% as compared to the leaves treated with water. The data indicate that the decrease in female fecundity after treatment with 1% sage extracts can be connected with shortened longevity as well as with a decrease in feeding activity. Similar decrease in fecundity of *T. urticae* as well as *T. cinnabarinus* females was found after application of 1% extracts prepared from *Taraxacum officinale* L, and *Achillea millefolium* L. on cucumber leaves (Tomczyk & Szymanska, 1995).

Mortality of spider mites during development

The data are presented in Fig 1.

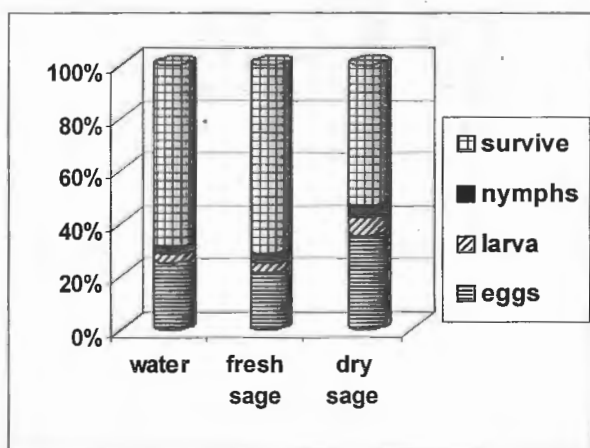


Figure 1. Mortality of different stages of *T. urticae* on Algerian ivy leaves treated and untreated with sage extracts.

It is shown that mortality of juvenile stages of *T. urticae* was highest on ivy leaves treated with extracts prepared from dry sage leaves but differences between these extracts and water were smaller than 15%. The most sensitive to sage extracts were eggs. Differences were also found in mortality of larvae, between larvae developed on leaves treated with dry material and with water. Extract made of fresh sage leaves did not affect any juvenile stage of mites more than distilled water. From the obtained data it is clear that mortality of juvenile stages of mites developing on extract treated leaves of ivy is not high. We obtained similar data after the use of extracts from *Taraxacum officinale* and *Achillea millefolium* for spider mite control on cucumber (Tomczyk & Szymanska, 1995).

Direct effect of extracts on spider mites mortality

The influence of sage extracts on the feeding of different *T. urticae* stages on *Hedera helix variegata* leaves is presented in Fig. 2

The highest toxic effect was observed after spraying feeding mites with fresh sage extracts. Significant differences were found between mortality of larvae as well as females treated with water or fresh *Salvia*. The extract made of dry *Salvia* material used directly on feeding females also increased mortality by 15%.

The obtained data indicate that extracts made of *Salvia officinalis* can significantly affect *T. urticae* biology and could be useful in spider mite control on ornamental plants.

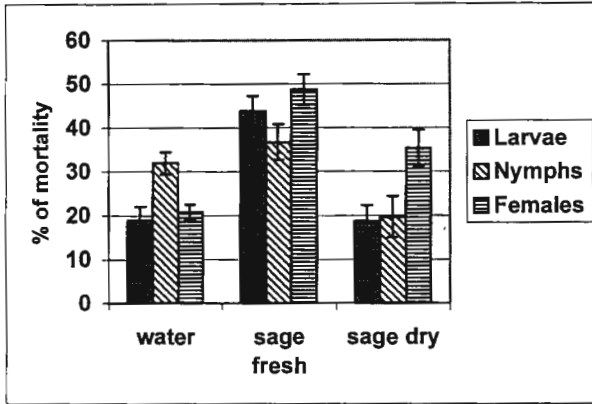


Figure 2. Toxic effect of sage extracts on *T. urticae* after direct application on spider mite feeding on ivy leaves.

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The impact of the exotic predatory mite *Neoseiulus californicus* (McGregor) on native phytoseiid species

Danuta Kropczyńska

Department of Applied Entomology, Warsaw Agricultural University, 02787 Warsaw, Nowoursynowska 166, Poland, E-mail: kropczynska@alpha.sggw.waw.pl

Abstract: A great tendency to interspecific predation on two indigenous species of phytoseiid mites was proved for *Neoseiulus californicus* in laboratory experiments. Within six weeks the predator completely displaced *Euseius finlandicus* and *Typhloctonus tiliarum* even when primary prey (*Tetranychus urticae*) was abundant.

Key words: *Neoseiulus californicus*, releases, local fauna of phytoseiid mites

Introduction

The predatory phytoseiid mite *Neoseiulus californicus* has been increasingly introduced into glasshouses to control two-spotted spider mites (*Tetranychus urticae* Koch). In Europe the predator is distributed in the Mediterranean Region. Recently it was shown that after numerous releases, *N. californicus* established in UK where it has been found in the south East and west England (Jolly, 2000). We expect that *N. californicus* will be widely used in Central Europe in nearest future both in glasshouses and in field crops. In Poland the predator was successfully applied against two-spotted spider mites on blackcurrant (Topa). Because the use of exotic species can bear some risk to the local environment, the need arises to evaluate to what extent these introductions can influence native species.

For evaluation of the consequences of *N. californicus* releases for indigenous phytoseiid species, a comparative experiment on the interaction of *N. californicus* and two predatory species *Euseius finlandicus* and *Typhloctonus tiliarum* has been conducted. Both indigenous predators are abundant on deciduous trees and bushes in Poland.

Material and methods

Neoseiulus californicus was supplied by Koppert B.V. Company, *Euseius finlandicus* and *Typhloctonus tiliarum* were collected from elm trees (*Ulmus laevis* Pall). Their colonies were maintained separately on bean plants (*Phaseolus vulgaris* L.) infested with two-spotted spider mites. The experiment was conducted on detached elm leaves. Leaves were placed on water saturated foam surrounded by wet tissue paper to prevent the mites from escaping.

Experimental arenas were kept in environmental chamber at 22-23°C, 65% RH and 16:8 h L:D photoperiod.

Phytoseiid females were chosen randomly from rearing units and placed in the arenas.

There were 5 variants in the experiment:

Test 1. 5 females of *N. californicus*

Test 2. 2 females of *N. californicus* + 3 females of *E. finlandicus*

Test 3. 5 females of *E. finlandicus*

Test 4. 2 females of *N. californicus* + 3 females of *T. tiliarum*

Test 5. 5 females of *T. tiliarum*

In all tests *T. urticae* served as a food. Bean leaves with mixed stages of prey were added to arenas every second day.

Ten replicates were run for each variant of the experiment. Arenas were checked every two days to follow changes in mite densities. The experiment lasted 6 weeks. At the end of the experiment all mites were collected and mounted for species identification.

Results and discussion

In all variants of the experiment, the curves illustrating changes in density of predators had similar trends. Two species separately developing on the leaves reached their maxima in the 5th week, with 23.2 mobile stages per arena for *N. californicus* and 21.4 for *E. finlandicus*. *T. tiliarum* had a peak with 20.2 active stages per arena in week 6. In the arenas where *N. californicus* was accompanied by one of the native species, mite densities attained the maximum starting from the 4th week of experiment (Fig. 1-2).

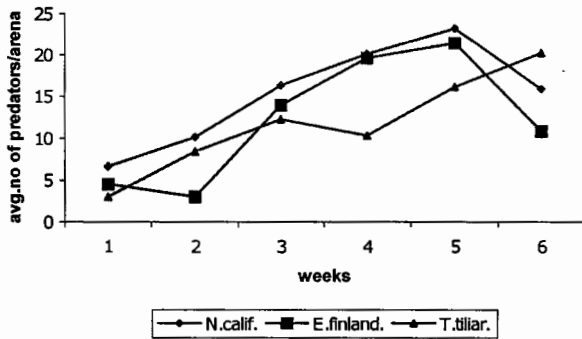


Figure 1. Density of predatory mites developing in the absence of other predatory species.

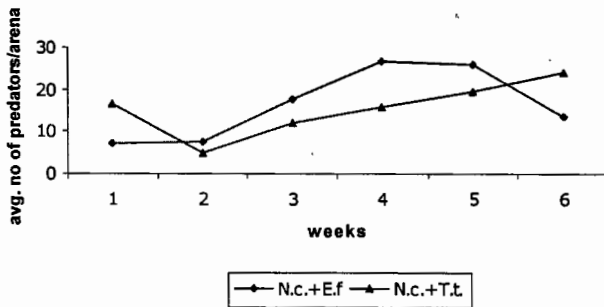


Figure 2. Density of predatory mites developing in the presence of other predatory species.

There were no significant differences in the average number of predators present on the leaves in the end of experiment in the series with single predatory species as compared to the series with two predatory species (Fig. 3).

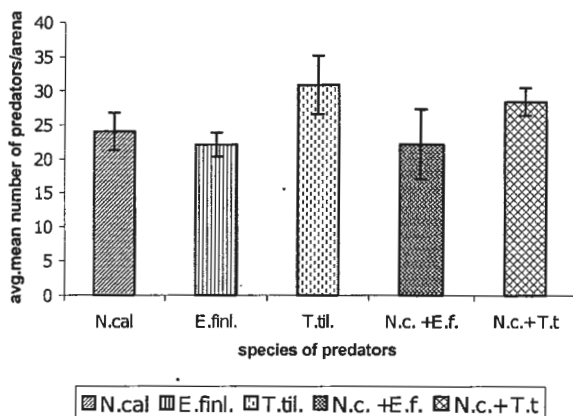


Figure 3. Mean number of predators (\pm SE) at the end of experiment.

N. californicus showed a great tendency to interspecific predation even when *T. urticae* was abundant. In variants of the experiment with *N. californicus* accompanied by *E. finlandicus* or *T. tiliarum* neither persisted to the end of experiment. A statistical analysis (one-way ANOVA) revealed no significant differences in the number of *N. californicus* on arenas without as well as with competitive predatory species. It can indicate that when its primary prey is abundant, interspecific predation does not influence rate of growth of *N. californicus* population.

The competitive displacement of one phytoseiid species by other was reported by Yao & Chant (1989), Croft & MacRae (1992), Duso & Pasqualetto (1993) and Schausberger (1998). For our experiment *E. finlandicus* was chosen as a species showing a great tendency to interspecific predation (Schausberger, 1997; Kaźmierczak, in press). It was shown that the predator could not compete with *N. californicus* when both occurred on the same leaves. The number of shrivelled dead bodies of *E. finlandicus* was the evidence of predation. In laboratory experiments *N. californicus* exhibited strong ability to compete with the two tested native phytoseiid species. If this behaviour will be observed in field experiments, it will be important to study whether frequent releases of *N. californicus* lead to changes in composition of the local phytoseiid fauna.

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An overview of biological control in ornamental greenhouses in Québec, Canada

Liette Lambert¹, Alain Cécyre², Thierry Chouffot³, Susan Johnson⁴, Andrée Roy⁵

¹Quebec Ministry of Agriculture, Fisheries and Food, 118 Lemieux, St-Remi, QC, J0L 2L0, Canada, E-mail: liette.lambert@agr.gouv.qc.ca; ²Plant Prod Quebec, 3370, Le Corbusier, Laval, QC, H7L 4S8, Canada; ³Koppert Biological Systems, 250 Principale, St-Étienne des Grès, G0X 2P0, QC, Canada; ⁴Biodôme de Montréal, 4777 Pierre de Coubertin, Montréal, QC, Canada, H1V 1B3; ⁵Quebec Ministry of Agriculture, Fisheries and Food, 1700 boul. Laval, bureau 500, 5^{ième} étage, Laval, QC, H7S 2J2, Canada

Abstract: The use of biological control in Québec ornamental greenhouses is limited by several constraints including structural problems and lack of resources. Despite these limitations, positive results have been achieved on a limited acreage in annuals, potted plants (hibiscus, poinsettia, orchids), cut flowers (roses), perennials, and interior plantscapes.

Key words: ornamental, biological control, Canada (Quebec), greenhouses, interior plantscapes

Introduction

The use of biocontrol in vegetable greenhouses in Québec is well established but its implementation in ornamental greenhouses has been slower to develop. It is being tried on a small scale by some producers with positive results and growers are becoming increasingly interested in producing ornamentals with few or no pesticides, mostly for reasons of health and well being. Moreover, several institutional greenhouses (schools, botanical gardens, zoos) use biocontrol. However, there are important constraints limiting the adoption of this technique on ornamental crops. These constraints, as well as an overview of the present state of biocontrol use in ornamentals in Quebec are covered.

Constraints

Pesticides

Compared with the United States and Europe, Canada has a limited number of registered pesticides compatible with beneficials and only one biopesticide is registered, the biofungicide, *Streptomyces griseoviridis* (Mycostop by Kemira). This lack reduces the grower's options, often forcing him or her to choose between biological control or chemical control. Pesticide residues on cuttings can seriously affect the success of a subsequent biological control program. Better communication between propagator and grower about what pesticides have been used on cuttings is needed.

Cost of biological control

Biological control can be more expensive than chemical control in ornamental production (1.5 – 4\$CDN/square metre) because the low tolerance threshold for pests often necessitates

repeated introductions of beneficials at relatively high rates. In addition, the cost of beneficials in Canada is often higher than in Europe or the United States.

Quality and availability of beneficials

Because the quality of beneficials is of ultimate importance, simple, rapid and dependable quality testing techniques for the grower are essential. A research project on this subject is underway in Canada. Because there are other factors that can affect the success of beneficials, such as ambient conditions, pesticide residues and predator interactions, it's important for the grower to be sure of the quality of the beneficials received. As the range of host plants and thus the range of pests expands, the predator availability becomes a problem. For example, there is no good predator for longtailed mealybug (*Pseudococcus longispinus*) or tarnished plant bug (*Lygus lineolaris*).

Instructions on use of beneficials

The directions for use of beneficials provided by companies could be improved. Some introductions of *Encarsia* have failed because cards were placed too high above the plants. Banal details can become important especially for beginners or when new beneficials are used. Also, pesticide-beneficial compatibility charts are often not consistent and the effect of such factors as ambient conditions on spray-introduction intervals are not always mentioned.

Training and technical support

Resources for training extension professionals and growers in greenhouse biocontrol are inadequate in Québec. There is a need for governments, schools and companies to work together to fill this gap. Instructors need to be trained and teaching materials need to be developed. Some progress has been made in this direction but more needs to be done.

Research

Most trials and technical fine tuning is done by growers in Québec due to an absence of subsidized research projects and resources for technology transfer. This increases production costs and slows implementation of biological control.

Marketing and public education

Consumers do not look for biologically protected ornamentals. However, it is possible to promote such a product for several crops. A logo identifying the product as 'biologically protected' needs to be developed to position the product on the market and to educate the clientele. Several growers have developed their own logo.

Monitoring

Monitoring techniques, and tolerance and economic thresholds are fairly inconsistent from grower to grower. Again, a lack of information and local testing are a problem. The use of indicator, trap and banker plants is starting but is still limited.

Crop overview

Annuals and hanging baskets

These short crops (2 – 4 months) are rarely severely infested. Except for fungus gnats, which infest seedlings, most pests arrive late and only create local infestations. Fungus gnat control using *Hypoaspis spp.*, introduced early, with applications of *Bacillus thuringiensis* var.

israelensis or *Steinernema* spp. if infestations develop, works very well for growers. Aphids and whiteflies arrive late in the production cycle and are usually controlled with chemicals. Localized spider mite hot spots are controlled successfully with *Phytoseiulus*. Thrips control is still dicey although *Amblyseius cucumeris* has given good control in most trials (e.g. zonal geranium, New Guinea impatiens). Application of hydrated lime below the benches is widely used to control thrips pupae and fungus gnats. Because thrips are INSV/TSWV vectors, populations are strictly controlled. The use of indicator plants such as petunias needs to be encouraged.

Trials on Draceana gave good results for thrips control with *Hypoaspis* spp., *A. cucumeris* and one introduction of *O. insidiosus*. Predators such as *Orius* and lacewings from outdoors were observed in the greenhouse and aphid and spider mite populations remained stable without deliberate introductions of predators.

Potted plants

Biocontrol has been tried in Québec on a few potted plants: hibiscus, orchids, and poinsettia. Hibiscus gave the best results despite a long list of pests: aphids, spider mites, thrips, whiteflies and even tarnished plant bug in some cases. The latter pest, for which there is no commercialised predator available, may disrupt biocontrol programs. Otherwise, results were very positive and costs were acceptable. Thrips were well controlled with slow release *A. cucumeris* and *Hypoaspis* spp. applied in the pots at the beginning of the crop. Removal of flowers also reduces thrips populations considerably. Fungus gnats and spider mites were well controlled on orchids but longtailed mealybug control was inconsistent and sometimes needed a spot spray. Pesticide residues and the presence of *Bemisia argentifolii* on poinsettia cuttings often make the control of whitefly (silverleaf and greenhouse) with *Encarsia* and *Eretmocerus* difficult and expensive. Few growers have succeeded even with preventive introductions. Recognizing *Bemisia* parasitised by *Eretmocerus* is difficult and good quality photographs would improve this situation.

Cut flowers

Few cut flowers are produced in Québec and roses remain the principal crop. Although the bent cane method of rose production has been shown to be favourable to predators, it is not popular in Québec because of marketing constraints. Thrips are the main problem. *Orius*, *Amblyseius cucumeris* and *Hypoaspis* spp. have not given adequate control. The cost of the beneficials and monitoring time are limiting.

Perennials

Few growers have succeeded in using biocontrol in commercial perennial crops. The cost can be high (3\$CDN/square metre). The diversity of crops and pests in the same greenhouse makes control difficult and low night temperatures generally are not favourable to beneficials. Predatory mites give good results. *Phytoseiulus persimilis* is the primary spider mite predator but *Amblyseius fallacis* can play a complementary role as it tends to establish on the plants. Castor bean as a banker plant for *A. degenerans* improved thrips control. *Orius insidiosus* did not give good results because of its long lifecycle and because it tends to leave the greenhouse. Use of indicator plants such as Viola for aphids, Ajuga and Filipendula for spider mites, Labiatae (Salvia, Lamium, Monarda, Eupatorium) for whitefly and castor bean and daylily for thrips was useful.

Whiteflies are controlled with preventive and continuous introductions of *Encarsia*. *Aphidoletes* did not work well for aphids (mostly *Myzus persicae*). It did not seem to establish even with supplementary lighting and releases in pails to encourage mating. *Aphidius* gave

good control except during hot days in the spring when it could not control exploding pest populations. Trials with *Harmonia* and *Hippodamia* did not work well. Where pesticides were not used, syrphid flies and the ladybeetle, *Coleomegilla maculata*, tend to migrate into the greenhouses. Otherwise, compatible or low residual spot sprays are used. Management of volunteer populations of beneficials surrounding the greenhouse could be a significant tool for improving the performance of biocontrol in the greenhouse.

Institutional greenhouses and interior plantscapes

Institutions in Québec (e.g. Biodome, Montreal Botanical Garden, horticultural schools) that have educational, research, production, collection and presentation greenhouses have been using biocontrol for years. Some private atriums have turned to biocontrol as well with excellent results. The best control is achieved on permanent plantings where plants are less stressed and beneficials establish, but many crops in production and educational greenhouses are also protected at least partially with biocontrol. Longtailed mealybug, citrus red mite (*Panonychus citri*) and thrips species other than *Frankliniella occidentalis* (e.g. *Echinothrips americanus*, *Hercinothrips femoralis*, *Selenothrips rubrocinctus*, *Heliothrips haemorrhoidalis*, *Liothrips sambuci*) and several scale species are the most problematical because of a lack of effective predators on the market. Institutions in North America are starting to network, to rear predators in-house and to exchange predators. Researchers are starting to show an interest in this sector, because the context is complex, interesting, and in close contact with the public thereby affording an important opportunity to market biocontrol to the public.

Conclusion

There is an enormous potential for the expansion of the use of biological control on ornamentals in Québec but much work needs to be done on education (consumers, growers, and professionals), training, research and technical support.

When native non-target species go indoors: a new challenge to biocontrol of whiteflies in European greenhouses

A.J.M. Loomans¹, I. Staneva^{1,2}, Y. Huang^{1,3}, G. Bukovinskić-Kiss¹, J.C. van Lenteren¹

¹Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH, Wageningen, The Netherlands, E-mail: Antoon.Loomans@Users.ENTO.WAU.NL; ²Department of Entomology, Plant Protection Institute, Kostinbrod – 2230 Bulgaria; ³Institute of Ecology, College of Life Sciences, Beijing Normal University, Beijing 100875, P.R.China

Abstract: Cabbage whitefly, *Aleyrodes proletella* (L.), has become a serious pest of various cabbage cultivars (in particular kale, Brussels sprouts, cauliflower, broccoli and savoy cabbage) in private garden complexes in The Netherlands. Since early 1999, it is causing some problems too in greenhouse grown gerbera crops (*Gerbera jamesonii*) in some parts of 'De Kring', near Rotterdam, the Netherlands. Occasionally, the strawberry whitefly, *Aleyrodes lonicerae* (L.), is found indoors as well. While evaluating non-target effects of exotic parasitoids released for the biological control of whitefly pests in greenhouses, we surveyed the parasitoid fauna of native *Aleyrodes* species outdoors and used them as test insects in our host specificity tests. An account is given on the whitefly species, their native and exotic natural enemies, prospects for biological control and non-target effects.

Key words: *Aleyrodes proletella*, *Aleyrodes lonicerae*, *Encarsia*, *Eretmocerus*, biocontrol, non-target

Introduction

Cabbage whitefly, *Aleyrodes proletella* (L.) and the strawberry whitefly *A. lonicerae* (L.) (Homoptera: Aleyrodidae) are the most common and widely spread native whitefly species in the Netherlands (Bink *et al.*, 1980). They are known from a wide range of host plants and have been considered as minor pests of vegetable crops outdoors (Martin *et al.*, 2000). Cabbage whitefly is mainly known from *Chelidonium majus* (Papaveraceae) and from cruciferous plants such as cabbage. In the 1970s it was considered an occasional pest in private gardens in fall in the south of the Netherlands (Bink *et al.*, 1980), but it was not thought of as a pest in large commercial field crops. During recent surveys in the Netherlands, however, we noticed that cabbage whitefly has become a major pest in private garden complexes across the country (Loomans *et al.*, unpubl. data). Because both species are common in cultural and natural ecosystems in and around greenhouse areas, we considered them as non-target species to study environmental effects of exotic parasitoids (*Encarsia formosa* (Gahan) and *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae)) mass-released for the control of whiteflies in greenhouses. Since early 1999, however, cabbage whitefly causes problems as well in greenhouse grown gerbera crops. Up to June 2000, about 10 nurseries were affected in parts of 'De Kring', near Rotterdam, the Netherlands. In addition, greenhouse grown cabbages, cultured at the University rearing facilities, have suffered from a major attack of cabbage whiteflies since the end of 1999. Occasionally the closely related strawberry whitefly, *A. lonicerae*, invades crops in unheated greenhouses as well, such as strawberry and brambles. Here we give an account of our surveys on the parasitoid fauna of these native whitefly species, and (conflicting) prospects for future biocontrol measures and using them in studies for non-target effects.

Field surveys

Whiteflies

Although *A. proletella* is considered as very polyphagous, mostly herbaceous plants are affected. The most common host plant is *Chelidonium majus* (Papaveraceae), but it has a marked preference for Brassicaceae (Cruciferae) and to a lesser extent for Compositae and Asteraceae (*Lactuca* spp.) (Martin *et al.*, 2000). During our surveys in private garden-complexes, large populations (>20,000 individuals per plant, >1000 per leaf), developed during late summer and fall, but in commercial field crops it was hardly found. In particular kale, Brussels sprouts, cauliflower and broccoli were infested, whereas white and red cabbage were hardly affected. The strawberry whitefly, *A. loniceriae*, on the other hand, even has a broader host range, in particular within Caprifoliaceae and Rosaceae (Martin *et al.*, 2000). During our surveys it was very common, but it was never found in large numbers outdoors, but reached pest-levels on strawberry in an unheated greenhouse in Oosterhout, the Netherlands. Eggs are laid on the abaxial side of young leaves, either in circles (*A. proletella*) or separated (*A. loniceriae*). Oviposition occurred from late March till late September. At low densities, eggs and juvenile stages are mostly found on the distal lobes of the (cabbage) leaf (*A. proletella*), but *A. loniceriae* distributes its eggs randomly on the leaf. In laboratory tests no eggs were laid on cucumber, tomato and sweet pepper, whereas on poinsettia incidentally a few larvae developed. Developmental time is 3 weeks from egg to adult at 25°C and almost 4 weeks at 21°C. Both species have 4-5 generations a year and overwinter as adult females on evergreen host plants or on shallow spots under fallen leaves (Bink *et al.*, 1980). Whereas in outdoor populations diapause is induced early fall, in greenhouse cultures this is not so.

Parasitoids

Nine parasitoid species have been reared from *A. proletella* in the Westpalaearctic Region (table 1), 6 species of which belong to the aphelinid genus *Encarsia*, 2 to the eulophid genus *Euderomphale* and there was 1 record from *Eretmocerus mundus* in Egypt. More species could be expected when intensively searched for. Most common and widely recorded species are the heteronomous hyperparasitoid, *Encarsia tricolor*, and the primary bisexual species *Encarsia inaron*. During our surveys 1999-2001, 4 native parasitoids attacked *A. proletella* (in private gardens and on *C. majus*): *E. inaron*, *E. tricolor* and *E. chelidonii*, as well as 2 exotics introduced for whitefly control in commercial greenhouses: *E. formosa* and *E. eremicus*.

Table 1. Parasitoids of cabbage whitefly, *Aleyrodes proletella* (L.) collected outdoors; ^{hh} = heteronomous hyperparasitoids, bold = introduced, *C.m.* = *C. majus*, *B.o.* = *B. oleracea*.

Species	Area	Host plants	Occurrence
<i>Encarsia davidi</i>	Egypt	<i>Lawsonia inermis</i>	Incidental
<i>Encarsia formosa</i>	Netherlands, UK	<i>C. m.</i> , <i>B. o.</i>	Incidental
<i>Encarsia inaron</i>	Westpalaearctic	<i>B. o.</i> , <i>Solanum nigrum</i>	Common
<i>Encarsia lutea</i> ^{hh}	Moldavia, Ukrain, Russia	<i>C. m</i> / <i>B. o.</i> ?	Incidental
<i>Encarsia pergandiella</i> ^{hh}	Italy	<i>Salvia splendens</i>	Incidental
<i>Encarsia tricolor</i> ^{hh}	Westpalaearctic	<i>C. m.</i> , <i>B. o.</i>	Common
<i>Eretmocerus mundus</i>	Egypt	<i>B. o.</i>	Incidental
<i>Euderomphale cerris</i>	Finland, Sweden, Rumania	<i>C. m.</i>	Regular
<i>Euderomphale chelidonii</i>	NL, Central Europe, Egypt	<i>C. m.</i> , <i>B. o.</i> , <i>S. nigrum</i>	Common

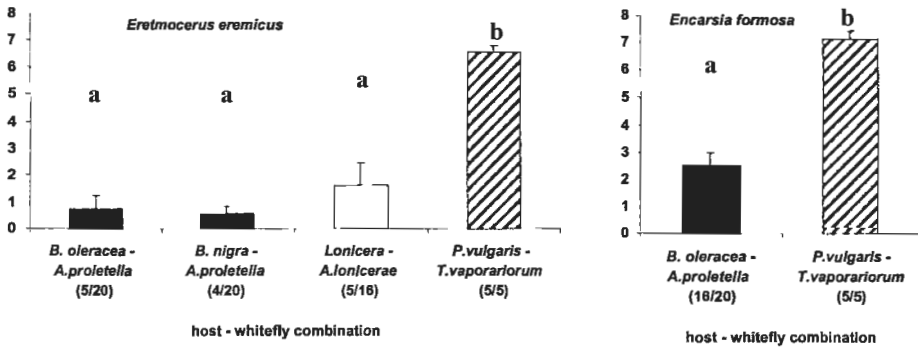


Figure 1. Parasitization of Aleyrodes species by *Eretmocerus eremicus* (left: 1♀/1 ♂, 2 days old) and *Encarsia formosa* (right: 1♀, 2 days old), when exposed for 24 hours to an abundance (>100 hosts) of larval host stages L3 (some L2-L4); *Aleyrodes proletella* on Brussels sprouts (n=20) and black mustard (n=20), *A. loniceriae* on *Lonicera periclyneum* (n=16), *T. vaporariorum* on bean (n=5) as a control; between brackets = females with offspring / total. Kruskal-Wallis test (left) and Mann-Whitney U-test (right), p<0.05.

From *A. loniceriae* nine species - 7 *Encarsia* and 2 *Euderomphale* - have been recorded in the Westpalaeartic Region, and species composition largely overlaps that known from *A. proletella*. In the Netherlands, *E. tricolor* and *E. chelidonii* were the most common parasitoids. *E. formosa* was reared from material collected from blackberry, strawberry and *Lonicera periclymenum* outdoors in greenhouse areas. In Norway *A. loniceriae* pupae collected on *Lonicera* outdoors contained *E. formosa* as well (Trandem, pers. coll.). In an unheated greenhouse on strawberry (Oosterhout, mentioned above), *E. tricolor* was the dominant parasitoid species, reaching parasitization levels up to 40% in fall 1999. *E. formosa*, originating from a commercial tomato greenhouse 500 meters south, had parasitized 1-3%.

Biological control

Van Vianen & Van Lenteren (1986) showed that *E. formosa* becomes larger and has more ovarioles when reared on large host species such as *A. proletella* and *A. loniceriae*, compared to those reared on *T. vaporariorum*. Attempts, however, to control pest outbreaks of *Aleyrodes* species in greenhouses, by using commercially available *E. formosa* and *E. eremicus*, have been unsuccessful so far: parasitization of *A. loniceriae* on strawberry (Trandem, pers. comm.) and of *A. proletella* in cabbage (Loomans, pers. comm.) was very low or even absent. A recent outbreak of cabbage whitefly on *Gerbera jamesonii* in some 10 nurseries in the Netherlands, could not be controlled by mass-releasing *E. formosa* and *E. eremicus* either: samples taken from 2 nurseries, showed no signs of parasitization. Although *E. formosa* is able to control greenhouse whitefly even in a complex system like gerbera, host plant species, different cultivars with different surface structures and plant architecture, are likely to effect biocontrol results. During our survey of native whiteflies, parasitization of *Aleyrodes* by native species sometimes reached high levels, and occasionally exotic parasitoids, primarily *E. formosa*, were recovered, but at low incidences. For *E. eremicus* only a single specimen was found.

When testing exotic parasitoids for host specificity in the laboratory, *E. formosa* readily accepted and parasitized *A. proletella*, whereas *E. eremicus* was highly inefficient attacking both cabbage and strawberry whitefly (Fig. 1). Direct observations on *E. eremicus* showed that, when searching for hosts on infested leaves, females rarely oviposited and sometimes even died within minutes after exposure, because of extensive coagulation of wax and honeydew to its legs. On heavily infested plants, honeydew and in particular wax particles produced by the adult whiteflies, limited successful parasitization by these species to a large extent. In a bankerplant system to facilitate the control of greenhouse whitefly, *T. vaporariorum*, *E. formosa* could sustain on *A. proletella* on the bankerplants (*Lapsana communis*: Van der Linden & Van der Staaij, 2001; *Brassica oleracea* cv. 'Acephala': Láska & Zelenková, 1988), whereas *E. eremicus*, failed to establish.

Native *Aleyrodes* species can be approached differently, depending on the researcher's point of view: an invasive pest on one side, non-target species and even beneficial on the other. When considered a non-target, it is desirable that when an exotic parasitoid released for the control of exotic pests, is unsuccessful in its attacks on a native species outdoors, being a pest or not. When considered a serious candidate for setting up a banker plant system for the biological control of whiteflies in unsuitable vegetable crops like cucumber, tomato or sweet pepper (Van der Linden & Van der Staaij, 2001; Láska & Zelenková, 1988), one has to be careful adopting this system to any crop. Therefore, efforts to control pest outbreaks of these and other whitefly species should be dealt with accordingly. When these native whitefly species go indoors, and parasitoids that are already used for whitefly control fail to control pest outbreaks of these species, native natural enemy species should be the first in line for further evaluation. In this way we can combine a maximum efficiency where it is needed and minimize the risk of newly combined exotics from going out and establish on native whiteflies in the release area.

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Heteronomous hyperparasitoids for biological control of whiteflies: balancing benefits and risks

A.J.M. Loomans¹, Y. Huang², G. Bukovinszkiné-Kiss¹, J.C. van Lenteren¹

¹Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH, Wageningen, The Netherlands, E-mail: Antoon.Loomans@Users.ENTO.WAU.NL; ²Institute of Ecology, College of Life Sciences, Beijing Normal University, Beijing 100875, P.R.China

Abstract: Secondary parasitoids, or hyperparasitoids, have been excluded from releases in biocontrol programs as a standard procedure. Heteronomous hyperparasitoids – parasitoid females that lay eggs in larvae of the same or of other primary parasitoid species to produce male offspring – , however, have been included in biocontrol programs for whitefly pests, but with variable results. The impact these hyperparasitic strategies may have on biological control applications as well as on non-target organisms is evaluated. We suggest that the introduction of any hyperparasitoid, which parasitizes other beneficials, is acceptable or permitted only under strict conditions.

Key words: intraguild predation, hyperparasitism, *Encarsia*, biocontrol, non-target effects

Introduction

Every year billions of exotic beneficial organisms are produced and released seasonally or inundatively to control various exotic and native insect pests in European greenhouses. Evaluation criteria previously used by biocontrol researchers for the selection of natural enemies as biological control agents for insect pests, were generally based on biotic, behavioural and abiotic characteristics, which enhanced the impact of the natural enemy over the target pest. In addition, trying to prevent negative effects with the introduction and release of such agents, particularly polyphagy was not appreciated, and undesirable organisms (such as hyperparasitic species) were largely excluded from being introduced (Van Lenteren & Loomans, 2000). Selectivity procedures differed, however, with respect to the target pest (weeds or insects) and to the type of release program which is used: classical, seasonal inoculative or inundative release programs. In weed control, host specificity always has been the key pre-release-criterion for evaluating the potential efficacy and risks (for beneficial plants) of exotic natural enemies, whereas in insect biocontrol, this was not so and often multiple agents have been released. In insect control, facultative autoparasitoids, or heteronomous hyperparasitoids, have been part of biocontrol programs of various insects pests, including whiteflies (Williams, 1996), although it is not always clear whether they enhance or disrupt existing biocontrol programs (Rosenheim *et al.*, 1995). The urgency of the economic problem always has been the overriding factor for the import and release of exotic natural enemies, but recently there is an increasing concern, that besides the benefits of these introductions for pest control, also potential ecological risks could be involved for non-target organisms and native natural enemies (Lynch & Thomas, 2000; Van Lenteren & Loomans, 2000). Here we address the impact that inoculative releases of heteronomous hyperparasitoids may have on the natural environment. We also discuss how introduction of these species may affect the local parasitoid and host population and under what conditions the introduction of such a hyperparasitoid, which parasitizes other beneficials, would be acceptable or permitted.

Intraguild interactions and biological control

Whereas multiple natural enemy interactions are common in natural ecosystems, they are much less in managed systems, in particular those in temperate areas. Greenhouse biological control mostly consists of direct, unidirectional trophic interactions (one-to-one relation of plant-pest-beneficial), which has proven to be rather effective. Various parasitoid (and predator) species have been screened for the control of whitefly pests, *Trialeurodes vaporariorum*, and more recently the tobacco whitefly, *Bemisia tabaci*. Primary exotic parasitoid species such as *Encarsia formosa* and *Eretmocerus eremicus*, the native *E. mundus* and the predatory mirid, *Macrolophus caliginosus*, currently are the main mass-produced natural enemies for the control of whitefly pests in Europe. The recent approach to release multiple natural enemy species for the control of each greenhouse pest has brought biocontrol measures more into a multi-trophic context. Intraguild interactions (IGP) have, as a result, become prevalent in a number of greenhouse crops and greenhouse areas. Introduction of additional guilds of natural enemies is not necessarily a risk to the extraguild (pest). Exotic beneficials interact and compete with native species that may spontaneously enter the greenhouse and vice versa with various outcome. In large areas of greenhouse grown crops, especially those in the Mediterranean region, where open windows and structures allow an easy entrance of those natural enemies prevalent in the natural surroundings and exotics to leave, IGP-interactions are quite common: natural control of pests has been and still is largely depending on natural enemies invading from the natural or managed environment during summer. Hyperparasitoids, obligate or facultative, are part of these natural invasions as well, partly to the benefit, partly to the risk of biocontrol measures (Rosenheim *et al.*, 1998; Brodeur & Rosenheim, 2000).

Heteronomous hyperparasitoids in whitefly control

In heteronomous hyperparasitoids females develop as primary parasitoids on whitefly hosts ('primary hosts'), whereas males develop as obligate hyperparasitoids, either on females of the same species ('conspecific') or on other ('heterospecific') primary parasitoids ('secondary hosts') (Hunter & Kelly, 1998). Facultative autoparasitoids act therefore at different levels in a multi-trophic system, as a competitor on the 3rd and as an IGP on the 4th level and even one single individual female can act as both. Hyperparasitoid species have been introduced for whitefly control either intentionally or by accident, but most of the exotic *Encarsia* species which established in Europe are hyperparasitic (Loomans & Van Lenteren, 1999).

Two hyperparasitic species have gained most attention as potential biocontrol agents for whitefly pests: *Encarsia tricolor* and *Encarsia pergandiella*. *E. tricolor*, which is native to the Westplaeartic region, has been studied as a biocontrol agent of the greenhouse whitefly, *T. vaporariorum*, in Italy, France and Spain. Although trials had promising prospects, it has never been used for seasonal inculative or inundative releases. Sometimes naturally occurring populations invade greenhouses, thereby rarely disrupting biological control by *E. formosa* (Del Bene & Landi, 1991). *E. pergandiella* was introduced in Italy in 1980 and meanwhile has established and spread throughout the Mediterreanean region (Italy, France, Spain, Tunesia, Macaronesia (Loomans & Van Lenteren, 1999)). Invasions by *E. pergandiella* occasionally contribute to biocontrol of greenhouse whitefly (Giorgini & Viggiani, 2000), directly or by ovipositional probing, during the early season or in regions which are climatically unfavourable for *E. formosa*, but it is reported as detrimental to a successful biocontrol program by primary parasitoids (Gabarra *et al.*, 1999). In spite of biological and behavioural features, which could put the balance in favour of heteronomous hyperparasitoids, mass-production has been a constraint for seasonal inoculative or inundative releases.

Biological control and non-target effects

When exotic primary parasitoids, such as *E. formosa*, are used for whitefly control indoors, invading hyperparasitoids preferentially allocate male eggs in the prevalent biocontrol agent and thus may severely hamper biological control by the primary. Gabarra *et al.* (1999) and Del Bene & Landi (1991) showed this for *T. vaporariorum* and *E. formosa* when invaded by the exotic *E. pergandiella* and native *E. tricolor* respectively. Simultaneous release of a primary (*E. formosa*) and a hyperparasitoid (*E. pergandiella*) provided better control than single releases in the case of *Bemisia tabaci* (Heinz & Nelson, 1996), but with *T. vaporariorum* as a host, *E. formosa* soon got extinct (Giorgini & Viggiani, 2000). Mills & Gutierrez (1996) in their theoretical model suggested that the introduction of a facultative autoparasitoid into a system where a whitefly pest is already controlled by a primary parasitoid, might disrupt biological control. Holt & Polis (1997) in their theoretical analyses on IGP interactions, suggest that as a general criterion for coexistence in IGP systems, the intermediate species (i.e. primary parasitoid) should be superior at exploitative competition for the shared resource, whereas the top species (the secondary parasitoid) should gain significantly from its consumption of the intermediate species. Empirical data on *E. formosa* parasitizing *T. vaporariorum*, mentioned above, collected under optimal conditions by Gabarra *et al.* (1999) and under suboptimal conditions (climate: Giorgini & Viggiani (2000); whitefly host species: Heinz & Nelson, 1996), seem to support this model, indicating a decreased risk for the extraguild (disruption of biocontrol) resp. substituted or additive effects of a multiple release, including a primary and (facultative) secondary parasitoid.

Although a lot is known on the impact of exotic species and host range inside the greenhouse, their impact and potential hazard for the native fauna still is a puzzle. Over 50% of the trials and tests on inundative methods actually show population level effects on non-targets (Lynch & Thomas, 2000), but most agents are considered 'safe' because they do not persist or disperse very far. When such agents, like hyperparasitoids, with a relative wide host range, are released and establish in natural ecosystems, there is no way back.

Balancing benefits and risks

Hyperparasitic species may play an important role in the biological control of whiteflies, but the direction of their impact, whether positive or negative, is still unpredictable. Relying on a system of natural control for greenhouse whiteflies by an exotic or native hyperparasitic species for a seasonal crop, is insecure as well: natural control agents often are coming in too late to be efficacious, but still early enough to disrupt already effective biological control measures. Future studies should include a better understanding of their ecology in managed as well as natural ecosystems: effects of the primary host (whitefly species; mixed infestations; relative densities), of secondary hosts (suitable developmental stage; vulnerability window) or combinations of both (absolute and relative host densities) for the exotic hyperparasitoid species involved. Understanding of their natural ecology is very poor, but is necessary to understand the basis for the transience or persistence of any non-target effects, and the conditions that will prohibit exotic agents to establish permanently. For parasitoids it covers also issues such as host specificity, dispersal, searching efficiency for a non-target and overwintering abilities. Modelling hyperparasitic-interactions (e.g. Holt & Polis, 1997 as a starter), including niche (climate, habitat, host) differentiation and density effects, could bridge our search for maximal biocontrol efforts and minimal non-target effects.

Except for risks to non-targets, heteronomous hyperparasitoids may also be of benefit to biological control releases: native (or established) hyperparasitoids may counteract primary

parasitoids that leave the greenhouse in which they were released. In this way environmental risks of primary exotics leaving the greenhouse can be diminished, thus lowering the probability getting established outdoors. As long as their role in managed and in natural ecosystems is not clear, we should be cautious with any intentional introduction of heteronomous hyperparasitoids, which should be done only as a last resort, after the supply of primary parasitoids, native or exotic, is exhausted.

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Evaluating environmental risks of biological control introductions: how to select safe natural enemies?

A.J.M. Loomans¹, J.C. van Lenteren¹, F. Bigler², G. Burgio³, H.M.T. Hokkanen⁴, M.B. Thomas⁵
¹Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH, Wageningen, the Netherlands, E-mail: Antoon.Loomans@Users.ENTO.WAU.NL; ²Swiss Federal Research Station for Agroecology and Agriculture, Zürich, Switzerland; ³Institute of Entomology "G. Grandi", University of Bologna, Italy; ⁴Department of Applied Zoology, University of Helsinki, Finland; ⁵CABI Bioscience, Silwood Park, Ascot, UK

Abstract: Over the past 30 years biological control of greenhouse pests has become a key component of sustainable horticulture in the world. In Europe, more than 100 natural enemy species have been imported and released for biological control of greenhouse pests and billions of exotic beneficials are produced, distributed and released seasonally or inundatively. Although no clear direct adverse effects have been found up till now, the potential non-target effects of these releases have been little emphasised. In this paper we summarise the current state of affairs with respect to selection procedures for importing, mass-rearing and releasing (new) exotic natural enemies. These will be based on protocols for risk assessment that are being developed within the EU funded project "Evaluating Environmental Risks of Biological Control Introductions into Europe" [ERBIC].

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

Introduction

Biological control of insects - the use of natural enemies to reduce pest numbers - in greenhouse systems, has been applied for more than 30 years with great success. During the past decades its use has increased considerably as it offers a sustainable, economical and environmentally attractive alternative for chemical pest control (Albajes *et al.*, 1999). In biological control, locally occurring natural enemies are used or exotic species are imported to control introduced and / or native insect outbreaks. Until now, introductions of hundreds of species of insect natural enemies are not known to have led to ecological problems when the procedures for selection, importation and release were carefully applied (Van Lenteren, 2001). In contrast with these beneficial introductions, many intended and unintended introductions of plants and phytophagous animals have resulted in very negative effects on the environment.

Currently, many countries have an interest in promoting biological control, which has led to a steep increase in both the number of natural enemy imports and the numbers released during the past decades. On the other hand there is a growing policy to regulate biocontrol, which makes the import and release of exotic species more and more difficult. Not only is there concern about the direct effects biocontrol might have on the native fauna through attacking native hosts or competition with native parasitoids or predator species, but also through indirect effects such as changes in the habitat. Within an OECD working group, guidelines are being developed for harmonised information requirements for the import and release of invertebrate biological control agents used in augmentative biological control (Van Lenteren *et al.*, 2002). In the course of 2002/2003 OECD will publish the full guidance document (Van Lenteren *et al.*, in prep.). In this paper, major strategies and new methodologies of research are indicated, which will lead to selecting efficacious, yet environmentally safe, exotic natural enemies.

Risk assessment

Risk assessment procedures for biological control agents are normally characterized by questions concerning four issues (Van Lenteren *et al.*, 2002): human health, characterization and identification of biocontrol agent, efficacy and environmental risks. Here we only address the last issue in detail. Risk of adverse environmental effects of release of a biocontrol agent is defined as 'hazard x probability'. Hazard in biological control is considered as any direct and indirect adverse effects on non-target organisms and the ecosystem. With probability, the likelihood is indicated that an adverse effect will be found (e.g. reduction in numbers of a non-target organism). In biological control the likelihood that an adverse effect will be found is often a matter of space (dispersal) and time (survival and establishment). Normally, for a risk evaluation, one will identify the hazards, and determine the probabilities that hazards will materialize. When more hazards are expected to occur we consider worst case scenarios with accumulation of risks (e.g. attack of other natural enemies, attack of non-target and threatened species, and ecosystem effects by the newly introduced natural enemy).

Risk evaluation protocols

As a starting point for development of a risk evaluation for biological control agents, we use the system as proposed by Hickson *et al.* (2000) for environmental risk management in New Zealand. In this system, four groups of risks are considered related to the release of exotic biological control agents: establishment, dispersal, direct and indirect non-target effects. In order to calculate the level of risk, the likelihood and the magnitude of adverse effects are estimated according their matrix of magnitude (minimal <<>> massive) x likelihood (very unlikely <<>> very likely). The European Union funded 'ERBIC' research project is still working on a system where the calculation of risk is done in a numerical way, but before proposing this system a large number of cases will have to be evaluated.

Any risk assessment protocol will start with a review of the available literature for taxonomical, biological and behavioural characteristics of the biological control agent with respect to its efficacy towards the target as well its non-target effects (Lynch & Thomas, 2000). Except for empirical data collection, modelling the risks of biological control for transient or permanent impacts, related to non-target organisms can complete a risk evaluation, but this will be addressed elsewhere. The four areas an empirical risk evaluation needs to consider are:

1. Determining direct effects

Host specificity: The central point in any evaluation program of exotics will be testing for host-specificity. Within the ERBIC research project, we worked out a sequential test, which is presented by Van Lenteren *et al.* (this volume) and which will be published in greater detail later this year. Non-target species are selected according to their (1) phylogenetic relationship with the target, (2) occurrence in the same micro-habitat and prone to attack, and (3) status as endangered species. The target (pest) species is the control. In the sequential procedure, biological (preferred stage) and behavioural (encounter and attack rates over time) traits of the exotic candidate will be checked, in the laboratory and (semi-)field:

1. *Petri dish non-choice black box test*, to determine attack on the non-target organism in the appropriate stage; the activity (searching or not) of the natural enemy should be verified at the start of the testing period.
2. *Petri dish non-choice behavioural test*, to determine the consistency in attacks to the non-target and a possible increase in *acceptance* due to increasing oviposition/predation pressure. Risks for direct effects can be relatively small (when the non-target is attacked

only at the end of the observation period) to considerable (when attacked for a constant fixed percentage).

3. *Petri dish choice behavioural test*, to determine host *preference*, eventual shifts in preference and a possible increasing attack pressure of usually not attacked hosts, when the target species is present, but no longer available.
4. *Large cage choice test*, to determine attacks on the non-target with the target species present in a *semi-natural situation*, i.e. large cages, on their natural host plants. Non-target species that are easily attacked on their host plants pose a very high risk for direct effects.
5. *Field test*, to determine attacks on the non-target when the target species is present in a *natural situation*. This test can only be done on site if the biological control agent cannot establish (see #2) in a target area.

Testing for competition and intraguild predation: At first literature can provide information whether intraguild predation, competition and displacement effects have already been indicated for a specific biological control agent or related natural enemy species. In particular biological and behavioural traits (primary vs. hyperparasitism; searching efficiency for a non-target) of the natural enemy can indicate if effects can be expected. Then proceed with investigations on a case by case basis, by listing qualitative and quantitative effects, estimating effects (positive, neutral or negative) and subsequently draw conclusions concerning risk. Potential for hybridization with indigenous strains or biotypes should be addressed, and an estimation of the likelihood of hybridization between the natural enemy with indigenous strains or biotypes of the same or very closely related natural enemy species.

Testing for herbivory: Effects of the natural enemy on plants should be provided if the agent is potentially a facultative herbivore. Literature records can indicate whether effects on the target crop and non-target plants are indicated for specific or related natural enemy species, or it can be concluded from the biology of the natural enemy if effects are expected. Then investigate case by case and list qualitative / quantitative effects and draw conclusions concerning any risk.

2. Potential for establishment

In case of import of natural enemies for biological control of pests in greenhouses or other controlled environments in temperate climates, it is important to know if the agent can survive and establish outside the greenhouse. If an agent cannot establish, the environmental assessment can be less extensive. Literature data may suffice, but it may be necessary to carry out laboratory and semi-field tests to prove non-establishment in the target area. Key questions are addressed with respect to abiotic factors (whether climates match between the area of origin and the area of release) and biotic factors (availability of non-target species suitable for reproduction, temporal and/or spatial matching of non-target organisms and biocontrol agent, diapause capabilities, winter survival) as well as combined biotic and abiotic factors (whether other resources are available for survival and reproduction).

3. Potential for dispersal

The potential of the dispersal ability of a biological control agent is important to determine the probability of temporal and spatial encounter between the agent and non-target species. This would be based on the mechanism of dispersal, life-span of the agent and the local climate and habitat conditions in the area of release. If the agent does not disperse actively or passively for more than 10 meters per season, no further studies or information are needed. If the agent does not establish, but does disperse, dispersal experiments can be done in the target area. Make an inventory of non-target species over time, space and habitat. Transect studies

on dispersal speed (distance over time) and numbers dispersing (numbers over time) are performed under normal climate and habitat conditions. An alternative approach could be, not to count natural enemy numbers dispersed, but number of hosts attacked. Check attack of non-target hosts in various habitats but also offer target insect on target host plant ('trap plants') in these habitats to check for presence of biological control agent, because then one can conclude there is a very low risk for non-target effect if they only have attacked the target. If the agent can establish, do similar experiments in country of origin to estimate dispersal capabilities. Provide any information on the possibility for secondary dispersal e.g. mechanical dispersal.

4. Determining indirect effects

Any known or potential indirect effects on individual species and/or ecosystem should be addressed. Indirect effects that act through the target organisms (e.g. lower numbers of native natural enemies as a result of reduction of target pest) are generally accepted, and not considered negative. From the indirect effects via non-target organisms on population and community level, each direct effect on non-target is expected to result in a multitude of indirect effects, and these can be positive, neutral or negative. Applicants will have to provide existing information, and/or will have to estimate these indirect effects. If the exotic biological control agent is expected to attack non-target species in high numbers, the direct and indirect effects will be considered too serious, and establishment too risky.

Conclusion

Frequency, number and timing of releases, the rate of reproduction, dispersal capacity and non-target host location capability all determine the basic risk of releasing a certain organism. However, any risk assessment process also has to address risk management, including risk mitigation and risk reduction, as well as the benefits, including a comparative performance of pest management methods, particularly based on environmental aspects.

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Biological control in France in greenhouse vegetables and ornamentals

Jean-Charles Maisonneuve

Ministry of Agriculture, DRAF SRPV BRETAGNE, 14, rue du Colonel Berthaud, 29283 Brest cedex, France, E-mail: Jean-Charles.Maisonneuve@agriculture.gouv.fr

Abstract: Biological control has been used in France, on vegetables since 1980, and on ornamentals since 1988; the result is acceptable because the number of hectares (ha) reached in 2001 1752 for vegetables and 50 ha for ornamentals. This year, about forty-five different species of beneficial were introduced on more than twenty-five different crop, thus showing that it is possible to develop this technique on a large scale. The efficiency of introduced beneficials is increased by using banker-plants on approximately 95 ha; it will certainly be possible to decrease the unit cost in this way.

Key words: biological control, France, areas, pests, beneficials, vegetables, ornamentals

Introduction

From 1980, biological control (BC) has been used in France in greenhouses, reaching 1750 hectares of vegetables and 50 of ornamentals. This alternative method of crop protection is mainly used on four vegetable crops, three cut flower crops and more than ten species of potted plants. As of this year, BC is used in public city greenhouses.

In this survey, an area is considered using BC when one introduction of beneficials was made once. It is possible for a lot of area in some parts of France, because the arrival of many natural beneficials was sufficient to protect the crop with no introduced beneficial and no chemical: this is the case for few areas in the South, and we consider them as BC.

Preoccupied by the quality of vegetables and environmental considerations, the Ministère de l'Agriculture et de la Pêche (MAP) surveys each year the areas using alternative systems of crop protection, including BC. The results obtained also show the realities and effectiveness of BC in different parts of France.

Materials and methods

More than hundred questionnaires were sent to each vegetable and ornamental producer organisations, technicians and scientists working on BC, as well as all companies selling beneficials.

The areas given are the physical areas of greenhouses (in hectares) 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. It is different in ornamentals, particularly for potted plants: the areas given are the areas of the pots just before sale. Therefore, it is possible to have, for example, 4000 m² of Begonia using BC, under 1000 m² of greenhouse.

Results and discussion

It was possible to evaluate the first areas using BC, in vegetables, in 1979 (Fig. 1), but from that date to 1982, the use was only experimental. The increase in area began in 1983, with a continuous development until 1990. The arrival in France of *Frankliniella occidentalis* and *Bemisia tabaci*, reduced the role of BC, until 1995. After this year, the growers used new beneficials, experimented with new strategies and wanted to develop a good quality for vegetables. All these reasons explain the new development of BC, with 1752 ha in 2001.

It is different for ornamentals where BC was introduced in 1988, and where development started in 1994 (Fig. 2). At present, 50 ha applied BC on various crops:

- cut flowers;
- potted plants;
- arboretum under greenhouses;
- bedding plants for cities;

A lot of difficulties slacken the development, because these crops ask a lot of technicity to succeed in a good crop protection; it is then necessary to develop the education of growers, workers and advisors.

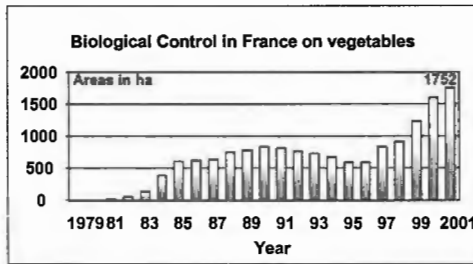


Figure 1. Biological control in France on vegetables.

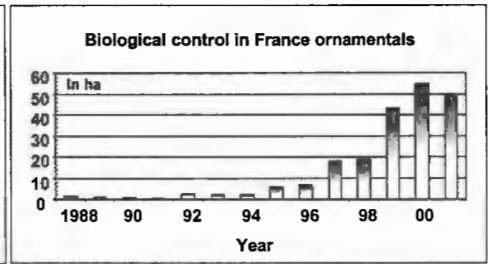


Figure 2. Biological control in France ornamentals.

Crops

BC is applied on five major crops (97%) in vegetables:

Tomato	: 1175 ha
Cucumber	: 219 ha
Strawberry	: 189 ha
Pepper	: 83 ha
Eggplant	: 47 ha

The other crops are: herbs, french bean, zucchini, raspberry etc. Even if tomato is the main crop, it is possible to consider that BC is also effective on a lot of other vegetables.

On ornamentals, the inquiry shows three cut flowers (rose, gerbera and alstroemeria) and a lot of potted plants (Kalanchoe, Pelargonium, Cyclamen, Chrysanthemum, Gerbera, Impatiens, Poinsettia, Begonia and Hibiscus). To this list can be added all the plants protected in arboretum and greenhouses producing bedding plants.

Beneficials

Twenty years ago, when BC began to be developed, only two beneficials were sold to the growers. Today, there are forty-five different species available to be used against pests (table 1). With this progress, it is possible to implement BC on various numbers of crops. Nevertheless, the main pests in vegetables are whiteflies and aphids, and in ornamentals thrips and aphids.

Table 1. Beneficials used in France in 2001.

	Vegetables	Ornamentals	Cities:bed.pl.	Arboretum
<i>Adalia bipunctata</i>				X
<i>Amblyseius californicus</i>	X	X	X	X
<i>Aphidius colemani</i>	X	X	X	X
<i>Amblyseius cucumeris</i>	X	X	X	X
<i>Amblyseius degenerans</i>	X	X		X
<i>Anagyrus fusciventris</i>				X
<i>Aphelinus abdominalis</i>	X		X	
<i>Aphidius ervi</i>	X	X	X	
<i>Aphidoletes aphidimyza</i>		X	X	X
<i>Bacillus thuringiensis</i>	X		X	X
<i>Chilochorus nigrinus</i>				X
<i>Chrysoperla carnea</i>			X	
<i>Chrysoperla kolthoffi</i>	X			X
<i>Chrysoperla lucasina</i>	X			X
<i>Coccinella septempunctata</i>			X	X
<i>Cryptolaemus montrouzieri</i>			X	X
<i>Dacnusa sibirica</i>	X	X		
<i>Delphastus pusillus</i>			X	
<i>Diglyphus isaea</i>	X	X		
<i>Eretmocerus eremicus</i>	X	X	X	X
<i>Encarsia citrina</i>				X
<i>Encarsia formosa</i>	X	X	X	X
<i>Feltiella acarisuga</i>	X	X		
<i>Fusarium antagonist (FO 47)</i>	X			
<i>Harmonia axyridis</i>			X	X
<i>Hypoaspis miles</i>		X	X	X
<i>Heterorhabditis bacteriophora</i>	X			
<i>Heterorhabditis megidis</i>	X			
<i>Leptomastix abnormis</i>			X	
<i>Leptomastix dactylopii</i>			X	X
<i>Leptomastix eponae</i>				X
<i>Macrolophus caliginosus</i>	X		X	X
<i>Metaphycus helvolus</i>				X
<i>Microterys flavus</i>			X	
<i>Orius insidiosus</i>	X		X	X
<i>Orius laevigatus</i>	X			
<i>Phasmarhabditis</i>				X
<i>Phytoseiulus persimilis</i>	X	X	X	X
<i>Podisus maculiventris</i>	X			
<i>Rhizobius lophantae</i>				X
<i>Steinernema feltiae</i>		X	X	
<i>Trichogramma brassicae</i>	X			
<i>Trichogramma evanescens</i>	X			
<i>Typhlodromus pyri</i>				X
<i>Verticillium lecanii</i>			X	

Banker plants

In order to increase the efficiency of BC, growers use a lot of banker plants in their greenhouses, on different crops. It is important to note that advisory services promote this solution in a lot of places and the results are interesting:

- on vegetables, barley, tobacco and eleusine (*Eleusine coracana*) are grown with Tomato, Cucumber, Egg-plant, Melon, Strawberry, Zucchini and herbs, on 87 ha.
- on ornamentals, barley, tobacco, eleusine and castor-bean (*Ricinus communis* L. cv. Impala), are grown with Rose, Kalanchoe, Pelargonium, Gerbera, Alstroemeria, Begonia, Cyclamen, Chrysanthemum and Hibiscus, on 8.5 ha.

When banker plants are introduced in a crop, they show a lot of advantages:

- decrease the cost of BC ;
- increase the efficiency of introduced beneficials;
- allow an early release of beneficials;
- permanent mini-rearing unit in the crop;

It is possible to compare banker-plants with slow release bags of mites sold by companies to control thrips.

Development of BC in France is a non-reversible process, despite many difficulties in ornamentals, the evolution of the surface areas prove it. Growers are convinced about the use of beneficials; nevertheless it is necessary that the cost stay at the same level as chemical protection. In that way, the use of banker-plants can help this approach.

Acknowledgements

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Biological control French greenhouse ornamentals

Jean-Charles Maisonneuve

Ministry of Agriculture, DRAF SRPV BRETAGNE, 14, rue du Colonel Berthaud, 29283 Brest cedex, France, E-mail: Jean-Charles.MAISONNEUVE@agriculture.gouv.fr

Introduction

From 1988, biological control (BC) has been developed in France in greenhouse ornamentals, reaching 52 hectares of these crops in 2001. Ten years ago, it was difficult to imagine this kind of crop protection. However, a constant increase of this area in that period has been observed.

Many crops can be protected by this alternative way of plant protection:

- cut flowers
- potted plants
- bedding plants
- cities greenhouses

So, these examples of application of BC on very different crops show that is possible to protect many crops and not only tomato or cucumber, like in the past, with an acceptable cost.

The main idea about this development can be thus summarised:

- in 2002 the beneficials sold are able to protect a large scale of ornamental crops.

General evolution

An inquiry, realised in France in 1988, showed the first data about BC on ornamentals, with about one hectare. At that time, it was the fact of Pelargonium, bedding plants and some violets. Later, the organisation of some conferences in France on BC (L.R.Wardlow in 1993) and the creation of an experimental network (ASTREDHOR), stimulated the development of BC everywhere in France.

For these reasons, the areas reached 52 hectares last year (Fig. 1); otherwise, this way of crop protection asks a lot of technicity to be effective:

Different explanations allow to understand this development:

- education and training courses for workers, growers, advisors and teachers
- the efficiency of BC is at least equivalent to chemical control
- the cost is, at the most, equivalent to chemical control (slightly superior the first year)
- it is possible to work all day long in the greenhouses
- the workers prefer to work in a greenhouse with BC
- no problem of destruction of empties
- whole respect of regulations and registration about phytosanitary products

The French Ministère de l'Agriculture et de la Pêche (MAP), Service de la Protection des Végétaux (SPV [Plant Protection Service]) wants to know each year the areas using

alternative means of crop protection, and try to promote this alternative way of plant protection, which is effective and respect all the environment.

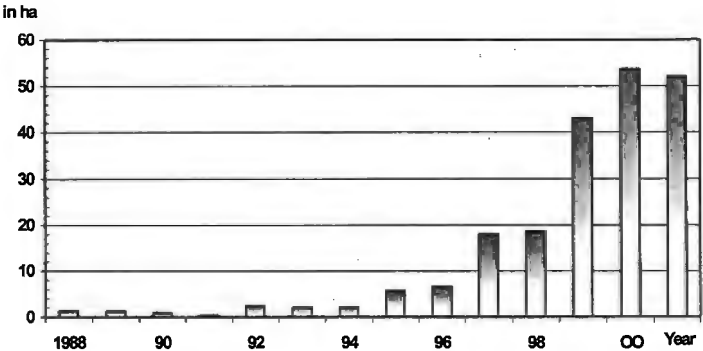


Figure 1. Biological control in French ornamentals.

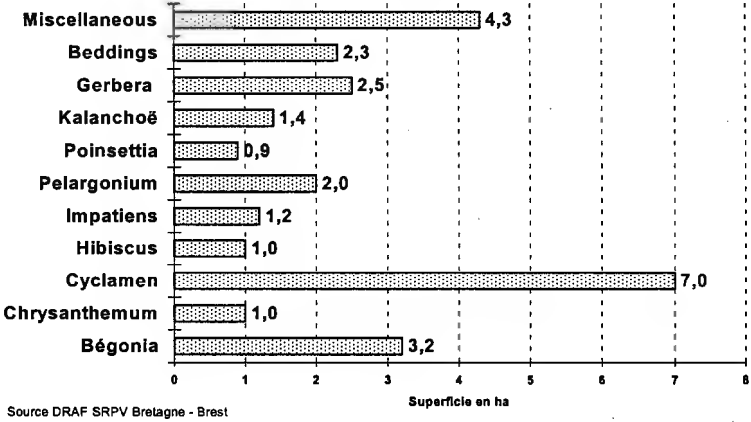


Figure 2. Biological control in France in 2001. Potted plants in greenhouses.

The crops

The 52 hectares of areas using BC are divided in the following way:

- cut flowers : 12.6 ha
- potted plants : 26.8 ha
- cities greenhouses : 12.6 ha

For cut flowers, the main crops are in 2001:

- Alstroemeria : 3.75 ha
- Rose : 5.60 ha
- Gerbera : 1.80 ha
- Violets : 0.60 ha
- Freesia : 0.10 ha
- Lilium : 0.10 ha
- Chrysanthemum : 0.10 ha
- Miscellaneous : 0.55 ha

Three years ago, there were only three main crops (Alstroemeria, Rose and Gerbera) showing how progress has taken place.

For potted plants, there were also a lot of crops very different which are recapitulated in Fig. 2; more than eleven crops are able to be protected by beneficials, showing thus their effectiveness on a large scale of crops, even the relatively small areas.

Different cities use beneficials to protect their greenhouses of bedding plants, as well as their protected botanical gardens. More than twelve cities develop BC, for example: Paris, Lyon, Nice, Angers, Caen, Nantes, Orléans, Blois, Toulouse, Besançon, etc.

It is important to observe the exemplary action of the city of Caen, which has developed a nursery of beneficials from twenty years: *Coccinella septempunctata*, *Adalia bipunctata*, *Cryptolaemus montrouzieri*, *Leptomastix dactylopii* and *Phytoseiulus persimilis*. They are used in the greenhouses of the city, released on some outside flowerbeds or distributed to the inhabitants.

Sometimes BC is used on open fields, with inoculative release. For example, to protect Eucalyptus for cut leaves against a psyllid *Ctenarytaina eucalyptii*, *Psyllaephagus pilosus* has been used. This beneficial has been released in the south-east and in the west of France in 1997. It acclimated under good conditions and protected more than 200 ha from one release point with few adults. Thus, it is possible to say that BC can be used outdoor with good results.

The beneficials

In table 1, the extreme diversity of the 41 different beneficials clearly appears. Without this large scale, it would not be possible to organise BC in so different crops. The results are acceptable for the growers and city gardeners. The growers are able to protect their crops for a comparable cost with chemicals, and the city gardeners obtain a good level of protection in substitution of chemicals. It is necessary to understand that the citizens want a decreasing of the used of phytosanitary products.

Today, these 41 beneficials, used in France in 2001, give an alternative answer, proving that it is possible to change of system of crop protection.

Table 1. Beneficials used in France in 2001 on ornamentals.

	Ornamentals greenhouse	Ornamen- tals Open fields	Cities Green- houses	Cities Bed flowers	Botanical gardens
<i>Adalia bipunctata</i>				X	
<i>Amblyseius californicus</i>	X	X	X	X	
<i>Aphidius colemani</i>		X	X		X
<i>Amblyseius cucumeris</i>	X		X		X
<i>Amblyseius degenerans</i>	X				X
<i>Anagyrus fusciventris</i>					X
<i>Aphelinus abdominalis</i>			X		
<i>Aphidius ervi</i>	X		X		
<i>Aphidoletes aphidimyza</i>	X	X	X	X	X
<i>Bacillus thuringiensis</i>			X	X	
<i>Chilochorus nigritus</i>					X
<i>Chrysoperla carnea</i>			X		
<i>Chrysoperla kolthoffi</i>					X
<i>Chrysoperla lucasina</i>	X	X			X
<i>Coccinella septempunctata</i>			X	X	X
<i>Cryptolaemus montrouzieri</i>		X	X	X	X
<i>Dacnusa sibirica</i>	X				
<i>Diglyphus isaea</i>	X				
<i>Eretmocerus eremicus</i>	X		X		X
<i>Encarsia citrina</i>					X
<i>Encarsia formosa</i>	X		X		X
<i>Feltiella acarisuga</i>	X	X			
<i>Harmonia axyridis</i>		X	X		X
<i>Hypoaspis miles</i>	X		X	X	
<i>Heterorabditis bacteriophora</i>	X				
<i>Heterorabditis megidis</i>	X				
<i>Leptomastix abnormis</i>			X		
<i>Leptomastix dactylopii</i>			X		X
<i>Leptomastix eponae</i>					X
<i>Macrolophus caliginosus</i>			X		X
<i>Metaphycus helvolus</i>					X
<i>Microterys flavus</i>			X		
<i>Orius insidiosus</i>			X		X
<i>Phasmarhadditis</i>				X	
<i>Phytoseiulus persimilis</i>	X		X		X
<i>Podisus maculiventris</i>	X				
<i>Psyllaephagus pilosus</i>		X			
<i>Rhizobius lophantae</i>					X
<i>Steinernema feltiae</i>	X		X		
<i>Typhlodromus pyri</i>				X	
<i>Verticillium lecanii</i>			X		
TOTAL = 41	17	8	23	9	21

The banker plants

In order to increase the efficiency of BC, growers use a lot of banker plants in their greenhouses, on different crop (table 2). It is important to note that advisor services promote this solution in a lot of place; results are interesting.

- in ornamentals, barley, tobacco, eleusine and castor-bean (*Ricinus communis* L. cv. Impala) are grown with Rose, Kalanchoe, Pelargonium, Gerbera, Alstroemeria, Begonia, Cyclamen, Chrysanthemum and Hibiscus, on 8.5 ha.

When banker plants are introduced in a crop, they show a lot of advantages:

- decrease the cost of BC
- increase the efficiency of introduced beneficials
- allow an early release of beneficials
- permanent mini rearing unit in the crop
- attract the wild beneficials

It is possible to compare these banker plants with the little bags of mites sent by companies to control thrips.

Table 2. Use of banker plants in French ornamentals in 2001 (in ha).

Banker plants Crop	Barley	Eleusine	Tobacco	Ricin	Total
Gerbera	0.21	0.228			0.438
Alstroemeria	0.2	0.2	1	1	2.4
Rose	0.032	0.0275			0.0595
Kalanchoe	0.405				0.405
Begonia	0.465	0.555			1.02
Cyclamen	1.467	1.055			2.522
Chrysanthemum	0.45	0.1			0.55
Hibiscus	0.1	0.1			0.2
Pelargonium		0.37			0.37
Mini rose tree	0.07	0.07			0.14
Bedding plant	0.07	0.23			0.3
TOTAL	3.469	2.936	1	1	8.40

These banker plants, to be easily used by the grower, must have a lot of quality:

- easy to cultivate
- different from the crop to protect
- if possible have a reduced size
- the pests and diseases must be different from the ones in the commercial crop

The first results showed a lot of advantages, and when the grower well understood the system, then it is possible to speak of sustainable biological control. Nevertheless, it is necessary to have advisors well educated on this system.

Conclusion

With 52 hectares of ornamentals in greenhouses using BC, the advisory services succeed a lot to develop this difficult alternative way of crop protection. More than forty beneficials are used and a lot of banker plants, but it is not often easy to use it all together, because there are also a large number of pests.

Nevertheless, there are no crops impossible to protect with BC, even partly; for these reason a lot of research are still necessary to increase the feasibility of BC on ornamentals.

Acknowledgements

To all advisory services and supplying companies for their help and data to realise this inquiry.

Biological control of aphids in early strawberries. Importance of *Chrysoperla kolthoffi* in greenhouses

Christine Marrec¹, Franck Lolivier², Géraldine Le Corre², Jean-Charles Maisonneuve¹

¹Ministry of Agriculture, DRAF SRPV BRETAGNE, 14, rue du Colonel Berthaud, 29283 Brest cedex, France, E-mail: Jean-Charles.Maisonneuve@agriculture.gouv.fr; ²FE.RE.DEC, 14, rue du Colonel Berthaud, 29283 Brest cedex, France

Abstract: The Ministry of Agriculture in Brittany (North-western France) has been studying IPM on strawberries with green lacewings since 1993. For one year, *Chrysoperla kolthoffi* has been studied on the early crop in greenhouses. Adult releases of *C. kolthoffi* were tested for the first time. *C. kolthoffi* eggs and larvae were observed in the crop for ten weeks after adult release. This indicates that green lacewing can establish in the greenhouse.

Key words: *Chrysoperla carnea*, *C. kolthoffi*, biological control, strawberry, aphid

Introduction

Aphids are major pests on early strawberries in Brittany. Many species appear on this crop including *Chaetosiphon fragaefolii*, *Acyrtosiphon rogersii*, *Macrosiphum euphorbiae*, *Aphis gossypii* and *Aulacorthum solani*.

The Ministry of Agriculture (MAP) has been studying IPM on strawberries with green lacewing since 1993. Six species of *Chrysoperla* occur in Europe comprising the *Chrysoperla carnea* complex (Thierry *et al.*, 1992). Results of biological control against aphids were interesting in late strawberries outdoors with *Chrysoperla lucasina* larvae (Maisonneuve & Marrec, 1999).

C. kolthoffi has been studied in early strawberries in greenhouses for one year. This second species predominates in the north of France (Thierry *et al.*, 1994). Greenhouse strawberry growing in Brittany has increased significantly during the past three or four years. Tabletop production is mainly used. Lacewing larvae are not adapted for this kind of crop because they often fall on the floor and then can not climb up the plant again. Releases of lacewing adults were therefore tested in this experiment.

Materials and methods

Mass rearing

C. kolthoffi was reared in the same manner as *C. lucasina*. The rearing procedure was described by Maisonneuve & Marrec (1999). The mass rearing unit is effective, and the process has been used by other laboratories: Agriphyto (France), National Institute of Horticulture (France) and Biodome de Montréal (Canada).

Strawberry crop

A heated and illuminated greenhouse of 3,500 m² was used. Peat grow bags were suspended from the ground at about 1.5 m from the floor, on gutters.

The crop was planted in the beginning of December. Plant density was 10 plants/m². The aphid population was high before the beginning of trial so the producer decided to apply endosulfan in week 2.

750 *C. kolthoffi* mature adults were released two weeks later, ready to lay eggs. They were fed a mixture of pollen, honey and yeast once a week.

12 yellow sticky traps suspended at the top of plants monitored the presence of adults in the greenhouse.

Beneficial organisms and pests were monitored weekly on 56 plants. The number of eggs, larvae and adults of *Chrysoperla* and the density of aphids were noted. During the trial, in week 9, the greenhouse was separated into 333 areas defined by structural posts (12 squares of 9.6 m² per greenhouse section). Three plants per square were observed to evaluate the number of predator eggs occurring in the greenhouse.

Results and discussion

Sticky traps

No lacewing adults were observed during the test on the plants or moving between plants. However, their presence on sticky traps showed that they were present in the greenhouse for nearly eight weeks (table 1).

Table 1. Number of green lacewing monitored on sticky traps each week.

	Yellow sticky trap								Blue sticky trap							
	5	6	7	8	10	11	12	13	5	6	7	8	10	11	12	13
Row																
3	4	3	0	0	+1	0	0	0	+3	+1	+2	0	0	0	0	0
7	10	1	+1	0	0	0	0	0	+4	+1	+1	0	3	0	0	0
12	3	0	+1	0	0	0	0	0	+5	0	0	+1	0	0	0	0
15	13	0	+2	0	0	0	0	0	+3	+2	0	0	0	0	0	0
20	2	0	0	+1	0	0	0	0	+5	0	0	0	+1	0	0	0
24	1	+2	0	0	0	0	+3	0	+7	0	0	0	0	0	0	0

Biological control of aphids with Chrysoperla kolthoffi

Aphid population

Aphid density remained very low during the trial. At the end of the test, the aphid population increased but only on one row, which did not effect production because the harvest was nearly finished.

Population dynamics of Chrysoperla kolthoffi

Eggs were observed for two months. The laying began three weeks after adults were released. The aphid population increased when eggs density was low (week 15 and 16) (Fig. 2).

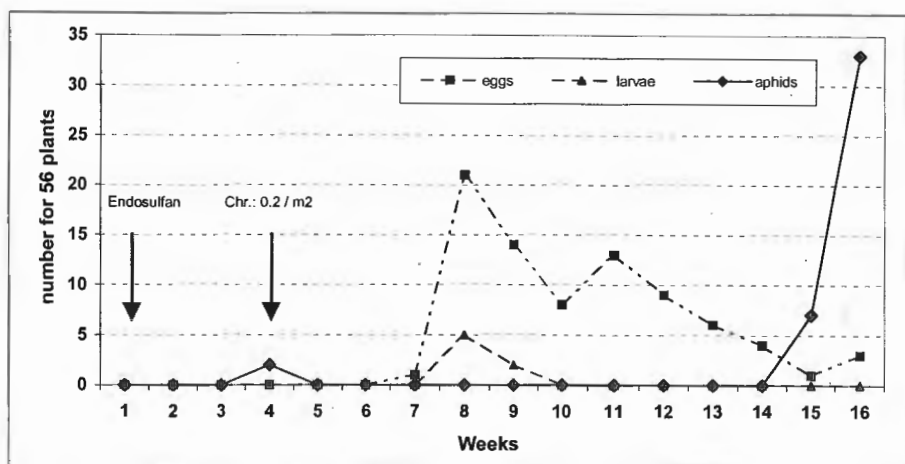


Figure 2. Population dynamics of *C. kolthoffi* (eggs and larvae) and aphids.

Percent egg laying per plant

The results showed that *Chrysoperla kolthoffi* laid eggs in 42% of the squares defined by structural posts. Egg-laying was not homogeneous in the glasshouse: 51% occurred on the sunny side and 34% on the shady side. The row where the aphid population increased was on the shady side.

Conclusion

The presence of *Chrysoperla kolthoffi* eggs and larvae for 10 weeks after release of adults in the strawberry crop indicated that it can establish in the greenhouse. However, because of the chemical spray applied at the beginning of the trial, it is impossible to conclude that *C. kolthoffi* was effective in protecting the crop from aphids.

New trials will be necessary to evaluate the capacity of this new species of lacewing in IPM.

Acknowledgements

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Regulations are necessary for biological control agents

Peter G. Mason¹, Ulrich Kuhlmann²

¹Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, 960 Carling Avenue, Ottawa, Ontario K1A 0C6 Canada, E-mail: masonp@em.agr.ca; ²CABI Bioscience Centre Switzerland, Rue des Grillons 1, 2800 Delémont, Switzerland

Abstract: Biological control is a keystone of pest management in greenhouse environments. Its continued success is linked to implementation of regulatory oversight that will govern which species can be used. Regulations are needed to safeguard biodiversity and protect biological control as a pest management tool. Host range and risk assessments will prevent the use of generalist species some of which have had significant impacts on biodiversity, thus negatively affecting the reputation of biological control. Harmonized regulations will facilitate commercial producers of biological control agents by minimizing costs to develop new agents. Participation of interested parties in developing regulations and in determining the agency responsible for oversight is imperative.

Key words: regulations, biological control, invasive species, biodiversity

Introduction

Biological control has become a cornerstone of pest management in many parts of the world. In the greenhouse industry, producers around the world began adopting biological control strategies in the late 1970's due largely to pesticide resistance in major pests such as greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, and twospotted spider mite, *Tetranychus urticae* (Koch) (van Lenteren & van Woets, 1988). Since then there has been an increasing reliance on the use of natural enemies in greenhouse environments. For example, in Canada, in 2000, about 15 pest species were managed by approximately 30 biological control agents (Floate *et al.*, 2002) and worldwide 125 species are commercially available (van Lenteren 2000). Augmentative biological control will continue to grow due to the demand from farmers moving away from the use of chemical pesticides (Waage, 2000). The future of biological control in greenhouses looks bright. However, debate is increasing on the need for stricter regulations of biological control agents, including augmentative agents which are the keystone for biological control in greenhouse environments.

Factors that have brought the need for regulation of biological control agents into the limelight include trade globalization and increased awareness of biodiversity. The increased globalization of trade has resulted in an astounding increase in the numbers of invasive species establishing in new habitats. It is estimated that invasive alien species are responsible for annual losses of US\$55-248 billion to worldwide agriculture (Bright, 1999). In the United States alone 79 exotic species were responsible for US\$97 billion in damage from 1906-1991 (Pimental *et al.*, 2000). More difficult to assess are the damaging costs to the environment through habitat loss or species extirpation or extinction caused by invasive species (Parker & Gill, 2002). Biological control is an important strategy for combating invasive alien species

and has enjoyed the status of environmental friendliness for more than 100 years. During the last decade as science and society have become increasingly aware of biodiversity and its importance to human well-being a darker side of biological control, particularly in island environments, has emerged (Howarth, 1991; Simberloff, 1992). This side of biological control, non-target/unintended impacts, has stimulated much debate (Schick *et al.*, 1996; Follett & Duan, 2000; Wajnberg *et al.*, 2001). Some, such as Strong & Pemberton (2001) have concluded that at least in the United States biological control regulation is archaic and “In the absence of reform rational as well as irrational opposition to biological control will grow. Only sensible reform will maintain public support for this powerful tool.” There is growing consensus that **all** deliberate introductions of non-indigenous species should be subject to impact risk assessment (Wittenberg & Cock, 2001). Further, regulations for biological control agents “... are needed to provide clear guidance as to what introduction can be made legally and to define procedures to resolve any conflicts of interest that may arise.” (Van Driesche & Bellows, 1996). Although it is clear that regulations for biological control agents are necessary for the preservation of biodiversity they are also necessary for the protection of biological control as a pest management strategy.

Preservation of biodiversity

Biodiversity is “all hereditary based variation at all levels of organization, from the genes within a single local population or species composing all or part of a local community, and finally to the communities themselves, that compose the living parts of the multifarious ecosystems of the world.” (Wilson, 1997). Biodiversity is important because it is essential to human survival (Raeburn, 1995; Lovejoy, 1997). Agricultural crops provide the clearest demonstration of this. For example, two species of perennial corn are known from Mexico, one, discovered only in the mid 1970's, had the same number of chromosomes as domestic corn. It is easier to transfer traits from species with the same number of chromosomes, making the long term goal of developing perennial corn and the short-term goal of developing disease resistance achievable (Lovejoy, 1997). In all, eight centres of diversity, representing the origins of the world's major crops, have been identified and are all loosely clustered around the equator (Raeburn, 1995). The cost of preserving these biodiversity centres is far less than the benefits that can be realized. A single seed sample containing resistance to Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), could be worth at least US\$100 million a year to American farmers (Raeburn, 1995).

The importance of arthropods in biodiversity is not well understood. This is not surprising since only a fraction of the world's species have even been described and taxonomists have determined that the number of extant species is even greater than previously thought (Huber *et al.*, 2002). However, we do know that the evolutionary struggle between insects and the plants they feed on, which produce chemical and biochemical defenses, is a source of a wide range of molecules important for medicine, e.g. salicylic acid (= aspirin) (Lovejoy, 1997).

Throughout the world the environmental community has recognized that invasive alien species pose particular threats to conserving biodiversity (Waage, 2000). World trade has been recognized as the primary driver of environmental decline due to invasive alien species (Bright, 1999). Deliberate introductions of alien species for biological control of invasive alien pest species has resulted in substantial savings in crop loss and pesticide use, however, a few biological control introductions have gone awry (Wittenberg & Cock, 2001) and it is these that have pushed the lobby for increased regulations. In Hawaii, the impact on biodiversity of rogue biological control agents has been well-documented by Howarth (1991),

Wong *et al.* (1991), Duan *et al.* (1996), Duan & Messing (2000) and others. In eastern North America, the tachinid fly *Comptosia concinnata* (Meigen), introduced against 13 pest species from 1906-1986, was found to cause 36, 70, and 81% mortality of the saturniid moths, *Hemileuca maia maia* (Drury), *Callosamia promethea* (Drury), and *Hyalophora cercropia* (L.), respectively (Boettner *et al.*, 2000). Publication of this paper resulted in headlines in scientific periodicals such as “Silk moth deaths show perils of biocontrol” (Jensen, 2000), “Fly may be depleting U.S. giant silk moths” (Milius, 2000), and “Safety data crucial for biological control agents” (Pemberton & Strong, 2000). The *C. concinnata* example illustrates the unintended non-target impacts of a generalist biological control agent not only on biodiversity but on biological control as a pest management strategy.

Protection of biological control as a pest management strategy

The continued use of arthropods for biological control of pests in greenhouses is essential to the survival of that industry. Regulation of these biological control agents is imperative otherwise, as Strong & Pemberton (2001) suggest, opposition will continue to grow and public support will be lost. The end result could be the elimination of biological control as a pest management strategy.

There is consensus that generalist predators and parasitoids have the greatest impact on biodiversity and these should not be approved as biological control agents. Regulations, with strict host-specificity requirements would prevent generalist predators and parasitoids from being approved for release. Mistakes, such as introduction of *Coccinella septempunctata* L. and *Harmonia axyridis* (Pallas) would be avoided. Both species are not only generalist predators but they are aggressive competitors and have contributed to the decline of native lady beetle species (Wheeler & Hoebeke, 1995, Gillespie *et al.*, 2002). Both species have been a lightning rod for conservationists interested in preserving biodiversity and *H. axyridis* has received lots of bad press because of its congregating habits and the perception that it will attack humans. For example, the headline ‘Asian invader is no lady, and won’t hesitate to bite’ (Rogers, 2001). Sadly, because both *C. septempunctata* and *H. axyridis* were introduced as biological control agents the image of biological control has been tarnished. This negative image can lead to biological programs involving exotic species being stopped or not initiated because of perceived non-target effects or a bureaucratic and severe regulatory system (Messing, 2000). A substantial number of augmentative biological control agents are alien to the habitats where they are released (van Lenteren, 2000; Waage, 2000) and through regulation these would be subject to increased host-specificity scrutiny. This will require some rethinking by the commercial biocontrol industry.

The decision to introduce non-native biological control agents rather than developing native species is usually associated with the availability of an existing ‘product’ and the costs associated with developing new agents rather than efficacy and safety (Waage, 2000). Regulations will certainly have an impact on this business strategy, particularly when generalist species are involved. Development of local strains of the same or a related species would be encouraged. However, local populations should be used only as source material for laboratory cultures not as a convenient supply. In North America, the convergent ladybird beetle, *Hippodamia convergens* Guerin, is collected from overwintering aggregations and shipped directly to buyers (Gillespie *et al.*, 2002). This is questionable because the practice has potential to reduce local *H. convergens* populations (i.e. affect biodiversity) and as Gillespie *et al.* (2002) point out wild populations may contain parasitoids which can inadvertently be sent to the recipient and reduce the efficacy of the biological control agent. Such practices do not favour biological control and must be stopped. Further, regulations

would encourage a better understanding of natural enemy-host systems and will lead to use of more biological control agents that show greater specificity, increasing successes.

A disadvantage of regulating biological control agents would be the increased costs and time lag to develop new biological control agents. While producers of biological control agents must invest more initially to develop new agents, these costs are likely to be passed to growers who buy biological control agents and ultimately to consumers who want to purchase 'pesticide-free' products. There is also the risk that a few producers of biological control agents will dominate the industry and the 'little guy' will be eliminated.

It is important that augmentative biological control is not oversold, that is, recommended when unnecessary or when not appropriate (Williams & Leppla, 1992). A spinoff benefit of regulating biological control agents will be the increased difficulty for unscrupulous individuals to sell products that are ineffective or inappropriate. While examples of this are not documented most people involved in biological control are aware of instances where this has happened. Another potential benefit would be greater protection of intellectual property. Thus, regulations would enhance reputable biocontrol agent manufacturers and sellers.

Regulating biological control agents

Worldwide, for FAO members the International Plant Protection Convention (IPPC) provides guidance for "securing common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control" (FAO, 1999). A 'Code of Conduct for the Import and Release of Exotic Biological Control Agents' (FAO, 1996) serves as a framework for regional and national plant protection organizations to develop guidelines/regulations that are appropriate for their jurisdiction. Regional organizations such as the European Plant Protection Organization (EPPO) and the North American Plant Protection Organization (NAPPO) have developed standards that outline information required for a submission to obtain a permit for release of exotic biological control agents. On a country basis, present regulation of invertebrate biological control agents is highly variable. Some countries have no laws while others, e.g. Australia and New Zealand, regulate biological control agents under separate acts. Initiatives are underway by countries, either alone or as groups, to develop regulations for biological control agents. To facilitate the commercial biocontrol industry it will be necessary for these regulations to require a single data set that is accepted by all participating jurisdictions. Such harmonized regulations would minimize costs for developing new agents.

A critical component of regulation is risk assessment which is often interpreted as meaning the greater the specificity of a biological control agent, the less the risk for non-target impacts. However, for arthropod biological control agents, specificity testing has lagged behind that for weed biological control agents because the concerns for non-target impacts on invertebrates has not been as great (Waage, 2000). Protocols used for assessing host range of weed biological control agents are well-defined but these are not necessarily appropriate for entomophagous biological control agents (Sands, 1998; Barratt *et al.*, 1999; Mason *et al.*, 1999; Kuhlmann *et al.*, 2000) and there is much still to be done. Thus, parties with an interest in biological control must participate in developing appropriate protocols.

The key to ensuring that augmentative biological control agents are appropriately scrutinized will be the agency (or agencies) in each country that oversees regulation. Depending on the agency charged with this responsibility, requirements and risk assessments could be based on models used for pesticides (as is the case for microbial agents) or even infectious diseases. More appropriate models are those already in place for regulating classical biological control agents of weeds and arthropods. These are based on ecological

theory and assessments are done by scientific experts. In North America, current turnaround from the time of petition submission until approval/rejection of the agent for release is less than one year.

Conclusions

Regulation of augmentative biological control agents is inevitable. International, 'harmonized' standards are already being developed and these should minimize the investment necessary to obtain approval for use (registration) in the global marketplace. The challenge to the greenhouse industry will be to ensure that the process is efficient and that the appropriate agencies have regulatory oversight. Hence, participation in the process to develop these regulations is in the best interests of researchers, manufacturers of biological control agents and greenhouse producers.

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Mating disruption of cabbage loopers (*Trichoplusia ni*, Lepidoptera: Noctuidae) and the response of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) to host pheromone in pepper greenhouses

R.R. McGregor¹, D.R. Gillespie², D.M.J. Quiring², M.R.J. Foisy²

¹Department of Biology, Douglas College, P.O. Box 2503, New Westminster, B.C., V3L 5B2, Canada, E-mail: mcgregorr@groupwise.douglas.bc.ca; ²Pacific Agri-Food Research Centre, Agriculture & Agri-Food Canada, P.O. Box 1000, Agassiz, B.C., V0M 1A0, Canada

Abstract: Matings of cabbage looper females were substantially reduced in sweet pepper greenhouses treated with the principal component of cabbage looper pheromone ((Z)-7-dodecen-1-ol acetate) compared to control greenhouses. In laboratory bioassays, *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) females displayed longer searching and residence times on pheromone-treated pepper leaves than on control leaves. In one greenhouse release, *T. brassicae* parasitized fewer eggs on artificial egg patches in a pheromone-treated greenhouse than in a control greenhouse. In a second greenhouse experiment, no difference occurred in the level of parasitism between pheromone-treated and control greenhouses.

Introduction

The cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae), is the major lepidopterous pest of greenhouse-grown vegetables in British Columbia (B.C.), Canada. Pheromone-based mating disruption of cabbage loopers has been shown to be effective for managing cabbage loopers in field crops (Mitchell *et al.*, 1997), but this method has not previously been attempted in vegetable greenhouses.

The sex-attractant pheromones of Lepidoptera are used as host-location cues by *Trichogramma* wasps (Noldus *et al.*, 1991a, 1991b; McGregor & Henderson, 1998). Female *Trichogramma* undergo an arrestment response and are "retained" in areas where host pheromone is adsorbed on leaf tissue (*i.e.* where host adults are actively mating and most likely to lay their eggs) (Noldus *et al.*, 1991a, 1991b). Under natural conditions, the response to pheromone serves to increase the searching efficiency of *Trichogramma* females (Noldus *et al.*, 1991a, 1991b; McGregor & Henderson 1998).

If host pheromone applied for mating disruption is adsorbed on foliage throughout a planting, *Trichogramma* females may be retained and continue searching in areas where no host eggs are present. If this occurs, the searching efficiency and biological control efficacy of *Trichogramma* could be reduced. Here, we report on laboratory bioassays of the response of *T. brassicae* to cabbage looper pheromone and on greenhouse experiments where cabbage looper pheromone and *T. brassicae* are concurrently applied to a pepper crop. Our objectives were: (1) to determine if mating disruption of cabbage loopers is feasible in pepper greenhouses, and (2) to determine the effect of cabbage looper pheromone on the searching behaviour and efficacy of *T. brassicae*.

Materials and methods

Laboratory response of T. brassicae to pheromone

Leaves excised from pepper seedlings (variety Spirit) were exposed to pheromone in 20 liter plastic pails containing 10 pheromone lures (each treated with 30mg of (Z)-7-dodecen-1-ol acetate) for 17 hours. Control leaves were placed in identical plastic pails with no pheromone present. Treated or untreated leaves were covered with plexiglas sheets that left a circular arena (diameter 5cm) of the leaf underside exposed. A *T. brassicae* female (24-72 hours old) was placed on the leaf undersurface and the time spent actively walking (searching time) and trial duration (residence time) of each wasp was recorded. Trials were terminated when the wasp walked or flew off the leaf section.

Greenhouse experiment 1

Rubber septa, each treated with 30 mg of the principal component of *T. ni* pheromone ((Z)-7-dodecen-1-ol acetate), were applied to 20 plants in a research greenhouse (12 x 6.4 m) planted in sweet peppers (variety Spirit). A second identical greenhouse was left untreated. Male *T. ni* were released, and female moths were placed in mating stations (plastic cups lined with Teflon tape), in pheromone-treated and untreated greenhouses on three separate occasions. The mating status of females was determined after 48 hours by dissection and observation of the spermatheca. *T. brassicae* were released and artificial patches of *T. ni* eggs (10-200 eggs per patch) on paper towelling were pinned onto the tops of 36 plants in both greenhouses. After 48 hours, egg patches were retrieved. After incubating for 5-7 days, the number of black (parasitized) eggs was recorded for each egg patch. Parasitism of *T. ni* eggs was measured on two occasions in each greenhouse.

Greenhouse experiment 2

Six weeks after removal of pheromone dispensers from the greenhouse treated in experiment 1, pheromone was applied to the previously untreated house using the method described above. The greenhouse treated with pheromone in experiment 1 was left untreated. *T. brassicae* were released in both houses, and egg parasitism was determined as described above.

Results and discussion

Laboratory response of T. brassicae to pheromone

Mean searching time on pheromone-treated leaves was significantly higher (0.75 ± 0.10 minutes) than on control leaves (0.60 ± 0.09 minutes, Mann-Whitney test: $U=942$, $p=0.03$, $n=50$). Similarly, mean residence time on pheromone-treated leaves was significantly higher (1.18 ± 0.28 minutes) than on control leaves (0.98 ± 0.22 minutes, Mann Whitney test: $U=910$, $p=0.02$, $n=50$). This result indicates that *T. brassicae* females exhibit an arrestment response and are retained on foliage in the presence of cabbage looper pheromone.

Mating disruption of cabbage loopers

After the pheromone dispensers were placed in the treated greenhouse, virtually no mating of female moths occurred (Fig. 1; Logistic regression analysis: $\chi^2=18.6$, $p<0.001$). This occurred despite the fact that males were accumulating in treated and control greenhouses as the experiment proceeded (*i.e.* because males were released in the greenhouses before each mating assessment). These results clearly indicate that pheromone applications can disrupt mating of cabbage loopers on sweet peppers in a greenhouse setting.

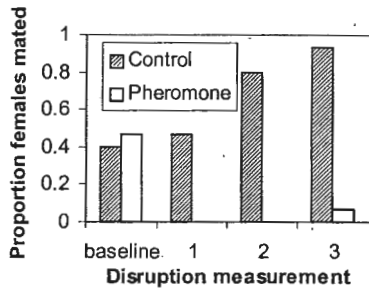


Figure 1. Disruption of cabbage looper mating.

Parasitism of cabbage looper eggs in greenhouses

In the first greenhouse experiment, the number of *T. ni* eggs parasitized per egg patch was higher in one measurement in the control greenhouse than in the pheromone-treated greenhouse, but not significantly so (Fig. 2; Mann-Whitney U=695, p=0.23). In the second measurement, the number of parasitized eggs was significantly higher in the control greenhouse than in the pheromone-treated greenhouse (Fig. 2; Mann-Whitney U=850, p<0.05).

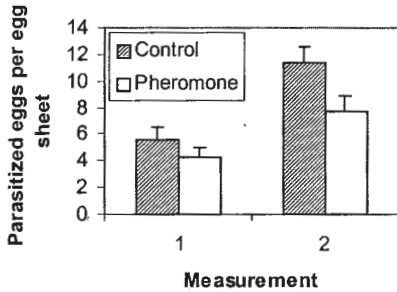


Figure 2. Parasitism of cabbage looper eggs in the first experiment.

In the second experiment, *T. brassicae* parasitism was assessed in the presence and absence of host pheromone without concurrent releases of *T. ni*. Treatment and control greenhouses were reversed relative to the first experiment. No differences were detected between the pheromone-treated and control greenhouses in the number of *T. ni* eggs parasitized per egg patch (Fig. 3; Measurement 1: Mann-Whitney U=608, df=2, p=0.57; Measurement 2: Mann-Whitney U=799, df=2, p=0.08).

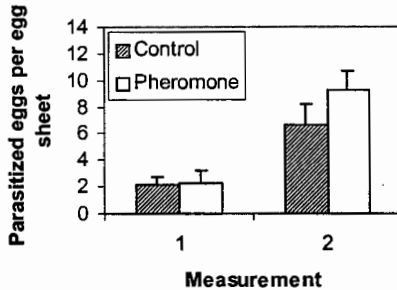


Figure 3. Parasitism of cabbage looper eggs in second experiment.

Conclusions

Mating of *T. ni* was disrupted in greenhouses treated with the principal component of cabbage looper pheromone. This indicates that there is a strong potential for using this method to manage cabbage looper populations in greenhouses. In one experiment, parasitism of *T. ni* eggs by *T. brassicae* was apparently disrupted by the presence of pheromone. This effect may have been caused by retention of *T. brassicae* females on leaves with pheromone present, but no eggs. In both experiments, *T. brassicae* successfully parasitized host eggs in the presence of pheromone. This indicates that mating disruption treatments and *T. brassicae* releases may be used concurrently for management of cabbage loopers in greenhouses.

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Introduction of the predatory mites *Phytoseiulus persimilis* and *Neoseiulus californicus* against *Tetranychus urticae* in outdoor roses

Judit Menyhért¹, Anton van der Linden

Applied Plant Research, Nursery Stock Research Unit, Rijnveld 153, 2771 XV Boskoop, The Netherlands, E-mail: A.van.der.Linden@PPO.DLO.NL; ¹Study address: Department of Systematic Zoology and Ecology, Eötvös Loránd University, Faculty of Sciences, Pázmány Péter sétány 1, 1117 Budapest, Hungary

Abstract: After intentional infestation of 12 plots of *Rosa* 'The Fairy' with spider mites *Tetranychus urticae*, the predatory mites *Phytoseiulus persimilis* or *Neoseiulus (Amblyseius) californicus* were introduced. The treatments were in fourfold. Four plots were not treated with predatory mites. The number of spider mites was high in July and decreased in August. Predatory mites were present in the samples, but their numbers were low. Other natural enemies were also present, including *Orius* spp., *Chrysopa* spp. and *Feltiella* spp. The predatory mites migrated also to untreated plots. Reduction of spider mites occurred in all plots. The reduction of spider mites was faster in the plots with *N. californicus* than in the plots with *P. persimilis*, and in the plots with *P. persimilis* it was faster than in the untreated plots. The effect of naturally occurring natural enemies is probably underestimated.

Key words: spider mites, biological control, integrated control

Introduction

In Dutch nursery stock, roses and rootstocks are grown on approximately 600 to 700 ha. The two-spotted spider mite, *Tetranychus urticae* Koch is named after the two dark spots on the abdomen. This mite is a major pest of many crops including rose. Because of their translucent skin the colour of the body can change due to the colour of digested food. Damage may occur as growth reduction and also as cosmetic damage. The mite is living on the undersides of the infested leaves, on which small yellow spots appear and lose their bright green colour, later turning into pale or bronze. These mites can also make webs, which sometimes can be seen also on the upper side of leaves. They prefer permanent hot and dry weather. The high temperature reduces the time of the lifecycle and low humidity makes it easier to remove waste products from their body.

In outdoor roses there is so far not much experience with biological control. Because natural enemies such as the predatory mites *Phytoseiulus persimilis* and *Neoseiulus californicus* are commercially available for release in glasshouses, it is obvious to test them first. Earlier results of biological control of the spider mites on hardy nursery stock showed a better performance of *N. californicus* than *P. persimilis* (Buxton, 1999). An other approach is to investigate which species of natural enemies occur naturally on roses outdoors and to find a method to encourage these species. The aim of the present study was to investigate the effect of released *P. persimilis* and *N. californicus* on spider mites on outdoor roses.

Material and methods

On the 29th July spider mites were released on 12 plots with 16 plants of *Rosa* 'The Fairy' by twenty infested bean plants which were put in the plots. These spider mites were collected on 11th April from *Rosa* 'Prophyta' from a glasshouse at Applied Plant Research, Naaldwijk. The mites were released on *Phaseolus* bean plants in Boskoop in order to scale up their numbers. On the 5th July from every plot leaves were taken to check the presence of spider mites. Almost on every sampled leaf spidermite eggs or motiles were found. After that the plants were colonized by the spidermites, on four plots (P) one predator, the *P. persimilis*, and on another four plots (A) the other predator, *N. californicus* were released on the 12th July. On four plots (O) no predatory mites were released. After this the plants were checked for spidermites every one or two weeks, together 9 times. On the first sampling date 4 leaves were taken from each plot, on the other dates 1 leaf per plant (16 leaves per plot) were collected and checked the next day in the laboratory. The number of the eggs and motile stages of both the spider mites and the predators were counted on each leaf.

Table 1. Plots of 16 rose plants each with three treatments in fourfold (I – IV). P=*Phytoseiulus persimilis*; A=*Neoseiulus californicus*; O=untreated.

IP	IO	IA	II O	II A	II P	III P	III O	III A	IV O	IV A	IV P
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P. persimilis was bought in a 100 ml bottle containing 2,000 predatory mites in wood-chips, while *N. californicus* was bought in a 500 ml bottle also with 2,000 mites. The volume of this small bottle was mixed with vermiculite to get 500 ml. Each plant received a small amount of wood-chips or wood-chips mixed with vermiculite. The number of predatory mites in the material was estimated by taking a sample of 10 ml and putting it in water with a detergent. The mites in these samples were counted. In 10 ml of *N. californicus* were 14 predatory mites while in 10 ml of *P. persimilis* this number was 9. That means 5.5 *N. californicus* per plant in the A plots, and 3.5 *P. persimilis* per plant in the P plots were released.

Results and discussion

The results of the countings of spider mites and predatory mites are presented in Fig. 1-3. The distance between the fields was 1 meter at the beginning. Later this distance was reduced due to the growing of the roses. This made the migration easier between the plots. After 4 weeks both in the treatments A and P the predatory mites were present and even appeared in the untreated blocks. It seems that mainly *N. californicus* migrated to the untreated plots, although *P. persimilis* was also identified from one of the O plots. This can be because *N. californicus* is a better competitor, or because of the position of two O plots between two A plots while the other O plots were between one A and one P plot. So *N. californicus* simply had more chances to colonize the untreated plots.

The conditions at the beginning of the trial were good, there were enough mites present in each plot according to a Metrical More Dimension analysis (MMDS). The fact that we did not find predatory mites in a certain treatment at a time does not mean that there were not any, but their numbers were low. It seems that *N. californicus* was present in higher numbers than the *P. persimilis* and the results suggest that *N. californicus* is a better colonizer. The result of

the statistical analysis after log transformation was that the reduction of the spidermites was faster in the treatments A than in treatments P and in treatments P faster than in O.

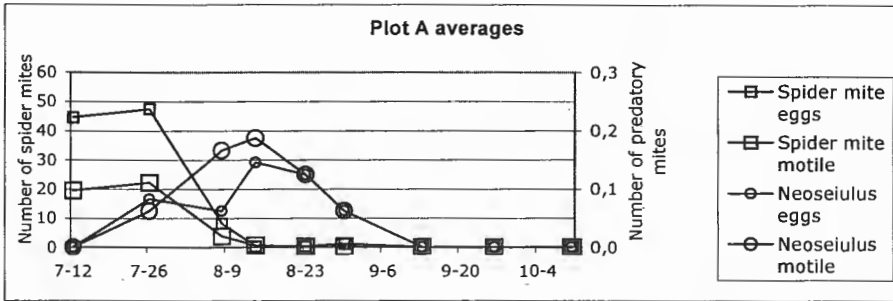


Figure 1. The average number of spider mites and *N. californicus* in 4 plots of 16 rose plants.

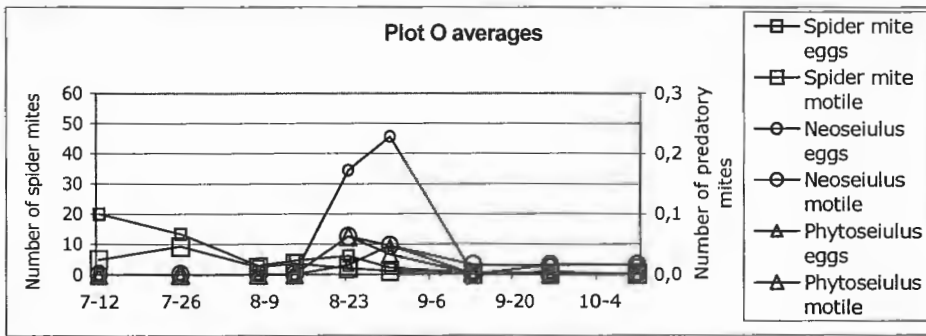


Figure 2. The average number of spider mites and predatory mites in 4 plots of 16 rose plants.

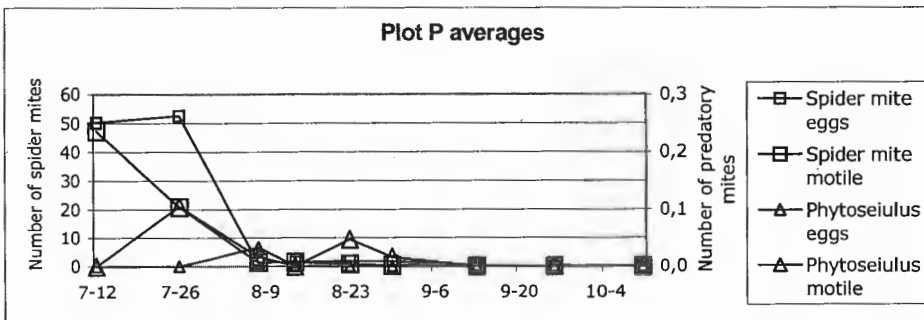


Figure 3. The average number of spider mites and *P. persimilis* in 4 plots of 16 rose plants.

In areas where the winter is cold the spider mites can overwinter as diapausing females. This is induced by the shortening of the days. The last date of sampling was in October so it is possible that mites already had started to enter diapause.

Besides the released predatory mites, other predators were present almost from the beginning. In order of importance they were *Chrysoperla* spp., *Orius* spp. and *Feltiella* spp. All of them feed on spider mites. According to the literature (McMurtry *et al.*, 1970) they can be very voracious predators of the mites. Likely they had a role in keeping the spider mite population on a low level, although their presence did not reach 5% of the sampled leaves on any of the dates. The presence of naturally occurring predators was probably encouraged by the diversity of plants in the nursery where the test was carried out. *Orius* spp., *Chrysoperla* spp. and *Feltiella* spp. reach their full potential when the spider mite population is high. *Chrysoperla* larvae and *Orius* nymphs are also able to move to another leaf searching for more spider mites. However, high densities of spider mites are preferably avoided in horticulture. To keep spider mites at a low level predatory mites are probably the best option. Some species, such as *N. californicus*, are able to maintain themselves for a longer period of time in the absence of prey. Many similar predatory species are also able to survive on alternative food, such as pollen, nectar, plant sap and other mites. Predatory mites, which were found on roses in the collection of Applied Plant Research in Boskoop were identified as *Euseius finlandicus* and *Amblyseius andersoni*. These may even be better candidates for spider mite control on roses than *P. persimilis* and *N. californicus*. These facts are a motivation to develop methods, which encourage predatory mites even when the pest population is low. *Phytoseiulus persimilis* is a specialist on *Tetranychus* spp., which makes it difficult to survive at a low density of its prey.

Conclusions

Both *Neoseiulus californicus* and *Phytoseiulus persimilis* established on outdoor roses. The reduction of spider mites was faster in the plots with *N. californicus* than in the plots with *P. persimilis*, and in the plots with *P. persimilis* reduction was faster than in the untreated plots. *N. californicus* showed a greater tendency to migrate than *P. persimilis*. The presence of other voracious natural enemies, such as *Orius* spp., *Chrysoperla* spp. and *Feltiella* spp. had probably also an important impact on the spider mites.

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Biological control of caterpillars with *Cotesia marginiventris* (Hymenoptera: Braconidae) in sweet pepper and tomato

Gerben Messelink

Applied Plant Research (PPO), Division Glasshouse Horticulture, P.O. Box 8, 2670 AA Naaldwijk, The Netherlands, E-mail: G.J.Messelink@ppo.dlo.nl

Abstract: *Cotesia marginiventris* was released in different densities in greenhouses with sweet pepper and tomato for testing its efficacy in controlling larvae of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) and *Spodoptera exigua* (Lepidoptera: Noctuidae). *C. marginiventris* was not able to control larvae of *C. chalcites* in tomato, even at high densities. In sweet pepper larvae of both *C. chalcites* and *S. exigua* were parasitized. About one female of *C. marginiventris* per suitable host larva of *C. chalcites* was needed to achieve complete control. The released parasitoids were able to locate caterpillars in all directions from the release point and could bridge a distance of at least 14 metres. In commercial greenhouses the braconid parasitoids *Cotesia plutellae* (Kurdj.) and *Cotesia vanessae* (Reinhard) were observed in association with larvae of *C. chalcites*.

Key words: *Chrysodeixis chalcites*, *Spodoptera exigua*, *Cotesia marginiventris*, *Cotesia vanessae*, greenhouse vegetables

Introduction

Larvae of Lepidoptera can cause serious damage in greenhouses in the Netherlands. The most harmful species in sweet pepper and tomato belong to the family of Noctuidae, with *Chrysodeixis chalcites* (Esp.) (Noctuidae: Plusiinae) as the most abundant species. *Spodoptera exigua* (Hb.) (Noctuidae: Amphipyrrinae) is less common, but more difficult to control. There is a strong demand for good biological control methods for these pests. Parasitic hymenoptera have high potentials for controlling caterpillars in greenhouses (van der Linden, 1996). In commercial greenhouses it would be ideal to have a parasitoid that parasitizes more than one stage of different pest species.

The solitary endoparasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) occurs abundantly on field populations of *S. exigua* (Alvarado-Rodriguez, 1987, Henneberry *et al.*, 1991) in Mexico and California and parasitizes young larvae and facultative eggs of *S. exigua* (Ruberson & Whitfield, 1996). This parasitoid also parasitizes larvae of the cabbage looper *Trichoplusia ni* (Lepidoptera: Noctuidae) (Henneberry *et al.*, 1991, Gillespie *et al.*, 1999), a species that belongs to the same subfamily as *C. chalcites* (Plusiinae). In this paper we describe some preliminary greenhouse experiments to investigate the parasitoids efficacy in controlling larvae of *S. exigua* and *C. chalcites* with *C. marginiventris* in sweet pepper and tomato. In addition, a survey was done of endemic parasitoids associated with *C. chalcites* in commercial greenhouses.

Material and methods

Insect rearing

C. chalcites was reared on small plants of tomato and sweet pepper. *S. exigua*, kindly supplied by Koppert B.V., was reared on an artificial diet. The parasitoid *C. marginiventris* was reared on *S. exigua* and delivered by Koppert B.V. and Biobest N.V. in plastic bottles with some honey.

Greenhouse experiments

In six experiments low and high numbers of caterpillars and parasitoids were combined in sweet pepper and tomato (table 1). Experiments in tomato were performed in two greenhouse compartments of 139 m² with unscreened ventilators at Applied Plant Research (PPO) in Naaldwijk. Each compartment had 252 tomato plants cv. Armata. Experiments in sweet pepper were performed in three greenhouse compartments of 151 m² with unscreened ventilators at Applied Plant Research (PPO) in Naaldwijk. Each compartment had 384 sweet pepper plants cv. Spirit. During experiment 3 (table 1) the windows of each compartment were screened (mesh size 1.0 by 4.0 mm). In all experiments L1-larvae of *C. chalcites* or *S. exigua* were introduced into the greenhouses by small brushes and put on marked plants. In most cases about 50 percent of the introduced larvae did not survive. In experiment 3 moths of *C. chalcites* were allowed to lay eggs very regularly on each plant. Numbers of caterpillars were determined by counting all larvae of five plants per compartment. Parasitoids were released in the centre of each greenhouse compartment in the morning one day after introducing the caterpillars. In control compartments, only caterpillars were released.

During all experiments, harvesting and crop treatments were omitted to exclude disturbance of the experiments. Other pests like spider mites, aphids and thrips were controlled with commonly used natural enemies, except for *Orius sp.* that was not used because of possible predation of caterpillars. Pesticides were not used during the experiments. Ten to fourteen days after releasing the parasitoids, larvae were recovered and reared singly in test tubes to determine whether they were parasitized. In experiment 3 we collected 280 caterpillars per compartment from 28 plants, in other experiments all larvae that could be recovered.

Table 1. Number of released parasitoids and number of noctuid larvae in different greenhouse experiments.

Experiment number	Crop	Lepidopteral pest	Number of successfully introduced larvae	Number of female <i>C. marginiventris</i>
1	sweet pepper	<i>C. chalcites</i>	20	15
1	sweet pepper	<i>C. chalcites</i>	20	150
2	sweet pepper	<i>C. chalcites</i>	30	15
2	sweet pepper	<i>C. chalcites</i>	30	150
3	sweet pepper	<i>C. chalcites</i>	6000	90
3	sweet pepper	<i>C. chalcites</i>	5650	17
4	tomato	<i>C. chalcites</i>	29	15
5	tomato	<i>C. chalcites</i>	31	31
6	sweet pepper	<i>S. exigua</i>	56	98
6	sweet pepper	<i>S. exigua</i>	56	374

Survey of parasitoids

During summer in 2001 parasitoids were obtained and identified from larvae of *C. chalcites*, collected in commercial greenhouses with sweet pepper in the Netherlands. Braconid species were identified by Dr. C. van Achterberg (Naturalis, Leiden). Parasitoids were reared on larvae of *C. chalcites* in a climate chamber at 22°C and 16:8 L:D.

Results and discussion

Greenhouse experiments

In sweet pepper parasitization rates were found to depend on the number of released females of *C. marginiventris* per larva (Fig. 1). It seems that about one female of *C. marginiventris* is needed per suitable larva of *C. chalcites* to achieve a 100 percent control in the first generation (regression analyses, Fig. 1), although higher densities do not guarantee this maximum efficacy (Fig. 1). Similar results were found in cucumber where 30 percent of larvae of *Trichoplusia ni* were parasitized when 0.3 females/larva were released (Gillespie *et. al.*, 1999). In experiment 3 in one compartment, about half of the larvae of *C. chalcites* was found to be parasitized by a spontaneously occurring *Cotesia plutellae* (Kurdj.). In other experiments larvae were only parasitized by *C. marginiventris*. In experiment 6 larvae of *S. exigua* were even parasitized in the control compartment. These parasitoids bridged a distance of at least 14 metres. Both release densities of parasitoids in this experiment gave a similar percentage of parasitized larvae, so nothing can be concluded about the minimum density needed to give a sufficient control (Fig. 1). The released parasitoids in sweet pepper were able to locate caterpillars in all directions from the releasing point.

In tomato even an 'overkill' of parasitoids gave only a minimum of parasitized larvae of *C. chalcites* (Fig. 1). For some reason this crop influences the behaviour of *C. marginiventris* in a negative way. Since this parasitoid utilizes both plant and host odours in order to locate hosts (Turlings *et. al.*, 1991), the strong smell of tomato plants may impede the search for feeding damage by the parasitoids. Another reason could be a toxic effect of the trichomes of tomato leaves (Levin, 1973).

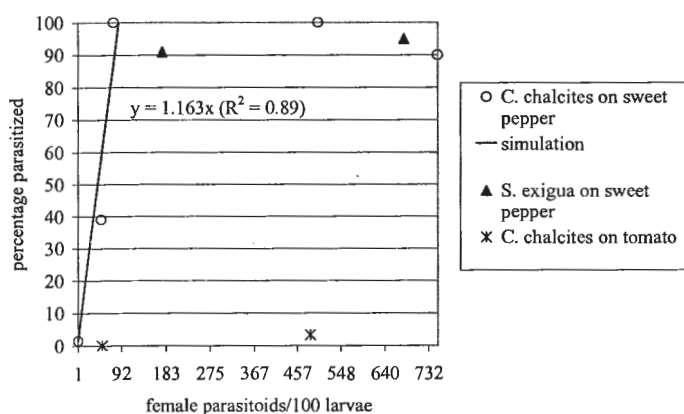


Figure 1. Efficacy of *C. marginiventris* in controlling L1-larvae of *C. chalcites* and *S. exigua* in sweet pepper and *C. chalcites* in tomato in greenhouses of respectively 151 m² and 139 m².

Summarizing we conclude that *C. marginiventris* is not able to control larvae of *C. chalcites* in tomato, but can be used to control larvae of *C. chalcites* and *S. exigua* in sweet pepper. However, using these parasitoids as a single control method will be rather expensive, due to huge amounts of caterpillars that can occur in commercial greenhouses. During this research it was found that caterpillars treated with *Bacillus thuringiensis* were not suitable for development of *C. marginiventris*. However, the parasitoids can be used together with insectivorous birds that mostly feed on late instar larvae (van der Linden, 1999).

Observed parasitoids

The most common endemic parasitoid of *C. chalcites* in greenhouses was found to be *Cotesia plutellae* (Kurdj.) (Hymenoptera: Braconidae). This species is a solitary endoparasitoid. A less common species was the gregarious endoparasitoid *Cotesia vanessae* (Reinhard) (Braconidae: Microgasterinae). This species is known as a common parasitoid of *Vanessa atalanta* (L.) and other Nymphalidae outdoors. One larva of *C. chalcites* hosted between 20 and 60 larvae of *C. vanessae*. At 22°C these larvae emerged 15 days after oviposition. Parasitoid adults emerged 8 days later. This gregarious species is probably interesting for commercial use because of the lower rearing costs, compared to solitary species. In one greenhouse we found a pupa of *C. chalcites* parasitized by a parasitic fly (Tachinidae). This species was not identified.

Acknowledgements

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The flower bugs, *Anthocoris nemorum* and *Anthocoris nemoralis*, voracity and prey preference for aphids in glasshouses

Nicolai Vitt Meyling^{1,2}, Annie Enkegaard², Henrik F. Brødsgaard²

¹University of Copenhagen, Zoological Institute, Department of Population Ecology, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark, E-mail: Annie.Enkegaard@agrsci.dk; ²Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark

Abstract: Voracity and prey preference were evaluated for female *Anthocoris nemorum* and *Anthocoris nemoralis* preying upon four species of aphids in 24-h experiments in climate cabinets at 20°C, 60-70% RH, L:D 18:6. Both predators accepted all four species and the numbers of aphids killed varied between 1 to 11 aphids per day. No difference in preferences was found between the two *Anthocoris* species. Both predatory bugs preferred *M. persicae* to the other species, the most accepted alternative prey were *A. gossypii*, *A. solani* and *M. euphorbiae* in descending order.

Key words: *Anthocoris* spp., aphids, voracity, prey preference, biological control, glasshouse pests

Introduction

Aphids are serious pests in many glasshouse crops and have proved difficult to control, mainly due to the rapid development of resistance to insecticides. Biological control has become a widely used control strategy, especially in vegetable crops, and presently a large array of agents for aphid control are available. However, there is still a need for evaluation of potentially new natural enemies of aphids to supplement the existing agents. The predatory bugs *Anthocoris nemorum* (L.) and *Anthocoris nemoralis* (Fabricius) (Heteroptera: Anthocoridae), acknowledged as natural enemies of aphids (e.g. Campbell, 1977, Hodgson & Aveling, 1988), are such candidates. In the present investigation, predator traits in terms of voracity and prey preference were evaluated for *A. nemorum* and *A. nemoralis* when offered four species of aphids, common as glasshouse pests.

Material and methods

Origin of experimental insects

A. nemorum adults were collected by sweep-netting herbaceous vegetation in late summer and autumn 2000. All collected females had entered reproductive diapause. Prior to usage, the animals were stored in large petridishes in a climate cabinet at 20°C, L:D 18:6, 60-70% RH and fed on eggs of *Sitotroga* sp. (Olivier) (Lepidoptera: Gelechiidae). Leaves of *Pilea* served as a source of humidity. *A. nemoralis*, originating from a mass rearing in Italy, was obtained from Borregaard Bioplant, Denmark. This species was kept in a similar fashion as described above. The females readily laid eggs in the *Pilea* leaves.

The aphid species were reared in netcovered glasshouse cages, 85x75x68 cm, at 22°C, L:D 16:8 in a glasshouse on sweet pepper (*Myzus persicae* Sulzer, *Macrosiphum euphorbiae* (Thomas), *Aulacorthum solani* (Kaltenbach)) or Chrysanthemum (*Aphis gossypii* Glover).

Voracity and preference

All experiments were conducted on pepper leaves in large petridishes (14 cm Ø) in climate cabinets at 20°C, L:D 18:6, 60-70% RH for a 24 hour period. Females of *A. nemorum* and *A. nemoralis* were starved in small petridishes (9 cm Ø) with filterpaper and a small piece of leaf for 24±1 h in a climate cabinet at similar conditions. Preference for aphid species was tested in pairwise combinations of similar sized instars with *M. persicae* as reference species in the following combinations: 20 4th instar *M. persicae* vs. 20 3rd instar *A. solani*; 20 4th instar *M. persicae* vs. 20 3rd instar *M. euphorbiae*; 40 2nd instar *M. persicae* vs. 40 4th instar *A. gossypii*. One starved predator was introduced to the set-up, which was subsequently placed in the climate cabinet. After the trial the predator was removed and the number of killed aphids was registered. Controls revealed no background mortality in any combination. Data analysis was performed by logistic regression (PROC GENMOD, (SAS Institute Inc., 1989)).

Results

Voracity

The results on voracity of *A. nemorum* and *A. nemoralis* are shown in table 1.

Table 1. Mean number of killed prey [95% confidence limits] in each combination of aphid prey for *A. nemorum* and *A. nemoralis*. Numbers of replicates were 20 in combination 1-2 and 25 in combination 3.

	<i>A. nemorum</i>	<i>A. nemoralis</i>	Test-statistic F	P
Combination 1				
<i>M. persicae</i>	4.5 [3.4; 5.8]	2.7 [1.9; 3.9]	F _{1,36} =4.864	0.0339
<i>A. solani</i>	2.1 [1.6; 2.7]	0.9 [0.6; 1.4]	F _{1,36} =12.575	0.0011
Prey killed in total	6.6 [5.2; 8.4]	3.6 [2.5; 5.1]	F _{1,36} =7.989	0.0076
Combination 2				
<i>M. persicae</i>	2.5 [1.9; 3.3]	4.0 [3.3; 4.9]	F _{1,36} =7.381	0.0101
<i>M. euphorbiae</i>	1.2 [0.7; 2.0]	0.9 [0.5; 1.6]	F _{1,36} =0.407	0.5277
Prey killed in total	3.7 [2.7; 4.9]	4.9 [3.8; 6.2]	F _{1,36} =3.002	0.0917
Combination 3				
<i>M. persicae</i>	10.8 [8.8; 13.2]	7.5 [5.7; 9.8]	F _{1,42} =4.517	0.0395
<i>A. gossypii</i>	7.2 [5.5; 9.4]	5.2 [3.7; 7.3]	F _{1,42} =2.287	0.1379
Prey killed in total	18.0 [14.7; 21.9]	12.7 [9.6; 16.6]	F _{1,42} =4.293	0.0445

The experiment with the combination of *M. persicae* and *A. solani* revealed that both *A. nemorum* and *A. nemoralis* accepted the two prey species when offered simultaneously. The total number of aphids killed differed for the two predators. In addition *A. nemorum* killed significantly more of each aphid species in this combination than did *A. nemoralis*. When the experiment was performed with *M. persicae* and *M. euphorbiae* in combination the voracity of *A. nemoralis* was not significantly higher than of *A. nemorum*. However, when considering voracity divided between the two prey species, *A. nemoralis* killed significantly more *M. persicae* than did *A. nemorum*, while no significant difference was found between numbers of *M. euphorbiae* killed. When *A. nemorum* and *A. nemoralis* were offered *M. persicae* in combination with *A. gossypii* both predators exhibited a high voracity, presumably reflecting

the smaller size of each prey individual compared with aphids in the other combinations. When all killed aphids of both prey species were included, *A. nemorum* killed more prey than did *A. nemoralis*. The number of killed *M. persicae* was also significantly higher for *A. nemorum*, while there was no difference between the numbers of *A. gossypii* killed by the two predators.

Preference

Manly's index of preference (Manly, 1974) was calculated for the different experimental combinations with *M. persicae* chosen as prey type 1, i.e. an index above 0.5 suggests preference for *M. persicae*. A comparison of the preference indices for the two predators within each prey combination revealed no significant differences. As a consequence, a compiled index for each prey combination was calculated and interpreted as the combined preference of both *A. nemoralis* and *A. nemorum* when offered the different aphid species (Fig. 1). In all cases, the preference indices were significantly different from 0.5, i.e. the anthocorids had a significant preference for *M. persicae* over the other aphid species.

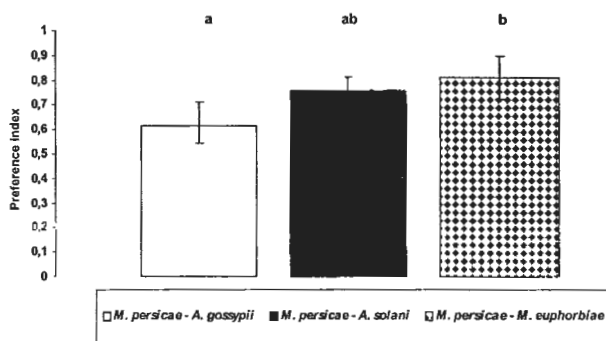


Figure 1. Median values of combined preference indices for both *A. nemorum* and *A. nemoralis* for each combination of aphid prey. Bars indicate approximate 95% confidence intervals. Different letters indicate significantly different indices.

Discussion

Voracity

The present study showed that all the four aphid species were killed by both *A. nemorum* and *A. nemoralis*, of which *A. solani*, *M. euphorbiae* and *A. gossypii* not earlier have been tested as prey for both predators. In two of the three experimental combinations, *A. nemorum* killed more prey individuals than *A. nemoralis*. Campbell (1977), who reared both predators on hop aphids *Phorodon humuli* (Schrank), found no significant difference in daily voracity between the two species of predators in neither any nymphal stage nor in the adult stage.

When reproductive diapause in *A. nemorum* is terminated an approx. 3-fold increase in voracity occurs (pers. obs., data not shown) indicating that caution must be taken when evaluating the effect of a predator. Increased voracity is likely caused by greater requirements for nutrition of the ovipositing females. Thus, the differences in voracity between *A. nemorum* and *A. nemoralis* reported here would possibly become larger, if experiments had been performed with non-diapausing *A. nemorum* females.

Numbers of aphids killed by *A. nemorum* and *A. nemoralis* in the present investigation were not as high as for several of the aphid enemies commonly applied as biological control agents (coccinellids, parasitoids). However, the voracity of the *Anthocoris* species seemed to be higher than found for *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) at 21°C, where the daily voracity was 2.9 large *M. persicae* nymphs (Uygun, 1971). Although voracity in the present investigation seemingly was lower than found among most of the established beneficials, *Anthocoris* spp. should not be ruled out as candidates for biological control. The predatory bugs could supplement the usually applied species.

Preference

The present results revealed no difference in prey preference between the two predators and *A. nemorum* and *A. nemoralis* thus had a similar preference for *M. persicae* in each experimental combination. Earlier studies on prey selection by *A. nemorum* found no evidence of preference between various prey (Dempster, 1963; Herard & Chen, 1985).

Both predators exhibited the strongest preference when *M. persicae* was combined with *M. euphorbiae*. A possible explanation for the relatively low predation on *M. euphorbiae* can be a difference in behaviour exhibited by the various aphid species. Thus, in the presence of the predator *M. euphorbiae* seemed to have a greater tendency to withdraw their stylets and walk away when disturbed, compared to *M. persicae* (N.V. Meyling, pers. obs.). A similar behavioural mechanism seemed to be present in *A. solani*. Consequently, the searching predator had a reduced possibility of encountering *M. euphorbiae* and *A. solani* individuals.

Conclusion

As revealed by the presented experiments both *A. nemorum* and *A. nemoralis* killed the different aphid species offered. Both predators showed a preference for *M. persicae* in all experimental combinations and this preference did not differ between the predators. Considering *A. nemorum* and *A. nemoralis* as future candidates for biological control, both predators showed potential as beneficials against *M. persicae* in glasshouses.

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Intraguild predation between the predatory flower bug, *Anthocoris nemorum*, and the aphid parasitoid, *Aphidius colemani*

Nicolai Vitt Meyling^{1,2}, Henrik F. Brødsgaard², Annie Enkegaard²

¹University of Copenhagen, Zoological Institute, Department of Population Ecology, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark, E-mail: Annie.Enkegaard@agrsci.dk; ²Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark

Abstract: Prey preference between *Aphidius colemani* parasitised *Myzus persicae* (mummy stage) and unparasitised aphids was evaluated for female *Anthocoris nemorum* in 24-h experiments in climate cabinets at 20°C, 60-70 % RH, L:D 18:6. *A. nemorum* preyed readily on the immature parasitoids contained within mummies and showed no preference for either of the two prey types.

Key words: *Anthocoris* spp., aphids, intraguild predation, prey preference, biological control

Introduction

In glasshouse crops, the availability of beneficials has increased substantially through the last decade (Eilenberg *et al.*, 2000), making it possible to release several different beneficial species in order to control one or more pest species simultaneously. Especially in ornamentals the use of multispecies assemblages is widespread, thus creating basis for a multitude of interactions, including intraguild predation, within and among both pests and beneficials – interactions which may alter the outcome of biocontrol (Brødsgaard & Enkegaard, 2001).

Aphid parasitoids are specialists which consume – and eventually kill – the host from the inside out (Starý, 1988). The immature parasitoid is vulnerable to predation both when the host is still alive and when the parasitoid larva has pupated within the emptied cuticle shell of the aphid, termed the ‘mummy’. This predation on an immature parasitoid could be performed by an aphid predator, making the interaction a case of intraguild predation.

Few experiments have evaluated the presence of this kind of intraguild predation among aphidophagous insects, which could be present in a programme of biological control of aphids in glasshouses. In the present investigation the prey preference of adult *Anthocoris nemorum* L. (Heteroptera: Anthocoridae) between unparasitised and parasitised green peach aphids (*Myzus persicae* Sulzer (Homoptera: Aphididae)) was evaluated. The aphids were parasitised by *Aphidius colemani* Viereck (Hymenoptera: Braconidae), which is a widely used parasitoid in biological control of aphids in glasshouses. Aphid control might be enhanced by the simultaneous use of both a parasitoid and a predator, but a preliminary evaluation of the interactions between the two types of beneficials is essential to select appropriate species compositions.

Material and methods

Origin of experimental insects

A. nemorum was collected on stinging nettle, *Urtica dioica* L., in early autumn 1999. To terminate reproductive diapause in the females, adults were cold-stored for approx. 80 days in

a climate cabinet at 5°C, L:D 16:8 and 75% RH in large petridishes provided with folded filterpaper for hiding, and leaves of *Pilea* and small pieces of water saturated cotton wool as sources of moisture. The source of food for *A. nemorum* was eggs of *Sitotroga* spp. (Olivier) (Lepidoptera: Gelechiidae). Once a week water and fresh *Sitotroga*-eggs were replenished. After the cold storage females were transferred to new large ventilated petridishes containing the same material and food source as described above. Leaves of *Pilea* served as a medium for oviposition. Leaves containing eggs were replaced with new ones twice a week and placed in small petridishes with folded filterpaper as hide out for hatching nymphs. Approximately 20 to 30 anthocorid eggs were placed in each petridish. Nymphs were reared on eggs of *Sitotroga* spp. until adult eclosion. All egg laying and rearing took place in climate cabinets at 20°C, L:D 18:6, 60-70% RH. The experimental insects used were adult females of this laboratory-generation which prior to experimentation had entered reproductive diapause.

The green peach aphid *M. persicae* was reared on pepper in netcovered glasshouse cages, 85x75x68 cm, placed in a glasshouse at 22°C, L:D 16:8. Only apterous nymphs were used for experiments.

The parasitoid *A. colemani* was reared on either cotton aphids *Aphis gossypii* Glover (Homoptera: Aphididae) on Chrysanthemum or *M. persicae* on pepper in separate netcovered glasshouse cages, 85x75x68 cm, placed in a glasshouse at 22°C, L:D 16:8. Additional parasitoids were supplied by EWH BioProduction, Denmark.

Experiments

Production of mummies. Between 50 and 60 2nd instar nymphs of *M. persicae* were transferred to a leaf of pepper placed vertically in a waterfilled plastic cup. Three female *A. colemani* were added to the set-up which was placed under a transparent plastic jar. The females were allowed to oviposit for three hours. Following removal of the parasitoids the set-up was incubated in a climate cabinet at 20°C and L:D 18:6, 60-70% RH for 8-9 days until mummies appeared.

Preference experiments. Excess mummies as well as unparasitised aphids were removed until 30 mummies remained on the leaf. Thirty 4th instar *M. persicae* nymphs were added to the leaf and one adult female *A. nemorum* introduced to the set-up which subsequently was placed in a climate cabinet at 20°C, L:D 18:6, 60-70% RH for 24 hours. Prior to experimentation the *A. nemorum* females had starved for 24 h in small petridishes in a climate cabinet at 20°C, L:D 18:6, 60-70% RH.

After the course of the experiment the predator was removed and sucked-out and dead aphids were counted. Since it was not possible by external features to distinguish between consumed and unconsumed mummies (N.V. Meyling, pers. obs.), the mummies were removed and subsequently opened with fine insect needles to determine the number of consumed immature parasitoids. Parasitoid prepupae and pupae that had been eaten were recognised as shrunken and emptied individuals. A control dissection of 27 mummies revealed that shrinking did not occur in the absence of a predator. 26 replicates of the experiment were made. Five controls of the experimental set-up were made to investigate background mortality of the aphids. Data analysis was performed by logistic regression (PROC GENMOD, (SAS Institute Inc., 1989)).

Results

In the experiment adult *A. nemorum* females had the opportunity to prey on two types of prey simultaneously, unparasitised fourth instar *M. persicae* nymphs and mummies formed by the parasitoid larvae of *A. colemani*. Each *A. nemorum* female killed a mean proportion [95%

confidence limits] of 9.4% [6.9%; 12.5%] of the offered immature parasitoids contained within the mummified aphids. In addition, each predator killed a mean proportion [95% confidence limits] of 12.1% [9.3%; 15.6%] of the offered unparasitised *M. persicae* nymphs. These proportions correspond to a mean [95% confidence limits] of 2.8 [2.1; 3.8] killed immature parasitoids within mummies and 3.6 [2.7; 4.6] killed unparasitised aphid nymphs, as presented in Fig. 1. No significant difference was found between these proportions ($F_{1,50}=1.674$; $P=0.2016$).

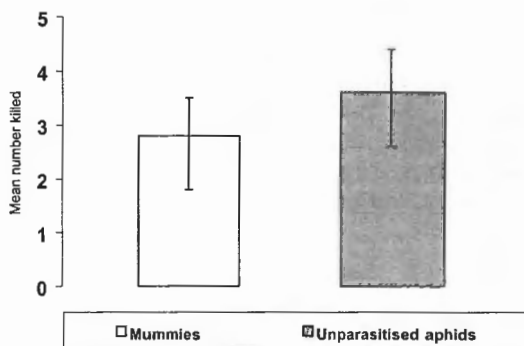


Figure 1. Mean numbers (95% confidence limits) of killed immature *A. colemani* parasitoids inside mummified *M. persicae* and unparasitised 4th instar *M. persicae* nymphs by 1 *A. nemorum* female during 24 h.

Another way of evaluating prey preference is by calculating Manly's index of preference (Manly, 1974) for unparasitised aphids. This yielded a mean index (\pm s.e.) of 0.579 (\pm 0.057). The lower and upper 95% confidence limit of the index was 0.462 and 0.696, respectively. Thus, the index was not significantly different from 0.5 and *A. nemorum* therefore did not exhibit a significant preference for either of the prey types.

Discussion

The present study has revealed that adult *A. nemorum* females will prey upon immature parasitoids contained within mummies of *M. persicae*, even in the presence of unparasitised *M. persicae* nymphs and that the predator do not discriminate between the two prey types consuming them in comparable amounts. Based on the results of the present investigation it is not expected that adult *A. nemorum* would discriminate between unparasitised aphids and parasitised aphids which are alive, but contain eggs or early instars of parasitoid larvae.

A. nemorum has preciously been reported to consume parasitised aphids in the mummy stage. Dixon & Russel (1972) found that immature parasitoids sheltered by mummies of sycamore aphids *Drepanosiphum platanoides* (Schr.) constituted an important food source for *A. nemorum* and *A. confusus* Reuter. The predation on mummies was observed more frequently than should be expected from random selection (Dixon & Russel, 1972). Thus, *A. nemorum* and *A. confusus* exhibited an evident preference for parasitised aphids which Dixon & Russel (1972) argued was due to the ease of handling an immobile prey as a mummy

compared to the very mobile adults of *D. platanoides*, which were the most abundant alternative prey. Adult individuals of *D. platanoides* are approximately of equal size to an adult *A. nemorum* while *M. persicae* is considerably smaller (Heie, 1982; 1994). Applying this theory to the present results, no difference between capturing and handling a fourth instar *M. persicae* nymph and a mummy formed by *A. colemani* should be expected. This is supported by relatively high success ratios of capturing fourth instar *M. persicae* nymphs by adult *A. nemorum* (N.V. Meyling, pers. obs.).

Other predators of aphids often applied in biological control have been found to engage in intraguild predation of parasitoid mummies. Brødsgaard & Enkegaard (2001) found that three coccinellid species (*Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* Pallas and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)) preferred unparasitised *M. persicae* over *A. colemani* mummies and that neither *Orius majusculus* (Reuter) (Heteroptera: Anthocoridae) nor the larvae of the lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) exhibited any preference between mummies or *M. persicae*. These findings suggest that coccinellids do not prey on mummies when alternative prey is present to the same extent as for instance anthocorid bugs do.

Conclusion

The investigation performed on intraguild predation revealed that adult *A. nemorum* females preyed indiscriminately on unparasitised *M. persicae* and immature *A. colemani* parasitoids within mummified *M. persicae*. This lack of preference for unparasitised *M. persicae* could obstruct a biological control programme where both *A. nemorum* and *A. colemani* were applied simultaneously. However, this would have to be evaluated by further experiments.

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The use of biological control in Canadian greenhouse crops

G.D. Murphy¹, G. Ferguson², Ken Fry³, Liette Lambert⁴, Margaret Mann⁵, Jim Matteoni⁶

¹ Ontario Ministry of Agriculture, Food and Rural Affairs, Box 7000, Vineland, ON, L0R 2E0, Canada, E-mail: graeme.murphy@omafra.gov.on.ca; ² Ontario Ministry of Agriculture, Food and Rural Affairs, Harrow Research Station, Harrow, ON, L0R 2E0, Canada; ³ Alberta Research Council, Postal Bag 4000, Vegreville, AB, T9C 1T4, Canada; ⁴ Quebec Ministry of Agriculture, 118 Lemieux St-Remi, QC, J0L 2L0, Canada; ⁵ New Brunswick Agriculture, Fisheries and Aquaculture, PO Box 6000, Fredericton, NB, E3B 5H1, Canada.; ⁶ British Columbia Horticultural Centre, Kwantlen University College, 12666 72nd Avenue, Surrey, BC, V3W 2M8, Canada

Abstract: The use of biological control in greenhouse crops in Canada was determined by a survey of 803 growers. Biological control is used on greater than 90% of tomatoes and peppers and on approximately 50% of cucumbers. For ornamental crops, biological control is used on 12% of the area and by 26% of growers. The results are presented and discussed.

Key words: biological control, Canada, greenhouses, survey

Introduction

Biological control as a component of Integrated Pest Management is increasingly important in the pest management programs of Canadian greenhouse growers. For many individual growers, industry associations and provinces, reducing pesticide use is a major objective. The use of biological control is an indirect indicator of pesticide use reduction. It is increasingly apparent that more growers are using natural enemies in greenhouse crop production, particularly in vegetable crops, but also in ornamentals. However, there has been no quantification of its usage. This paper quantifies the use of biological control in greenhouse production in Canada in 2001.

Materials and methods

Extension specialists, consultants and researchers collaborated on the development of a survey form that was sent to growers in each province. Various methods of contact, including mailings, facsimile and interview by telephone were used to maximize coverage. The length of the survey was limited to a one-page form to encourage its completion. This had obvious implications on the amount of data that could be collected. Information was requested on:

- Greenhouse location, area and crops grown
- The current or previous use of biological control
- Reasons for using (or for no longer using) biological control
- Natural enemies used and total area under biological control
- A rating of the success of control for each major insect/mite pest

Results and discussion

Survey forms were sent to growers in all major greenhouse production areas in Canada including the provinces of Ontario, British Columbia, Quebec, and Alberta, and smaller production regions in New Brunswick and Manitoba. Completed forms were received from 803 growers (table 1).

Table 1. Summary of completed survey forms received (number of returns).

	Province					Area covered by the survey	% of the Canadian industry surveyed	% Biocontrol use (by area)
	Ontario	British Columbia	Quebec	Alberta	Other*			
Vegetables	115	14	112	58	3	499 ha	61%	83%
Ornamentals	198	12	148	148	5	353 ha	32%	13%

*Includes growers in New Brunswick and Manitoba

Adoption of biological control in ornamental crops is slower than in vegetable crops and it can be more difficult to define the extent of its use. In vegetables, biological control is almost always used on the total crop. In ornamentals, it is more common to find partial use. Growers may use natural enemies to control one pest only and use pesticides for other pests. They may use biological control on one crop only and not on other crops, or they may use it only at certain times of the year and revert to pesticides in the summer when pest pressure is at its peak.

Evidence of these differences in use patterns, can be seen by comparing the use of biological control in individual greenhouses. For growers of ornamental crops where biological control was used, it was on only 44% of their total area. For vegetable crops, the area using biological control is almost the same as the total area of the greenhouses reporting its use.

Vegetable crops

Biological control of insects and mites in greenhouse vegetable crops is well-established and in crops such as tomatoes and peppers, is used almost exclusively. In cucumbers however, it is used in just over half the total area grown (table 2). This statistic concurs with the finding that 64% of growers, who had discontinued use of biological control, were cucumber growers.

Table 2. Use of biological control, in greenhouse vegetable crops in Canada.

Crop	Total area surveyed (No. growers)	Area using biocontrol (% of total area)
Tomato	260 ha (165)	241 ha (93%)
Cucumber	122 ha (120)	62 ha (51%)
Pepper	104 ha (52)	102 ha (98%)
Other	13 ha (37)	7.5 ha (58%)
TOTAL	499ha	412.5 ha (83%)

Between provinces, there are some differences (table 3), although direct comparisons are difficult. Ontario and British Columbia are the major producers of greenhouse vegetables in Canada and show similar use patterns except for cucumbers in which BC reported 100% use

of biological control. However, the BC figures are based on a sample size of only five cucumber growers. Use of biological control in Alberta and Quebec is less than in Ontario and BC, but their industries are smaller, and the average size of individual greenhouses also smaller.

Table 3. Use of biological control in greenhouse vegetables in Canadian provinces.

	Provinces				
	Ontario	B. Columbia	Quebec	Alberta	N. Brunswick
Total area of greenhouse vegetables	493 ha	209 ha	84 ha	36 ha	2 ha
Area surveyed (no. of reports)	330 ha (115)	89.5 ha (14)	46.7 ha (112)	19.8 ha (58)	0.6 ha (3)
Area using biocontrol (no. of reports)	282 ha (89)	89.5 ha (14)	36.9 ha (80)	11.5 ha (34)	0.3 ha (2)
% area using biocontrol	85%	100%	79%	58%	50%

The most commonly used natural enemies included *Amblyseius cucumeris*, *Encarsia formosa*, *Phytoseiulus persimilis*, *Aphidius* spp., *Aphidoletes aphidimyza*, *Bacillus thuringiensis*, and *Hypoaspis* spp. For thrips, slightly more than 50% of tomato and cucumber growers were satisfied with control versus almost 80% of pepper growers. A similar breakdown occurred for aphid control. Control of spider mites was fair with 53% and 65% of tomato and pepper growers, respectively, expressing satisfaction. However, only 47% of cucumber growers reported good control of mites, and many growers in Ontario felt that spider mites are a major limiting factor to the success of biological control in general. Improved control of caterpillars is also needed with approximately 60% of respondents satisfied. For whiteflies, about 65% of cucumber and pepper growers, and 75% of tomato growers were satisfied with the level of control. Biological control of fungus gnats was most successful with 73% of all growers giving a highly positive response.

Ornamentals

A summary of the ornamental growers surveyed is given in table 4. Bedding plants and potted plants have been combined, because many growers grow both crops and often no distinction was made between the use of biological control in the two crops. Potted plants includes potted flowers, foliage and tropical plants; and bedding plants includes annuals and perennials.

Between provinces there were differences in the use of biological control. Ontario is the largest producer of greenhouse ornamentals in Canada with more than 50% of overall production, but biological control is used on less than 10% of its total area. Quebec growers reported 16% use and Alberta 20%. British Columbia reported 52% of their greenhouse area as using biological control, but those figures are based on a sample size of only 12 growers.

Table 4. Summary of biological control in greenhouse ornamental crops in Canada.

	Total area surveyed (# growers)	Total area using biocontrol (% of total)	# growers using biocontrol (% of total)
Potted, bedding plants	286 (434)	32 (11%)	108 (25%)
Cut flowers	67 (69)	12 (18%)	23 (33%)
TOTAL	353 (495)	44 (12%)	130 (26%)

A feature of biological control in ornamentals is the number of growers who have used the strategy previously but for various reasons have stopped. At the time of the survey, 26% of all growers were using biological control and another 24% had previously used it. It is encouraging that half of the industry has used natural enemies at some time; however, there is concern that so many growers have reverted to using pesticides. The most commonly given reasons for stopping the use of biocontrol were a lack of effectiveness and/or consistency.

The mean size of greenhouses in the survey was 7,124 m². For growers using biological control, the mean size was 3,415 m² and for previous users the mean size of greenhouses was 8,790 m². The reasons for this discrepancy are unclear. It may be related to ease of management in the different size greenhouses or the possibility of a greater tolerance for pests in a smaller greenhouse (local markets compared with export). If greenhouse size plays a role in the success of biological control, it may explain the low usage in Ontario where average greenhouse size is almost 12,500 m², compared with Quebec and Alberta (3,578 m² and 2,184 m² respectively).

The pests most successfully controlled were aphids with 58% of potted/bedding plant growers and 63% of cut flower growers being satisfied or very satisfied with the level of control. Approximately 50% of all growers felt that good whitefly control was achieved. For thrips and spider mites there were differences between potted/bedding plant growers and cut flower growers. Only 32% of potted/bedding plant growers reported good control of thrips compared to 57% of cut flower growers. For mites, 35% of potted/bedding plant growers and 64% of cut flower growers reported good control. These differences between potted/bedding plant growers and cut flower growers are reflected in the overall usage of biological control shown in table 4.

Conclusions

Biological control in Canada is the primary pest control strategy in the greenhouse vegetable industry. It also plays a significant role in pest management in greenhouse ornamentals although pesticides are still the major component of pest control in those crops. This survey provides baseline data that can be used to measure progress in the use and adoption of biological control.

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Biological and integrated control in ornamentals in North America: successes and challenges

G.D. Murphy

Ontario Ministry of Agriculture, Food and Rural Affairs, 4890 Victoria Ave N., Vineland Station, ON, L0R 2E0, Canada, E-mail: graeme.murphy@omafra.gov.on.ca

Abstract: A summary is presented of some of the challenges and some of the factors associated with success of biological control programs in greenhouse ornamental crops in North America.

Introduction

Biocontrol in greenhouse ornamentals presents different challenges to those faced by growers of greenhouse vegetables. It is not the purpose of this paper to compare use of biological control in ornamentals and vegetables; nonetheless it is difficult to discuss reasons for success and failure without making some distinctions between the two. For vegetable growers, the ultimate issue is one of economics. It does not make economic sense for vegetable growers not to use biocontrol and take advantage of ensuing benefits such as: the use of insect pollinators; potential yield increases; reduced pesticide residues; and marketing advantages. For growers of ornamental crops, economic incentives are not usually the motivating factor.

In North America, the adoption of biological control in ornamental crops has been a slow process. Growers first began experimenting with natural enemies in the late 1980's and early 1990's. A survey of Canadian growers in 2001, showed that biological control is currently being used on 12% of the total area and by 26% of all growers (Murphy *et al.*, 2002). In the USA, there is less information available upon which to base an estimate of usage, but it is unlikely to be much higher than in Canada. In Mexico, use of biological control in ornamentals is limited to entomopathogenic fungi (R. Bueno, pers. comm.). The challenges faced by growers using biological control and the reasons they succeed or fail, are many. No attempt is made here to address all issues affecting the efficacy of biological control, but a number of contributing factors, illustrated by case studies, are discussed.

Challenges

The large number of growers who have unsuccessfully attempted biological control (Murphy *et al.* 2002), is cause for concern. The most commonly given reason for failure of biological control programs in Canada was lack of efficacy. From a grower's perspective, this is a valid reason to discontinue biological control, but it is a simplistic assessment that ignores the actual underlying causes. Poor efficacy may occur for various reasons (e.g. incorrect rates of introduction, poor timing, quality control issues, pesticide residues). However, there are also other fundamental reasons why biological control may not succeed.

Why do growers use biological control? The reasons a grower may initially consider using biocontrol can have a bearing on its chances of success. Many growers begin using natural enemies because of poor control with a pesticide-based program. However, if pesticides have not been effective, it has probably resulted in high pest populations and crops with excessive pesticide residues, both of which can compromise biological control. In effect, the decision to use natural enemies has been precipitated by circumstances outside the grower's control.

Growers who adopt biological control for health, environmental or philosophical reasons are doing so for reasons over which they have control. For example, a grower of potted flowering plants in Ontario began using biological control in 1996 because of personal health concerns. Despite variable levels of control over and ongoing high introductions of natural enemies, the grower continues to persist, because he feels that he does not have other options. Self-motivated change is more likely to succeed than that which is forced by external factors.

As biocontrol becomes more accepted, growers will begin using it simply because other growers do. Many vegetable growers begin using biological control because it is a standard crop management practice used by the industry (Murphy *et al.*, unpubl.data), the implication being that alternatives (i.e. pesticides) are not even considered. Peer pressure is a powerful tool for change and may help explain the success of the study groups discussed below.

Pesticide issues. Lack of availability of compatible pesticides has been often identified as an obstacle to biological control in ornamental crops, and until recently, there has been validity to this argument. In Canada, which has a paucity of compatible pesticides, there is still reason to hope that registration of new products may provide impetus to increased use of natural enemies. However, in the USA, there are now many products registered that are much more compatible with natural enemies than the older products they are replacing. Microbial pesticides, insect growth regulators and new chemistries with fewer non-target side-effects have become available in recent years. In some situations, such products have been instrumental in increased use of biological control (e.g. the California rose industry discussed below). However, in general, the availability of such products does not seem to have stimulated any major progress in adoption of biological control.

A second concern is the registration of highly efficacious products and the impact of these on the use of biological control. When imidacloprid was registered in North America in the mid-1990's, the product was so effective and persistent that the need for biological control was completely negated. There is a concern that the same attitude will prevail with the recent registration of spinosad for thrips control.

Thrips. Western flower thrips, *Frankliniella occidentalis* remains the most difficult of the major pests to control (either with pesticides or biologically). In both Canada and the USA, WFT (and its associated tospoviruses) have been identified as the major pest management research priority. Why are growers of ornamental crops failing to control thrips on a consistent basis? Or more relevant, why do some growers achieve control of thrips in similar situations to those in which other growers fail? One of the concerns with biological control of thrips is their cost of control compared to other pests. If growers try to save either by reducing rates or frequency of introductions, then control can be compromised (R. Valentin, pers. comm.). Growers more likely to succeed are those most willing to commit the necessary resources.

Successes

For biological control to be successful, various obstacles initially must be overcome, including:

- Pesticide residues that may have built up over years of prolonged use
- Established pest populations that may increase rapidly before the introduced natural enemies are able to establish and exert control
- The grower's own inexperience in working with natural enemies

These factors combined can make the first 6-12 months of a biological control program difficult. For the same reasons, costs for biological control are highest when growers initially put a program in place, reducing as growers gain more experience and the natural enemies become established (Gullino & Wardlow, 1999).

When faced with the question of why so many growers have been unsuccessful in their use of biological control, the immediate reaction is to seek reasons for the high failure rate. More important, however, is to ask what successful growers are doing, that their unsuccessful counterparts have been unable to do? Are they better prepared prior to introducing natural enemies, so that pesticide residues and high pest populations do not play as large a role? Do they have technical support that lessens the impact of their own inexperience? Are there other factors involved in whether or not a grower will be successful? The following describe examples of successful use of biological control in ornamental crops.

Group support/partnerships. The University of California, Davis in partnership with growers, and the biological control and pesticide industries, has developed an IPM program for cut roses focusing on the major pests of that crop, twospotted spider mites, western flower thrips and powdery mildew (Parrella, 2001). Rose growers in California began using biological control in the late 1990's in an attempt to improve control of spider mites and with few exceptions, have eliminated the use of miticides which used to account for up to 40% of pesticide use (H. Petersen, pers. comm.). However, as with many other ornamental crops, control of thrips is ultimately critical to the overall success of the program. In California, the use of the pesticide spinosad on the upper canopy of the crop has allowed growers to control thrips with minimal impact on the efficacy of the mite predator *Phytoseiulus persimilis*. Removal of unmarketable buds and liberal use of blue sticky tape also play a major role in thrips control. It is estimated that more than 750,000 m² of rose production in California is now grown using biological control. Success of the program is attributed to factors such as the growers' willingness to share information through regular meetings and on-site visits, and the technical support provided by industry and research partners.

In British Columbia, Canada, a coordinated effort has been made to promote the use of biological control. Growers who use biological control attend monthly focus groups to discuss their programs, and share information. Provincial ministry staff, university staff, and biological control suppliers also attend and provide technical support and advice. Individual growers host the meetings, which involve a tour of the crop and a discussion of their successes and failures. General issues are also discussed such as control strategies, new pest species, and updates on new natural enemy products. The meetings are an excellent way to address common problems and to promote communication among growers.

Technical support. A common failing of biological control when growers first began using the strategy, was the introduction of natural enemies by growers without adequate preparation and without follow-up technical support. Growers were often sceptical of biological control to

begin with, and to experience rapid failure soon after the initial introductions, deterred many of them from further use. Since the mid-1990's, most major biological control producers have employed technical staff to support their customer base in the field. This has had a noticeable impact on the attitudes of growers towards biological control and their confidence in the strategy.

From a government extension perspective this change has also had an impact. Prior to these changes, a significant amount of extension time was spent with individual growers, providing the support now offered by private companies. There were insufficient resources (both time and personnel) to adequately address the needs of the industry. Now, more time is spent working with and assisting company representatives than with individual growers.

Technical support is an excellent resource for growers. However, growers often buy based on price and may purchase natural enemies from several companies, possibly following several different management strategies. For the grower, this is a wonderful opportunity to gain as much information as possible. For the biological control suppliers however, it is an inefficient use of resources that strains their ability to provide the necessary technical backup for growers.

Conclusions

There are many reasons why growers use biological control, why it fails and why it succeeds. While this paper has not attempted to comprehensively discuss or even identify all of them, it does examine several key factors that may contribute to success or failure. There is concern that more effort needs to be directed to why growers succeed with biological control, rather than why they fail. Often, success is determined by the grower's commitment and motivation, whether it comes from within the individual, or is fostered by support from a larger like-minded group.

Acknowledgements

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Spinosad as a new compound for integrated control of *Frankliniella occidentalis* Pergande on cucumber and tomato in greenhouse

Bożena Nawrocka

Research Institute of Vegetable Crops, P.O. Box 110, 96-100 Skierniewice, Poland, E-mail: bnawroc@inwarz.skierniewice.pl

Abstract: In 2001 two experiments with Spinosad 480 SC in control of *Frankliniella occidentalis* on cucumber and tomato under greenhouse condition were done. The obtained result was very good, both on cucumber and tomato. Spinosad 480 SC – a naturally derived biological control product – was more effective than Talstar 100 EC – a synthetic pyrethroid – in thrips control.

Key words: *Frankliniella occidentalis*, cucumber, tomato, control, Spinosad

Introduction

Spinosad is the first active ingredient in the Naturalyte class of insect control products. It has a very low mammalian toxicity and is safe to beneficial insects. Spinosad is especially very selective with the respect to predators and the toxicity to parasitoids is much less than synthetic insecticides (Bret *et al.*, 1997; Peterson *et al.*, 1997). In that respect Spinosad is a very good new compound in integrated control of *Frankliniella occidentalis*.

Material and methods

The investigations were carried out in greenhouse cucumber and tomato in the growing season of 2001 in Poland. The general principles of investigations were in accord with EPP0 standards (Anon., 1997).

Tested combinations: Spinosad 480 SC – 0.02 %, Spinosad 480 SC – 0.03%, Talstar 100 EC – 0.05% (as a standard) and untreated plants. The size of the plot for cucumber was 4.5 m² and 3.5 m² for tomato. Four replications were made in randomised block design. To determine the efficiency of the examined insecticide 10 plants per plot were observed. The following records of pests were done: 24 h before and 3, 5, 7, 10 and 14 days after treatment. The efficiency of Spinosad in control of *F. occidentalis* was presented as a percentage pest mortality. Conversion of data to percentage of mortality based on the original count at each application was done with the Abbot equation (Abbot, 1925).

Results and discussion

In two experiments on cucumber (table 1) and tomato (table 2) Spinosad 480 SC gave very good results in control of *F. occidentalis*. Both the lower (0.02%) and higher (0.03%) rates of Spinosad were more effective in control of the examined pest than Talstar 100 EC. The differences were not very high but statistically significant. It was very obvious in 14 days after treatment. Spinosad in both rates still showed the highest effectiveness with over 99% thrips mortality. Effectiveness of Talstar went down and mortality of thrips were lower than 85% (table 1 and 2).

The obtained results showed that Spinosad 480 SC has relatively high suppressive effect on *F. occidentalis* populations.

Spinosad has a low toxicity to non-target species, especially to predators and parasitoids (Bert *et al.*, 1997) and is used or proposed to be used in Integrate Pest Management programs (Peterson *et al.*, 1997; Olszak & Pluciennik, 1999; Bylemans & Schoonejans, 2000). As a consequence of all mentioned above characteristic Spinosad 480 SC seems to be very suitable for integrated control of *F. occidentalis* on vegetables in greenhouse.

Table 1. Efficiency of Spinosad 480 SC in control of *Frankliniella occidentalis* Pergande on cucumber in greenhouse. Skierniewice 2001.

Combinations	Avg. number of thrips per plant before treatment	Average percent of thrips mortality in following observation days			
		3	5	7	14
Spinosad 480 SC – 0.02%	13.0	84.2a*	99.1b	97.0a	99.6b
Spinosad 480 SC – 0.03%	18.5	100.0b	100.0b	99.8a	99.5b
Talstar 100 EC – 0.05%	10.0	93.4b	90.5a	94.6a	84.6a

*Newman – Keuls test, $\alpha = 0.05$

Table 2. Efficiency of Spinosad 480 SC in control of *Frankliniella occidentalis* Pergande on tomato in greenhouse. Skierniewice 2001.

Combinations	Avg. number of thrips per plant before treatment	Average percent of thrips mortality in following observation days			
		3	5	7	14
Spinosad 480 SC – 0.02%	6.1	92.7a	100.0b	100.0b	100.0b
Spinosad 480 SC – 0.03%	4.0	100.0b	100.0b	100.0b	100.0b
Talstar 100 EC – 0.05%	5.2	90.2a	91.8a	92.0a	83.7a

*Newman – Keuls test, $\alpha = 0.05$

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***Macrolophus caliginosus* affected by a fungal pathogen**

Barbro Nedstam

Swedish Board of Agriculture, Plant Protection Centre, Box 12, S-23053 Alnarp, Sweden, E-mail: barbro.nedstam@sjv.se

Abstract: Populations of *Macrolophus caliginosus* in tomato decrease during autumn. Dead adults and nymphs are found on abaxial leaf surfaces and on stems. On their malformed abdomina conidia of an *Entomophthora* species are being produced. Symptoms can be seen while the insects are still alive. The appearance and development of the disease have been followed in 2000 and 2001 tomato greenhouses. Some negative influence on the biocontrol capacity of the predator has been noticed.

Key words: *Macrolophus caliginosus*, *Entomophthora* sp., tomato, biological control

Introduction

The polyphagous predator *Macrolophus caliginosus* (Heteroptera: Miridae) is being widely used in Swedish tomato production in greenhouses. It is introduced in low numbers, less than 0.5 per m², early in the season (February to March). The main target is the greenhouse whitefly, *Trialeurodes vaporariorum*, but the predator also helps controlling the two-spotted spider mite, *Tetranychus urticae* and the tomato leafminer, *Liriomyza bryoniae* (Nedstam & Johansson-Kron, 1999). Due to the slow build-up of the mirid population, other, specialized, natural enemies are also being used against these pests: *Encarsia formosa*, *Phytoseiulus persimilis* and leafminer parasitoids, respectively.

By the end of June *M. caliginosus* has normally reached fairly high numbers, around 1 – 5 per plant, and there is a steady increase during July, towards 50 bugs per plant in some cases. In August, and for the rest of the season, populations generally decrease again. Adults and nymphs infested by a fungal disease can then be found. Symptoms start developing while the insects are still alive. This disease was first noticed in 1998, generally occurring wherever *M. caliginosus* was established. Probably the entomopathogen was present even earlier.

The fungus has been identified as *Entomophthora* sp. (Zygomycetes: Entomophthorales). Two species of this genus have been described from Miridae: the North American *E. erupta*, on several phytophagous hosts, and the European *E. helvetica* on *Notostira elongata* (Ben-Ze'ev et al., 1985). The former has also been found on *Creontiades pallidus* in Israel (Ben-Ze'ev et al., 1988). Klapwijk (1999) reported on collapse of populations of *M. caliginosus* during summers in greenhouses in the Netherlands, also caused by an Entomophthorales fungus. Chemical intervention, especially for whitefly control, will then sometimes be needed.

The development of the disease and the effect on the number of predators was followed in some tomato crops in Sweden during 2000 and 2001.

Materials and methods

Four tomato greenhouses were studied each year. Every second week, starting end of June, a random sample of 100 leaves at 1.6 m level was checked by visual examination for healthy and symptom-carrying (both dead and alive) adults and nymphs of *M. caliginosus*. Dead insects were collected and examined under a stereo microscope. The number of dead

predators due to the disease will surely be underestimated with this leaf sampling method, as quite many cadavers were found on the tomato stems, attached by the proboscis. Some few mirid bugs had been killed by spiders on the leaves. These mirids could be with or without the fungus, and have not been included in the results.

Results and discussion

Symptoms

The abdomen of infested nymphs (mainly later stages) and adults swell into a saucer-like structure dorsally, where conidia are produced. Often the colour changes from a normal, yellowish green into a pale, bluish hue while the bugs are still alive. After death the colour becomes more brownish. Dead nymphs and adults sit on tomato stems or on abaxial leaf surfaces with proboscis fastened to the substrate. Most of these traits are in concordance with the pathobiology described by Wheeler (1972) for *E. erupta* infesting the alfalfa plant bug, *Adelphocoris lineolatus*. The presence of resting spores was noticed in some of the dead adults, most often in September. As greenhouses nowadays are cleaned mainly with hot water only, without chemical disinfectants, there is a good opportunity for the resting spores to carry over the disease to the following season.

2000

During 2000 three of the four greenhouses were sprayed with pyrethrum in July, and only results for the unsprayed crop are presented here (Fig. 1). *Entomophthora* sp. was first noticed in the middle of August, which coincided with the onset of diminishing predator numbers. In the other greenhouses the disease also made its first appearance during August, except in one case where it started mid July. The reason for spraying pyrethrum was actually to reduce populations of *M. caliginosus*, as growers had realized the risks for plant damage and even reduced yields of tomato due to the sometimes phytophagous habits of this predator (Sampson & Jacobson, 1999).

2001

In this season all four growers refrained from spraying against *M. caliginosus*, as they now take it for granted that the entomopathogen will stop the predator from reaching damaging levels. No other insecticide treatments were carried out. The main prey for *M. caliginosus* was leafminers and/or whiteflies. In addition all greenhouses harboured spider mites. All pests were controlled by various natural enemies in addition to the mirid bug.

The major disease problem was grey mould, *Botrytis cinerea*. All growers used limited spot treatments with fungicides on lower parts of stems, except one who sprayed repeatedly on whole plants. This greenhouse is excluded from the presentation of results, as it could be expected that the treatments will also affect the entomopathogen.

Entomophthora sp. first appeared in July, except in one greenhouse (A) where it was not seen until early September (Fig. 2). This is an older greenhouse of the free-standing, single gable type, with a less humid climate than in the other, modern, gutter connected buildings. The difference in humidity was reflected in stem infections of *B. cinerea*: House A had in September only 2.5 stem infections per 100 plants, while B, C and D had 41, 9 and 22.5 respectively (T. Hansson, pers. comm.). It seems that climatic conditions favouring infections of *B. cinerea* are also good for the *Entomophthora* sp. House B, having big problems with grey mould this year, also had the heaviest infestation of the entomopathogen, while house C was intermediate (Fig. 3) and house A was almost free from both fungi.

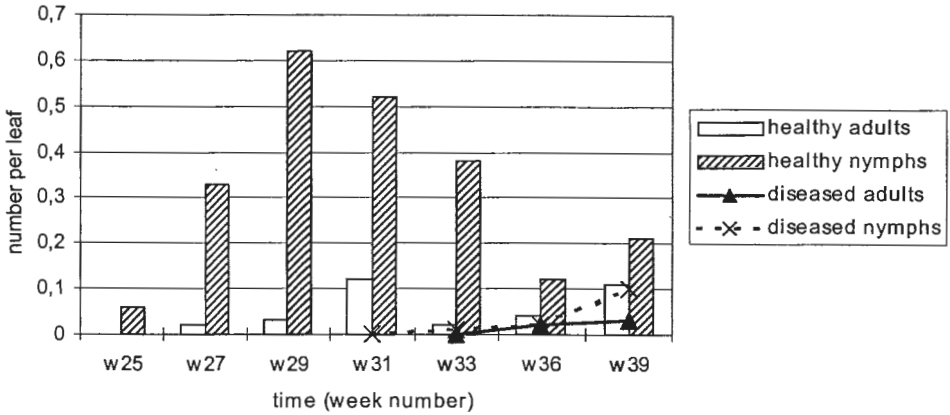


Figure 1. Number of healthy and diseased adults and nymphs of *M. caliginosus* in a tomato crop, June - September 2000.

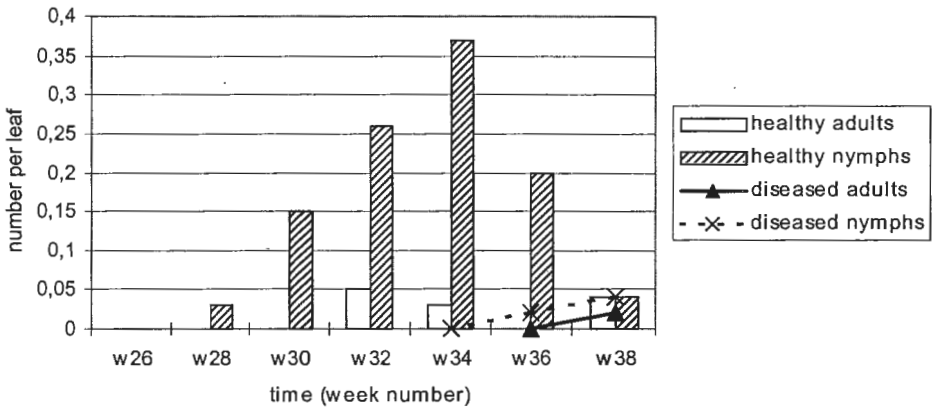


Figure 2. Number of healthy and diseased adults and nymphs of *M. caliginosus* in a tomato crop; free-standing greenhouse (A), June - September 2001.

There were no major problems with resurging pests during autumn, but a slight increase in whitefly and leafminer activity could be noticed. For leafminer control this can be important, as it increases pressure on the following crop (Nedstam & Johansson-Kron, 1999). In the Swedish situation so far, the entomopathogen on *M. caliginosus* is looked upon as mainly a regulating force, hindering the mirid from reaching high and maybe damaging levels.

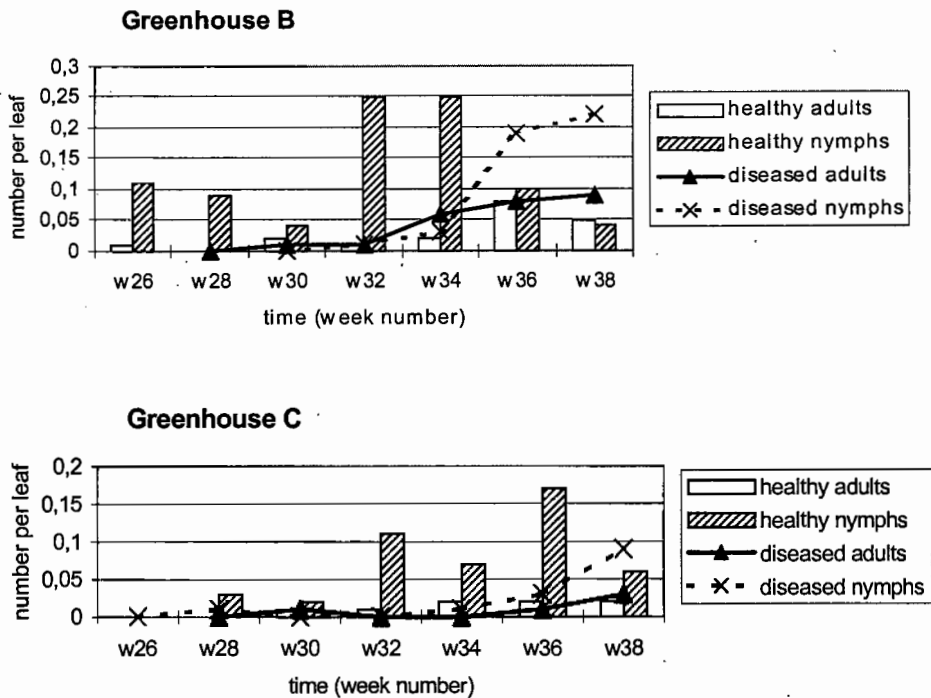


Figure 3. Number of healthy and diseased adults and nymphs of *M. caliginosus* in two tomato crops, June - September 2001.

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Biotechnology and its potential effect on the development and implementation of biological control/IPM strategies in greenhouses

Michael Parrella

Department of Entomology, University of California, One Shields Avenue, Davis, CA 95616 USA, E-mail: mpparrella@ucdavis.edu

Abstract: Biotechnology can be defined as the application of our knowledge and understanding of biology to meet practical needs. By this definition, biotechnology is as old as the growing of crops and the making of wines and cheeses. Today's biotechnology is largely identified with molecular biology and its applications in medicine and agriculture based on our understanding of the genetic code of life. While controversy swirls around this technology in both scientific and lay circles, one cannot doubt that we are in the midst of a biological revolution. Various aspects of this technology including the advent of transgenic crops and utilizing the tools of biotechnology for studies of arthropods and pathogens will be reviewed and discussed with respect potential impact on development and implementation of biological control and IPM in glasshouses.

Key words: biotechnology, transgenic crops, biological control, IPM

Introduction

Molecular biology has emerged during the last decade as one of the most profound developments in the biological and biomedical sciences. The fundamental approach of molecular biology is the study of life through genomics (i.e., the study of many genes) and is leading to the creation of a universal periodic table of life that will reflect common genetic properties and patterns of ancestral and functional affinities among genes of both plants and animals. This will unlock the record of 3.5 billion years of evolutionary innovation (Lander & Shork, 1998). Comparison of related organisms will reveal regulatory regions and key architectural features of proteins that can be used as Rosetta Stones for translating and understanding informational pathways and for deciphering biological complexity. Ultimately the field is leading to the creation of global tools of genomics that will revolutionize the medical, health, environmental and agricultural sciences (Collins *et al.*, 1998; Carey, 2000). Broad acceptance of this new technology is having a major impact on how scientific research is funded and conducted at the national and international levels. At many universities around the world, there is a race to hire faculty working in the area of 'genomics'; this follows major funding trends but often comes at the expense of more 'traditional' positions in organismal biology. For example there is concern over the reorganization and loss of Botany Departments across the US and that this will ultimately result in a weakening of the discipline and contribute to a decrease in public understanding of plants (Sundberg, 2000). In some situations, Botany Departments have evolved into departments or divisions of plant science with a decidedly molecular emphasis. The ultimate determination of gene function and the importance of that function are in the area of organismal biology. The field of molecular

biology was initiated with an all-out assault on determining the full genomic sequence of organisms. Obtaining a full genomic sequence today is viewed as technical work and only the starting point. The exciting areas of molecular biology are comparative and functional genomics, proteomics, metabolomics, and systems biology that provide a blueprint to the basic biology and functioning of the organism. The most important reason for determining the full genetic sequence of an organism is that it is an archive for the future, containing all the genetic information required to make the organism. However, the greater part of how this all fits together is not yet understood (Anon, 1998).

For many individuals, the term biotechnology and/or molecular biology is synonymous with genetic transformation of an organism and this is reinforced by the use of a multitude of terms in the literature including: genetic engineering, genetic transformation, transgenic technology, genetically modified organisms (GMOs), recombinant DNA technology, and genetic modification technology. However, biotechnology extends far beyond transforming an organism. A few of the areas where biotechnology has application beyond creating GMOs includes: advancing our knowledge in the area of systematics and evolution, enhancement and targeting of traditional plant breeding programs, and development of rapid and more accurate diagnostic techniques.

Clearly a major focus for biotechnology companies has been in the area of genetic modification where novel transgenic crop varieties have been developed. Many millions of acres of transgenic crops such as soybean, cotton, tobacco, potato, and maize have been grown annually in a number of countries including the USA (28.7 million ha in 1999), Canada (4 million), China (0.3 million), and Argentina (6.7 million) (James, 1999). There is considerable debate over the risk and benefits that may result from the planting and use of such crops. However, from a global perspective the need for such crops is overwhelming. By 2030, it is estimated that eight billion persons will populate the world, an increase of 2 billion people from today's population. Today it is estimated that 800 million people (18% of the population in the developing world) do not have access to sufficient food to meet their needs (Pinstrip-Anderson, 1999). Furthermore, malnutrition plays a significant role in the half of the nearly 12 million deaths each year of children under five in developing countries (UNICEF, 1998). In addition to lack of food, deficiencies in micronutrients (especially Vitamin A, iodine and iron) are widespread. Achieving the minimum necessary growth in total production of global staple crops – maize, rice, wheat, cassava, yams, sorghum, potatoes and sweet potatoes – without further increasing land under cultivation, will require substantial increases in yield per acre. Currently there is intense research on developing transgenic plants to satisfy this growing need for staple foods and to improve the nutritional content of these crops. In addition, research is being conducted on developing resistance to viral, bacterial and fungal diseases; modification of plant architecture (e.g. height), and development (e.g. early or late flowering or seed production); tolerance to abiotic stresses (e.g. salinity, drought); production of industrial chemicals (plant based renewable resources); and the use of transgenic plant biomass for novel and sustainable sources of fuel. Other potential benefits from transgenic plants include increased flexibility in crop management, decreased dependency on chemical insecticides and soil disturbance, enhanced yields, easier harvesting and higher proportions of the crop available for trading. Recently, the Brazilian Academy of Science, the Chinese Academy of Sciences, the Indian National Academy of Science, the Mexican Academy of Science, the National Academy of Sciences (USA), the Royal Society (UK) and the Third World Academy of Sciences issued the following statement, “genetic manipulation technology, together with important developments in other areas, should be used to increase the production of main food staples, improve efficiency of production, reduce

the environmental impact of agriculture, and provide access to food for small-scale farmers” (Anon, 2000). While such a statement generates international excitement over the benefits of genome-related biotechnology, many questions need to be addressed before this technology can be applied to benefit human health in developing countries (Singer & Daar, 2001). Biotechnology, through the appearance of transgenic crops and diagnostics, has already manifested itself in glasshouse production.

Transgenic crops

Transgenic approaches to modifying crop plants have similar disadvantages as traditionally bred crops. First, the period needed for development of transgenic plants – for such steps as finding appropriate genes, tissue culture selection process, developing plant transformation protocols, and backcrossing – can be as long as traditional plant breeding methods. This of course will be a function of the crop under study. Second, although much progress has been made in the discovery of new genes for introduction into plants, the ability to introduce the genetic material has surpassed the ability to discover new genes to engineer. Very few genes have been found for control of nematodes, sucking insects, and mites. In cotton for example, transgenic plants with Bt genes provide growers with another alternative for control of *Heliothis*, but the need for products that control whiteflies, mites, and lygus bugs remain. Cholesterol oxidase, an enzyme from the microorganisms *Streptomyces* is toxic to boll weevils and has been engineered into cotton plants: the enzyme is not effective on other pests. The targets for biological control in the glasshouse are usually the softbodied sucking arthropods which appear (at least for the time being) as not being amenable to control via transgenic plants. Lepidoptera, which can pose problems for crops grown in the glasshouse, may be the first area where plants engineered to ward off insect attack will appear in the glasshouse. Transgenic plants, whether engineered to contain insecticidal proteins, such as an endotoxin protein from Bt or a chitinase gene to control root rot pathogens, appear to have the same advantages as traditionally bred pest-resistant crops. For example, cotton engineered with a Bt Cry IA (c) or (b) protein are selective for Lepidoptera, increases the persistence of Bt for season long control, is compatible with the environment (by reducing the use of more toxic pesticides) and is compatible with other pest management techniques, such as the use of natural enemies (Verkerk *et al.*, 1998). Many broad-spectrum insecticides are detrimental to biological control agents that help to control insect and mite pests. Studies to date have indicated that Bt-corn and cotton are compatible with biological control and that these transgenic crops have little effect on natural enemies of pests. However, by keeping pest populations at extremely low levels, Bt crops can starve natural enemies as these beneficial insects need a small amount of prey to survive (Altieri, 1998). In addition, natural enemies could also be affected directly through inter-trophic level interactions. Evidence from studies conducted in Scotland suggest that aphids were capable of sequestering the toxin from Bt crops and transferring it to its coccinellid predators, in turn affecting reproduction and longevity of the beneficial beetles. Altieri (1998) goes on to suggest that there is the potential for Bt toxins to move through food chains which would pose serious implications for natural biocontrol in agroecosystems. Strong *et al.* (1990) have argued that transgenic techniques need to be refined so that toxins are only expressed in a subset of crucial tissues and at specific developmental stages and they must be integrated into an ecological framework if they are to be effective and contribute to biological control. Finally van Emden & Wratten (1991) have suggested that resistant crop varieties developed through transgenic techniques are often based on the allelochemical mechanisms (antibiosis) which might be damaging to natural enemies.

Initial choices of transgenic plants that contain a single Bt gene were termed 'first generation' plants and these are being followed by more sophisticated 'second generation' and 'third generation' plants with greater flexibility for use in IPM programs (National Research Council, 2000). Advances are likely to include plants with inducible and tissue-specific expression systems whereby expression of the protein is 'turned on' in response to herbivory or some other stimulus. Induced resistance involves plant-mediated changes associated with initial attack by herbivores and pathogens that negatively influence subsequent attackers. The jasmonate pathway (i.e., the octadecanoid pathway) and the salicylate pathway (conditioning systemic acquired resistance, SAR) are two of the biochemical response mechanisms that can be triggered by various attackers. The components of these pathways, jasmonic and salicylic acids (JA and SA, respectively), act as signals that trigger naturally occurring chemical responses that protect the plant from insect and pathogen invaders (Thaler *et al.*, 1999). There are already products that can be sprayed on plants to induce resistance. Bion® (marketed in Europe) or Actigard® (marketed in the USA by Syngenta) has the active ingredient benzo [1,2,3] thiadiazole-7-carbothioic acid-S-methyl ester or benzothiadiazole (BTH). BTH acts like SA and elicits systemic acquired resistance (the salicylate pathway). It has been tested for control of bacteria and fungi. Messenger (Eden Bioscience Corporation) has the active ingredient harpin (a protein derived from the bacteria *Erwinia amylovora*). Harpin triggers a cascade of responses that stimulate the salicylate pathway and the jasmonate pathway. It has also been shown to stimulate nutrient uptake and photosynthesis. When it is effective, the response is initiated within 10 minutes of treatment and depending on the plant species, may continue for several weeks.

Agrochemical companies have already introduced crops stacked with Bt and herbicide tolerance. Crops with multiple genes for proteins with different modes of action (for example, gene 1 containing Bt is combined with gene 2 containing a protease inhibitor) will be useful to prevent or delay the development of resistance by a pest. Regardless of the molecular strategy used to kill pests, transgenic plants should never be put into place without the adoption of IPM (Gould, 1988). Strategies designed to limit the development of resistant pests on the basis of understanding pest population dynamics (such as studies on pest population establishment and growth, genetics, movement, behavior, number of generations required to develop resistance, and generation time) are critical for optimal and sustained use of transgenic plants in IPM systems. This research may be even more important in the glasshouse, where conditions for the development of resistance are more severe and where strategies used to reduce the development of resistance in field crops (such as the planting of refugia) may not be possible.

A great deal of research is currently focused on the search for new genes for plant pathogen control. Within the next ten years, agriculture will see the introduction of plants engineered to resist fungal and bacterial pathogens. Virus resistant crops for field agriculture are already on the market and progress is being made on developing resistant floriculture crops using molecular tools against both the virus (Sherman *et al.*, 1996, 1998) and thrips (Annadana, 2001). Transgenic plants expressing resistance to the virus will allow the more effective use of biological control strategies on virus susceptible crops.

Transgenic and paratransgenic insects

Research aimed at pest management based on genetic engineering has focused primarily on the genetic manipulation of crop plants, economically important tree species (Raffa, 1989) and biological control agents (Hoy *et al.*, 1997). Little emphasis has been placed on the engineering of weeds, pathogens and arthropod pest species in ways that would decrease

economic damage. An important step in the genetic engineering process is to develop a full genomic sequence of the organism in question. Although insects cause more than \$26 billion dollars in damage annually to crops and livestock all over the globe, funding to discern insect genomes does not appear to be on the horizon (Pennisi, 2001). There are very few insect genomes being deciphered and these are either related to human health or are 'model' organisms. For example, work has begun on the malaria mosquito, *Anopheles gambiaea* and the genome of the fruit fly, *Drosophila melanogaster*, was completed in March 2000. Major efforts are underway to secure funding to determine the genome of the honeybee that is critical for pollination and is used as a model organism in neurophysiology/genetics/behavior. From a biological control perspective success in deciphering the honeybee genome would provide some information on Hymenoptera in general. Another focus is on the silkworm, *Bombyx mori*, which could shed some light on the genome of Lepidopterous pests. As indicated earlier, funding for these projects has not been secured (Pennisi, 2001). Despite the lack of complete genomic sequences, recent success in the stable transformation of mosquitoes based on the *Hermes* and *Mariner* **transposons** (Coates *et al.*, 1998) has prompted renewed interest in the genetic control of pests. The concept of modifying pests by the use of classical genetic manipulations dates back at least to the 1940s where it was suggested that chromosomal abnormalities and hybrid sterility could be used for insect control. Feldman (1980) suggested that incompatibility resulting from a cross between a translocation hemizygous male and a wild type female twospotted spider mite could reduce population fertility sufficiently to effect control. More sophisticated genetic manipulation, such as conditionally lethal genes (Davidson, 1974) and chromosomal translocations (Asman *et al.*, 1981) have received considerable attention. Conditional lethals could spread into populations during favorable times but induce a genetic load if they inhibit proper diapause initiation or hindered survival at high temperatures. Release of strains with single or double translocations could impose a genetic load on a native population while replacing native genes with those of the released strains. Theoretically, a population of virus transmitting thrips could be replaced with an artificially developed non-vector strain. Support for this approach has declined because of the lack of success stories. However, this method has had important implications in facilitating the mass rearing of the Mediterranean fruit fly in sterile insect technique (SIT) programs. A sex-linked temperature sensitive lethal (tsl) strain of *Ceratitis capitata* has been developed. Since female eggs are sensitive to high temperatures (Franz *et al.*, 1996) rearing conditions are modified so that only males are produced and released in SIT programs.

More recently, researchers have turned their attention to transposable elements (such as the P element in *Drosophila*) with the idea that they could be used in a manner similar to, but more efficient than, the use of translocations. It is theoretically possible that only a relatively small number of insects that had transposable elements (about 10% of the native population) would be necessary to replace the native insect strain (Ribiero & Kidwell, 1994). A number of laboratory experiments with *Drosophila* have shown that a new P element introduced into a population spreads rapidly and is usually fixed in the population within 10 generations. Following this logic, a transposon loaded with a gene for vector incompetence (inability to transmit malaria) could be used to transform *Aedes aegypti* and then this transformed strain could be released into the wild. The native malaria-transmitting strain of this mosquito would then become refractory. The same approach could be taken for insect transmitted plant diseases. This approach is not without its critics (Spielman, 1994). Another mechanism for driving desirable genes into native populations involves the use of rickettsial symbionts in the genus *Wolbachia*. When a male insect carrying *Wolbachia* mates with a female that does not carry *Wolbachia*, no offspring are produced because of cytoplasmic incompatibility. The

reciprocal cross is fertile, so the insects bearing the microorganisms spread through the population (Sinkins *et al.*, 1997). Because *Wolbachia* is maternally inherited, any other maternally inherited trait present in the initial *Wolbachia*-carrying insects will 'hitch-hike' to fixation. With this approach, the insect does not have to be genetically transformed – the focus can be on the genetic alteration of its symbiont creating a paratransgenic insect. This approach appears very promising for the control of Chagas disease in South America. This disease is caused by the parasitic protozoan *Trypanosoma cruzi* and transmitted by insects in the family Reduviidae, subfamily Triatominae, commonly known as kissing bugs. Because these insects feed throughout their entire developmental cycle on vertebrate blood, they harbor populations of symbiotic bacteria in their intestinal track that produce nutrients that are lacking in the insects' limited diet. It is possible to cultivate these bacteria, genetically modify them, and place them back into their insect host, thus generating a paratransgenic insect. This procedure has allowed the expression of antitrypanosomal gene products in the insect gut, thereby resulting in insects that are incapable of transmitting Chagas disease. A method has been developed that would allow introduction and spread of genetically modified symbionts into natural populations of kissing bugs, thus leading potentially to a transgenic intervention tool for use as a part of an integrated vector control approach. (Beard *et al.*, 2002). This approach (development of a paratransgenic insect) is being intensely investigated in California as a potential strategy to combat Pierce's Disease in grapes vectored by the glasswinged sharpshooter.

In addition to its use to develop novel control strategies based on transposons and microorganism-based incompatibility, molecular biology could be used to improve the efficiency of classical genetic control strategies such as the introduction of conditional lethal genes or sex-ratio distortion genes. Once the transformation of a diverse group of insects becomes more routine, it will be possible to transform a variety of species with appropriate genes.

Summary

The rapidly advancing field of molecular biology will likely have a dramatic impact on arthropod and disease control throughout the world, including glasshouse crops. Diagnostic kits are already available for virus detection using polymerase chain reaction (PCR) and this technique is far more sensitive than ELISA and can be just as easy and quick. The advent of transgenic plants with resistance to pathogens will expand the potential for IPM and biological control in the glasshouse. As noted earlier, transgenic plants should never be put into place without the adoption of IPM. Depending on the type of resistance employed, plants engineered for arthropod resistance will need to be evaluated for their compatibility with natural enemies just as new pesticides are screened for compatibility. Finally, we are still on the frontier of developing and utilizing transgenic and paratransgenic arthropods in control programs. The regulatory hurdles that must be overcome prior to any field evaluations are considerable. However, just as the SIT technique has exciting potential in the glasshouse (see Kaspi & Parrella, this volume), the glasshouse ecosystem offers a wonderful opportunity to evaluate some of the concepts and ideas underlying the use of transgenic and paratransgenic insects in control programs.

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Microbial control of greenhouse pests in Belarus

Ludmila Prischepa, Helen Yankovskaya

Belarussian Institute of Plant Protection, Priluki, Minsk region, Belarus 223011, E-mail: prplant@mshp.minsk.by

Abstract: The data on the efficiency of biological preparations based on local strains of entomopathogenic fungi and bacteria against greenhouse pests are stated. The efficiency at different initial number of phytophages is studied.

Key words: biological control, greenhouse pests, two-spotted spider mite, *Tetranychus urticae*, greenhouse whitefly, *Trialeurodes vaporariorum*, entomopathogenic fungi, entomopathogenic bacteria, *Paecilomyces fumoso-roseus*, *Bacillus thuringiensis*

Introduction

The greenhouse pests (onion thrips *Trips tabaci* Lindemann, two-spotted spider mite *Tetranychus urticae* Koch, greenhouse whitefly *Trialeurodes vaporariorum* Westw., cotton aphid *Aphis gossypii* Glov., peach aphid *Myzus persicae* Sulz.) cause significantly harm to the greenhouses vegetable crops in Belarus.

The last years scientists have paid a tremendous attention to the entomopathogenic fungus *Paecilomyces fumoso-roseus* (Wize) Brown et Smith, which is highly pathogenic for some whitefly species (Lindquist, 1996; Sosnowska, 1997; Vidal *et al.*, 1997) and for other greenhouse pests (Kalvish & Rakshaina, 1984; Castenieras *et al.*, 1996; Gindin *et al.*, 1996; Borisov & Uschekov, 1997) and to the entomopathogenic bacteria *Bacillus thuringiensis* spp. (Samersov & Prischepa, 1996).

A technology of preparation of the following natural bacterial and fungal preparations based on local highly virulent strains is developed in Belarus: baciturine – a bacterial bioinsecticide based on *Bacillus thuringiensis subst. darmstadiensis* and paecilomycine-B – a fungal bioinsecticide based on the entomopathogenic fungus *Paecilomyces fumoso-roseus*. The results of research on the evaluation of baciturine and paecilomycine efficiency in relation to main pests of protected crops are given in this report.

Material and methods

Evaluations of the efficiency of biological preparations were carried out in the Zhdanovich commercial greenhouse (Minsk region). Crops were cucumbers and tomatoes. The technology of cultivation is small-volume hydroponics on mineral substrate. The preparations were applied by surface spraying method.

Pest number records were done on 25 leaves taken evenly from 25 plants of every variant. Records were done before treatment and 3, 7, 10, 14, 21 and 29 days after the start of trial.

The biological efficiency of the preparations was determined taking into account the phytophage number change in the control.

Results and discussion

Research on evaluation of bacitaurine efficiency for two-spotted spider mite control was carried out in the Zhdanovichy greenhouse on cucumbers (cv.NIIOCH). The pest number on cucumber before treatment was 267 individuals per 1 record leaf. Bacitaurine was used in 2% concentration three times with 3 days interval. The biological insectoacaricide phytoverm (0.1%) was used as a standard. The biological efficiency in the variant with bacitaurine application on the third day after the second treatment was 98.1%, phytoverm – 100% (Fig. 1).

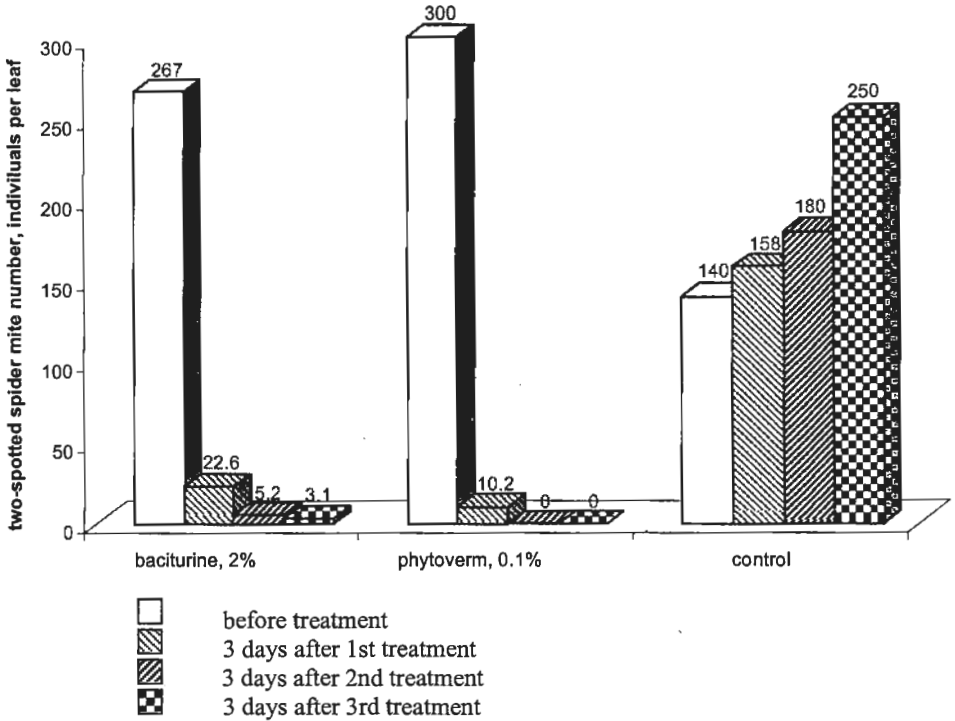


Figure 1. Bacitaurine treatment influence on two-spotted spider mite number.

The pest number on tomatoes (cv. Maeva) before treatment was 4 individuals per 1 record leaf. The preparation was used in 1% concentration two times with 3 days interval. The pest death on the third day after the first treatment was 50%, after the second – 100%.

As a result of search in natural biocoenoses 11 local *Paecilomyces fumoso-roseus* strains were isolated. The evaluation of resistance of greenhouse pest number (greenhouse whitefly *Trialeurodes vaporariorum* Westw., two-spotted spider mite *Tetranychus urticae* Koch., cotton aphid *Aphis gossypii* Glov., leaf-miner fly *Liriomyza* sp.) to *P. fumoso-roseus* was carried out.

By the results of the evaluation of insecticidal activity on the laboratory test-object (wax moth caterpillars *Galleria mellonella* L.) and then on pest species (greenhouse whitefly larvae), *P. fumoso-roseus* 3/1 strain was selected. The experimental samples of microinsecticidal preparation based on *P. fumoso-roseus* 3/1 strain were prepared by cultivation on solid substrate. This samples were tested under greenhouse conditions against whitefly.

The efficiency of a fungal application was evaluated at different initial pest number levels. The results of testing showed that the application of the entomopathogenic fungus at the initial stage of plant colonization suppressed the pest number. By two times application of *P. fumoso-roseus* (the working suspension 1.5 mlrd. conidia/ ml) after single whitefly imago appearance there was no pest on record leaves in the course of two weeks.

Three times treatment by a fungus (1.5 mlrd. conidia per ml, 7 days interval) promoted pest number stabilization at the same level, when the initial pest number was 10-23 larvae per one leaf (Fig. 2). An increase of larvae number was observed in the control variant and on the 29th day whitefly number was two times more then the same in the experimental variant.

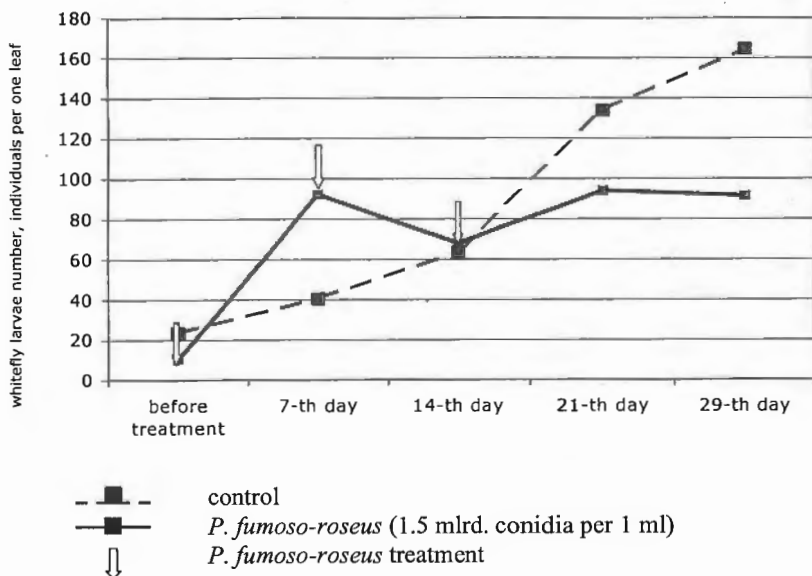


Figure 2. *P. fumoso-roseus* treatment influence on greenhouse whitefly number.

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Pest occurrence and control in organic year-round production of chrysanthemums

P.M.J. Ramakers, R.H.M. Maaswinkel

Applied Plant Research, division Glasshouse Horticulture, P.O. Box 8, NL-2670 AA Naaldwijk, The Netherlands, E-mail: P.M.J.Ramakers@ppo.dlo.nl

Abstract: Organic growing of chrysanthemums was continued for three years without using synthetic insecticides. A complex of 4 aphid species and Western Flower Thrips were the prevailing problems. The lack of natural insecticides for controlling these pests was the bottleneck for compiling a biological control program. Available botanical insecticides are not selective enough for being used in combination with natural enemies. Accepting new microbial insecticides would make organic production feasible for organic growers.

Key words: organic, chrysanthemum, pest, aphid, thrips

Introduction

Organic production is rarely applied in commercial greenhouses. Ideologically motivated consumers may reject greenhouses for being 'unnatural', or at least the energy input associated with intensive production. Mainstream consumers may be willing to pay an adequate price for edible products of organic origin, but hardly for ornamentals.

The Dutch Ministry of Agriculture has therefore decided to stimulate integrated production as a more realistic option for the vast majority of growers in the near future (Anon., 2001). Still, support of organic growers was defined as a secondary policy, aiming at an acreage of 10% organic for the total Dutch agriculture in the year 2010 (Anon., 2000). Growers' organisations consider this an unrealistic figure, while representatives of the organic movement fear that this aim will be achieved by stretching definitions rather than by changing practices.

Organic production of vegetables is already practised by a handful of glasshouse growers. Our institute was asked to study the feasibility of organic production in ornamentals. Chrysanthemum was chosen as a pilot crop, not just because of its economic significance, but especially because this is one of the few crops still grown in soil, which is a prerequisite in organic farming. The project has been running since May 1999 without interruption, and appears to be continued for another three or four years. At the time of this conference the 11th growing cycle has been completed.

Material and methods

Facilities

A glasshouse of about 750 m² was divided into 3 plots. The situation in a commercial year-round production was simulated by replanting one plot each month. With a growing cycle of 3 to 4 months, this means that at least 2 plots were covered with plants at all times. Noxious, beneficial as well as indifferent insects were thus free to move from the old plants to the new ones and *vice versa*, as in a commercial operation. On a total of about 30,000 plants, 24 plots

of 100 plants were used to compare the suitability of chrysanthemum cultivars for organic growing.

The soil had been steam-disinfected a few months before the start of the project, but this was not repeated since. Ventilators were covered with 400 mu screens to reduce insect immigration.

An insect-proof propagation house with an ebb-and-flood system on a concrete floor was available for rooting the cuttings without using insecticides. Theoretically, insects as small as thrips could penetrate this house through the screened ventilators. However, the numbers of thrips actually observed were close to zero, and were negligible compared to the numbers permanently present in the main compartment. Cuttings were acquired from outside, and might thus have contained some pesticide residues.

Fertilisation

Following the directions of SKAL for organic farming (Anon., 1999), plant nutrition was based on organic and 'natural' materials only. A base dressing with a mixture of manure and compost was given 2 times a year, and top dressings with blood-meal and potassium sulphate before each planting.

Growth regulation

Synthetic growth regulators used to reduce the size of the flower stalks (actually the length of the internodes) are not acceptable for organic farmers. Alternative methods used in this project were (in order of decreasing efficacy) a temperature drop at the end of the night (Cuijpers & Vogelesang, 1992), cultivar selection and (occasional attempt) frequent shaking of the plants.

Disease control

Problems with *Pythium* were avoided by minimising the water-supply at the start of each planting. Young plants were watered only after showing the first signs of withering. This method was effective, but at the cost of some reduction of the initial growing rate. White rust was successfully prevented by avoiding condensation on the leaves via appropriate climate control.

Pest control

For controlling thrips, the predatory mite *Amblyseius cucumeris* was introduced twice on each planting, once on the cuttings in the propagation house and once 4 weeks after transplanting. Occasionally *Orius laevigatus* was introduced shortly before flowering.

For aphid control, the parasitoids *Aphidius colemani* and *Aphidius ervi* and the predator *Aphidoletes aphidimyza* were released when necessary. Populations of these natural enemies were supported continuously by offering cereal aphids on wheat seedlings as substitute hosts.

Encarsia formosa or (in summer) *Eretmocerus eremicus* were introduced whenever whiteflies were recorded by yellow sticky traps, which was seldom the case.

Insecticides of botanical or microbial origin were used for controlling pest outbreaks, mostly against aphids and often against thrips.

Results

Western flower thrips, *Frankliniella occidentalis*, was the most persistent pest. Numbers in winter were often on a level that was considered acceptable for organic farming, but too high in summer and autumn. Aphids were sometimes absent, but if present the most difficult pest to

control. Four species were encountered: *Aphis gossypii*, *Aulacorthum solani*, *Myzus nicotianae* and *Brachycaudus helichrysi*. Aphids and thrips were the only species for which interference with insecticides was necessary. Three control programs were tested:

I. Chemical control in propagation house, biological control in production house

Since organic production of chrysanthemum as a cut flower is not practised, it is difficult for growers to acquire pesticide-free cuttings. SKAL regulations therefore accept the use of 'regular' cuttings for the time being. Using this 'escape', the roots of the cuttings were drenched into a solution of the systemic insecticide imidacloprid just before transplanting. In this way, a rather persistent aphid control was obtained with minimal use of insecticides and little effect on the natural enemies operating in the production house (van de Veire *et al.*, 2002a). If aphids were found in the last weeks of the production, a final treatment with Spruzit was applied on that plot only in order to deliver an aphid-free final product. Usually, this treatment also sufficiently suppressed thrips.

This program was found both convenient and compatible with the use of natural enemies. However, it is to be expected that in future SKAL will forbid the use of insecticides while the cuttings are rooting and even demand that the mother stocks are grown organically. Therefore, for organic farming program no. I offers an only temporary solution.

II. Repairing sprays with botanicals

Few insecticides are both registered and acceptable for organic farming. In the Netherlands Spruzit (pyrethrine + piperonylbutoxid) is the only botanical registered, and in some other European countries also NeemAzal (azadirachtin) is available. Program II was based on supervised control, using Spruzit in case of aphid outbreaks and NeemAzal when thrips numbers surpassed the action threshold.

This program was moderately effective, but resulted in a rather high spraying frequency, often higher than in well-supervised traditional growing. Besides, there was little evidence of any remaining activity of the natural enemies being introduced. Hence, this program was considered in line with the regulations but conflicting with the spirit of organic farming.

III. Repairing sprays with botanical and microbial insecticides

Some insecticides are produced with microorganisms. Products based on *Bacillus thuringiensis* are widely accepted by organic farmers. There is no logical reason why modern insecticides like avermectine (*Streptomyces avermitilis*) and spinosad (*Saccharopolyspora spinosa*) should not. In this program we assumed that such components may become accepted in the near future by the more rational type of organic farmers appearing on the market.

Neither *B.t.* nor avermectine were actually used, since the target pests (noctuids resp. spider mites and leafminers) did not occur in this project. Spinosad, which has a good IPM profile (van de Veire *et al.*, 2002b), provided excellent thrips control. Aphid outbreaks were controlled with Spruzit. The lack of selectivity of this compound was the main drawback of program III.

Discussion

Lack of efficient and selective insecticides is the Achilles' heel of organic farming. Accepting modern microbial insecticides should be considered. It is not clear whether this would conflict with a somewhat obscure rule in today's SKAL regulations: "Materials should not be purified in an unacceptable way".

It is somewhat precarious to draw generalising conclusions from the ABSENCE of pests in a project like this one. The absence of noctuids, tortricids, leafminers and mirids might be

explained by the hygienic standards including the use of insect-proof screens. The control programs would have been highly complicated by more 'difficult' pests than the ones observed, like *Spodoptera exigua*, tomato spotted wilt virus or symphylids. This risk seems much higher in a big commercial operation. Since the tools for eliminating such pests are not available, compartmentalising, preferably with each planting in a separate compartment, is therefore a wise (but expensive) recommendation for organic farming.

No control agents are available for soil-borne pests like nematodes and symphylids. The fact that these problems did not occur in the present study may be explained by the nature of the local soil, and again should not be generalised. Accepting soil steaming in organic farming is under discussion, since it conflicts with the current ideas about maintaining biological stability. Allowing steam treatments after proven necessity may be a workable compromise.

Spider mite control constitutes a considerable problem for commercial chrysanthemum growers. The absence of any problem with spider mites in this project was an aspect that drew considerable attention from the supervising committee. Since this greenhouse has been used for growing chrysanthemums since it was built, it seems unlikely that this was just a coincidence. The explanation of this phenomenon will be subject of further studies.

Spontaneous occurrence of predatory flies of the genus *Coenosia* may have contributed to the control or absence of slowly flying insects such as leafminers and whiteflies.

Comparing available cultivars did not produce encouraging results. Since organic farmers will probably have to tolerate some thrips on their crops, it seems wise to avoid varieties that are particularly susceptible to deformation of young plant parts caused by thrips. However, finding varieties that are suitable for being cultivated without chemical growth regulators is a much higher priority than insect resistance.

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Current status of biological control of diseases in greenhouse crops – a commercial perspective

Willem Ravensberg¹, Yigal Elad²

¹Koppert Biological Systems, R & D Microbials, P.O. Box 155, 2650 AD Berkel & Rodenrijs, The Netherlands, E-mail: WRavensberg@koppert.nl; ²Convenor, Phytopathogens Working Group at the IOBC WPRS, Department of Plant Pathology, The Volcani Center, Bet Dagan 50250, Israel

Abstract: Control of plant diseases in greenhouse crops is still largely carried out by chemicals. A number of microbial pesticides, however, have been developed and are starting to penetrate the market. An overview of the most important products is given. The development and success of these products are highlighted with regard to the market, the biopesticide industry and governmental factors with emphasis on registration.

The future prospects for microbial fungicides are discussed from the industry point of view.

Key words: biological control, biopesticides, commercialisation, disease, market, registration

Introduction

It is still early days for biological control of plant diseases in protected crops. Relatively few products for managing diseases have been developed, registered and successfully marketed, despite a great deal of research on biological control of diseases. Biological control of insects and mites, on the other hand, has enjoyed more than thirty years of success. The initial use of natural enemies such as *Phytoseiulus persimilis* and *Encarsia formosa* that were not compatible with broad-spectrum insecticides stimulated research and development of additional natural enemies. Today over forty beneficials are commercially available and play a key role in the control of arthropod pests in glasshouse crops world-wide.

Chemical fungicides have been used to treat diseases, soil-borne as well as foliar, for much of the twentieth century and still dominate the market today. As their mode of action is targeted to bacteria and fungi, their compatibility with beneficial insects and mites is generally satisfactory and there has been no real need to develop biological products for disease control. Nor has there been significant pressure from the market to develop alternatives (Elad, 1990). Recent changes in the regulatory climate may stimulate some uptake of alternative products, however.

The working group of Biocontrol of Fungal and Bacterial Plant Pathogens at the IOBC WPRS was established in 1991 in order to promote the implementation of biocontrol of plant diseases. It deals with biocontrol of plant diseases at research, extension and industry levels. During the decade of activity the subject was followed and discussed and it is evident that in the recent years more and more commercial products are presented and followed either on a research level or on commercial basis (Elad *et al.*, 2001).

This article will discuss the status and commercial future of microbial products as seen from an industry point of view. A distinction is made between products based on micro-organisms and products based on natural components. The potential for these products will be considered in light of market incentives and governmental policy. Finally, the question of

whether there can be any real progress expected in biological control of diseases will be addressed. For an excellent overview of biocontrol of diseases from a research perspective, see Paulitz & Bélanger (2001).

Microbial disease control products

Only a small number of products has actually been registered and are commercially used by greenhouse growers. Below the most important microbial disease control products, defined as products based on a micro-organism, are given with some specific information on their importance and registration status. Only the products that are viable for the North American and the European – Mediterranean regions are discussed. For many products it is difficult to find detailed information as the producer is a very small company. Often, these products are not registered and are only sold locally. In addition it is hard to distinguish between “good” products and products of “lower benefit” to growers. Data is not always available to back up the claims on the packaging or advertised claims. Most likely there are valuable products in this category, but to investigate this is not an easy task. Therefore, they have been left out of this overview. More information on microbial pesticides is given by Butt *et al.* (1999), Fravel *et al.* (1999) and Paulitz & Bélanger (2001).

AQ10, produced by Ecogen Inc, USA is based on *Ampelomyces quisqualis* (strain M-10) and sold as a biofungicide for the control of powdery mildews. It is used in grapes, and many other crops, including greenhouse crops. It is registered in the USA, pending for inclusion in Annex I in Europe and provisionally registered in Italy. The use in protected crops is still restricted.

Binab, produced by Binab Bio-Innovation AB, Sweden, is based on *Trichoderma harzianum* and *T. polysporum* and sold as a biofungicide for control of diseases in field crops (strawberries) and greenhouse crops: tomatoes, cucumbers and ornamentals. It was the first biofungicide registered in an European country, *i.e.* in France in 1976 for wounds on trees. In 1985 it was registered in Sweden for use in greenhouse crops, followed by Denmark. It is aimed at soil-borne fungal diseases in greenhouse vegetables, ornamentals and other crops, as well as *Botrytis* in strawberries. In this crop bumblebees are used to vector the antagonists to the flowers and many growers are using this technique. There are four formulations and these are sold as a plant growth enhancer, a biofungicide and a soil amendment.

Contans, produced by Prophya GmbH, Germany is based on *Coniothyrium minitans* and sold as a biofungicide for control of *Sclerotinia sclerotiorum*, *S. minor* and *S. trifoliorum*, in high value agricultural and horticultural crops. In greenhouses it can be used in lettuce, celery, beans, tomatoes, cucumbers and ornamentals. The registration is pending in the EU as a new active ingredient, and it has been sold in Germany on a preliminary registration since 1997 for lettuce in greenhouses. Registration is expected soon in some other EU countries once the A.I. will be placed on Annex I. It is registered in Hungary, Poland, Switzerland and the USA.

Mycostop is probably the best-known commercial biological fungicide for greenhouse crops. Produced by Kemira Agro Oy in Finland, it has been on the market since 1990 and is available in the greatest number of countries. This product is based on *Streptomyces griseoviridis* (K61) and sold for control of damping-off, root and stem rot diseases in greenhouse cucumbers, tomatoes, peppers, lettuce, and ornamentals. While it mainly targets

Fusarium, it also controls or suppresses *Pythium*, *Phomopsis*, *Rhizoctonia*, *Phytophthora*, *Botrytis*. It can be used as a seed treatment, on seedlings and in the crop itself.

It is registered in Bulgaria, Canada, Denmark, Estonia, Finland, Hungary, Iceland, Italy, Latvia, the Netherlands, Norway, Russia, Spain, Sweden, Switzerland and USA.

Plantshield/Rootshield, produced by Bioworks Inc., USA, is based on *Trichoderma harzianum* (T22) and sold as a biofungicide in the USA for control of damping-off diseases and root diseases in greenhouse and other crops for control of *Fusarium*, *Rhizoctonia*, *Pythium* and others. It is registered in the USA and registration is pending in Canada. There are two formulations, a wettable powder and a granulate, to be mixed with the growing medium. The same product is called *Trianum* in Europe, and will be distributed by Koppert BV and sold as a plant strengthener in greenhouse vegetables and ornamentals. Registration is pending in a number of European countries. It is already available in Spain, the UK and Norway and has been sold previously in some other countries as TRI 002 and 003. Plantshield/Rootshield is probably the best selling biofungicide at the moment.

Polyversum, produced by Biopreparaty, Czech Republic, is based on *Pythium oligandrum* and sold as a defence inducer against diseases in crops such as grapes, wheat, hops and vegetables. It is also a plant growth promoter. It has been available for six years in a number of European countries, but its registration status is unclear.

Prestop, produced by Kemira Agro, Finland is based on *Gliocladium catenulatum* (J1446) and is sold as a biofungicide for the control of *Pythium*, *Rhizoctonia*, *Phytophthora* and for foliar diseases, such as *Didymella bryoniae* in cucumber. It has been registered in the USA since 1999 and is pending EU Annex I inclusion as a new active ingredient. It is already sold in Finland since 2001 based on a preliminary approval. This product will replace Gliomix.

Serenade, produced by AgraQuest, USA, is based on *Bacillus subtilis* (QST 713) and sold as a biofungicide for control of many soil-borne and foliar fungal and bacterial diseases in field and protected crops. It is a preventative product that needs to be applied as a foliar spray. It is registered in the USA and in the EU the active ingredient is pending inclusion in Annex I.

SoilGard, produced by Certis USA, USA, is based on *Gliocladium virens* and sold for control of *Pythium*, *Rhizoctonia* and *Fusarium*. It is registered in the USA.

Taegro, produced by FZB Biotechnik GmbH in Germany and distributed by Taensa Inc., USA and various Bayer subsidiaries in Europe is based on *Bacillus subtilis* (FZB 24) and sold as a plant growth enhancer, suppressing also *Rhizoctonia* and *Fusarium* on many crops, including greenhouse crops. It is sold in Germany and the USA (registered) as a plant strengthener and is pending registration in some other European countries.

Trichodex, produced by Makhteshim Agan, Israel, is based on *Trichoderma harzianum* (T39) and sold as a biofungicide for control of *Botrytis cinerea* in grapes and greenhouse crops. It is also reported to be active against *Sclerotinia sclerotiorum* and some more diseases. It is registered in Denmark, Germany, Greece, Hungary, Israel, Italy, Romania, USA, several other countries in the Southern Hemisphere, and pending in the Netherlands.

Natural disease control products

An endless list can be made of other products claiming disease control. These products are based on many different compounds and are here called natural control products. Often research and registration approval is lacking and these products are usually sold directly to growers by very small companies. It is therefore very difficult to know what is on the market and the range of products and names is constantly changing. They are used for control of foliar as well as soil-borne diseases, and sometimes even against insects. These natural control products can be based on one of the following groups of components or even on every thinkable combination(s) of them: algae, enzymes/proteins, milk (extracts), minerals, mixtures of bacteria (incl. metabolites) and/or fungi, oils (mineral, vegetable or essential), plant or seed extracts, salts, soaps/fatty acids, sugars, sulphur and vitamins. Claims connected to these products can be: plant strengthener, plant-growth promoter, soil-improver, induced resistance, etc. It is impossible to give a detailed overview of these products. We have tried this once in the Netherlands and over 150 products were found. Most of them, however, are not accompanied by any serious information on (active) ingredients, mode of action or results.

Some of them, however, have been developed based on extensive research and could be useful products for control of diseases. As an example, *Milsana*, a plant extract from *Reynouria sachalinensis* can be mentioned. This was developed by the BBA in Darmstadt, Germany. Surely, many more products could be developed into good and reliable tools for growers, but it is a difficult area in which to get institutional research financed. Registration and results in the field will ultimately sieve out the useful products.

The biopesticide market and industry

The world market for pesticides is about \$ 30 billion and of this less than 1% is biopesticides, which is about \$ 300 million. This includes all non-synthetic chemicals, such as microbial pesticides, beneficial arthropods, natural pesticides and pheromones. In microbials, most of this is accounted for by *Bacillus thuringiensis* (B.t.) for caterpillar and mosquito control. In Europe in 2000, sales of biopesticides including beneficials, microbial pesticides and pheromone products were \$ 97 million. This is about 2% of the total European pesticide market. Sales of microbials were \$ 25 million, representing 26% of the total biopesticide sales in Europe. Most of the biopesticides are used in countries which export vegetables, fruit and ornamentals. In the category of microbials also "soft" pesticides are included, such as fatty acids (Frost & Sullivan, 2001). The authors state that the new registration procedure in the EU has severely limited the availability of microbials, whereas B.t.'s were already available before 1993 and did not suffer from this difficulty. They expect a yearly growth of 11.7 percent leading to about \$ 210 million in 2007 for all biopesticides. The beneficials sector has attained a high percentage of saturation, so it is obvious that an increase of sales of microbials is expected. Jarvis (2001) estimates the global biopesticide (real microbials) market at \$ 160 million in 2000 and stated that over 90% of this was accounted for by B.t.'s. This leads to about \$ 16 million for microbial pesticides world-wide, excluding B.t. sales.

If we compare the figures from Frost & Sullivan - \$ 25 million for all microbials, incl. B.t., in Europe - with Jarvis - \$ 16 million for all microbials world-wide excl. B.t. (ca. 90%) - and try to estimate the non-Bt microbial pesticide market for Europe (10-20%), this might be between \$ 2.5 - \$ 5 million. Still it is a very small amount, and considering it is the total of ca. 20-30 microbials (viral insecticides, fungal insecticides and fungal / bacterial fungicides) one can see that the turnover per microbial is very low. On top of this, these sales are spread over a number of countries for most products, giving small turnovers per country and

relatively high marketing costs. Taking into account development costs and registration costs it is apparent that the biopesticide industry is having a hard time.

Jarvis (2001) estimated development costs for a microbial around \$ 3 million, and if registration costs of at least \$ 0.5 million are added, total costs will come to about \$ 4 million. So development costs of a product are around 10 – 20 times higher than a yearly turnover. It is easy to see that profitability on microbials is difficult to reach, if not impossible at the moment. This is reflected by the failure of many companies who have started in this business. Lisansky (1997) mentioned that many have tried, more than 175 over the last twenty years, and that few succeed. This is partly due to faulty estimations on the development costs and time involved before a product will generate some return of investment, and partly due to a mistaken perception of the market.

At the moment about 35 companies in Europe produce and/or sell microbial pesticides. Many of these are very small companies and provide local markets with their products. Often there is no registration approval for these products or the local legislation allows the sale of them under specific conditions. In the EU context, however, this is not allowed and with the notification of microbials in 2002, most of these products will either have to apply for registration or will no longer be allowed.

If we look at the agro-chemical industry we have seen a strong consolidation of the industry with few companies still being active in that sector. A similar trend may be expected for companies involved in microbial pesticides. Is this a maturation of the industry or survival strategy? It is probably both and inevitable for the microbial industry.

Some microbials may do a better job if production costs are low, shelf-life is long, efficacy is good and the application simple. Microbials do have a number of drawbacks compared to chemicals, which are hard to overcome. These include a higher price, lower effectiveness, more complicated use, narrow spectrum and dependency on environmental conditions. B.t.'s and, to some extent, viruses come the closest to chemicals which may explain their success. Perhaps the market is not yet ready for microbials and only if real incentives develop will microbials be able to become profitable alternatives for the biopesticide industry and growers.

Registration and governmental policy

Registration procedures and costs are clear hurdles for the development of biological pesticides. Considering the market for a product and the development costs, as discussed above, it is obvious that registration costs are often too high to justify the investment. On top of this, procedures are not very clear and it is hard to estimate the costs involved up front.

In the EU there are 17 micro-organisms registered as plant protection products. These were on the market before July 1993, the date of the enforcement of the EU Directive 91/414, and are considered as "old" active ingredients. If a micro-organism is not on that list of 17 it is considered a "new" active ingredient and registration has to take place through the EU Directive 91/414. The first one to be applied for was *Paecilomyces fumosoroseus* in 1994, even though the requirements for microbial pesticides only recently were established.

P. fumosoroseus was recently included in the Annex I – the list of approved new A.I.'s in the EU. Six others are waiting for inclusion also.

In the USA there are 58 micro-organisms approved and the procedure here is much clearer and faster than in the EU which is indeed reflected in the number of approvals. Another very important difference between the two procedures is efficacy. In the EU almost every claim of disease control has to be proven by many official trials for each crop and for

each disease. In the USA this is not required and this makes a registration much cheaper and faster.

As a consequence of the high registration costs we see many products on the market in the EU that are used illegally, without any registration. These are microbial as well as natural plant protection products. Some countries have a separate regulation in which products are more or less just notified and then approved for use on crops. In Germany about 170 products are on this list and widely used. Austria has something similar and in the Netherlands some products are also exempt of registration, although these are primarily used in the home and garden sector. Other EU countries have again other definitions in their pesticide legislation, which sometimes allow the sale of non-registered pesticides, depending on the claim. Also submission and evaluation costs differ enormously between countries. Recently the UK increased the fee for a microbial from £ 13,000.-- to £ 40,000.--. So even today within the EU, registration legislation is far from harmonised.

There are attempts within the OECD (Organisation of Economic Co-operation and Development, The Pesticide Forum Working Group) to harmonise data requirements for microbials and pheromones. An EU attempt to set data requirements on a category "plant strengtheners" is on hold because of disagreements between countries on this issue. So we can conclude that registration is still a very difficult part of launching a microbial, despite all political rhetoric to stimulate the use of them. Many governments are now and already for more than over ten years striving to reduce the use of chemicals and the dependency on them for well-known reasons. Success has been achieved in reducing chemicals, with insecticides and soil-disinfectants. But if we look at fungicides, no real reduction has been achieved.

Only if governments develop some real tools to stimulate the development and registration of microbials might things change: for instance, lower and harmonised data requirements, lower submission and evaluation costs, faster procedures, and subsidies to stimulate companies taking risks in this field. Communication with the registration authority is often difficult, unless an application is submitted. In the Netherlands this bureaucracy was overcome by creating a "Helpdesk" and this certainly facilitates communication in advance of a real submission.

The data on efficacy can be reduced, which will make a big difference in regard to costs. A microbial will perform in a certain environmental situation and/or climate and may not have to be tested in each crop. We suggest testing major crops/sectors and extrapolating to a broader spectrum of application. In the USA no efficacy data are needed, but this leads to label recommendations that are not really supported by the product's ability. An in-between set of data seems a reasonable compromise for producers as well as regulatory bodies and will give users a proper idea on product performance.

Future perspective

Is the future really so bright for commercial microbial products? If the industry wants the products to succeed, they will have to be competitive with existing chemicals. As long as conventional fungicides are cheaper than microbial disease management products, they will continue to dominate the market for pesticides. Efficacy and user-friendliness must be improved if we are to move beyond the niche markets. There is a need to improve our marketing as well, and convince both the grower and governments that these products are desirable. Part of the larger problem is declining returns in agriculture. Growers need to receive better returns for their product if they are going to spend more capital on their inputs, such as more biological and natural solutions for their pest and disease problems.

It is unlikely that the success of beneficial arthropods will be followed by similar trends in microbial products. A much longer lead time and greater investments preclude this. Moreover, the major producers of natural enemies are barely achieving profitability and development potential is limited. Also, many big players in the agro-chemical world have invested significant amounts of capital in this market and have largely withdrawn. The microbial pesticide industry is learning from the hard lessons of the past. Consolidation is foreseen which will hopefully lead to some long-lasting successful products and companies.

Collaboration between scientists is crucial in order to develop these products and contacts between these two groups are necessary from the outset of the development of a microbial. A good example is given by Paulitz & Belanger (2001) for the development of a powdery mildew control product, based on *Pseudozyma flocculosa*. In order to improve the prospects for implementation of biological disease control there is a need for intensive research in subjects that will assist the minimising the inconsistency and maximising the efficacy of microbial biocontrol agents. This includes the study of integration of biocontrol agents among themselves as was done for biocontrol of *B. cinerea* (Guetsky *et al.*, 2001), taking into account the effect of environmental factors on the activity of biocontrol agents and integration with other control strategies. It is likely that in the future we will face microbial products with longer shelf life, better survival in the plant environment and higher efficacy. Much has also been learned looking at the chemical world regarding formulation and marketing of new products.

Governments have a central role to play, in ensuring a level playing field in terms of registration. Scientists need to speak out forcibly against continuing cuts in research and development in the public sector. And industry has to improve its communication with all stakeholders and develop reliable microbial products. Real progress nevertheless, will only occur when registration is harmonised and the market demands non-synthetic chemical solutions for the production of food.

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Fecundity and survival of mass reared *Phytoseiulus persimilis* (Acari: Phytoseiidae)

David A. Raworth¹, Susan Bjørnson²

¹BBA, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany, E-mail: raworthd@EM.AGR.CA; ²University of Gent, Department of Crop Protection, Laboratory of Agrozoology, Coupure Links 653, B-9000 Gent, Belgium

Abstract: Short-term fecundity and survival of *Phytoseiulus persimilis* Athias-Henriot were determined on receipt of product from six commercial sources, and after rearing in the laboratory for 30 days (4 generations). In 5 day trials mean fecundity and survival on receipt were 1.6±0.09 eggs per female per day and 4.7±0.05 days, respectively (74% of the females survived to Day 5). After 30 days in laboratory cultures, these values were 1.7±0.12 and 4.2±0.11, respectively (62% of the females survived to Day 5). During lifetime trials, fecundity was about 4 eggs per female per day from Day 2 to 14 (days after mating), after which it declined steadily until Day 23. Survival was close to 100% for 6 days and then declined steadily to 0 at Day 26. The results of a simulation model incorporating these data suggest that there is a biological potential for improved efficacy against spider mites if fecundity and survival of *P. persimilis* released in a greenhouse were closer to that measured for young females.

Key words: *Phytoseiulus persimilis*, fecundity, survival, model, efficacy

Introduction

During the last 10 years, considerable effort has been directed at developing quality control guidelines for *Phytoseiulus persimilis* Athias-Henriot (Steinberg & Dale, 1998). The recommended standard for fecundity and survival given a specific protocol is ≥10 eggs per female per 5 days and at least 80% survival ($n = 30$). We adapted the protocol slightly in order to study the relationship between fecundity and abdominal discoloration in stocks from four insectaries (Bjørnson *et al.*, 2000). Mean fecundity was 8.7±0.2 (SE) eggs per female per 5 days (range between insectary stocks, 6.8 to 10.6) and survival was 50% at Day 5 (range between insectary stocks, 39% to 54%) ($n = 563$). Our laboratory estimates of fecundity and survival appeared low considering the following three points. First, the age distribution of the adult female predators in a vigorous culture should be skewed towards young females. Second, many age specific oviposition curves in the literature reveal peak fecundity in young female *P. persimilis* above 3 eggs per day (for example: Amano & Chant, 1977, at 23°C; and McClanahan, 1968, at 20°C). Third, Sabelis (1981) showed that starvation, which occurs during collection and shipment of *P. persimilis*, did not affect total eggs produced by a female as long as the female is less than 50 days old.

The following describes studies aimed at determining why Bjørnson *et al.* (2000) observed low estimates of fecundity and survival for *P. persimilis* shipped from insectaries. We conducted a 5 day fecundity trial on material from six commercial sources. We then reared the predators for 30 days (4 generations) in the laboratory, repeated the 5 day fecundity trial, and determined lifetime fecundity and survival, rearing predators from the egg stage. Laboratory rearing eliminated several insectary procedures, including starvation of females during the collection phase, handling, storage, and shipping. We expected the fecundity of *P. persimilis* on receipt of a shipment would be low (mean < 10 eggs per female per 5 days), and

fecundity of laboratory reared females would be greater. Also, for laboratory reared females, we expected no difference between daily fecundity of young females in the lifetime trials and daily fecundity in the short term trials.

Material and methods

Mite rearing

Tetranychus urticae Koch were collected from shade houses at the Pacific Agri-Food Research Centre (Agassiz, BC) and reared on 4 week old bean plants within cages in an isolated rearing room (16L:8D; $25 \pm 2^\circ\text{C}$; $170 \mu\text{Es}^{-1}\text{m}^{-2}$; 50% RH).

Phytoseiulus persimilis was received from six commercial sources. A colony from each source was reared using the flotation method in a sealed rearing unit on detached spider mite infested bean leaves, the petioles of which were immersed in water. Rearing units were made from styrofoam shipping containers (40.0 cm L x 24.3 cm W x 13.2 cm H). A 12 cm L x 4 cm W opening was cut in each lid and covered with screen to facilitate air movement. A thin bead of Vaseline® petroleum jelly was placed 1.5 cm above the water level so that mites could not crawl up the sides of the container and escape.

The colonies of *P. persimilis* from different sources were reared in a growth chamber in separate, sealed containers (chamber conditions: $25^\circ\text{C}:20^\circ\text{C}$; 16L:8D; $107 \mu\text{Es}^{-1}\text{m}^{-2}$; 40% RH; internal container conditions: 20 to $25^\circ\text{C} \pm 0.5^\circ\text{C}$; 50% mean RH (range: 40 to 60%)). Colonies were started by placing 25 gravid *P. persimilis* females on infested leaves within each container, 30 days prior to being used in fecundity trials.

Experimental protocol

On receipt of a product from an insectary, 50 gravid female predators were selected at random. Each was placed in an observation dish (54 mm dia.) with a spider mite infested leaf disc (27 mm dia.) (for details see Bjørnson & Keddie, 1999). Dishes were placed in a large, plastic container within a growth chamber ($25^\circ\text{C}:20^\circ\text{C}$; 16L:8D). The predators were checked once daily for 5 days: eggs were counted and removed; survival was noted; and leaf discs were replaced as needed to maintain excess prey. The same procedure was followed 30 days later, randomly selecting 30 gravid female predators from each of the six colonies and observing fecundity and survival over a 5 day period.

After being reared for 30 days, lifetime fecundity and survival were determined for 22 females from one colony and 18 from another. Adult female predators were randomly chosen and placed individually on a spider mite infested leaf disc within an observation dish. Eggs were collected after 24 h. Each egg was isolated on a spider mite infested leaf disc and observed daily. Newly hatched immature mites were sexed on the Day 6. On Day 7, a single male and female were isolated on a fresh leaf disc for 24 h. The male was then removed. Leaf discs were replaced every 4 days. Fecundity and survival were assessed daily.

Results and discussion

Fecundity estimates for *P. persimilis*, on receipt of a shipment, were consistent with those in a previous study (Bjørnson *et al.*, 2000), but they did not improve when the predators were selected from a laboratory colony maintained for about 30 days ($P > 0.05$) (Fig. 1a). Mean fecundity on receipt and after 30 days was 1.6 ± 0.09 and 1.7 ± 0.12 , respectively. Likewise, survival was low on receipt, but it was less, ($P < 0.01$) when females were taken from the laboratory colony (Fig. 1b).

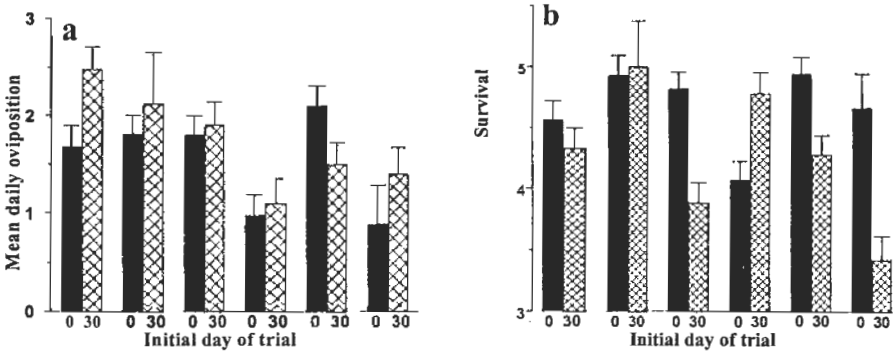


Figure 1. (a) Mean daily fecundity and (b) survival of *Phytoseiulus persimilis* (+SE) from six commercial sources, on receipt of product (Day 0) and after 30 days in rearing.

Mean survival on receipt and after 30 days was 4.7 ± 0.05 and 4.2 ± 0.11 days, respectively; survival to Day 5 was 74% and 62%, respectively. Age specific fecundity and survival, determined after 30 days were much higher than observed in the 5 day trials, and matched that expected from the literature (Fig. 2a).

The 5 day fecundity trials in this and previous studies (Bjørnson *et al.*, 2000), provide a consistent picture of what a grower could expect from predators released into a spider mite infestation. Egg production for 4 to 5 days will certainly initiate a population of predators, and subsequent generations should have fecundity and survival equivalent to that observed in the lifetime trials. However, a simple simulation of the predator-prey interaction suggests that efficacy would improve if fecundity and survival of the introduced predators were closer to that observed in the lifetime trials (Fig. 2b). This simulation underestimates prey survival at low prey density, but is adequate when prey are in excess of predator requirements.

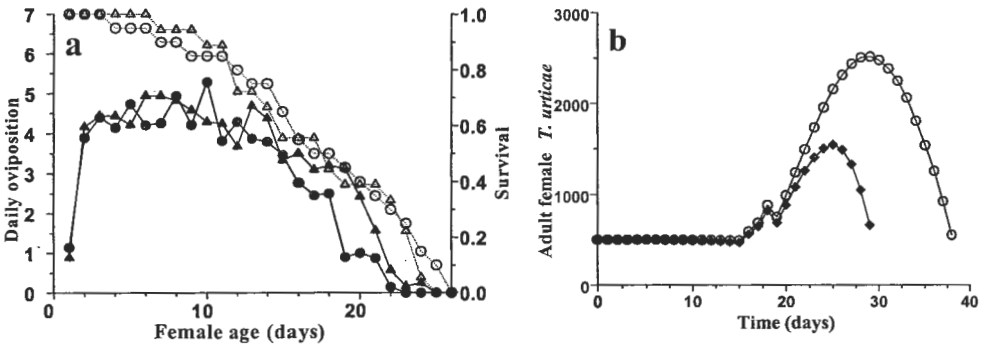


Figure 2. (a) Lifetime fecundity and survival of *Phytoseiulus persimilis* from two commercial sources. (b) Simulated population trends of female *Tetranychus urticae* when *P. persimilis* with lifetime fecundity and survival as in Fig. 2a are introduced (diamonds), versus the trends when predators with fecundity and survival as in Fig. 1 are introduced, followed by lifetime fecundity and survival as in Fig. 2a in subsequent generations (circles).

Fecundity and survival did not improve during the 5 day trials after the predators were reared for 30 days in the laboratory, despite the elimination of insectary procedures that might affect fecundity and survival. However, during the laboratory rearing, the colonies were once subjected to limited prey. Under these conditions, cannibalism of the young stages becomes a major factor that could affect age distribution within a population (Sabelis, 1981; Wheatley & Boethel, 1992). Therefore, we may have harvested older females with reduced daily fecundity. Insectary cultures are often starved in order to encourage female predators to move off leaves and into collection units. The potential effects of starvation on the age distribution of predator populations warrant further study.

Quality assurance tests for *P. persimilis* as described by Steinberg & Dale (1998) are useful in indicating immediate expectations from the predator culture, including the effects of age distribution, collection and storage protocols, and other factors. In the interest of assessing problems with protocols or cultures, the test should be modified to eliminate the effects of age distribution. This could be done by conducting the 5 day test as outlined by Steinberg & Dale, and extending the test to include a 5 day age specific measure of fecundity and survival for the first progeny of the test females. The difference in the two measures would indicate the potential for improvement in shipped material.

Acknowledgements

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Influence of greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) Vuillemin for control of greenhouse pests

Les Shipp¹, Yun Zhang¹, David Hunt¹, Gillian Ferguson²

¹Agriculture and Agri-Food Canada, Greenhouse and Processing Crops Research Centre, Harrow, Ontario, Canada NOR 1G0, E-mail: shipl@em.agr.ca; ²Ontario Ministry of Agriculture, Food and Rural Affairs, Greenhouse and Processing Crops Research Centre, Harrow, Ontario, Canada NOR 1G0

Abstract: The influence of humidity on the infection level of the entomopathogenic fungus, *Beauveria bassiana*, was determined for three greenhouse pests (*Aphis gossypii*, *Trialeurodes vaporariorum* and *Frankliniella occidentalis*) using small-scale research and commercial greenhouse trials. The trials were conducted on greenhouse cucumber and tomato using the commercial formulation Botanigard ES. The bioassay method consisted of using screened cages to confine the sprayed insects to the bottom side of plant leaves and assaying the sprayed insects for infection levels of the fungus after various post-spray application periods. Greater infection levels occurred at the top of the crop canopy compared to the middle of the canopy. Infection levels ranged from 65-91% depending upon the pest species under humidity conditions of 64-81% RH in commercial production greenhouses.

Key words: *Beauveria bassiana*, greenhouse pests, humidity, greenhouse vegetables

Introduction

Beauveria bassiana (Balsamo) Vuillemin (Hyphomycetes) is one of the most extensively studied entomopathogenic fungus species and is the active agent in many entomopathogenic products currently in use or under development worldwide (Feng *et al.*, 1994). Humidity plays a critical role in the germination/sporulation of *B. bassiana* (Wraight *et al.*, 2000). Very little information has been published on the use of *B. bassiana* on greenhouse crops, such as the infection level of *B. bassiana* on greenhouse pests, the effect on beneficial insects and the use of greenhouse microclimate to improve the efficacy of *B. bassiana*.

To use this fungus successfully in the greenhouse, the optimal microclimate conditions around inoculated host insects need to be determined. The objective of this study is to investigate the infection levels of *B. bassiana* on greenhouse pests under different humidity conditions in both small-scale and commercial greenhouse trials in order to evaluate the potential use of this entomopathogenic species for integrated pest management (IPM) on greenhouse vegetable crops.

Materials and methods

Greenhouse trials were conducted at the Greenhouse and Processing Crops Research Centre (GPCRC), Harrow on cucumber and at the commercial greenhouses (Cornies Farm, Kingsville [cucumber]; Cumberhill Farm, Leamington [cucumber]; and Ernesto Delciancio, Ruthven [tomato]) in the spring and fall of 2000. At the GPCRC, two adjacent high gutter, double-polyethylene covered greenhouse compartments (9 × 7.3 m) with an overhead misting system were used for the small scale trials. The commercial greenhouses were high gutter,

double-polyethylene houses, but without any misting systems. In all the trials, only the Botanigard ES formulation (Emerald BioAgriculture Corp., Salt Lake City, Utah, USA) was used. Laboratory trials showed that Botanigard ES was more effective against greenhouse pests than Botanigard 22WP.

Climate control and monitoring

At the GPCRC, temperatures in the two compartments were controlled at 23-25°C, and humidity was maintained at either 75-80 or 90-95% RH. Temperature and humidity were monitored both at the top and middle of the crop canopy. In the commercial greenhouses, greenhouse environment was maintained at production conditions and microclimate (temperature and humidity) was monitored around the test plants.

Leaf cage trials at two heights in the plant canopy in the research greenhouses

To reduce the effect of factors other than humidity, such as spray coverage, all trials were conducted by confining the sprayed insects to leaves using fine-mesh screen leaf cages. Cucumber seedlings were transplanted into the greenhouses in early March 2000. After complete canopy development, six plants in each greenhouse were randomly chosen as sample plants. Three top leaves (top three) and three middle leaves (around 1.22 m from the ground surface) on each plant were used as sample leaves for three post-spray time periods. Adult and immature stages of *Aphis gossypii* Glover, *Trialeurodes vaporariorum* (Westwood) and *Frankliniella occidentalis* (Pergande) were used in the greenhouse trials. Twenty-five host insects were placed on the bottom leaf surface and then sprayed until runoff with Botanigard ES (0.5 ml/100ml) or water, respectively, using a hand held sprayer. The sprayed insects were maintained in place with screen cages on the leaves. Three leaf cages with different insect species and the identical post-spray time period were attached to the same sample leaf. The sprayed insects were removed and surface sterilized 2, 4 and 7 days post spraying. The sterilized insects were placed on water agar plates and incubated for 4-5 days at 25°C before determining the infection levels.

To evaluate the infection levels of *B. bassiana* on immature *T. vaporariorum* under greenhouse conditions, 40 adults were released into a leaf cage on sample leaves 2 weeks before a trial and removed 24 h later. On the trial day, *T. vaporariorum* scales were sprayed with *B. bassiana* using the same protocol as stated above.

Leaf cage trials in commercial greenhouses

Leaf cage trials were conducted in the commercial greenhouses using a similar methodology as at the GPCRC. Two trials were conducted on a cucumber crop at Cornies Farm, and one trial at Cumberhill Farm and at Ernesto Delciancio. Only the adult stage of *F. occidentalis* and *T. vaporariorum* were tested in the commercial greenhouses. Samples were collected for surface sterilization at 4, 7, and 11 days post-treatment.

Results and discussion

Small-scale greenhouse trials

Air and leaf temperatures and humidity for the top and middle canopy levels are presented in table 1. Due to the limited downward diffusion of moist air from overhead misting in a full canopy, humidities decreased to 85.6 and 69.5% RH at the middle canopy levels for the high and low humidity greenhouses, respectively. The top leaves under the high humidity conditions were slightly warmer than those under the low humidity conditions due to the reduction in evapotranspiration cooling of the leaves in the humid air.

Table 1. Average microclimate condition (air temperature Ta, relative humidity RH, and leaf temperature Tl) at the leaf surface during leaf cage trials in two greenhouse compartments (GH) controlled at high humidity (HH) and low humidity (LH) conditions.

GH	Leaf location	Ta (°C)	RH (%)	Tl (°C)
HH	top canopy	23.45±0.04	91.65±0.07	24.23±0.07
	middle canopy	24.70±0.03	85.63±0.12	23.63±0.03
LH	top canopy	23.36±0.04	77.09±0.11	23.06±0.05
	middle canopy	23.92±0.05	69.54±0.21	23.74±0.05

The infection levels of *B. bassiana* in the adult stage of the target pests increased by 15-21% at both the top and middle leaves by raising the humidity (table 2). The sprayed insects on the top canopy leaves showed an average of 17.8% greater infection level than that at middle canopy regardless of the humidity treatment between the two greenhouses. The effect of leaf height on infection level was greater for adult *F. occidentalis* and *T. vaporariorum*. The post-spray exposure time required for maximum infection level varied among the three insect species. *A. gossypii* and *F. occidentalis* had maximum infection levels within 2-4 days post spray application. *T. vaporariorum* showed increased infection level over time reaching a maximum after 7 days.

Table 2. Infection levels (mean±se for six replications) for Botanigard ES for the adult stage of pest insect species released on the bottom surface of plant leaves in greenhouses with high (HH) and low humidity (LH) conditions for 2, 4 and 7 days post-spray time periods.

Host insect	GH	Top canopy			Middle canopy		
		2 days	4 days	7 days	2 days	4 days	7 days
<i>A. gossypii</i>	HH	82.0±1.3a	91.5±5.2a	96.1±1.5a	66.4±3.8a	84.8±2.7a	88.1±2.2a
	LH	68.7±1.6b	77.5±3.4b	71.6±3.9b	60.6±6.0a	67.4±2.1b	61.0±4.5b
<i>F. occidentalis</i>	HH	89.0±1.6 a	80.4±1.9a	81.6±2.9a	69.7±1.3a	72.9±3.9a	62.8±5.2a
	LH	66.5±6.1b	69.3±2.5b	71.0±3.8b	57.7±5.1b	52.4±4.4b	40.5±5.0b
<i>T. vaporariorum</i>	HH	56.8±1.4 a	79.1±7.1 a	92.6±3.9 a	37.7±3.6 a	44.5±4.4 a	52.6±4.6 a
	LH	27.1±2.4b	58.6±4.8b	74.5±9.9 a	29.6±3.1 a	29.6±2.0b	35.2±2.1b

^z means followed by different letters within each column for same insect are significantly different ($P<0.01$; SNK multiple range test).

For the immature stages, the maximum infection levels for *A. gossypii* (83 and 70%; 73 and 69%) and *F. occidentalis* (71 and 70%; 67 and 78%) occurred 2 days post spray application at both top and middle canopy heights for the high and low humidity treatments, respectively.

Infection levels for *T. vaporariorum* increased over time with the maximum occurring 7 days post spray application for both levels at the high and low humidity treatments (95 and 65%; 76 and 68%).

Commercial greenhouse trials

The microclimate around the sprayed cucumber leaves was T = 21.5-21.6°C, RH = 70.8-81.0% at Cornies Farm and T = 21.6°C, RH = 64.4% at Cumberhill Farm, and T = 17.0°C, RH = 73.1% around the tomato leaves at E. Delciancio. Infection levels were within the same range as observed during the cage trials at the GPCRC. Infection levels for both *F. occidentalis* and *T. vaporariorum* at the Cornies and Delciancio greenhouses were slightly higher (15-24%) than at the Cumberhill greenhouse. Maximum infection levels for *F. occidentalis* were observed 4-7 days post spray application and for *T. vaporariorum*, 7-11 days post spray application.

This study demonstrated the potential for including *B. bassiana* in an IPM program for *A. gossypii*, *F. occidentalis* and *T. vaporariorum* in greenhouse vegetable production. In Ontario, the humidity in commercial greenhouses usually reaches 64-81% RH during the spring, summer and fall, which was the level observed in the low humidity greenhouse trial at the GPCRC. Humidity levels will vary throughout the vertical canopy profile of the crop and thus, multiple applications of the fungus will be required for effective control.

Acknowledgements

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Mite movement and biocontrol: A virtual approach

Dave Skirvin

Department of Entomology, Horticulture Research International, Wellesbourne, Warwick, UK,
CV35 9EF, E-mail: David.skirvin@hri.ac.uk

Abstract: A combined experimental and modelling framework has been produced to allow rapid development of stochastic, spatially explicit models for biological control in ornamental crops. Application of this framework to the simulation of the dynamics of spider mites, *Tetranychus urticae*, and predatory mites, *Phytoseiulus persimilis*, has shown that the movement and dispersal of mites are crucial to the success of biological control. This paper describes the application of the novel technology of virtual plants to modelling of mite movement within the complex plant canopies associated with ornamental crops.

Key words: biological control, natural enemy, movement, dispersal, virtual plants

Introduction

Due to increasing pressure to reduce pesticide inputs, ornamental growers in the UK are increasingly turning to the use of biological control to reduce pest damage. Biological control has been used successfully on protected edibles in the UK (Hussey *et al.*, 1965, Nachman, 1981, Kropczynska & Tomczyk, 1996). However, due to the low tolerance to pest damage, diversity of crops grown, often in close proximity, and the range of different growing practices used, the approach to biological control taken in edible crops cannot be transferred directly to ornamental crops.

For biological control to succeed on ornamental crops in the UK, it will be necessary to focus on preventing pest establishment, through the use of prophylactic releases of natural enemies. For this strategy to work, it is necessary to have a detailed understanding of the tritrophic interactions involved in biological control, particularly in relation to the movement of natural enemies and pests within and between crops.

Initial modelling work has identified the movement of natural enemies as a crucial factor in determining the success of a prophylactic release programme for biological control in ornamental crops. Experimental work on the movement of *P. persimilis* on *Choisya ternata* has shown that the number of connections between plants has a significant impact on the dispersal of natural enemies, which is in agreement with the work of Zemek & Nachman (1998, 1999). In order to implement prophylactic releases of natural enemies for biological control, it is therefore extremely important to have a good understanding of the number of connections between and within plants in a canopy, and this will depend upon the structure of the crop plant. The novel technology of virtual plants (Room *et al.*, 1996, Hanan, 1997, Prusinckiewicz *et al.*, 1997) provides a useful tool for gaining this understanding. This paper presents a brief overview of virtual plant technology, and the way in which it may be utilised to aid biological control in ornamental crops.

Virtual plants

What are virtual plants?

Virtual plants are computer-generated models of the three-dimensional structure and growth of plants, based on the mathematical concept of Lindenmeyer systems (L-systems). L-systems are essentially a mapping that describes how a structure changes from one time step to the next. L-systems can also include time delays, so that one structure, e.g. a bud, may transform into a new structure, e.g. a flower, after a set time interval. Plant parts in a given state are represented by symbols, and the way in which they change over time is defined by a set of rules, known as productions. For example, the growth of a simple flower can be represented as follows, where A represents an apical bud, I an internode, L a leaf, B a flower bud, F a flower, and $[]$ encloses a branch

Starting with an apical bud, A, the following rules are implemented in each time step:

A \rightarrow IL[B]A (each apical bud becomes an internode + branch with apical bud + leaf + flower bud)
I \rightarrow I I (each internode doubles in length)
B \rightarrow F (flower bud becomes a flower)

In successive time steps, the virtual plant is represented as:

Step 0: A
Step 1: IL[B]A
Step 2: IIL[F]IL[B]A
Step 3: IIIIL[F]IIL[F]IL[B]A
Step 4: IIIIIIL[F]IIIL[F]IIL[F]IL[B]A

The angles of branching for branches, and leaves, and the width of lines are represented by other symbols, and these have been omitted from this example for ease of illustration.

A graphical representation of the plant at time step 4 is shown in Fig. 1 below.

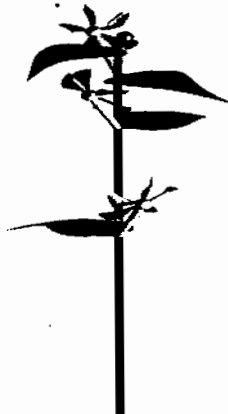


Figure 1. Graphical representation of the virtual plant from time step 4.

Creating a virtual plant

For the work on ornamental crops, the virtual plants are being created through the digitisation of real plants using a sonic digitiser and the Floradig software from the Centre for Plant Architecture and Informatics (CPAI), Brisbane, Australia. The sonic digitiser consists of a triangular array with a microphone at each apex, a probe which has two miniature electrodes which emit a sound milliseconds apart and a metal tip, and a box of electronics that calculates the three dimensional point at the end of the tip. To digitise a point, the tip of the probe is placed on the point and a trigger pressed to fire the electrodes, and the co-ordinates of the point are returned to the computer. These points are then passed to the Floradig software, and defined as either a node, leaf point, bud or flower. Leaves are digitised as a set of points defining the main edges of the leaves.

Once all the plant structures have been digitised, the software is able to calculate the branching angle, internode length, leaf size and other related information that is necessary to create the virtual plant model as an L-system. At present this information can only be converted into an L-system manually, although CPAI are currently developing software to automate the generation of L-systems rules from Floradig information. Having developed the L-system, the virtual plant can then be visualised, using the L-studio software from the University of Calgary, Canada, on a PC.

Virtual crop canopies: creating and analysing

For the current project on canopy structure, 16 *C. ternata* and 16 Chrysanthemum plants have been digitised, and the information on branching angles, internode lengths, leaf angles, lengths and widths exported into a Microsoft Access database. These data are currently being analysed to determine the relationship between the position of the structures on the plant and the mean angle, length or width. Once this has been done, a stochastic L-system will be created to grow the plants, with the internode lengths and branching angles being chosen from fitted statistical distributions.

Having developed an L-system for a single plant, the stochasticity of the L-system will enable multiple plants, each with a slightly different structure, to be created to form a model of a crop canopy. Having created a model of the crop canopy, it can then be analysed to determine the number of connections between leaves, both within and between plants. This is done using a simple collision detection (CD) algorithm. The leaves are represented as a set of triangles, and then the co-ordinates of the apices of the triangles are passed into the CD algorithm for each leaf, which then tests each of the triangles in different leaves for collisions with triangles from other leaves. If a collision is detected, the leaf with which a collision is detected is returned back to the L-system. A list of leaves that are touching is then stored in an array defined within the L-system, along with the plant to which the touching leaves belong. In this way, all the touches within and between plants can then be calculated, and related back to the structure of the individual plants.

How does this help biological control?

As mentioned previously, natural enemy movement is a determining factor of the success of prophylactic approaches to biological control in ornamental crops, and this movement is dependent upon the number of connections between plants. Therefore by understanding how individual plant structure affects the number of connections between plants in a canopy, we can determine the introduction strategies that will lead to successful biological control.

The models of canopy structure that have been developed can now be combined with models of the movement and searching of natural enemies, and since all the connections both

within and between plants are known, from the collision detection process, it should be possible to model the movement of natural enemies within the canopy. The combined virtual canopy – natural enemy movement model can then be used to determine how quickly natural enemies are able to locate sparsely distributed prey patches under a range of different pest distribution and natural enemy introduction scenarios.

The results of the modelling work will then provide us with the most effective introduction strategies for prophylactic releases of natural enemies, which rely on the normal movement and searching behaviour of the natural enemies. This should lead to more robust biological control strategies that can then be scheduled into crop production systems.

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Development of a new thrips predator, *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae) indigenous to Australia

Marilyn Steiner, Stephen Goodwin

National Centre for Greenhouse Horticulture, Horticultural Research and Advisory Station, Locked Bag 26, Gosford, New South Wales 2250, Australia, E-mail: Marilyn.Steiner@agric.nsw.gov.au

Abstract: A phytoseiid mite indigenous to Australia shows promise as an effective predator of thrips in warm greenhouse conditions. *Typhlodromips montdorensis* has a high rate of thrips consumption and intrinsic rate of natural increase, does not diapause under normal greenhouse conditions, colonises crops rapidly at temperatures over 20°C, and operates effectively on a range of ornamental and vegetable crops. In Australia it has shown promise in chrysanthemum, gerbera, capsicum, strawberry and cucumber crops. It also has potential against broad mite and tomato russet mite. Information on biology, usage, sensitivity to pesticides and crop usage is detailed.

Key words: phytoseiid mite, thrips, integrated control

Introduction

In 1994 we began a search for indigenous predators and parasitoids for management of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), in Australia, casting a wide net across all States and Territories and over a range of native and crop vegetation types (Goodwin & Steiner, 1996, Steiner & Goodwin, 1995, Steiner & Goodwin, 1998a). On the basis of thrips consumption rate, intrinsic rate of increase at 25°C, lack of diapause, establishment in a variety of crops and ease of rearing, two species of phytoseiid mites were identified as primary candidates (Steiner & Goodwin, 1998b). Commercial agreements were entered into with a European and Australian company to ensure adoption and development. The warm-temperate species, a native *Typhlodromalus*, has not progressed recently because of difficulties in large-scale rearing. The other, *Typhlodromips montdorensis* (Schicha), a warm-temperate/subtropical species, is presently being trialled and marketed in Europe by Syngenta Bioline™ as Amblyline-M®.

Initial small greenhouse trials established that both species controlled western flower thrips at very low release rates (2/plant or 10/m²) on cucumber, capsicum, strawberry, chrysanthemum and gerbera. Seasonal differences in control on commercial properties and in research greenhouses prompted more extensive studies into the rate of development under a range of temperature regimes in order to establish limits for use. Trials have continued also to better establish usage protocols (Steiner & Goodwin, 2001).

We report here information on the biology and crop usage of one of these species, *T. montdorensis*.

Biology and crop usage of *T. montdorensis*

Effects of temperature on development and survival

The minimum temperature for development is approximately 11°C. Development is normal within a range of 15 to 30°C, with time to egg laying ranging from 27 days at 15°C to 6 days

at 30°C. Variable diurnal temperature at 15°C (10°C/20°C) decreases development time from 27 to 20 days. The slow development rate at low temperatures precludes effective use of *T. montdorensis* at mean daily greenhouse temperatures much below 20°C.

There was no diapause under short day conditions of 25°C/8h:10°C/16h. The thermal death point for rapid cooling was between 0°C and 5°C. In its natural range minimum temperature is generally >8°C.

Fecundity and intrinsic rate of natural increase

Lifetime fecundity averaged 53 eggs, with maximum production of 1 egg/day at 15°C and 3.25 eggs/day at 30°C. The intrinsic rate of natural increase, r^m , at 25°C was 0.38. The sex ratio is >65% female. Under warm conditions this species can therefore increase very rapidly.

Thrips consumption rate

At 25°C, consumption of 1st stage thrips larvae averaged 14/day, with a maximum for an individual of 20/day. The high kill rate is not necessarily a consumption rate. Second stage larvae are rarely attacked by individuals, but gang attacks on older larvae and adults have been observed.

Host range

Typhlodromips montdorensis is also a predator of broad mite, tomato russet mite, two-spotted mite, and other small arthropods. It can survive and reproduce on pollens such as plantain and cattail.

Relative humidity requirement for egg hatch

The critical humidity for 50% egg hatch is 70% RH (VPD=0.95kPa). This species prefers humid environments for optimum performance.

Sensitivity to pesticides

Treatments were either a 30 second immersion in label rate insecticide or fungicide, or confinement on a Potter Tower-treated leaf surface. Assessment of treatment effect was based on five days post-treatment egg laying and survival of known age young adult females. *Safe or low toxicity*: Eco-Oil®, Torque®, Avatar®, AzaMax®, Natrasoap®, Pirimor®, Bayleton®WP, Rubigan®, Rovral®, Bravo®, Foli-R-phos®, Tilt®, Saprol®, Systhane®. *Intermediate toxicity*: Sulfur. *High toxicity*: Vertimec®, Vapona®, Regent®, Confidor®, Thiodan®, Malathion®, Dithane-DF®, Lannate®, Pyrethrum®, Success®. Several high toxicity products (Thiodan®, Regent®, Confidor®) were not toxic by immersion and were also repellent, so that in the field there may be survival. Sulfur and Dithane-DF® reduced egg laying. Success® was only slightly toxic at half label rate (5 gai/100L). The effects of pyrethrum, Vapona® and Vertimec® were relatively short-lived. Some tolerance to Lannate® was noted.

Information on crop use

Strawberry: Three years trial data in a commercial hydroponics operation (Steiner 2002, this Bulletin) indicate that *T. montdorensis* provides good control of thrips, including WFT, at mean temperatures >20°C, but not in cool weather. Predators are frequently found under the calyx on both green and red berries, particularly in periods of low humidity. Once temperatures are favourable, suggested introduction rates are 10-20/m² at weekly intervals for four weeks.

Cucumber: Recent trials in a 500m² greenhouse (Steiner & Goodwin, 2002, this Bulletin) have seen excellent control of onion thrips, *Thrips tabaci* Lindeman, and WFT at weekly introduction rates of 3-10/m² over a seven week period. Predators colonised leaves throughout the crop strata and fed also on two-spotted mite. *Phytoseiulus persimilis* does not appear to be impacted and is still required to prevent spider mite outbreaks.

Capsicum: Small greenhouse trials in capsicum with a single release of *T. montdorensis* at 2/plant demonstrated that predators establish good populations on leaves and also feed on thrips larvae under the calyx of fruit. They do not appear to inhabit flowers.

Tomatoes: Predators are able to establish good populations on tomatoes and are a serious problem in our tomato russet mite colony. Official trials against this pest are also being conducted. Releases have been made into commercial crops in South Australia and Victoria but the results have not been well monitored.

Gerbera: Releases have been made over the past two years in three commercial cut flower gerbera properties in NSW and QLD and to a crop on Station. Excellent results have been achieved with rates as low as 10/m² fortnightly for four releases. A low rate of Success® (spinosad) has occasionally been applied in the commercial crops against windblown influxes of plague thrips, *Thrips imuginis* Bagnall, or WFT from outside the greenhouse. When directed only on buds and flowers, spinosad appears to have minimal impact on IPM programs. Low winter temperatures reduce effectiveness but predators have overwintered successfully and resumed good populations in the spring. Partial control of two-spotted mite has also been achieved.

Chrysanthemum: Releases of *T. montdorensis* have been made over two years to both commercial stock plants in NSW and cut flower crops in QLD. Weekly releases of 10/m² are recommended for stock plants as cuttings with predators are removed frequently. Weekly releases have also been made in the cut flower crops (large WFT migration problem on other cut flower crops outside the greenhouse) and are credited with reducing pesticide applications for WFT to an occasional application of Success®.

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Management of thrips on cucumber with *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae)

Marilyn Steiner, Stephen Goodwin

National Centre for Greenhouse Horticulture, Horticultural Research and Advisory Station,
Locked Bag 26, Gosford, New South Wales 2250, Australia, E-mail:
Marilyn.Steiner@agric.nsw.gov.au

Abstract: Western flower thrips, *Frankliniella occidentalis* (Pergande), causes major losses in greenhouse cucumbers in Australia through leaf and fruit damage. A newly developed indigenous phytoseiid mite species, *Typhlodromips montdorensis* (Schicha) provided excellent control of both western flower thrips and onion thrips, *Thrips tabaci* Lindeman, when introduced into a cucumber crop at a weekly rate of 10/m² over a seven-week period. Western flower thrips was detected in low numbers on yellow sticky traps but was essentially eliminated within six weeks. Maximum larval thrips population density (onion thrips) reached only 4/leaf on lower leaves, despite trap catches of as many as 40 adults/trap. Other pests were also controlled successfully by natural enemies, with no chemical intervention necessary.

Key words: cucumber, thrips, phytoseiid mites, integrated control

Introduction

The greenhouse cucumber industry in NSW is comprised of relatively low technology operations, and it has been next to impossible to convert growers to IPM practices in these circumstances. Western flower thrips, *Frankliniella occidentalis* (Pergande), arrived in Australia in 1994 and has not ignored cucumbers. There are very limited chemical options available for their control. In 2001, construction of two research and demonstration greenhouses (500 m² and 600 m²) with good environmental controls at the Horticultural Research and Advisory Station at Gosford has given us the opportunity to trial a new indigenous phytoseiid mite with commercial potential, *Typhlodromips montdorensis* (Schicha), developed at the Station. During the past few years, extensive information has been generated on its life history, temperature preferences, sensitivity to pesticides and performance in small greenhouse situations on a range of ornamental and vegetable crops. It has the ability to multiply rapidly at temperatures between 20°C and 30°C and RH >70%, it does not diapause, it disseminates rapidly under warm conditions and occupies all parts of the plant canopy. In laboratory trials it has consumed as many as 20 thrips larvae/day (mean 14/day), and it can maintain good populations on two-spotted mite and broad mite. Preliminary releases of this predator into winter cucumber crops grown in the new greenhouses gave very encouraging results. A program was therefore set in place to incorporate *T. montdorensis* into an IPM program for the spring/summer crop.

Material and methods

Treatments

The crop consisted of approximately 500 Lebanese cucumbers and 200 of Japanese cultivars in a 500 m² polyhouse. It was planted on 5 October 2001 into either coir peat bags or NFT

channels and removed on 19 December 2001. Minimum and maximum temperatures were set at 16°C night and 26°C day temperature. Overhead misting was deployed at 70% RH. *Typhlodromips montdorensis* reared on the Station was distributed in fine vermiculite at a rate of 2/plant in Weeks 1 and 2, and 10/m² in Weeks 3-7. Mites were placed on one leaf of each plant. Releases were discontinued on 21 November once most plants reached the overhead wire, and thrips larval populations were clearly declining. *Encarsia formosa* was released weekly after 2 November for greenhouse whitefly at 2/plant, *Stratiolaelaps ?scimitus* (Hypoaspis) once at 100/m² at planting for fungus gnats, and *Phytoseiulus persimilis* and *Aphidius colemani* as necessary for spider mites (*Tetranychus ludeni*, *T. urticae*) and aphids (*Myzus persicae*), respectively.

Determination of thrips and predatory mite population levels

The crop was walked and leaves randomly examined weekly looking for signs of pest activity and pest presence. Upper, middle and lower leaves (26 of each, two from each strata/row) were removed randomly every week and washed through screens to extract pests and beneficials. Four yellow sticky traps were hung and checked weekly to monitor population levels of thrips and other flying pests.

Results and discussion

Thrips and predatory mite populations

Onion thrips, *Thrips tabaci* Lindeman, was the main thrips species infesting the cucumbers; all were female. They primarily infested lower leaves and caused minor feeding damage. Western flower thrips was detected only on the sticky traps (Fig. 1), from 9 November until 23 November only. They were primarily males, indicating a low in-house population rather than a migrant population.

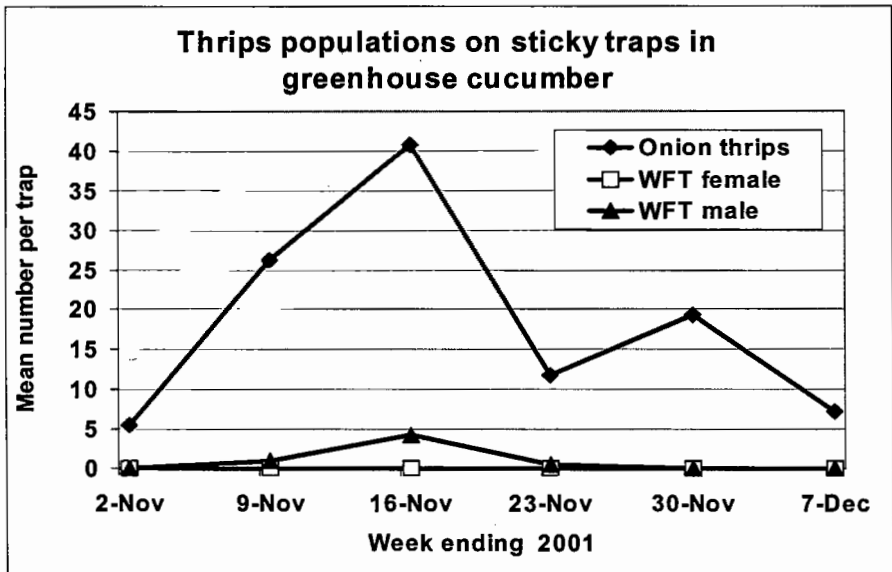


Figure 1. Yellow sticky trap catches of thrips in greenhouse cucumbers, 2001.

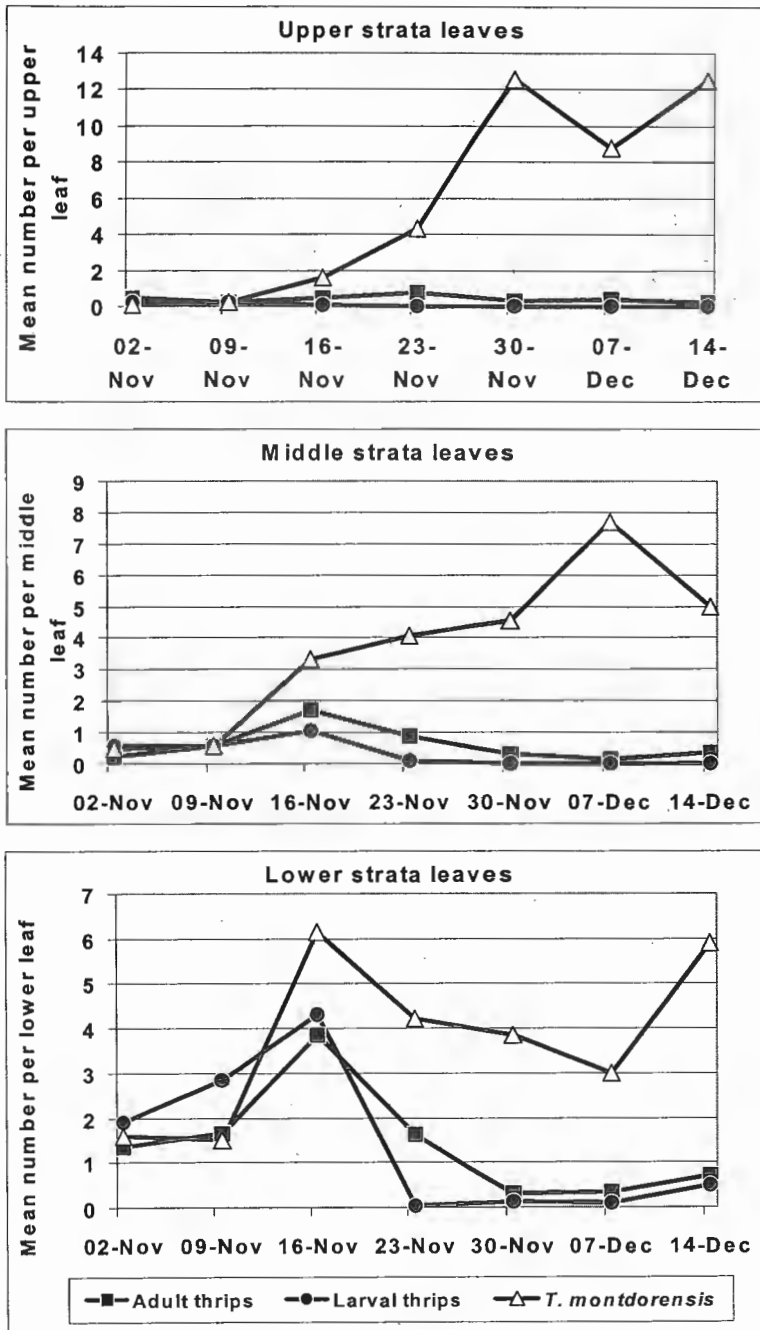


Figure 2. Thrips and predatory mite populations on greenhouse cucumber, 2001.

Populations of thrips and *T. montdorensis* in the three leaf strata (Fig. 2), as derived from leaf washes, show onion thrips population density initially increasing and then rapidly declining to remain low and below damaging levels. Despite the very low thrips densities, *T. montdorensis* numbers remained high and far in excess of thrips numbers, so that further introductions were not required. It is probable that fewer introductions of *T. montdorensis* would have been adequate, as predators were present on all leaves examined prior to the final release. Small greenhouse trials have used as few as two predators per plant with excellent results, but one of the primary aims in this crop was to prevent damage to the main stem fruit and interference with cultivar evaluation. Control of other pests such as fungus gnats, whitefly, aphids and two-spotted mite was excellent, but *T. ludeni* (bean spider mite) required more frequent intervention with *P. persimilis*. No pesticide applications were required.

Acknowledgements

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Progress towards integrated pest management for thrips (Thysanoptera: Thripidae) in strawberries in Australia

Marilyn Steiner

National Centre for Greenhouse Horticulture, Horticultural Research and Advisory Station,
Locked Bag 26, Gosford, New South Wales 2250, Australia, E-mail:
Marilyn.Steiner@agric.nsw.gov.au

Abstract: Thrips annually cause major crop losses in field and greenhouse strawberry crops in several States in Australia. A three-year study has made good progress in establishing damage caused by thrips and action threshold levels, and in identifying phytoseiid mites that are effective against thrips within an IPM program. Western flower thrips, *Frankliniella occidentalis* (Pergande) and plague thrips, *Thrips imaginis* are the two most important species. In a hydroponic crop in the third year of study, targeted sprays of spinosad when thrips populations reached a level of 40% of flowers with 10 or more adult thrips has been effective in keeping fruit damage to below economic threshold levels. Only two applications were necessary in four months. This strategy was combined with releases of the native predatory mite *Typhlodromips montdorensis* (Schicha), which establishes good populations on strawberries under summer conditions. It was effective in maintaining low thrips numbers on fruit during mid to late summer. Environmental factors impacting on degree of damage were also determined.

Key words: strawberries, thrips, integrated control

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande), arrived in Australia in 1994; in recent years it has caused major berry losses in field and hydroponic strawberries in Victoria, Western Australia, South Australia and New South Wales. Plague thrips, *Thrips imaginis*, a native migrant species, is a long established pest of strawberries in all States except the Northern Territory. Growers regularly apply insecticides, generally at an arbitrary 5 thrips/flower. While damage symptoms caused by the two species are similar, management strategies differ. Plague thrips is often unpredictable, but it generally appears early in spring as large numbers of windblown adult females, whereas western flower thrips builds up slowly, is more predictable, and is a mid to late summer pest in hot conditions (early December to March).

Thrips are blamed for many types of damage to strawberries, including flower and fruit abortion, fruit distortion, bronzing, 'seediness', shrivelling, and leaf damage. A project initiated in 1999 aimed to identify the types of damage caused by western flower thrips, and treatment and economic threshold levels. It also aimed to evaluate two indigenous phytoseiid mites already showing promise for WFT management in a range of crops, and to make management recommendations that would lead to more effective control and less chemical use. Most of the trial work has been conducted in a hydroponic strawberry operation near Sydney, NSW. Visits to field grown strawberries in Victoria and South Australia were made in January 2001. Further laboratory trials have been conducted at HRAS to examine the effect of temperature, humidity and population density on severity of damage, and leading on from this, the effect of overhead misting on damage severity and population density is being studied. Progress to date is reported in this paper.

Material and methods

Damage caused by thrips

Laboratory trials isolating WFT larvae (2nd stage) or adult females with either individual flowers, green (50% seed cover) berries or red berries was conducted in small, screened plastic containers, with the base of plant parts set in 1% agar covered with a thin layer of solid paraffin. Containers were placed in incubators for 72 h and temperature, relative humidity and thrips numbers were varied. Damage to berries was scored on a scale of 1-5.

Determination of thrips and predatory mite population levels, and damage levels

The primary investigation was conducted in a commercial greenhouse ('shed') that was plastic-covered with roll-down sides and top-vented but otherwise not temperature regulated. The shed held two blocks each of 6,000 plants, hydroponically grown in inverted v-shaped PVC pipes. The cultivar was Osso Grande in Year 1, Osso Grande and Camerosa in Year 2, and Camerosa in Year 3. In 1999 the site (6 sheds) had experienced a three-year history of total crop loss from WFT by mid summer. In Year 1, thrips counts were conducted in flowers, on red berries, and on all leaves of 12 plants per treatment. Predatory mites were counted only on leaves. There were two chemical treatment blocks and two where either *Typhlodromus montdorensis* (7/plant total, 30 Sep-9 Dec) or a combination of *T. montdorensis* (1.7/plant total, 30 Sep-7 Dec) and *Typhlodromalus lailae* (Schicha) (1.9/plant total, 21 Sep-9 Dec) were released. A wash through screens of various plant parts was conducted on 3 February 00 to examine distribution across plant parts. Having shown good establishment of both predatory mite species, in Year 2, treatments were either *T. montdorensis* alone (total 3.5/plant, 28 Sep-9 Nov), *T. montdorensis* + *T. lailae* (total 1.75/plant of each species, same dates), or *T. lailae* alone (total 3.5/plant, same dates). For each treatment there were four blocks, assessed weekly. Flowers, week-old small green fruit, green fruit (50% seed cover), ripe fruit and leaves (10 reps/treatment) were returned to the laboratory for washing and counting of thrips and predators. In Year 3, only *T. montdorensis* was released (two rates, total 2.5 or 5/plant, 26 Sep-14 Nov). There were four blocks and five reps per treatment category, and flowers, green fruit, red fruit and leaves were examined *in situ*. Thrips and predatory mites were pootered up as they were counted and species checked in the laboratory. Because *T. montdorensis* is a warm weather mite, it does not develop into high populations until mid summer, so spinosad was used to control thrips in flowers early in the season. Yellow sticky traps were also set out and thrips counted weekly.

Action thresholds

As most damage is caused by larvae moving from flowers to green fruit, flowers were selected as the target of action thresholds in Year 3. On the basis of previous data comparing damage levels with thrips populations, and a high degree of variance between flowers, the treatment level was set at 40% of flowers with five or more WFT adults/flower, or 10 or more plague thrips/flower. When this level was reached, a single application of spinosad was applied at 5 g ai/100L, directed at flowers and fruit. The low rate was intended to reduce thrips and have minimal impact on predatory mites, *Encarsia formosa* and other beneficials.

Results and discussion

Damage caused by thrips

Laboratory experiments confirmed field observations that thrips cause two types of damage to fruit, and that WFT and plague thrips cause the same type of damage. Thrips (primarily

larvae) originating from flower infestations feed on tissue between seeds of green berries, causing bronzing. In older green berries this is evident as a networking on the high side of the flesh. In a ripe berry this results in dull, rough or soft fruit with reduced shelf life. As fruit turns colour, a second type of damage occurs as thrips (again, primarily larvae) feed in the crevice around the seed. This type of damage is far less noticeable and does not affect shelf life to the same degree. In flowers, high populations of thrips often caused little noticeable damage except petal bronzing. Very high adult populations may damage anther bases and prevent pollen maturation, otherwise thrips appear to be good pollinators. Powdery mildew was the major cause of fruit deformation and flower abortion, not thrips.

Damage to green or red fruit by WFT larval or adult populations was only significant at populations of 10 or more, at relative humidity greater than 60%, and at high temperature (25°C night and 35°C day). Overhead misting appears to reduce damage.

Determination of thrips and predatory mite populations, and damage levels

Year 1. Both *T. montdorensis* and *T. lailae* established well on strawberry leaves from early December and built up to high population levels on leaves by late summer. Maximum density reached was ~95/plant for *T. lailae* and 61/plant for *T. montdorensis* (leaf counts). *Typhlodromips montdorensis* establishment was a month behind *T. lailae*. The plant wash found that both predator species were also found on fruit at all stages, but very few in flowers. During January, adult WFT population density in flowers in the blocks with predatory mites was half that in the chemical blocks, and damage to both green and red fruit considerably less.

Year 2. Both phytoseiid species established about three weeks earlier than in Year 1, with *T. lailae* establishing earlier on leaves (maximum 8.5/leaf for *T. lailae* and 14/leaf for *T. montdorensis*). On fruit, *T. montdorensis* density was much higher than that of *T. lailae*. No advantage of using both species together was evident. WFT did not appear in this shed, but plague thrips was numerous in spring and caused major fruit damage in October before being killed by *Entomophthora* and later predators.

Year 3. A cool early season delayed good establishment of *T. montdorensis* until late November. Predator density on green and red fruit increased rapidly to a mean of approximately 6/red fruit and 3/green fruit by late December, despite an exceptionally dry summer. Fruit damage has been minimal all season due to a combination of two early season spinosad treatments on 24 October and 5 December 2001 at the action threshold and good predator populations subsequently. Western flower thrips was found in very low numbers in early October and early November on yellow sticky traps and in flowers, but has not been found since. The primary thrips species was plague thrips. *Typhlodromips montdorensis* was released in three other sheds on the same property early in the season and has established and controlled thrips there also. The use of spinosad has not apparently affected *Encarsia formosa*, *Phytoseiulus persimilis* or other natural enemies successfully controlling whitefly, aphids and two-spotted mite. *Campylomma liebknechti* and *Ceranisus menes* also assisted in thrips control from mid December. The crop will continue to be monitored until it is pulled, generally late January.

Action thresholds

Implementing the action threshold level for plague thrips of 40% of flowers with 10 or more adult thrips by applying spinosad (adulticidal and larvicidal action) at 5 g ai/100L successfully prevented economic fruit damage in hydroponic strawberries. This threshold would likely be set at 5 or more adult thrips for WFT, a larger and more damaging species, particularly during hot weather. Where this treatment can be combined with the use of natural enemies such as *T. montdorensis*, capable of controlling thrips on berries, the tolerance level for thrips in flowers increases.

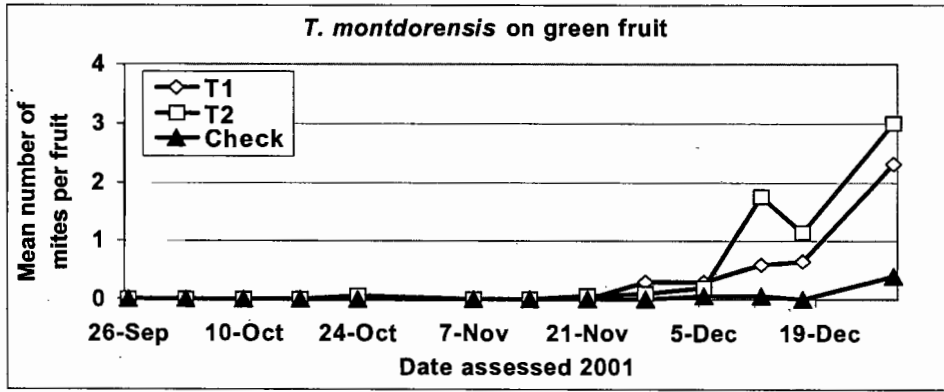
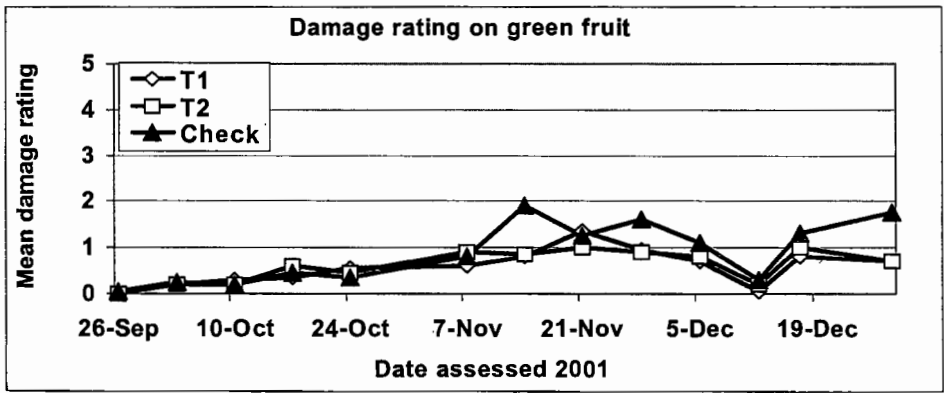
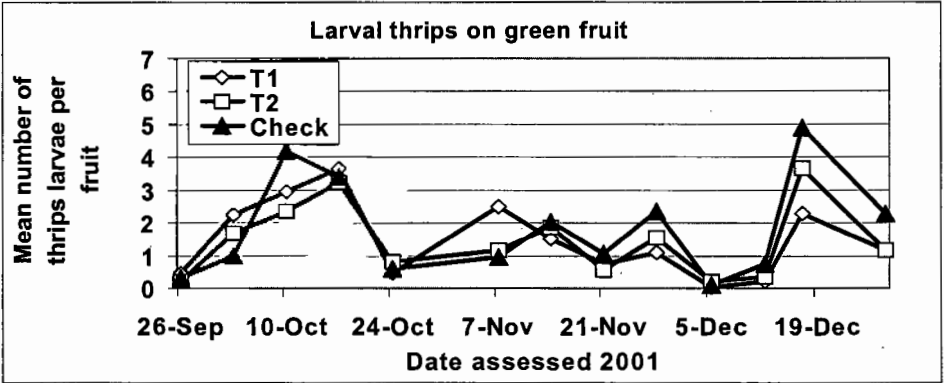


Figure 1. Populations of larval thrips and *T. montdorensis* on green strawberries and damage rating (1-5) based on percentage of surface damaged. A rating of more than two results in degraded fruit. Spinosad was applied on 24 October and 5 December. T1=*T. montdorensis* at 2.5/plant total and T2=*T. montdorensis* at 5/plant total, introduced during late September to early November.

Antagonistic properties of Mycostop (*Streptomyces griseoviridis*) to diseases agents in greenhouses plants

Elena Surviliene

Lithuanian Institute of Horticulture, Babtai, Kaunas distr., LT-4335 Lithuania, E-mail: Apsauga@babtai.lsd.lt

Abstract: Mycostop was efficient against root rot in greenhouse ornamentals and vegetables and it did not differ in efficiency in comparison with Previcur. The study was done on species composition of micromycetes and their spreading in greenhouse substrate, reaction to chemical component propamokarb hydrochloride (Previcur 607 SL) and interaction with the antagonistic microorganism *Streptomyces griseoviridis* (Mycostop). Both chemical and biological treatments changed the number and composition of micromycetes in substrates. From infected and non-treated substrate there were isolated and identified 52 fungi species belonging to 28 genera. Most prevailing fungi species were from *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Pythium*, *Rhizoctonia* and *Verticillium* genera. The systemic fungicide Previcur reduced and inhibited the development and spreading of micromycetes in the substrate most efficiently. The biofungicide Mycostop suppressed many pathogenic fungi species. The similarity and disparity coefficient by Sørensen of microflora in comparison with the non-treated substrate is reliable: in tests with Previcur – 30.38%, with Mycostop – 38.04%.

Key words: cucumber, gerbera, root rot, soil-borne fungi, Sørensen coefficient, *Streptomyces griseoviridis* (Mycostop), propamokarb hydrochloride (Previcur)

Introduction

Various fungi species can function in the greenhouse substrate and a considerable part of them are pathogenic and conditionally pathogenic fungi (Lugauskas, 1988). Poor species composition of soil microorganisms causes ecosystem instability in greenhouse. There is an opinion that pathogenic properties of fungi depend to a large degree on environmental conditions (Mirzink, 1988).

As the application of fungicides often produces a harmful effect on ecosystems, searching for antagonists to plant pathogens and introduction of them to agroecosystems is one of the decisions in modern plant protection (Utkhede, 1996). There are many reports on the application of potential microbial antagonists to control soil-borne pathogens (Nicolaev *et al.*, 1997; Pięta, 1998; Utkhede, 1996; Elad *et al.*, 1999). Among other fungi, the strains of *Trichoderma harzianum* were recommended as agents for biological control of plant diseases (Harman, 1998). Kortemaa *et al.* (1997) studied the effect of soil-spraying time on root-colonization ability of antagonistic *Streptomyces griseoviridis*.

Biological agents are much more sensitive to different conditions than chemicals. Soil pH, aerob or anaerob circumstances, availability of certain nutrients, temperature, humidity, all have an effect and may substantially determine the efficacy and the persistence of the biopreparate. Anyway, efficacy of biological agents never reaches 100% – even it would not be good. They should decrease the damage below certain threshold – but they should not change the soil microflora significantly – as chemical pesticides do (Dormanns-Simon, 1995).

Materials and methods

The trials were conducted in a commercial unheated greenhouse. Cucumber seedlings with 5-6 leaves were transplanted in a soil bed, 3.5-4 plants/m². The soil in the greenhouse was treated with a chemical fungicide, Previcur 607 SL (propamocarb hydrochloride), at the rate of 80 ml/100 m² and in other cases the surface of soil around seedlings was sprayed with the biofungicide Mycostop (active substance – *S. griseoviridis*, min. 10⁸ c.f.u./g) at the rate of 12 g or 24 g/100m². Rockwool for gerbera *Bianca* was watered three times on May 12, June 12 and July 16 by Mycostop at the rate of 30 mg per plant and Previcur at the rate of 0.45 ml per plant.

In the mycological tests a highly infected and not disinfected (non-treated) greenhouse substrate was treated by chemical and biological means. The substrate in vegetative boxes (0.6 x 0.4 x 0.1 m) was poured by Previcur (9.6 ml/0.024 m³). In other treatments the substrates were sprayed with Mycostop (0.12 g/0.024 m³). Fungi were isolated before and after substrate treatments. For mycological analysis the following agar media (pH 4.0-4.5) were used: malt extract, potato and Czapek. The cultural and morphological peculiarities of the fungi were studied employing microscopical methods and identification was performed according to different manuals (Domsch & Gams, 1970; Domsch *et al.*, 1980; Ellis, 1976; Arx, 1981 and others) at the Institute of Botany, Laboratory of Biodeterioration Research. Percentage similarity of the complexes of fungal species was evaluated using T. Sørensen coefficient (Lugauskas, 1988).

Results and discussion

Mycostop on vegetables

In the treatments, where the substrate around a cucumber was sprayed with Mycostop 0.01% suspension (12 g/100m²), plants were affected by root diseases 2.3 times less than plants in the control. A higher rate of Mycostop (24 g/100m²) depressed prevalence of root rot even more efficiently. The number of affected plants was by 3.1 times lower. Cucumber plants treated with Previcur (80 ml/100m²) were less affected by root rot as well, in comparison with the control fungicide decreased disease prevalence by 2.6 times (table 1). Prevalence of diseases influenced cucumber yield. By 2.8-4.7 kg/ m² higher production was obtained from the plots treated with Mycostop than from control plot. Biological efficiency of Mycostop reached 55.9-67.9% and Previcur 60.9%.

Table 1. Influence of Mycostop on root rot prevalence and cucumber *Crispino* yield.

Treatment	Healthy plants, %	Injured by root rot, %	Yield, %
Untreated (control)	26.1±3.0	69.0±3.9	100
Mycostop 12 g/100m ²	58.4±3.5	30.1±4.3	140
Mycostop 12 g/100m ²	67.0±3.0	22.1±2.0	168
Previcur 80 ml/100m ²	65.7±2.8	26.7±2.3	155

Mycological analysis

From non-treated substrate there were isolated and identified 52 fungi species belonging to 28 genera and *Mycelia sterilia*. Prior to substrate treatment there were 49,200 propagules/1 g of dry soil. After the treatment with the fungicide Previcur the concentration of colony forming units was 26,700 from 1 g d. m. of soil and 37,400 c.f.u./1g d. m. of soil in combinations with the antagonistic organism *S. griseoviridis*.

In non-treated substrate the following fungi were isolated most frequently: *Aspergillus fumigatus*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Cladosporium herbarum* and various species of *Fusarium*, *Mortierella*, *Penicillium* and *Trichoderma* genera. Such frequently isolated fungi as *Penicillium decumbens*, *P. expansum*, *P. paxilli*, *P. spinulosum*, *Humicola grisea* and *Rhizopus stolonifer* var. *stolonifer* (= *R. nigricans*) can cause intensive maceration of root tissues. Also typical soil-borne fungi, *Mortierella* spp. and *Trichoderma* spp. were abundant in investigated substrate. In many cases pathogenic *Botrytis cinerea*, *Fusarium oxysporum*, *F. solani*, *F. solani* var. *argillaceum*, *Phoma betae*, *P. lingam*, *Pythium debaryanum*, *Rhizoctonia solani*, *Thielaviopsis basicola* and *Verticillium albo-atrum* were isolated from non-treated substrate. These species are known as agents of root infection of many crops.

Activity of some organisms is depressed partly or completely, others can adapt and grow (Lugauskas, 1988; Mirczink, 1988). Microorganisms functioning in propamocarb hydrochloride-contaminated substrate were found and 27 fungi species belonging to 17 genera and *Mycelia sterilia* were identified. The systemic fungicide Previcur affected many of the soil-borne micromycetes. In the disinfected substrate there were 25 fungi species less than in the non-treated one. There is an opinion that, when a number of saprotrophic organisms remained, parasitic fungi, such as *B. cinerea*, *F. oxysporum*, *P. betae*, *P. lingam* and *R. solani* beyond competition can spread.

From the substrate treated with *S. griseoviridis* there were isolated and identified accordingly 40 and fungi species ascribed to 23 genera. In this case complex lignin-cellulose prevailed that destroyed micromycetes from *Trichoderma*, *Aspergillus*, *Mortierella* and *Mucor* genera. The antagonistic organism *S. griseoviridis* was more aggressive against *F. oxysporum*, *F. solani*, *F. solani* var. *argillaceum*, *P. betae*, *P. debaryanum*, *R. solani* and *V. albo-atrum* species.

It should be noted, that all substrates contained the following fungi species: *Acremonium alternatum*, *Aspergillus clavatus*, *A. fumigatus*, *Cladosporium cladosporioides*, *Doratomyces microsporus*, *Mortierella isabellina*, *Penicillium lividum*, *P. paxilli*, *P. spinulosum* and *T. harzianum*. These micromycetes are found abundantly in soil and they destruct various organic compounds. It is noted, that these fungi adapt under extreme nutrition conditions and can exist on the effect of chemical and physical factors.

The obtained data show that different microorganisms species and their compositions can function in variously treated substrates. The similarity and disparity coefficient by Sørensen of microflora in comparison with the non-treated substrate is reliable: in tests with Previcur – 30.38%, with *S. griseoviridis* – 38.04%.

Mycostop on ornamentals

Mycostop and Previcur did not produce negative effect on gerbera growth. Therefore, it is possible to assume that biological fungicide Mycostop rate of 30 mg/plant (during all training period) is not phytotoxic for gerbera. The employment of Mycostop produced good results, though its biological efficiency was only 54.6%. The rather low efficiency of Mycostop in this case may be explained, that gerbera has already been grown for a year, a part of them was injured by root rot and biofungicide Mycostop treatments were not started immediately after transplanting. In our opinion Mycostop can be applied in greenhouses for protection of ornamental plants against root rots, seedling rots, damping off etc. However, it is important, that the first treatment is made immediately after transplanting and then repeated monthly or at 3-6 week intervals.

During the first inspection of the trial gerbera plot we established, that from 650 gerbera, which were planted one year ago 38 plants were affected by *Pythium* root rot and 66 were dead and removed. In the disintegrated tissues of plants roots there were found insects *Collembola*. The fact that gerbera has already been grown for a year and a part of them had been infected, affected the trial results. It must be admitted, that we failed to stop the

prevalence of root rot in gerbera in any treatment. The number of healthy plants decreased in all treatments. But in the plots with Mycostop and Previcur there were by 25-36% more healthy gerbera plants than in the untreated control (table 2).

Table 2. Efficiency of Mycostop against *Pythium* root rot in gerbera *Bianca* in rockwool.

Treatment	Infected plants, %	<i>Pythium</i> root rot, %	Biological efficiency, %
Untreated	44.6	45.8	-
Mycostop 30 mg/plant	19.5	20.8	54.6
Previcur 0.45 ml/plant	8.4	18.5	59.6

During three trial months in the control gerbera affected by *Pythium* root rot increased more than 10 times. Meanwhile, in the treatment, where gerbera were watered with Mycostop 0.01% suspension three times (30 mg/plant), the number of injured plants increased only 5 times and in the treatment with Previcur 0.15% (0.45 ml/plant) 1.7 times.

In addition root rot intensity in gerbera was different depending on treatments. Applications of Mycostop and Previcur affected quality of gerbera flowers. According to data of three calculations there were more of non quality flowers in the untreated plots. In the treatments with Mycostop and Previcur the percentage of non quality flowers decreased and reached 10.4-16.7%.

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Biological and integrated control in ornamentals: successes and challenges

Mette Skovly Svendsen, Erik Wermund Hansen

EWB BioProduction, Centervej Syd 4, DK-4733 Tappernoeye, Denmark, E-mail: mette@bioproduction.dk

Abstract: Denmark was one of the first European countries to implement biological control in ornamentals starting in 1987 with the introduction of *Verticillium lecanii* in cuttings. In 2001 the number beneficial organisms had increased to about 50-60 biological products. Today biological control is used on 30-35% of the area producing ornamentals. The complex pest-system in ornamentals makes a demand for a large range of biological/integrated solutions and more beneficial species are sought for to solve specific pest problems. Two new candidates in ornamentals, the ladybird *Stethorus punctillum* and the gallmidge *Feltiella acarisuga*, may have a promising potential in ornamentals. Factors influencing successes and challenges in Danish ornamentals are discussed.

Key words: biological control, integrated control, ornamentals, interactions, monitoring, successes, challenges, monitoring, new beneficials, *Stethorus punctillum*, *Feltiella acarisuga*, Denmark

Introduction

The area of pot-plant production in Denmark is around 350 ha. The main ornamental crops are pot roses, Poinsettia, Kalanchöe, Gerbera, Hedera, and Chrysanthemum. They are attacked by a wide range of pest species, the most important ones being spider mites, aphids, whiteflies, thrips and fungus gnats.

From the mid 70'ies successful biocontrol methods were developed and used in various vegetable crops and this valuable knowledge has since the late 80'ies been adapted to pest control in ornamentals. In 1990 biocontrol was used on 10% of the area with pot flowers, which had increased to 30-35% in 1999. The number of biocontrol agents available for Danish pot-plant growers has increased to 50-60 species (Enkegaard *et al.*, 1999) (selected species, table 1), and new agents are discovered.

The pest complex in ornamentals is often large since growers commonly have several plant species in different stages in culture side by side in the same glasshouse. This combined with low damage thresholds has delayed the implementation of biocontrol in ornamentals compared to vegetables (Brodsgaard & Enkegaard, 1997). However, within the last 5 years there seems to be an increasing tendency to separate the different stages of pot plants more and more, as well as to grow monocultures of ornamentals hereby reducing the risk of contamination between plant stages/species (pers. obs.).

Due to the fact that many ornamentals are attacked by six to ten pest species a wide range of beneficials must be introduced to achieve sufficient pest control. The abundant number of species in the pest-beneficial system increase the possible interactions. Only few experiments with focus on the consequences of interactions among species have been made and more knowledge is needed. Although many challenges have been solved successfully, some must still be overcome to improve biological control in ornamentals and still more complex biological control programs are needed to predict successful control (Brodsgaard & Enkegaard, 2001).

Table 1. The main beneficial species used in Danish ornamental productions. (+) the beneficial is used, (+) no use in the respective crop, (♦) new candidates in ornamentals, (●) the beneficial has recently been introduced in ornamentals with good results (Hansen, 1995; Brodsgaard & Enkegaard, 1997; Svendsen *et al.*, 1999; pers. obs.).

Beneficials	Ornamental production in Denmark (2001)					
	Pot roses (25 ha)	Kalanchöe (25 ha)	Gerbera (2 ha)	Poinsettia (20 ha)	Hedera (50 ha)	Other crops (300 ha)
<i>Phytoseiulus persimilis</i>	+	+	+	÷	+	+
<i>Hypoaspis miles</i>	+	+	+	+	+	+
<i>Hypoaspis aculeifer</i>	+	+	÷	÷		+
<i>Amblyseius cucumeris</i>	+	+	+	÷	+	+
<i>Amblyseius californicus</i>	+	+	+	÷		+
<i>Feltiella acarisuga</i>	÷	÷	♦	÷	♦	♦
<i>Aphidoletes aphidimyza</i>	+	+	÷	÷	+	+
<i>Orius majusculus</i>	+	+	+	÷	+	+
<i>Orius laevigatus</i>	+	+	(+)	÷	+	+
<i>Stethorus punctillum</i>	÷	÷	♦	÷	♦	♦
<i>Harmonia axyridis</i>	+	+	÷	÷	÷	+
<i>Hippodamia convergens</i>	+	+	÷	÷	÷	+
<i>Cryptolaemus montrouzieri</i>	÷	÷	÷	÷	÷	+
<i>Encarsia formosa</i>	+	+	+	+	+	+
<i>Eretmocerus eremicus</i>	●	÷	●	÷	÷	●
<i>Aphidius colemani</i>	+	+	+	÷	+	+
<i>Aphidius ervi</i>	+	+	+	÷	+	+
<i>Steinernema feltiae</i>	+	+	+	+	+	+
<i>Heterorhabditis</i> spp.	÷	÷	÷	÷	÷	+
<i>Verticillium lecanii</i>	+	÷	÷	+	+	+
<i>Trichoderma</i> sp.	+	+	+	(+)	(+)	+

Biological and integrated control in ornamentals

Few ornamental growers apply pest control based solely on biological means. Integrated strategies are used in most cases. Thus biocontrol is used in connection with mechanical reduction of pests (sticky traps), with physical reduction of fungal spores in the recycling water in order to control various soil borne diseases, or in combination with selective pesticides e.g. Pirimor (Pirimicarb), Applaud (Buprofezin) or non-selective pesticides e.g. Pyrethroids. In the last case biological control can not be used for a longer period. Monitoring is one of the key elements to avoid pest explosions and to grow ornamentals with efficient IPM.

Successes

Release strategies – preventive and “keep-down”

In order to avoid pest “hot spots” many growers apply several less costly products preventively, and when the first pest are observed more expensive products are taken into use. When the “keep-down” strategy is used high numbers of beneficials are released inundatively throughout the growing period to keep pest populations below the very low damage threshold (Brodsgaard, 1995).

Different products for different plant stages

It is a must to start an ornamental production with clean cuttings. In e.g. pot roses *V. lecanii* is used under the humid conditions when cuttings are rooting to kill insects on the plant material. After the rooting period the plants grow under lower humidity levels and predatory mites (*Amblyseius cucumeris*, *Hypoaspis miles* and *Phytoseiulus persimilis*) are released preventively against thrips and spider mites, respectively. These products are relative cheap and not costly to use preventively. In addition, the parasitoids *Aphidius ervi*, *A. colemani* are used preventively (often as banker plant systems) for aphid control. To avoid damage on flower settings the more expensive ladybirds *Harmonia axyridis* and *Hippodamia convergens* are released late in the growing cycle to control aphids in the flowers (pers. obs., 2001). Treatments with chemicals or CO₂ are often used just before sale to kill off all insects, pests and beneficials alike.

Factors influencing effective pest control

A high level of hygiene, clean plants (cuttings), the right advice and schedules for releases of beneficials, continuous monitoring of pests, having a person in charge of biocontrol, communication among researchers, growers, and advisory companies are important for successful biological/integrated control.

Challenges in ornamentals

Biological/integrated control programs

With the increasing use of more beneficial species simultaneously it is of great importance to offer safe and sound biological/integrated control programs to help growers determine which species to use, as well as the time of application and the quantities and frequencies of release. Control programs are adapted to the specific ornamental crop by advisers.

Better distribution systems

Some beneficials fly and locate pests in the crop easily. However, others e.g. mites crawl and disperse slowly necessitating time consuming releases throughout the greenhouse. A “mite gun” with a gentle radiating air flow releasing predatory mites with large efficacy has been developed and tested with *A. cucumeris* with good results in several ornamentals (Hibiscus, pot roses and Gerbera). This treatment is, however, harmful to the predatory mite *Phytoseiulus persimilis*. Slow release bags with *A. cucumeris* are often used with introductions every six weeks. The mites disperse from the bags over a long period (hereby saving time for the grower) and spread easily where plants are in contact.

Development of new biocontrol agents in ornamentals e.g. *Feltiella acarisuga* and *Stethorus punctillum*

With the large pest-complex in ornamentals there is an increasing demand from the growers for new and more efficient beneficials. In cases where good biocontrol has been achieved in vegetable crops, it is obvious to test the beneficials’ potential in ornamentals. However, the cultural conditions and the very low damage thresholds in ornamentals make high demands on the pest control.

A combination of the gallmidge *Feltiella acarisuga* and the predatory mite *P. persimilis* efficiently controlled spider mites in cucumber in 2001. The adult gallmidges are able to disperse up to 25 m per week from the releasing point and to locate even small "hot spots" of spider mites where eggs are laid (pers. obs.). After hatching the larvae feed upon all stages of spider mites with large appetite both at high and low humidity levels (Svendsen *et al.*, 1999). Use of *F. acarisuga* in ornamentals is new and it is advisable only to release this beneficial where spider mite "hot spots" are tolerated. In the early January 2002 *F. acarisuga* was released in a propagation section of a production of Danish cut roses, and a fine establishment was observed ultimo January 2002. The development and spider mite control will be followed.

The ladybird *Stethorus punctillum* is new on the market. It feeds upon several species of spider mites including *Tetranychus urticae* and *T. cinnabarinus* and is exceptionally good at detecting small spider mite infestations. The ladybird may be a welcome supplement in ornamental crops when spider mite "hotspots" occur.

Price and pesticides

Some growers chose to use pesticides in ornamental crops when biological control is difficult or more expensive than chemical solutions. It is a challenge to make growers refrain from the use of new and old (illegal) non-selective pesticides, hereby avoiding disruption of well-working biological programs. This is linked to the key issue: Competitive prices of beneficials compared to the use of pesticides.

Pest control in ornamentals in the future

The use of biocontrol in ornamentals is far below its potential and when growers have good experiences by using biocontrol they are more likely to continue. With increasing number of biocontrol agents being available and increasing knowledge of intra- and interspecific interactions more precise solutions can be found. In addition, there is a large potential for the use of microbial products against fungal diseases. A potential of 100 million DKK (~14 million EURO) can roughly be estimated if biological control is used on the whole ornamental area against all pests. This is, of course, an ideal situation, even less would be welcome.

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Can *Tetranychus urticae* be controlled by *Macrolophus caliginosus* in glasshouse tomatoes?

Marc Van de Veire, Evy Cornelis, Luc Tirry

Ghent University, Department of Crop Protection, Agrozoology Unit, Coupure Links 653, B-9000 Ghent, Belgium, E-mail: Marc.vandevaire@rug.ac.be

Abstract: The functional response of the predatory bug *Macrolophus caliginosus* to the twospotted spider mite *Tetranychus urticae* was studied. Adults and nymphs of the predator were offered increasing numbers of spider mite deutonymphs or adults. The number of attacked mites increased when prey density increased. The predation rate at high prey densities was very high. When *T. urticae* and *Trialeurodes vaporariorum* preys are offered simultaneously to *M. caliginosus* females, the latter attack proportionally more spider mites than whitefly larvae at any proportion of the 2 prey species. The high predation rate and the affinity of *M. caliginosus* for *T. urticae* in the presence of greenhouse whitefly larvae may explain why *T. urticae* populations in greenhouse tomatoes are not able to expand, when growers use the predatory bug for whitefly control.

Key words: *Macrolophus caliginosus*, *Tetranychus urticae*, *Trialeurodes vaporariorum*, functional response, prey preference

Introduction

In North European glasshouse tomatoes, the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) is controlled with the parasitoid wasp *Encarsia formosa* (Gahan) and the predatory mirid bug *Macrolophus caliginosus* Wagner. *M. caliginosus* is a polyphagous predator, attacking a wide range of arthropod preys; at lower temperatures, which are unfavourable for *E. formosa*, the bug remains active (Fauvel *et al.*, 1987; Trottin-Caudal, 1994). Due to its slow population build up, the bug must be introduced soon after transplant, at low whitefly population densities, to back up *E. formosa*.

Tedeschi *et al.* (1999) studied daily prey consumption and mean developmental times of *M. caliginosus* nymphs on different preys (*Myzus persicae* and *Aphis gossypii* adults, *Frankliniella occidentalis* adults, *Tetranychus urticae* adults, *Spodoptera exigua* eggs). In laboratory choice and no choice tests, no clear prey preferences were found; all prey types were consumed. However, the developmental time was twice as long on *T. urticae* adults, compared to other prey types. Nevertheless, it is interesting to mention that individual nymphs killed an average of more than 500 spider mite adults during their nymphal developmental period. Also, many tomato growers in Belgium and The Netherlands observed only small or no twospotted spider mite problems in glasshouse tomatoes after the introduction of *M. caliginosus* for additional whitefly control. This was also noted earlier by Sampson & King (1996) in glasshouse tomatoes in England.

We have tried to evaluate the potency of *M. caliginosus* as a biocontrol agent for *T. urticae* in glasshouse tomatoes. Currently, the functional response of *M. caliginosus* nymphs and adults, fed on *T. urticae* adults and nymphs, was studied. We also carried out a prey preference study with *T. urticae* adults and *T. vaporariorum* larvae.

Materials and methods

Insects and test cages

M. caliginosus adults and nymphs were collected from a laboratory colony, maintained at $25 \pm 3^\circ\text{C}$, 50% RH and a photoperiod 16/8 (L/D) h. They were fed *Ephestia kuehniella* eggs; small spanish pepper plants (*Capsicum annuum* L. cv. Amando F1) were used as moisture source and oviposition substrate. *T. urticae* adults and nymphs were reared on *Phaseolus vulgaris*. Two to 3 days prior to the tests, adults or nymphs of *M. caliginosus* were transferred to the test cages, fed on a mixture of *E. kuehniella* eggs and *T. urticae* (different stages), and put in a growth chamber at $25 \pm 1^\circ\text{C}$, 60 % RH and a photoperiod of 16/8 (L/D) h. The latter conditions were used in all experiments.

The test cages consisted of 3 parts: 1) a cylindrical Plexiglas ring (\varnothing : 9 cm; height: 3.5 cm), with 6 ventilation holes (diameter: 1 cm) covered with nylon gauze and one opening (diameter: 0.8 cm) for the introduction of the predators; 2) a round Plexiglas plate (\varnothing : 9 cm) to cover the top of the cylinder, with filter paper taped to the underside to absorb condensation water and 3) a Petri dish (\varnothing : 9 cm) to form the bottom of the cage. The bottom of this Petri dish was covered with wet cotton wool, on which a rectangular piece of sweet pepper leaf (*Capsicum annuum*) (2.8 x 2.8 cm) was placed as food source for the mites. The edges of the leaf cutting were covered with wet filter paper to avoid mite migration. Adults of both *T. urticae* and *M. caliginosus*, used in tests, were less than 48 h old.

Functional response experiments

Experiment 1: combination *T. urticae* adults – *M. caliginosus* N2. Fifty, 100, 150, 250 or 300 *T. urticae* adults were transferred to the test cage on the sweet pepper leaf cutting. Four replicates were used per amount. Then, 1 *M. caliginosus* nymph (N2) was introduced in each cage. The number of surviving spider mites was counted after 48 h. Three test cages containing 50 *T. urticae* each were used to check for the natural mortality of the mites. Killed prey was not replaced.

Experiment 2: combination *T. urticae* adults – *M. caliginosus* females. The set-up was similar to experiment 1, except that 1 adult female *M. caliginosus* was introduced per test cage.

Experiment 3: combination *T. urticae* deutonymphs – *M. caliginosus* adult females. The set-up was similar to experiment 2, but instead of adult *T. urticae*, increasing amounts (10, 20, 30, 40, 50, 75, 100, 150, 200 and 250 per container) of *T. urticae* deutonymphs were offered. Five replicates were used per amount.

Prey preference experiment

Individual *M. caliginosus* females were offered a mixture of 3rd instar *T. vaporariorum* larvae and *T. urticae* adults in different proportions; the total number of preys was 280. The experimental set-up was similar to that used in the functional response tests except that *Phaseolus vulgaris* leaf cuttings, which were more suitable for the greenhouse whitefly, were used, and that the number of surviving spider mites and whiteflies was counted after 24 h.

Results and discussion

Functional response experiments

Fig. 1a shows the results of experiment 1 and 2. In both experiments, the number of attacked mites increased with increasing prey density. In the case of *M. caliginosus* females, the number of attacked mites still seemed to be increasing, even at the highest mite density tested (300 *T. urticae* adults). When using *M. caliginosus* nymphs, the number of attacked mites seemed to have reached its maximum. The calculated regression curves resemble a type II functional response curve (Holling, 1959).

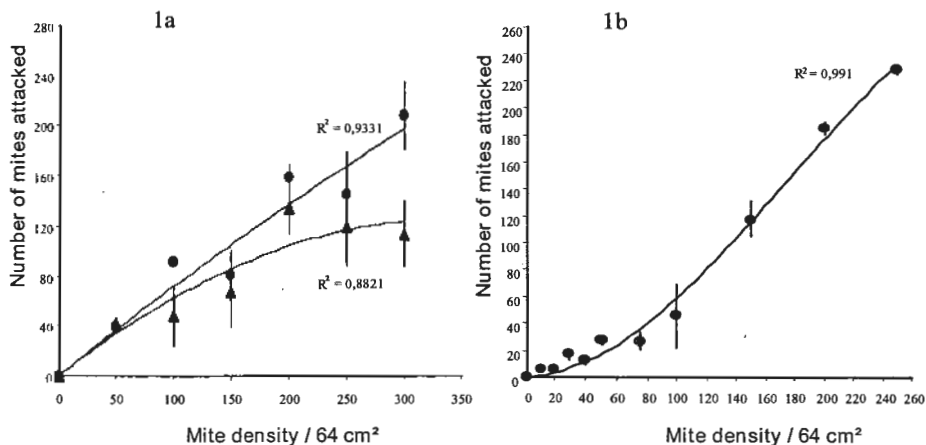


Figure 1a. Functional response of *M. caliginosus* N2-nymphs (▲) and females (●) to increasing densities of *T. urticae* (adults). Data are given for a 48 h predation period. (Mean ± SE). Figure 1b. Functional response of *M. caliginosus* females (●) to increasing density of *T. urticae* (nymphs). Data are given for a 48 h predation period. (Mean ± SE).

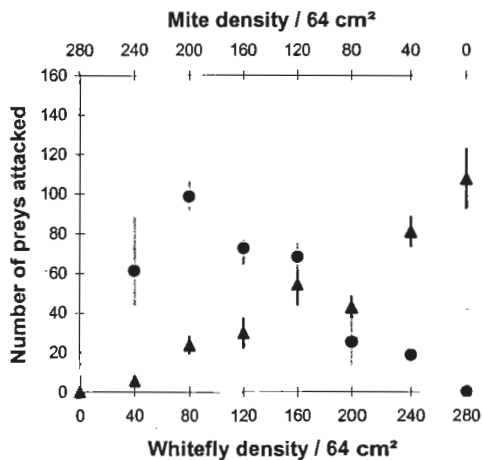


Figure 2. Predation of *M. caliginosus* females on different proportions of *T. urticae* adults (●) and *T. vaporariorum* (L3) (▲) (total: 280 preys). Data are given for a 24 h predation period. (Mean ± SE).

It was clear that *M. caliginosus* nymphs (N2) attacked less prey than adults. The maximum number of attacked prey (by adults of *M. caliginosus*) was 260 out of 300 in 48 h.

Fig. 1b shows the results of experiment 3: functional response of *M. caliginosus* females to spider mite deutonymphs. In this experiment, the number of attacked prey also increased with increasing prey density. However, as could be expected, *M. caliginosus* females attack more *T. urticae* deutonymphs than adults.

Prey preference study

Fig. 2 shows the predation of *M. caliginosus* females on spider mite adults and 3rd instar whitefly larvae, which are offered simultaneously in different proportions, but with a constant total of 280 preys.

The number of attacked prey per prey species increased with increasing prey density; for none of the 2 preys, a plateau has been reached. The experiment also showed that spider mites were still attacked even if its proportion on the total number of prey offered, was low. However, looking at the predation per species, the percentage attacked spider mites of the total number of spider mites offered is higher than the percentage attacked whiteflies of the total number of whiteflies offered, irrespective of the proportion of preys.

Conclusions

The predatory bug *M. caliginosus* does attack and feed on *T. urticae* adults and nymphs. The number of prey eaten by female adult predatory bugs, after 48 h, was so high that a plateau in the functional response curve was not yet achieved by offering 250 to 300 spider mite nymphs or adults. During the same period, more spider mite nymphs were attacked than adults.

M. caliginosus nymphs attacked less spider mite adults than *M. caliginosus* adults.

When spider mite adults and whitefly larvae were offered simultaneously to female adult *M. caliginosus*, they attacked proportionally more spider mites than whiteflies at any prey species ratio.

The very high predation rate of *M. caliginosus* on *T. urticae* and the affinity of *M. caliginosus* to *T. urticae* in the presence of *T. vaporariorum*, may be responsible for the efficient spider mite control in tomatoes or for the fact that tomato growers using *M. caliginosus* for whitefly control often encounter little or no problems with spider mites in their crop.

However, we should keep in mind that the tests were done on small arenas, and that the effect of *T. urticae* as prey on the reproduction of *M. caliginosus* was not yet studied in these experiments. Further studies, including semi-field and field experiments are needed to confirm the laboratory results.

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State of integrated crop protection in Dutch nursery stock and future prospects

Anton van der Linden

Applied Plant Research, Nursery Stock Research Unit, Rijnveld 153, 2771 XV Boskoop, The Netherlands, E-mail: A.van.der.Linden@PPO.DLO.NL

Abstract: An overview is given of experiences with IPM in nursery stock in The Netherlands. So far, the most important use of natural enemies are the entomophagous nematodes *Steinernema* spp. against Sciaridae and *Heterorhabditis* spp. against the black vine weevil. On a smaller scale the predatory mites *Amblyseius californicus* and *Phytoseiulus persimilis* are being introduced against spider mites, and *Aphidius* spp., *Aphelinus* spp. and *Aphidoletes aphidimyza* against aphids. In outdoor crops natural control is of more importance. Other experiences are reviewed and some future expectations are outlined. The experience with biological control in glasshouses and the observation of natural control in crops outdoors seems of mutual benefit.

Key words: pests, diseases, integrated control, natural enemies

Introduction

Nursery stock production includes many different types of crops and cultures, with approximately 20,000 plant species and cultivars grown. As a result of governmental policy the number of registered pesticides is and will be further reduced. Moreover, the area of nursery stock is relatively small on an agricultural scale. This makes it economically less interesting for companies to register new pesticides. Growers need alternative reliable means of controlling pests and diseases.

This review gives the areas of Dutch nursery stock production, recent experiences with regard to IPM in nursery stock and information from practical advisors.

Review and prospects

Nursery stock area

An overview of different types of nursery stock (LEI / CBS, 2001) is given in table 1, which shows that the total area of nursery stock is still increasing every year. The percentage of protected cultivation (glasshouses and tunnels) in nursery stock is small compared with the total areas of nursery stock, but it is a substantial area compared with that in many other countries.

Many crops suffer from similar pests such as aphids, mites and caterpillars. Beneficial insects and mites can be applied against these pests in nursery stock under protected cultivation, which had an area of 369 ha in the year 2000 (table 1). The wide variety of crops, and thus pest species in nursery stock makes IPM complex and challenging. In outdoor nursery stock crops natural control will probably be more important than the release of natural enemies. The coexistence of both outdoor and protected cultivation gives an interesting exchange of possibilities for pest control. The Dutch experience with biological control in glasshouses, which started in vegetable crops about 30 years ago now also benefits other

glasshouse crops. In many situations natural enemies can be used very successfully. The occurrence of natural enemies on outdoor crops may also give new ideas for biological control of pests in glasshouses.

In outdoor cultures releases of natural enemies might be less effective. One important factor is the number of generations pests and natural enemies are able to complete outdoors. In outdoor nursery stock the encouragement of beneficial insects, mites and even predacious vertebrates might have a greater impact on pests than releases of cultured natural enemies. Although the role of field boundaries, hedges, flower strips and banker plants are not yet completely understood, they may serve as reservoirs of natural enemies. The entomophagous nematodes *Steinernema* and *Heterhorhabditis* spp. against sciarid flies and vine weevil, are currently more widely used than predatory mites against spider mites in nursery stock. The natural occurrence of predators deserves much attention, because this gives an indication which natural enemies are on the right spot. These natural enemies select any particular situation according to habitat, hostplant, prey or host. This might give new ideas for natural enemies against pests in glasshouses, because several crops are grown both outdoors and under protection. However, methods for encouraging natural enemies outdoors will need to be developed so that pests are kept under control.

Table 1. Areas (ha) of Dutch nursery stock (LEI / CBS, 2001).

	2000	1999	1998
Lane and Park trees	3158	3008	2955
Forest, Hedge, Public garden plants	2362	2333	2277
Ornamental conifers	2340	2074	1822
Ornamental shrubs and climbing plants	1787	1745	1658
Fruiting trees and rootstocks	1251	1368	1330
Perennials	1207	1108	947
Roses and rootstocks	612	676	716
Total outdoor culture	12717	11714	9775
Cultures in pots and containers	975	903	805
Protected cultivation	369	315	324

Integrated control in nursery stock

An overview of experience with IPM in nursery stock is extracted from Dolmans (1994) and from Van der Horst & Van Tol (1995):

In containers in tunnels and glasshouses, *Phytoseiulus persimilis* was released against spider mites on *Aralia elata*, and *Aphidoletes aphidimyza* with *Aphidius colemani* were released against aphids on *Campis. Magnolia*, *Choisya ternate*, *Photinia fraseri* and *Viburnum plicatum* had thrips and aphids as prominent pests. *Verticillium lecanii*, *Orius insidiosus* and *Amblyseius cucumeris* were applied with good result. *Choisya* and *Photinia* were provided with extra natural enemies, because these crops are not deciduous and cosmetic damage is not acceptable. *Aphelinus abdominalis* and *Aphidoletes aphidimyza* were succesful against *Macrosiphum euphorbiae* on *Photinia* and *Viburnum*. Before cuttings were taken pirimicarb

was applied. Greenhouse whitefly *Trialeurodes vaporariorum* was controlled with *Encarsia formosa*.

In outdoor container fields with *Passiflora*, *Vaccinium* and *Cornus canadensis* the main problems were fungal diseases. *Phytophthora* in *Vaccinium* was controlled with fosetylaluminium. *Euonymus fortunei* cv. "Dart's Blanket" was utilized as an indicator plant for *Otiiorhynchus sulcatus*. There have been good results with entomophagous nematodes *Heterorhabditis* spp. against the black vine weevil *Otiiorhynchus sulcatus*. Spider mites occurred in *Daphne*, *Cornus*, *Magnolia*, *Wisteria* and *Cercis*. Usually fenbutantinoxide and hexythiazox gave good control. On *Aralia elata*, *Morus latifolia*, *Prunus subhirtella* and *Prunus sargentii* the natural control by the predatory mite *Amblyseius potentillae* (Garman) (= *Amblyseius andersoni* (Chant)) kept the spider mites at low numbers. Damage by aphids varies according to the crop and species of aphid. *Periphyllus californiensis* was very damaging to *Acer*, while *Betula* was able to bear large numbers of *Euceraaphis puntipennis*. Aphids on *Aesculus*, *Amelanchier*, *Betula*, *Caragana*, *Catalpa* and *Corylus* were controlled by natural occurrence of Coccinellidae, Syrphidae and Chrysopidae.

Further integrated control measures that were developed are a decision model for powdery mildew, *Sphaerotheca pannosa* in rose. For the very polyphagous *Pratylenchus penetrans*, suitable crops for crop rotation, including *Tagetes erecta* were investigated.

Present research in nursery stock

IPM in nursery stock has been introduced on several Dutch nurseries and undoubtedly the area will grow in the years to come. So far, there are no reliable data on the area with biological control so a provisional estimation was made with the help of suppliers and practical advisors in Dutch nursery stock. In 2001 the total area treated with *Steinernema* spp. against sciarids was approximately 30 ha, *Heterorhabditis* spp. against black vine weevil 10 ha, *Amblyseius californicus* against spider mites 10 ha and *Aphidius* spp., *Aphelinus* spp. and *Aphidoletes aphidimyza* against aphids 10 ha. Natural control is probably even more important than released natural enemies, but when a pest does not become a problem the action of natural enemies is not recognized.

During 2000 and 2001 good control of aphids in *Magnolia* propagation in the glasshouse was achieved using *Aphidoletes aphidimyza*. Good control of spider mites and aphids in *Acer* propagation in the glasshouse was achieved after introduction of *Amblyseius californicus* followed by natural occurrence of *Feltiella acarisuga* and aphid parasitoids, such as *Praon volucre*.

Other important naturally occurring predators against spider mites and rust mite *Eriophyes macrotrichus* in *Carpinus betulus* were *Euseius finlandicus* and *Amblyseius andersoni*. In *Buxus*, *A. andersoni* and *A. californicus* were found as predators of the gall mite *Eriophyes canestrinii*, with more predatory mites observed in high gall mite densities. It would be useful to evaluate banker plants to increase the numbers of these predatory mites.

In *Rosa* a test has been carried out with *Phytoseiulus persimilis* and *Amblyseius californicus* against spider mites. The number of spider mites was reduced in both the treated and the untreated plots. Numbers of spider mites decreased faster in plots with *A. californicus* than in plots with *P. persimilis*, and in the plots with *P. persimilis* faster than in untreated plots. *A. californicus* showed the strongest tendency for migration. Other really voracious natural enemies of spider mites, i.e. *Orius* sp., *Chrysopa* sp. and *Feltiella* sp. contributed to the control and their role in the suppression of spider mites was probably underestimated. For early or low infestations or starting infestations of spider mites, predatory mites still seem to be the best option to keep the pest under control.

In nurseries with young apple trees, *Orius minutus* was found in leaves curled by larvae of the apple leaf gallmidge, *Dasineura mali*. *Orius* was found in larger numbers together with lower numbers of apple leaf gallmidge in a 'biological' field than in a 'regular' field. It is possible that planting *Ulmus* nearby could serve as a reservoir for *Orius*, as it has been observed that *Orius* sp. can be abundant in *Ulmus* in the absence of prey.

Further work is being done on early warning systems for pests and diseases, biopesticides, mechanical weed control, the application of mulches, etc.

Future prospects

In glasshouses with nursery stock, biological control can give good results. In ornamental cultures it is necessary to avoid any cosmetic damage, which may make it more difficult to achieve adequate control. Many outdoor cultures may tolerate some damage as long as growth is not hampered. For outdoor cultures, natural control will be of more importance than the release of natural enemies. It will be important to investigate optimal conditions for encouraging and establishing beneficial insects, mites and other animals, such as field boundary strips, hedges and banker plants. Experiences from other horticultural crops may give tools to accomplish this and likewise, experiences in nursery stock may be mutually beneficial for other horticultural crops.

Beech aphid *Phyllaphis fagi*, oak midge *Arnoldiola quercus*, *Stephanitis* spp. on Rhododendron and *Pieris* are pests with a limited host range, but often giving problems on these crops. It will be a challenge to develop a method of integrated control for them. *Stephanitis* bugs are also on a list for special attention by the European Plant Protection Organisation (EPPO). These, and other exotic pests as well, could be interesting to study for classical biological control.

A goal for the coming years will be to establish and maintain contacts between nursery stock researchers from different countries. Good contacts between researchers in horticultural crop protection and nursery stock in particular are very important in order to make the best progress with integrated crop protection.

To get a better view of the area on which integrated crop protection is applied in nursery stock an annual enquiry amongst the biological control suppliers will be set up.

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Greenhouse trials in Massachusetts and New York with *Amblyseius cucumeris*: effects of formulation and mechanical application

Roy Van Driesche¹, Suzanne Lyon¹, John Sanderson², Tina Smith³, Paul Lopes³, Susan MacAvery⁴, T. Rusinek⁴, Gary Couch⁴

¹Department of Entomology, University of Massachusetts, Amherst, MA, 01003, USA, E-mail: vandries@fnr.umass.edu; ²Department of Entomology, Cornell University, Ithaca, NY, 14853, USA; ³Massachusetts Extension, University of Massachusetts, Amherst, MA, 01003, USA; ⁴Cornell Cooperative Extension of Orange and Ulster Counties, NY, USA

Abstract: Trials in spring bedding plant crops in 2000 and 2001 in Massachusetts and New York commercial greenhouses measured the ability of *Neoseiulus (Amblyseius) cucumeris* to control western flower thrips, *Frankliniella occidentalis*. In 2000, the effect of formulation (mites in bran vs. sachets) was examined at three businesses. At all three sites, we found that sticky card catches of adult thrips were lower in greenhouses receiving mites formulated in bran vs. sachets. In 2001, we compared western flower thrips densities in greenhouses in which *N. cucumeris* releases were made either via hand application (sprinkle) of mites formulated in bran or mechanical application of the same material with a battery powered air blower ("mite gun"). Results suggested that the two application methods did not differ in their ability to suppress thrips populations.

Key words: western flower thrips, predacious mites, formulation method, application method, *Neoseiulus (Amblyseius) cucumeris*, *Frankliniella occidentalis*, biological control, bedding plants

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is the most serious pest of greenhouse bedding plants in New England. In California, releases of *Neoseiulus (A.) cucumeris* (Oudemans) on chrysanthemum at 2.5 mites per leaf were unable to reduce thrips densities below 2-7 per leaf (Hessein and Parrella, 1990), an unacceptable level. In Maryland, Gill (1994) found that *N. cucumeris* in a slow release formulation reduced pesticide applications needed for thrips control in bedding plants from 3.6 to 0.4. We report results of trials in commercial greenhouse bedding plant crops in Massachusetts and New York that examined the ability of *N. (A.) cucumeris* to control western flower thrips. A 2000 trial compared two formulations (mites formulated loose in bran ["bulk release"] vs. paper sachets ["slow release"]) and the 2001 trial examined mechanical application versus hand sprinkling of mites in bran.

Material and methods

2000 trial on formulation

In this trial there were three treatments: sprinkle application of mites formulated loose in bran (bulk release), sachets, and the chemically-treated grower check. The trial was run at three growers, two in Massachusetts and one in New York. At both Massachusetts sites there were three greenhouses, one for each treatment. Greenhouses were filled with flats of diverse

bedding plant species. In New York, there were only two greenhouses, one a bulk release and one a sachet greenhouse, both of which contained only potted dahlias.

In the Massachusetts greenhouses receiving bulk releases of *N. cucumeris*, we followed the manufacturer's recommended release rate of 106 mites/m² (10,000 mites per 1,000 sq. ft.) and we made five releases (in weeks 1, 2, 3, 5 and 7 of the 10 week crop), averaging of 53 mites/m²/wk (=5,000 mites/1,000 sq. ft./wk). In the New York greenhouses, the release rate was twice as high (212/m² or 20,000 mites per 1,000 sq. ft.) and releases were made in weeks 1, 3, 5, 7, 9, 11, 13 of a 14 week crop, giving an average of 106 mites/m²/wk (10,000 mites/1,000 sq. ft./wk). For mites applied using the sachet formulation, in Massachusetts we applied 1 sachet per 2.5 m² (=37 sachets per 1,000 sq. ft., or 1 per 27 sq. ft.), as recommended by product producer for preventative control. Sachets are reported by manufacturers to last 8 weeks, but replacement is recommended after 6 weeks for bedding plants. In Massachusetts, two deployments of sachets were made, in weeks 1 and 6. In New York, sachets were deployed in week 1 at a rate of one per 2.7 m² (40 sachets per 1,000 sq. ft.). Subsequently, one third of the sachets were replaced in weeks 3, 5 and 7, and again in weeks 9, 11, and 13. In both the sachet and bulk release greenhouses, we allocated 5% of the total material to be released for placement into hanging baskets. In the sachet greenhouses, this was achieved by tearing open some sachets and placing the contents in the baskets. In both states in all but the chemical checks, one release was made of *Hypoaspis miles* Berlese at a rate of 106 mites/m² (10,000 per 1,000 sq. ft.) onto the media (or in the pots) at the beginning of the crop.

In Massachusetts, we assessed the quality of mites formulated in loose bran by counting live mites per 0.25 g in 10 samples from each shipment. To assess quality for sachets, we retrieved 3 sachets from each grower weekly and placed them flat on a 15 x 25 cm sticky card on a greenhouse bench. After one week, cards were collected, sachets removed and mites counted. Mite counts from aged sachets were compared to that for new sachets just received from the supplier and held under the same conditions.

To measure treatment effects on WFT control, we counted western flower thrips caught on yellow sticky cards, made by cutting standard sized cards (7.6 x 12.7 cm) in half. Each half card sample unit was counted on both sides and replaced weekly. There were 20 cards per greenhouse, which were held up by clips on sticks stuck into pots or flats. Cards were distributed evenly throughout the greenhouse, placing cards in most kinds of bedding plants present. Counts were made in the greenhouse with a head-mounted optical magnifier (Optivisor®), supplemented as needed with a 10X hand lens.

2001 trial on application technique

In this trial, there were two treatments: (1) application via hand sprinkling of *N. cucumeris* in a bran formulation and (2) mechanical application of the same with a custom-made, battery-operated, air-powdered application gun. Application rates and patterns were the same as in the 2000 Massachusetts bulk release greenhouses. The mite gun application device (created by Warren Sargent of AgAttac® in Visalia California) consisted of a pvc pipe (10 cm dia) that acted as the gun barrel. Attached to the barrel was a stopcock onto which the product bottle could be fastened. Flow of bran into the barrel was by gravity, and was regulated by the degree of opening of the stopcock. Air movement down the barrel was produced by a battery-driven fan (10 cm dia). Mites were blown 1-2 meters and survivorship of mites collected in pans was 100%.

We assessed the quality of the product received from commercial suppliers before release by counting the number of live mites in ten 0.1 g samples from each shipment. We evaluated the efficacy of each method of application by means of sticky trap catches of adult thrips in each greenhouse as was done in the Massachusetts greenhouses in the 2000 trial, described earlier. We also measured the number of minutes needed to treat a standard area of greenhouse bedding plants (94.6 m² =1000 sq. ft.) with each of the application methods.

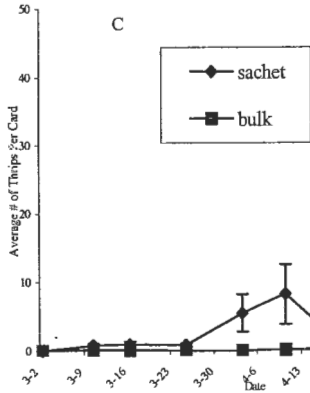
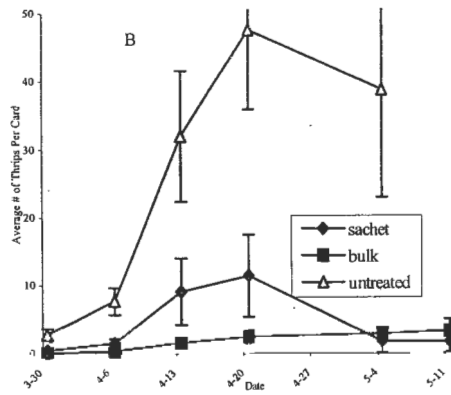
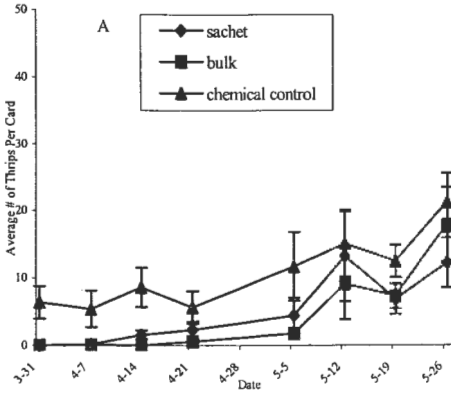


Figure 1. Count of western flower thrips on sticky cards for two *N. cucumeris* formulations at three sites in MA (A,B) or NY (C) in 2000.

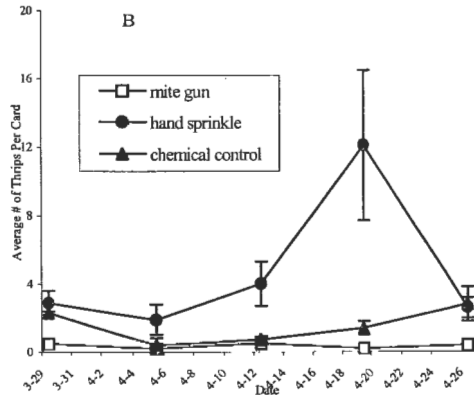
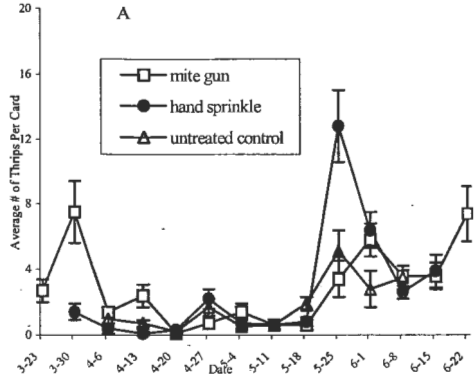


Figure 2. Counts of western flower thrips on sticky cards for mechanical vs. hand application of *N. cucumeris* at two sites in MA in 2001.

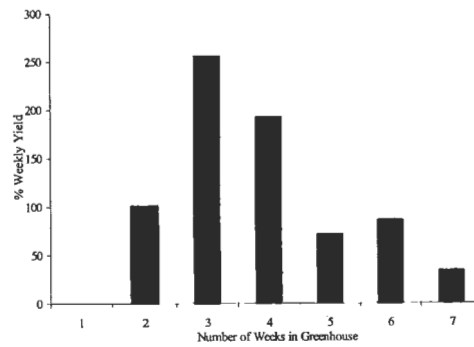


Figure 3. Yield of *N. cucumeris* aged sachets per week relative to the weekly yield of a fresh sachet.

Results and discussion

Formulation trial (2000)

At grower 1 (Fig. 1a) there was little difference in thrips catch among treatments, but the chemical control greenhouse consistently had the highest catches, followed by the sachet greenhouse. The greenhouse in which mites formulated loose in bran were applied had the lowest thrips catches on 5 of 8 sample dates (with numbers on the other three dates being the same as in the sachet greenhouse). At grower 2 (Fig. 1b), the grower did not make any pesticide applications in the grower check greenhouse and numbers of thrips reached 48 per card. The greenhouse receiving mites formulated loose in bran had the lowest counts on 3 of 6 sample dates and on the other three dates was not different from trap catch numbers in the sachet greenhouse. (Parts of both sachet and bulk greenhouses were treated with acephate on 27 April for aphids). At grower 3 (Fig. 1c), trap captures of thrips were lower in the greenhouse receiving mites formulated loose in bran than sachets. We conclude that the *N. cucumeris* formulated loose in bran is more effective than sachets in bedding plant crops.

Mechanical vs. hand application trial (2001)

At grower 1 (Fig. 2a), thrips captures in greenhouses where mites were applied by hand vs. by an air-powered gun differed only on two of 14 sample dates, but in opposite directions. At grower 2 (Fig. 2b), thrips captures in greenhouses in which mites were applied by the mite gun were consistently lower than in either the greenhouse in which mites were applied by hand sprinkling or in the grower check (chemically treated) greenhouse. However, at this location, the greenhouse that was treated with the mite gun had a higher proportion of plants, such as Coleus, that didn't flower and were thus less attractive to thrips. These findings suggest that the mite gun performed as well as hand sprinkling, but not necessarily better. Time to apply mites was reduced 47% by mechanical application (1.5 min. to treat 94.6 m² vs. 3.8 min. by hand).

Mite quality

For mites formulated loose in bran, in 2000 the number received per shipment ranged from 59 to 187% of the number ordered and averaged 118%. In 2001, the average number received was 73% of the number ordered. For mites in sachets, in 2000 we found that numbers of mites emerging per week remained at or above levels from fresh sachets for 3 weeks and then decline to 34% of the emerged from a fresh sachet by week 7 (Fig. 3).

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Invasive species as pests in greenhouses: forecasting, preventing and remediating future invasions

Roy Van Driesche

Department of Entomology, University of Massachusetts, Amherst, MA, 01003, USA, E-mail: vandries@fnr.umass.edu

Abstract: Greenhouses are susceptible to invasions from local species pools. Some such invaders have characteristics permitting them to be moved readily by the shipment of plant materials between businesses within the greenhouse industry, allowing species to become internationally distributed pests, sometimes in relatively brief periods. Such new pests disrupt pest control systems and require new responses. Greenhouse operators need to predict, prevent and remediate such invasions by deliberate collective action. Prediction should flow from crop-specific, country-specific inventories of current greenhouse pests to identify pests of greenhouse crops still confined to local areas, but which have features conducive to further spread. Prevention should be fostered by development of sophisticated, web-based identification systems focused on the high risk potential invaders identified in the prediction phase. Remediation requires application of classical biological control, an approach not familiar to greenhouse operators. This approach requires search for new natural enemies in the native ranges of new pests, study of host ranges of new agents, obtaining legal permissions for release of new agents in multiple countries, and finally development of systems for production of new species. Production of some species will be uneconomical for insectaries, but might be achieved through mutual aid networks of entomologists at conservatories and other facilities. These activities require the development of a new body, an international consortium of greenhouse operators, which can pool contributions of member businesses and hire staff with appropriate technical expertise to conduct the activities mentioned above. Such a coordinated effort to confront invasive species in greenhouses is likely to be more effective than the current fragmented system of local response.

Key words: greenhouses, invasive species, prevention, forecasting, classical biological control

Introduction

Greenhouses are subject to invasions of non-native pests. These typically arrive on plants obtained from greenhouses from other countries, where the pest occurs as a local native insect or previous invader. The greenhouse habitat is especially susceptible to exotic species invasions because small pieces of the habitat (individual greenhouses) are dispersed around the world, yet linked by plant movement between the fragments of the habitat. Greenhouses are easily entered by local species. Those local species that are able to feed on plants grown in the greenhouses may establish breeding populations on the crop. This allows these species to then be shipped internationally on plants moved between greenhouses. Greenhouses can thus be thought of as a trap crop, pulling species from local faunas wherever greenhouses are built, into the greenhouse habitat, which, while fragmented, spans international space. As greenhouse industries are established (for purposes of international trade) in more and more countries, the pool of local species from which the greenhouse habitat can recruit new pests, will expand. We should therefore expect new invasions on a regular, even increasingly frequent, basis due to globalization of the industry and its expansion into new locations in search of lower production costs. In addition, greenhouses may be entered by exotic species that invade countries by routes other than international trade between greenhouses. Citrus

leafminer (*Phyllocnistis citrella* Stainton), for example, entered Florida by means other than the greenhouse trade, but is now a species that can enter greenhouses in Florida if suitable plant species are produced.

Management of the invasive species problem has several components. First we should attempt to forecast near term likely invaders at the international level by compiling detailed lists of greenhouse pests country by country, especially in new production areas that are physically isolated by oceans or deserts from traditional production areas. Second, we should attempt to prevent the spread of these pests by developing specific educational materials for growers on the recognition of the most probable invaders so that they can be spotted early and eradicated as they begin to move into new countries. Third we should remediate damage from exotic invaders by using classical biological control in greenhouses. We should do this with a three prong attack. (1) We should educate growers and extension agents on how classical biological control works and how it could be used in greenhouses. (2) We should work at the international level to identify candidate natural enemies for various invaders and develop host specificity data needed to support the introduction of new natural enemies. (3) We should develop a system of shared quarantine laboratories for production of colonies of natural enemies for introduction. This should be modeled after efforts currently being made by a consortium of entomologists working to share natural enemies of scales, mealybugs and other pests in North American plant conservatories. We should not expect that the insectory industry will provide the needed natural enemies for classical biological control of new pests.

Forecasting future invasions

Invading species start as local species that enter greenhouses in one or a few locations and only later begin to spread internationally. We can and should identify future potential invaders likely to become internationally important pests at this early stage. To do this, we in IOBC should inventory pests currently found in greenhouse crops in each country with a greenhouse industry. This effort should focus particularly on the greenhouse “frontier” – those countries that have only recently begun to have a greenhouse industry engaged in international trade. The pools of local pests in such places are likely sources of new international pests simply because they have previously lacked the means to move internationally on greenhouse products. The inventory should indicate which pests are present, which plants are produced, what is shipped and where it is shipped to. Pest species will vary as plants in trade vary. Even traditional greenhouse areas such as Europe and North America may contribute new pests to the international invader pool as new plant species come into vogue and are produced as crops. This is especially true for plants grown as ornamentals because these species are often not yet as ubiquitous as the food crops grown in greenhouses. Country-specific lists of greenhouse pests should be updated formally every three years, and informally as invasions occur.

Preventing future invasions

Lists of local greenhouse pests not yet in international circulation generated by the international survey discussed above should then be used to develop identification and educational tools to sensitise growers to the appearance of the riskier species. A website should be developed on which high quality photographs of the life stages of these species are posted, together with information on their biology, crop species attacked, known geographical distribution, and other pertinent information. Growers then should study this database to familiarize themselves with dangerous species. The website should provide links for growers

to obtain updates on recent invasions and to report any suspicious infestations they encounter in their own greenhouses on newly received plants. The latter should be a simple list of countries, each with a direct link to the proper agency that should receive information about such observations in any particular country. This process should allow for greater odds of early encounter and eradication, before a pest becomes so dispersed in a country as to be firmly established within a country's greenhouses.

Remediating invasions: classical biological control in greenhouses

Greenhouse operators, even though used to employing biological control, are not familiar with classical biological control. Rather, greenhouse operators are accustomed to employing augmentative biological control, based on purchases of natural enemies from commercial suppliers. This model is not likely to provide optimal natural enemies for invasive species. Instead, there will be a need for greenhouse operators to work with national or university groups in particular countries to participate in government-led classical biological control projects that import, distribute and establish new, specific natural enemies for the particular new species of invasive pests. (Concurrent with this approach, commercial insectaries are likely to offer whatever available natural enemies they are already rear to help combat a new invader. In a few instances, these may be a good enough fit to be effective, but in many cases, these will simply not be the right species. They are likely to be generalists with limited efficacy).

To more effectively suppress invasive species, the model of classical biological control should be followed. This will have to be a government-run effort, involving foreign exploration for new species of natural enemies tightly tied to the invader in its native range. These natural enemies will have to be found and then their safety established by estimating their host ranges relative to fauna in particular countries in which introductions are to be made. This requires both access to quarantine facilities and knowledge of this type of research. Once release has been approved for new natural enemies, the commercial insectaries may wish to mass produce and market the new species, but this will be based on their estimates of likely profitability of doing so. Furthermore, such new natural enemies will have to be approved for release in each new country where the species might be sold. This fragmentation of the potential market is a large problem retarding the development and use of new natural enemies. The greenhouse industry should help solve this problem by forming an international consortium for the purpose of developing information needed to obtain permission for release of new species of natural enemies in all the member countries. Such a consortium will require persons with expertise in legal matters governing importation of new species in each participating country. The consortium will also require biologists able to conduct host range tests (or let subcontracts to university scientists to do so) and evaluate national faunas to determine if host range estimates imply adequate safety for particular countries. Finally, consortium employees should make necessary importation requests for particular countries and follow through with the process to the final decision.

Once new natural enemies have been found and their release permitted, there will be a need to develop a delivery system. Two models are possible. For species with high demand and low rearing cost, commercial insectaries are likely to be interested in producing and selling the agents. For other species, there may be sufficient demand, but rearing costs may be too high. In such cases, it may be possible for university scientists or insectary employees to develop less expensive rearing procedures. Such species eventually might feasibly be produced through the commercial insectary system. A second case exists, however, of species for which the demand, while important, is too small to support profitable commercial production. Such natural enemies are orphans, much in the same sense as some effective

medical drugs whose markets are too small to attract commercial producers. For such orphaned natural enemies, I propose that the model currently being used by entomologists running North American plant conservatories be adopted. This system is based on the voluntary maintenance of colonies of selected natural enemies (to date, mostly parasitoids of various scales and mealybugs) by the conservatory managers. Starter cultures from these colonies are made available free or at low cost to persons needing them, on a mutual aid basis. If coupled with work by the greenhouse consortium mentioned above to obtain needed host specificity data and legal permission for importation and release in member countries, such a system could supply small quantities of a wide variety of agents. Small grants from the consortium (from money raised from membership dues in the consortium) could be made to persons or businesses maintaining colonies, to help reduce the costs of doing so.

After new natural enemies are obtained, growers will likely need to reconsider methods of natural enemy conservation that they could use to preserve populations of these key natural enemies in their greenhouses on a permanent basis. This would be valuable by avoiding annual costs to purchase natural enemies to re-establish them in each succeeding crop. Methods for conservation are a current subject of biological control research and this information, while not developed for greenhouses, should be applied to them.

Effect use of classical biological control in greenhouses will be possible only with a high degree of interaction between university-based biological control biologists, government employees overseeing review of release petitions, the insectaries, and end-users (the commercial greenhouses). Each will have an important part and coordination of the parts will be needed. The consortium mentioned above should be structured with one of its goals being to support such coordination, as well as conducting the initial prediction and prevention activities discussed here.

An international overview of invasive greenhouse pests, from data received via email from greenhouse entomologists, will be published in June 2002, issue of *Sing* (newsletter of the IOBC/WPRS WG "Integrated Control in Protected Crop, Temperate Climate").

Risks of importation and release of exotic biological control agents: how to determine host specificity?

J.C. van Lenteren¹, F. Bigler², G. Burgio³, H.M.T. Hokkanen⁴, M.B. Thomas⁵

¹Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH, Wageningen, The Netherlands, E-mail: Joop.vanLenteren@users.ento.wau.nl; ²Swiss Federal Research Station for Agroecology and Agriculture, Zürich, Switzerland; ³Department of Agroenvironmental Sciences and Technologies, University of Bologna; ⁴Department of Applied Biology, University of Helsinki, Finland; ⁵CABI Bioscience, Silwood, Ascot, UK

Abstract: In the past 30 years many exotic natural enemies have been imported, mass reared and released as biocontrol agents for greenhouse pests. Negative effects of these releases for greenhouse biological control have not been reported yet. The current popularity of biological control may, however, result in problems, as an increasing number of projects will be executed by persons not trained in identification, evaluation and release of biocontrol agents. Therefore, a working group of OECD is developing a guidance document for registration requirements of exotic natural enemies. This guidance document is based on protocols for risk assessment that are being developed within the EU project "Evaluating Environmental Risks of Biological Control Introductions into Europe" [ERBIC]. In this paper, the state of affairs concerning these developments is summarized.

Introduction

In the past 30 years many exotic natural enemies have been imported, mass reared and released as biocontrol agents for greenhouse pests (Albajes *et al.*, 1999). As far as we know, hardly any problems have occurred concerning negative effects of these releases for greenhouse biological control (Lynch *et al.*, 2000; van Lenteren, 2000). The current popularity of biological control may, however, result in problems, as an increasing number of projects will be executed by persons not trained in this field of pest control. An increasing number of countries now apply risk assessment procedures before a new natural enemy can be imported or released. Within an OECD working group and in collaboration with the EU-ERBIC project, guidelines are being developed for harmonised information requirements for import and release of invertebrate biological control agents used in augmentative biological control.

The first activity of the group was to collect, study and summarise the risk assessment procedures that are currently used by about 25 countries (N.B. less than 10% of all countries are using some form of regulation concerning import of exotic biocontrol organisms). Some procedures (e.g. those of Australia, New Zealand and Hawaii; see articles in Lockwood *et al.*, 2001) are so strict that hardly any natural enemy can be introduced. Other countries have no regulations at all, so any species can be imported and released. The aim of the OECD group is, on the one hand, to come up with guidelines that will prevent serious mistakes with importations and, on the other hand, to be able to proceed with safe forms of biocontrol.

Next, code of conducts and guidelines produced by other organisations (e.g. FAO, EPPO, NAPPO, CABI) were studied and summarised. It was decided that the OECD guideline would be based on, and for a large part be similar to the FAO and EPPO guidelines. However, these guidelines are not very specific concerning criteria and methodology, so it was decided to develop clearer guidelines including methodology and criteria based on work of the EU-

ERBIC project. Risk assessment procedures for biological control agents are normally characterized by questions concerning four issues:

1. Health risks (these are for invertebrate natural enemies much easier to determine than for chemical agents)
2. Characterization and identification of biocontrol agent (classical methods or molecular techniques, voucher specimens to be deposited, DNA fingerprinting in case of taxonomic problems)
3. Environmental risks (this is the most difficult part for invertebrate biocontrol agents)
4. Efficacy (will be treated different to cases with chemical control; as biocontrol agents often form part of IPM programme, it is often not necessary to reach 90-100% control by the biocontrol agent alone, as long as the total IPM programme results in sufficient reduction of pest or disease; efficacy of a biocontrol agent is defined as the ability to cause a significant reduction in number of pest organisms, direct and indirect crop damage, or yield loss)

In this paper only the environmental risks assessment developed by the EU-ERBIC group is discussed.

Assessment of potential hazards posed to the environment

If one intends to release an exotic natural enemy, one has to:

1. Identify any potential hazards posed to the environment including:

- a. available information on the role of organism in original ecosystem (type of natural enemy (parasitoid, predator, pathogen), type of organisms it attacks, effect of attack, intraguild effects, higher up trophic level effects, effects on ecosystem)
- b. available information on existing natural enemies of the target organism in the area of release
- c. available information on non-target effects from previous use in biological control
- d. available information and /or data on possible direct effects:
 - on non-target host/prey related to target host (phylogenetically or ecologically related)
 - on non-related non-target hosts, like threatened and endangered species
 - concerning competition or displacement of organisms
 - concerning potential for interbreeding with indigenous natural enemy strains or biotypes
 - on plants (target crop and non-target plants)
- e. available information and/or data on potential of establishment and dispersal of biological control agent
- f. available information and /or data on possible indirect effects
- g. available information (from rearing facility; in the field) on ability to vector viruses or micro-organisms which can negatively affect non-target organisms.

2. Summarize risks and environmental benefits e.g. beneficial effects of natural enemy release compared to alternative control methods

3. Report any adverse effects on non-targets to regulatory authorities after releases have been made

In the above list, there are two types of requests: (1) available information on, and (2) data on. If only available information is requested, the applicant has to provide a summary of what is known about the natural enemy in the literature. When data are requested, specific

information from the literature, or when not available, data from new experiments have to be provided. The main criterion on which a decision for release will be made is host specificity.

Measuring host specificity

The following sequential test for host specificity (van Dijken *et al.*, 1986; Follett *et al.*, 2000; Sands, 1988) was developed in the EU-ERBIC project:

Step 1: Petri dish non-choice black box test. The aim of the test is to answer the question: does the biological control agent attack the non-target organism in the appropriate stage? The control species is the target species. The non-target species are selected according (1) to their phylogenetic relationship with the target, (2) occurrence in the same micro-habitat and prone to attack, and (3) their status as endangered species (Lonsdale *et al.*, 2000). If none of the non-targets is attacked and the pest (control) species is attacked, one can stop testing, and no direct effects on non-target species in field are expected. If non-target species were attacked, go to step 2. Long-term behavioural observations are not done in step 1, but it is suggested to check the activity (searching or not) of the natural enemy at the start of testing, and after a sufficiently long interval to be sure that lack of attack in tests is not the effect of poor condition of natural enemies, but of rejection of the non-target.

Step 2: Petri dish non-choice behavioural test. The aim of the test is to answer the question: does the biological control agent attack the non-target organism consistently? The control species is the target species. Check encounter and attack rate over time for non-target species to determine possible increase in acceptance due to increasing oviposition/predation pressure. If non-target is not attacked at all and the pest (control) species is attacked, one can stop testing for that species, and no direct effect on that non-target species in field is expected. If non-target is only attacked at the end of the observation period, then the risk of direct effects on that species is relatively small. If non-target host is attacked for a constant fixed percentage, then the risk might be considerable. For non-target species that are attacked, go to step 3. This no-choice test can also be done with sequential alternate exposure of target and non-target to avoid risk of “involuntary” oviposition in non-target, and thus to avoid inclusion of false positives in the list of non-target species. Observations can, when behaviour of natural enemy is known, be automated.

Step 3: Petri dish choice behavioural test. The aim of the test is to answer the question: does the biological control agent attack the non-target when the target species is present? Choice test with target and non-target host. Control is target host only. Check encounter and attack rate over time for non-target and target, to determine host preference, eventual shifts in preference and a possible increasing attack pressure of usually not attacked hosts, because the preferred host is no longer available. No or low attack of non-target and no shift in host preference over time: low risk for direct effects on non-target. If non-target is attacked in choice test, but not in no-choice test, this may be a spillover effect (‘confusion’ of natural enemy), and non-target is likely to be outside host range, but do check whether it can develop on the attacked non-target. If non-target is easily attacked either from start onwards, or later during the observation, go to step 4. Observations can, when behaviour of natural enemy is known, be automated.

Step 4: Large cage choice test. The aim of the test is to answer the question: does the biological control agent attack the non-target when the target species is present in a semi-natural situation? Present multiple host plants with various non-target and target hosts to biological control agent in a large cage. Offer target and non-target hosts in as natural a situation as possible and on their natural host plants. Control test is done in the same type of cage with only target host. Determine encounter and attack rates over time. For interpretation

of results, see step 3. Non-target species that are easily attacked on their host plants pose a high risk for non-target effects.

Step 5: Field test. The aim of the test is to answer the question: does the biological control agent attack the non-target when the target species is present in a natural situation? This test can only be done if biological control agent cannot establish in target area (e.g. agents from tropical areas to be used in greenhouses in temperate climates)! Release natural enemy in non-target habitat, determine attack of non-target species. Control: put target species on target host plant in the non-target habitat. If target species is easily attacked, and no or low attack of non-target occurs: low risk for direct effects on non-target. Non-target species that are easily attacked on their host plants in their habitat pose a very high risk for non-target effects.

Discussion

The topic of implementation of a registration procedure for natural enemies is hotly debated by the biocontrol industry and regulators. The biocontrol industry foresees lengthy, cumbersome procedures leading to high costs, and, thus, in some cases the impossibility to market an interesting natural enemy because of too high costs. Regulators within ministries of environment and agriculture want to prevent unnecessary and risky releases of exotic organisms. The history of arthropod biocontrol shows that very few mistakes have been made until now. This is a point in favour for the biocontrol industry, and is in strong contrast with the problems that have been created by accidental importation of pests and diseases on infested plant material by others. The current work by, among others, the EU-ERBIC project will hopefully result in a light and harmonized registration procedure that is not prohibitive for the biocontrol industry and will result in the pre-selection of safe natural enemies.

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Biological control and survival of *Echinothrips americanus* in pepper

Jeroen van Schelt, Hans Hoogerbrugge, Yvonne van Houten, Karel Bolckmans

Koppert B.V., Veilingweg 17, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands,
 E-mail: JvSchelt@koppert.nl

Abstract: Ten different predators: *Franklinothrips vespiformis*, *Franklinothrips orizabensis*, *Amblyseius limonicus*, *Macrolophus caliginosus*, *Orius laevigatus*, *Orius majusculus*, *Aeolothrips tenuicornis*, *Dicyphus hesperus*, *Geocoris punctipes* and *Chrysoperla carnea* were tested as potential biological control agents against *Echinothrips americanus*. All predators had their impact on the thrips population, however *M. caliginosus* had the strongest and most long lasting effect. This corroborates with field observations that growers who use *M. caliginosus* never have problems with *E. americanus*. The 50% survival of *E. americanus* at 5 and 10 degrees was one and three weeks, respectively. This indicates that a good sanitation between crop change is very important.

Key words: *Echinothrips americanus*, *Franklinothrips vespiformis*, *Franklinothrips orizabensis*, *Amblyseius limonicus*, *Macrolophus caliginosus*, *Orius majusculus*, *Aeolothrips tenuicornis*, *Dicyphus hesperus*, *Geocoris punctipes*, *Orius laevigatus*, *Chrysoperla carnea*, sweet pepper, survival

Introduction

Echinothrips americanus Morgan has been observed in Dutch glasshouses since 1993 (Vierbergen, 1998). From the greenhouse vegetables, sweet pepper, cucumber and egg plant are the most preferred host plants, but also many ornamentals are attacked. Especially in the western part of the Netherlands where vegetable and ornamental growers are often found at close distance, this thrips species is present in the area at a year round basis. An estimated 40% of the pepper growers will find this thrips in the crop during the season.

In pepper the thrips is living low in the crop on the old leaves and is easily overlooked. *E. americanus* pupates on the leave in contrast with the other most common pest thrips species. For an overview of the biology of *E. americanus* see Oetting & Beshear (1993) and Opit *et al.* (1997). Opit *et al.* (1997) tested different potential biological controls (*Orius insidiosus*, *Amblyseius cucumeris*, *Amblyseius degenerans*). *O. insidiosus* was found to be superior to the predatory mites. Because *E. americanus* is larger in size than WFT it is likely that these mites can only prey on the first instars of *E. americanus*. Ramakers *et al.* (2000) compared *Orius laevigatus*, *Franklinothrips vespiformis* and *Franklinothrips orizabensis*. In this trial results are difficult to interpret because of contamination with WFT and predators in the different compartments. It seemed that *Franklinothrips* was somewhat better in controlling hot spots, but that in the long run *O. laevigatus* gave a more stable control. Intraguild predation from *O. laevigatus* on *Franklinothrips* was suspected. Also *O. laevigatus* was a better control agent on WFT than *Franklinothrips*.

The objective of this study was to screen a high number of possible natural enemies from different families on *E. americanus* in a set of glasshouse cage trials. Of the mites only *Amblyseius limonicus* was tested, because in preliminary experiments it was shown to be the only mite to prey on second stage larvae of *E. americanus*. From the Mirid family the European *Macrolophus caliginosus* and the North American *Dicyphus hesperus* were used. The latter one seems to be less phytophagous than *M. caliginosus*. Furthermore two predatory thrips species: *F. vespiformis* and *Aeolothrips tenuicornis* (collected in Spain), a North American Hemipteran, *Geocoris punctipes* (Lygaeidae) and the chrysopid *Chrysoperla*

carnea were tested. The last two were chosen because of their behaviour to stay low in the crop.

In addition, we assessed the survival of larvae, pupae and adults of *E. americanus* at cold temperatures. Because in the Netherlands most sweet pepper growers change their crop at the end of November in a two week period, it was suspected that old leaves which are temporarily dumped outside the glasshouse can serve as a reservoir for new infestations. Five and ten degrees were chosen as representative test temperatures.

Material and methods

Cage trials with predators

In three consecutive cage trials the different predators were tested. Species and numbers released are given in table 1 and 2. In the third trial 15 *C. carnea* per plant were released. All three trials had one control cage. In each cage 10 sweet pepper plants were placed. The plants were around 1 meter high and flowering when the trials started. Each plant was infested with 15 larvae and 5 adults of *E. americanus* one week before the release of the predators. Just before the release of the predators the number of thrips was counted and adjusted if necessary (by removing leaves). The total *E. americanus* population per plant was around 75 when the predators were released. The average temperature during the trial was 22°C. Every week all cages were counted. From every plant three leaves (low, middle and top of the plant) were checked for thrips and predators.

Table 1. Number of released predators in the first trial.

Treatment	# predators per plant	
	Female	Male
<i>A. limonicus</i>	32	2
<i>M. caliginosus</i>	8	2
<i>O. majusculus</i>	4	2
<i>F. vespiformis</i>	4	0
Untreated	-	-

Table 2. Number of released predators in the second trial.

Treatment	# predators per plant	
	Female	Male
<i>O. laevigatus</i>	2	1
<i>O. majusculus</i>	2	1
<i>M. caliginosus</i>	2	1
<i>D. hesperus</i>	2	1
<i>G. punctipes</i>	2	1
<i>A. tenuicornis</i>	2	1
Untreated	-	-

Survival of *E. americanus* at low temperatures

At two different temperatures (5 and 10°C) the survival of larvae, pupae and adults was assessed. As a bioassay a 7 cm sweet pepper leaf disc was placed on saturated wet cotton pads. From every life stage 25 individuals were placed on the disc. Trays were closed with a ventilated lid. After 4, 7, 14 and 21 days the mortality was assessed. All experiments were conducted in threefold.

Results and discussion

Cage trials with predators

In the first trial all predators could suppress the thrips population during the first three weeks (Fig. 1). After that period the thrips in the *A. limonicus* cage increased sharply, in all the other cages the thrips was exterminated. In all cages reproduction of the predators was observed.

In the second trial an effect of all predators was shown (Fig. 2). However, *M. caliginosus* suppressed the thrips population already after three weeks to a level which was not reached by the other predators even after 7 weeks. *O. laevigatus*, *M. caliginosus* and *D. hesperus* reproduced well during the trial. *O. majusculus* and *G. punctipes* reproduced badly and *A. tenuicornis* did not reproduce at all during this trial.

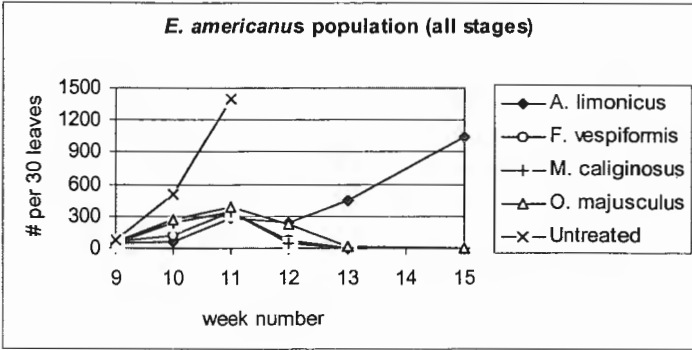


Figure 1.
E. americanus population in the first trial.

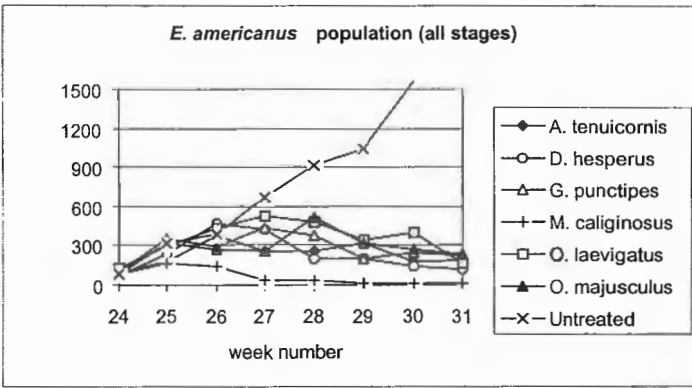


Figure 2.
E. americanus population in the second trial.

C. carnea managed to reduce the *E. americanus* population during the first 8 days (Fig. 3). After 8 days all the *C. carnea* larvae pupated and the *E. americanus* population increased.

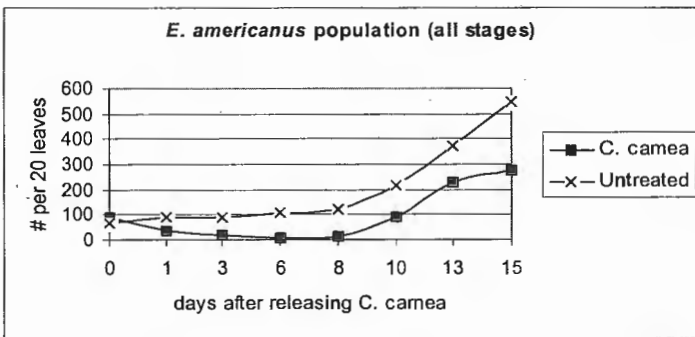


Figure 3. *E. americanus* population in the third trial.

In table 3 overall results for the different predators are summarised. Spatial overlap is an important factor because the thrips is found so low in the plant. In this trial the plants were only 1 meter high but e.g. *O. laevigatus* was observed more in the top than *M. caliginosus*.

As an overall conclusion, *M. caliginosus* seems to be the most promising biological control agent against *E. americanus* in sweet pepper. In the season 2000-01 at 60 hectares of sweet pepper in the western part of Holland *M. caliginosus* was introduced. None of these growers have had problems with *E. americanus*.

Table 3. Overview of the different predators.

	Direct impact on <i>E. americanus</i>	Long impact on <i>E. americanus</i>	Spatial overlap	Reproduction	Availability /rearing cost	Overall score
<i>A. limonicus</i>	+	-	-	++	-	-
<i>M. caliginosus</i>	++	++	+	+	+	++
<i>D. hesperus</i>	+	+	+	+	+	+
<i>G. punctipes</i>	+	-	+	-	-	-
<i>C. carnea</i>	+	--	+	-	+	+/-
<i>O. majusculus</i>	+	+	+/-	+/-	++	+
<i>O. laevigatus</i>	+	+/-	-	+	++	+/-
<i>Franklinothrip spp.</i>	++	++	+	++	-	+
<i>A. tenuicornis</i>	+	-	?	-	--	--

Survival of *E. americanus* at low temperatures

A substantial number of the three tested thrips stages can survive for a period of 3 weeks at 10°C or a period of 1 week at 5°C. (Fig. 4, 5). Only a small percentage of the thrips pupae is able to survive a period of two weeks at 5°C. Oetting & Beshear (1997) determined the survival rate at 5 and 10°C after 1 and 5 days. After 5 days at 10°C, survival was around 65%. At 5°C 50, 20 and 70% survival for larvae, pupae and adults was found. Our results especially for the pupae are higher. These results indicate that thrips from the last year's crop, which is left outside the glasshouse, can be a source of infection for the new crop. An effective sanitation between the growing seasons is therefore recommended.

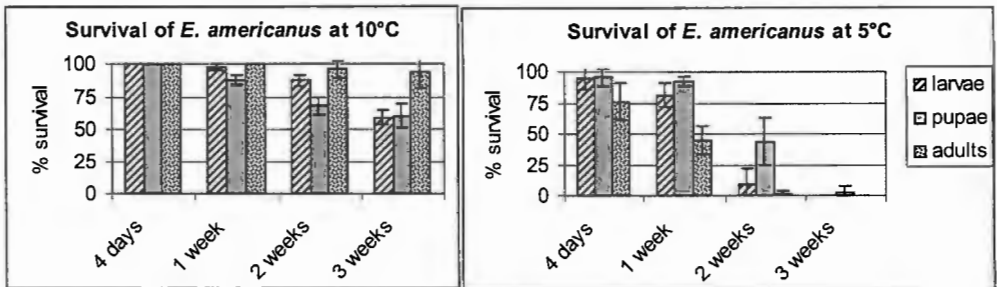


Figure 4 and 5. Survival of *E. americanus* at 10 and 5°C.

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Performance of *Neoseiulus cucumeris* as a biocontrol agent of the Western Flower Thrips in cut roses

Irene Vänninen, Marika Linnamäki

Agrifood Research Finland (MTT), Plant Production Research, Plant Protection, FIN-31600 Jokioinen, Finland, E-mail: Irene.Vanninen@mtt.fi

Abstract: *Neoseiulus cucumeris* is the most commonly used thrips predator in greenhouses, despite that it may not be the best option on all crops. We tested the performance of this mite against the Western Flower Thrips (WFT) on cut roses in spring/summer conditions of Finland. Biweekly application rates of 1000-2000 predators per m² (from controlled release sachets, or CRS) in four 38 m² greenhouse compartments in May-September resulted in a cumulative flower crop (Escimo) that was 72-89% free of thrips damage in three compartments of four. In a commercial greenhouse, predators applied preventatively every six weeks between January and June did not disperse from the CRS to leaves or flowers of roses before the middle or end of April. Our results have three implications: (1) the CRS-method may not be the best option to release *N. cucumeris* against WFT in cut roses; (2) *N. cucumeris* may not be the best predatory mite species for use in cut roses; and (3) light conditions during the winter months in northern Europe appear to slow down the dispersal of *N. cucumeris* to roses from CRS, a phenomenon that may interfere with the desired effect of preventative WFT control in this crop.

Key words: predatory mites, thrips, *Frankliniella occidentalis*, roses, biological control

Introduction

The Western Flower Thrips (*Frankliniella occidentalis*) continues to be a destructive pest of cut roses in Finland and elsewhere, and the recent years have witnessed an increased interest toward its integrated and biological management in this valuable crop (e.g. Casey *et al.*, 1999; Teerling & Murphy, 2000; Parrella, 2001). Despite not being a very efficacious thrips predator (Van Houten, 1996) and the increasing understanding that predator performance is dependent on host plant species (Skirvin & De Courcy-Williams, 1999; cf. also Beard & Walter, 2001), *Neoseiulus cucumeris* is widely used for thrips control in many greenhouse crops owing to the favourable economics of its production which enable inundative release rates, developments in application methods (Sampson, 1998) and proven efficacy against thrips when used appropriately (Jacobson, 1997). Some biocontrol agent producers have recommendations on the use of *N. cucumeris* for thrips control in cut roses (Biobest, 2002), but detailed studies on the performance of this mite as a thrips biocontrol agent in this crop are lacking or few. We addressed this knowledge gap by testing the performance of *N. cucumeris* on a thrips-sensitive rose cultivar in spring/summer conditions, and by monitoring predator dispersal from controlled-release-sachets (CRS) in year-round production of cut roses.

Material and methods

Biocontrol of WFT with Neoseiulus cucumeris in experimental greenhouses

White Escimo-roses were grown in four 38 m² greenhouse compartments. There were two conventional, 9.6 m² trickle-irrigated beds per compartment, one of open peat (Kekkilä Oy)

and one of rockwool bags (Pargro). Roses were grown by bending the canes. Temperature was set at 20-22°C. Relative air humidity varied between 60 and 75%. Artificial lights were on between 2 am and 10 pm. Powdery mildew was kept in check with weekly sprayings of 40% carbon fertilizer containing ethanol (Kekkilä Oy). An average of 0-0.1 thrips per flower were found at the start of the experiment in week 21. *Neoseiulus cucumeris* (Biobest) were applied (CRS hung to the erect stems) at the following rates per m²: 500 (weeks 21 and 23), 1,000 (weeks 25 and 27), 1,000, 2,000 or 3,000, depending on the compartment (week 29), and 500 or 1,000 (week 33). Thrips and predator numbers were monitored weekly from 20 flowers per compartment. Flowers were harvested every 2-3 days and rated for thrips damage as undamaged; mildly damaged, but saleable in bunches; and unsaleable.

Dispersal of *N. cucumeris* from CRS to roses in the winter and spring months of 1999

Predators (Koppert) were applied preventatively to Saphir (800 m²) and Kardinal-roses (1,000 m²) starting in week 2 and then every six weeks at the rate of 225 per m² until week 20. The roses were grown with the bent cane method in rockwool bags under artificial lights in raised beds. Predator and thrips numbers from 1-2 flowers per bed (30 per compartment) were monitored weekly until week 23. Additionally, 10 leaves of bent canes and 10 of erect canes per 250 m² were checked for predators and thrips. Each week 5-10 CRS hung in the latest reapplication week were checked to verify the presence of living predators. The roses had not been treated with pesticides harmful for beneficials since October 1998. During the experiment, Saphir received a spot treatment of abamectin in three beds in week 15. Kardinal received a blanket treatment of imidacloprid in week 2 and one of penconazole in week 15. Powdery mildew was kept in check by occasionally keeping sulphur fumigators on for 3-4 h per night.

Results and discussion

Biological control was relatively successful in three compartments of four, with a maximum number of 0.6-0.9 thrips per flower compared to that of 1.5 in the fourth compartment, and 72-89% of the cumulative crop was clean of thrips damage compared to 41% in the fourth compartment (Fig. 1). However, when looking at the dynamics of crop quality (data not shown), the combined proportion of unsaleable and mildly damaged flowers decreased below acceptable level (90%) during 3 or more weeks when WFT numbers were at the highest.

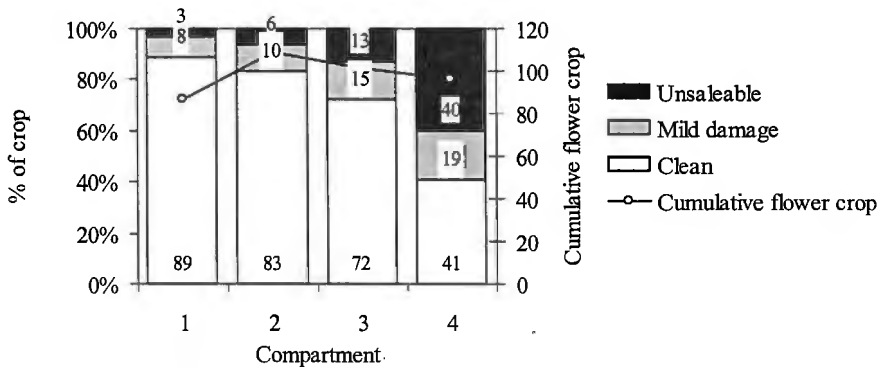


Figure 1. Quality of cumulative flower crop in four greenhouse compartments under biological control of WFT with *Neoseiulus cucumeris*.

The control efficacy might have improved by harvesting all flowers daily, resulting in decreased population growth of WFT (cf. Teerling & Murphy, 2001). Even without this, bearing in mind that the experiment was run in warm seasons, the overall control level achieved on a thrips-sensitive cultivar was encouraging, although costly (but smaller release rates were shown to be ineffective in this cultivar; see Vänninen *et al.*, 2001). It is likely that the release rates used here would have better protected more tolerant rose varieties from WFT. We observed that despite biweekly re-introductions of predators, their numbers in flowers started increasing only a week after increase in thrips numbers. Application methods other than CRS that more quickly result in colonization of flowers by predators should be developed and studied, and predatory mites that are more prone to colonize rose flowers and that are adapted to roses in general should be searched and compared with *N. cucumeris*.

In the dispersal experiment, *N. cucumeris* was found on leaves (of bent canes) only once in weeks 2-16, when less than 1% of leaves were colonized (data not shown). Mites were seen outside the CRS, but they hid below the cartoon piece used to hang the sachets in the crop. In week 16 and 17, predators started to disperse to the crop (Fig. 2). They were first found on leaves of bent canes, two weeks afterwards on leaves of erect stems, and only five weeks afterwards in flowers. The results were similar in both compartments, although predator numbers were lower in the second one due to mice that destroyed part of the CRS. *Frankliniella sp.* (not WFT) from outdoors were caught in small numbers in yellow sticky traps from week 18 onwards, but were never found on plants. Spider mites were present on roses in both compartments throughout the experiment, providing a potential prey source for the predators.

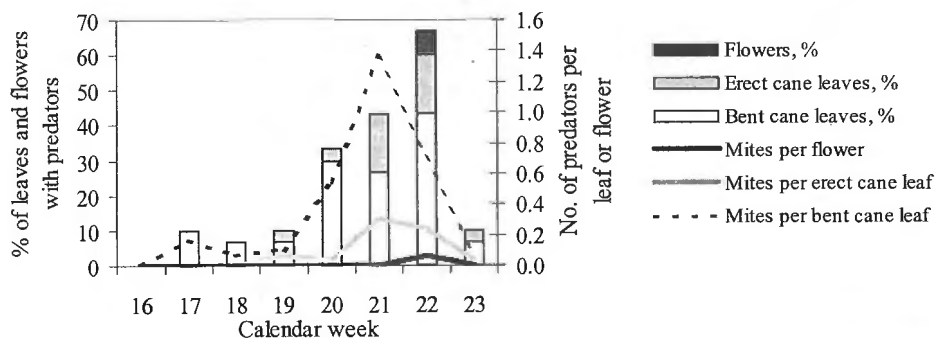


Figure 2. Colonization of rose leaves (cvar. Saphir) by *Neoseiulus cucumeris* during spring months of 1999 in a commercial rose cultivation.

CRS hung in the bent canes is a practical way of applying predators to roses grown in raised beds, because then the sachets do not interfere with harvesting or pruning activities. Year-round production of cut roses in raised beds is becoming increasingly common in Finland and other Scandinavian countries. The slow dispersal of predatory mites from CRS to the plants and particularly to flowers raises concern, however. In our conditions, the source of WFT infestation is usually from the greenhouse itself, thus preventative control is important from early on. Our results suggest that light and/or temperature conditions in the compartments were not conducive for dispersal of *N. cucumeris* onto the two rose varieties in the winter or early spring months. WFT development is also hampered by our winter conditions, but should it appear, say, in the

beginning of April, there may be the danger of predatory mites not being able to respond to its presence quickly enough by colonizing the whole plant and particularly flowers which WFT infests first. More detailed studies are needed on the dispersal of *N. cucumeris* from CRS to roses in winter months both in the presence and absence of prey and in varying abiotic conditions, particularly at different light levels in respect to crop type, as we suspect these were the major factors explaining the pattern of observed dispersal. The same dispersal problem has not been observed in year-round cucumber or is not as pronounced, thus the tendency to disperse may be host-plant dependent.

Acknowledgements

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Influence of a biofungicide Trichodermin-BL on growth and development of plants

Dmitry V. Voitka

Belarussian Institute of Plant Protection, Priluki, Minsk region, Belarus 223011

Abstract: The stimulating action of a biological preparation Trichodermin-BL on seed qualities of vegetable crop seeds: tomato, fodder beet and carrot was determined. Trichodermin-BL use in the technology of cucumber, tomato, spring wheat makes more active growth and development of plants, promotes their productivity increase. The application of a biopreparation in purple *Echinacea* plants increases the output of medicinal raw material.

Key words: biocontrol, diseases, antagonists, *Trichoderma* spp., sowing qualities, stimulating effect, tomato, beet, carrot, cucumber, spring wheat, medicinal crops

Introduction

There are numerous data on positive influence of soil microorganisms on growth of uppermost plants. The use of preparations having growth-stimulating properties makes it possible to realize more completely the genetic potential of plants.

The fungi of *Trichoderma* Pers ex Fr. genus are widely used in the agricultural practice for diseases control. Apart from the antagonistic properties in relation to disease agents of agricultural crops, these fungi are able to stimulate plant growth and development. In a number of works of domestic and foreign authors the stimulating effect of *Trichoderma* spp. is reported.

Michalikova (1995) noted a decrease of root rot infection, an increase of height and dry weight of surface parts, as well as numbers, length and dry weight of hypocotyls of winter wheat roots.

Trichoderma spp. fungi have an ability to induce plant stability, thereby promoting their growth and development (Harman, 2000).

Trichodermin-BL application in soil with fibre flax sowing gave an opportunity to get 116.2 c/ha of flax straw (in the control 65.7 c/ha); the number of heads of 100 plants was 855 (in the control 479). Trichodermin-BL application in the substratum during cucumber seedlings growth in clay pots promoted plant weight increase in 8 days after full seedlings stage up to 19.0 (in the control 6.5 g). By Trichodermin-BL soil treatment in hotbeds the number of male and female flowers per plant increased up to 83 and 14 (in the control 53 and 5, respectively), yield increase to the control was 46.4% (Methodical manual, All-Russian Institute of Plant Protection, 1965).

Pristchepa & Voitka (1999) and Voitka (2000) noted phytoprotective and growth stimulating effect of Trichodermin-BL while growing tomato by small-volume hydroponics on organic substrates based on upland moor.

Products of metabolism of various *Trichoderma* spp. strains are nonidentical, which testifies to their selective action rendered on the process of agricultural plants growth. A selective character of active products of different *Trichoderma* spp. species and strains is

revealed not only within the limits of plant families which they influence, but also their varieties (Seyketov, 1982).

Now the preparations based on highly active strains of *Trichoderma* spp. are at the centre of attention of researchers. The problems of antagonism of *Trichoderma* spp. are studied in relation to phytopathogenic microorganisms (Tong *et al.*, 1994; Michrina *et al.*, 1995; Khramtsov, 1998; Voitka & Lakida, 2000) and through practical use of biopreparates in plant protection (Elad *et al.*, 1998; Grinko, 2000).

We evaluated the growth-stimulating effect of Trichodermin-BL (strain-producer of *Trichoderma lignorum* T 13-82) on growth and development of open and protected ground plants.

Materials and methods

Pre-sowing Trichodermin-BL treatment (20 g/kg) of tomato, beet and carrot seeds was done according to "the Methodical recommendations..." (Mukhin, 1979). The trial variants included pre-sowing seed powdering by the preparation and control (without treatment). The preparation titer was 6 mlrd spores/g. The power of germination and laboratory seed germination was taken into account.

Spring wheat seeds were treated before sowing. The application rate of a preparation was 5 kg/t of seeds. The trial variants included control (without treatment), Trichodermin-BL (powdering), Trichodermin-BL + greatly swollen polymer hydrogel for adhesion (SSPG).

The biometric parameters were taken into account according to the recommendations of All-Russian Scientific-Research Institute of Plant Protection (1998).

While cucumber growing in soil and subsoil, Trichodermin-BL was used by the following technology: seedlings watering with a suspension of a preparation (2 g/250 ml of water/plant), two times plant watering by the preparation suspension in 20 days after the previous treatment (5 g/250 ml of water/plant).

Purple Echinacea plants were treated by Trichodermin-BL 3 times (200 g of preparation/10 l of water). As a chemical standard azofos, 75% p. (spraying - 100 g of preparation/10 l of water) was used.

Results and discussion

It was determined, that pre-sowing tomato, sugar beet and carrot seed treatment raised their seed qualities (table 1).

The use of a biopreparation for tomato seedlings treatment (3 g/100 ml of water/plant) promoted better growth and plant development. So, the plant height in Trichodermin-BL variant was 9.5 cm (in the control – 7.1 cm), number of leaves – 4.9 (in the control – 3.6), stem diameter in root column area – 0.35 cm (in the control – 0.27 cm).

Trichodermin-BL application on summer-autumn rotation cucumbers in soil and subsoil resulted in a 11.7% yield increase compared to the control.

In open ground Trichodermin-BL was used for pre-sowing spring wheat seed treatment (variety Bango). The application rate of a preparation was 5 kg/t of seeds. The stem length and rootlet root length were recorded.

The results demonstrated, that when Trichodermin-BL and Trichodermin-BL with greatly swollen polymer hydrogel for adhesion were applied, the biometric parameters of wheat plants were higher than in the control (Fig. 1).

Table 1. Influence of a biopreparation Trichodermin-BL (*Trichoderma lignorum* T 13-82) on sowing qualities of vegetable crops.

Crop	Trial variant	Method of pre-sowing treatment	Power of germination, %	Laboratory germination, %
Tomato (variety Kalinka BelSRIVG)	control (without treatment)	-	72.5	77.5
	Trichodermin-BL (powdering)	20 g/kg of seeds + water 0.5 l/kg of seeds	97.5	100.0
	20 g/kg of seeds			
Beet (variety Bordeaux)	control (without treatment)	-	52.5	70.0
	Trichodermin-BL (powdering)	20 g/kg of seeds + water 0.5 l/kg of seeds	65.0	77.5
	20 g/kg of seeds			
Carrots (variety Kollisto)	control (without treatment)	-	70.0	65.0
	Trichodermin-BL (powdering)	20 g/kg of seeds + water 0.5 l/kg of seeds	70.0	80.0
	20 g/kg of seeds			

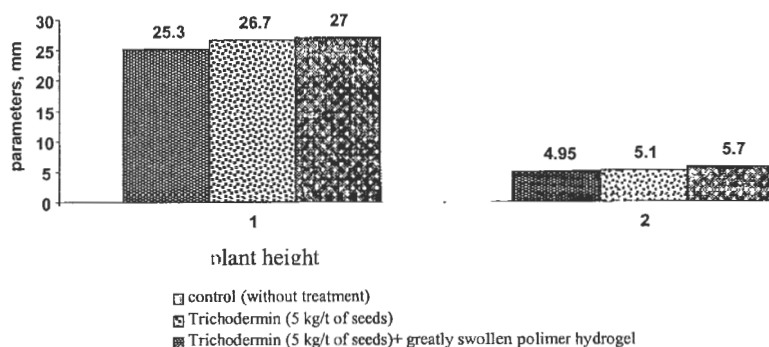


Figure 1. Influence of pre-sowing treatment by a biological preparation Trichodermin-BL on biometric parameters of spring wheat variety Bango plants (field trial, 2000).

The evaluation of a stimulating Trichodermin-BL action on purple *Echinacea* plants showed that the average flowers number per plant in Trichodermin was 5.8; in azofos variant – 3.6; in the control – 4.9.

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Intraguild predation (IGP) between the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus* and the effects on their population dynamics

Andreas Walzer¹, Peter Schausberger²

¹Institute of Zoology, Department of Evolutionary Biology, University of Vienna, Austria, E-mail: andreas.walzer@relay.bfl.at; ²Institute of Plant Protection, University of Agricultural Sciences, Vienna, Austria

Abstract: Predation, mortality, development and oviposition of *Phytoseiulus persimilis* and *Neoseiulus californicus* was examined when provided with con- or heterospecific prey. Irrespective of the type of prey, both species completed juvenile development. *P. persimilis* was more cannibalistic than *N. californicus*, whereas *N. californicus* exhibited stronger intraguild predation than *P. persimilis*. Oviposition was only possible for *N. californicus* preying on heterospecifics. *N. californicus* preferred to prey on heterospecifics when given the choice between con- and heterospecifics. When confined to detached bean leaves, *P. persimilis* was outcompeted by *N. californicus* within 30 days, which was attributed to asymmetric intraguild predation. The effects of IGP and competition for food between *P. persimilis* and *N. californicus* and the consequences on the combined release of the two species are discussed.

Key words: *Phytoseiulus persimilis*, *Neoseiulus californicus*, *Tetranychus urticae*, intraguild predation, competition for food

Introduction

The phytoseiid mites *Phytoseiulus persimilis* A.H. and *Neoseiulus californicus* McGregor (Acari, Phytoseiidae) are released singly as well as in combination (in expectation of additive and/or synergistic effects) in biological control of spider mites in greenhouse crops. Related to their life-styles *P. persimilis* and *N. californicus* are different types of natural enemies of spider mites (Acari, Tetranychidae) (McMurtry & Croft, 1997). The diet-specialist *P. persimilis* is characterized by strong functional and numerical responses to changes in spider mite densities, which allows rapid suppression of spider mite populations. *P. persimilis* depends on spider mites for sustained oviposition and usually disappears after prey depletion. Consequently, releasing *P. persimilis* alone often guarantees only short-term spider mite suppression. The diet-generalist *N. californicus* utilizes various animal and non-animal food substances and has a lower intrinsic rate of increase (r_m) with tetranychid prey than the diet-specialist *P. persimilis*. The broad food range of *N. californicus* should allow persistence at low or diminishing spider mite densities. In perennial greenhouse ornamentals spider mites are often permanently established and due to the high rate of increase the populations build up rapidly. The combined release of *P. persimilis* and *N. californicus* could enhance both immediate and long-term control of spider mites. However, potential interactions between the two predators and their consequences on their population development may influence the success in spider mite control. We conducted experiments at the individual level to study IGP and cannibalism of *N. californicus* and *P. persimilis*. Then we examined the consequences of IGP and competition at the population level in a simplified ecosystem (detached leaves).

Materials and methods

Even aged cohorts of eggs or larvae were obtained by placing gravid females on detached bean leaves (*P. persimilis*) or on plastic tile arenas (*N. californicus*). Closed cages consisting of plexiglass cells (15 mm Ø, 3 mm h) with a fine mesh screen at the bottom were used in experiment 1 to 3 (Schausberger, 1997). Experimental arenas consisting of a bean leaf (4×4 cm) put upside down on water saturated foam in plastic boxes were used in experiment 4. Water saturated cellulose strips (2 cm h) at the edge of the leaf arena prevented the mites from escaping. In all experiments, treatments were replicated 9 to 14 times. The experiments were conducted in a climate chamber at 25±1°C, 65±5% RH and 16:8 L:D photoperiod.

Experiment 1. Predation, survival and oviposition. Gravid females of *P. persimilis* and *N. californicus* were chosen randomly from the rearing units and placed singly in closed cages, and each was provided with 6 con- or heterospecific larvae. Every 24 h, predation rates, survival and oviposition were recorded and the prey was renewed for consecutive 12 days.

Experiment 2. Juvenile development. Newly laid eggs of *P. persimilis* and *N. californicus* (later regared as predators) were placed singly in the cages. 6 con- or heterospecific larvae (later regarded as prey) were added into each cage after hatching of the predator. For treatments with conspecific prey, larvae and protonymphs of the predator were marked with a tiny watercolor point at the dorsal shield to distinguish them from prey. Cages were checked twice daily in intervals of 12 h to record predation, survival and development of the predator. Prey was replenished daily.

Experiment 3. Predation preference. Singly caged gravid females were provided with 4 con- and heterospecific larvae. After the start of the experiments, cages were monitored for the first successful attack of the female predator within 12 consecutive h.

Experiment 4. Population development. Four gravid females and two males of *P. persimilis* or *N. californicus* (single-species system) or two females and one male of either predator in combination (two-species system) were placed onto the leaf arena. Every 24 h, surplus mixed stages of *T. urticae* were added and all predator life stages were recorded. The experiment lasted 30 days.

Results

Experiment 1. Predation, survival and oviposition

N. californicus consumed more heterospecific prey and gained higher nutritional benefits from IGP in terms of oviposition and survival than did *P. persimilis*. In contrast, cannibalism was more pronounced in *P. persimilis* than in *N. californicus*. *N. californicus* but not *P. persimilis* was able to sustain oviposition by IGP. Oviposition of both species was negligible with conspecific prey (table 1).

Experiment 2. Juvenile development

Both species reached adulthood by feeding on con- or heterospecific prey. Predation rates of *N. californicus* were higher on hetero- vs. conspecifics, whereas *P. persimilis* consumed similar amounts of con- and heterospecifics (table 2).

Experiment 3. Predation preference

The number of cases in which the first prey attack was observed within 12 h was equally balanced between con- (7 attacks) and heterospecifics (9 attacks) for *P. persimilis*, whereas *N. californicus* attacked and fed more often on hetero- (12 attacks) than conspecifics (3 attacks).

Experiment 4. Population development

In the single-species system the populations of both species persisted at high densities until the end of the experiment. Overall, the bean leaf arenas allowed a higher density of *P.*

persimilis than *N. californicus*. In the two-species system *N. californicus* completely displaced *P. persimilis* within 30 days (Fig. 1).

Table 1. Predation, oviposition, mortality and survival of adult *P. persimilis* (PP) and *N. californicus* (NC) females caged singly and provided with con- or heterospecific larvae for 12 days.

Predator	Prey	Predation (larvae/female/day)	Oviposition (eggs/female/day)	Mortality (proportion)	Survival (days)
PP	PP	2.31	0.06	0.50	8.50
	NC	1.90	0.03	0.70	6.55
NC	PP	3.68	1.22	0.11	11.50
	NC	0.71	0.01	0.33	10.50

Table 2. Development, predation and mortality of immature *P. persimilis* (PP) and *N. californicus* (NC) caged singly and provided with con- and heterospecific larvae for 8 days.

Predator	Prey	Developmental time (days)	Predation (larvae/day)	Mortality (proportion)
PP	PP	5.30	0.79	0.00
	NC	5.66	0.58	0.43
NC	PP	5.72	0.90	0.07
	NC	7.63	0.44	0.14

Discussion

The diet-specialist *P. persimilis* and the diet-generalist *N. californicus* exhibited contrasting behavioural patterns concerning IGP. This may have a strong influence at the population level (Schausberger & Walzer 2001; Walzer *et al.*, 2002). In our experimental system with limited space and no chance to disperse the diet-specialist *P. persimilis* was outcompeted by the diet-generalist *N. californicus*. Predation by *N. californicus* on *P. persimilis* was frequently observed and was obviously responsible for the displacement of *P. persimilis*. IGP between *N. californicus* and *P. persimilis* is strongly asymmetric in favor of *N. californicus*. First, given the choice between con- and heterospecific prey, *N. californicus* preferred to prey on the latter, whereas the specialist *P. persimilis* did not show a preference. Second, *N. californicus* preys on eggs, larvae and nymphs of *P. persimilis* despite the presence of its primary prey (spider mites) (Walzer & Schausberger, 1999a). Third, *N. californicus* gains more nutritional benefit from heterospecific phytoseiid prey with respect to development and oviposition than does *P. persimilis* (Walzer & Schausberger, 1999b). The implications for the combined release of *N. californicus* and *P. persimilis* in greenhouse-grown ornamentals are restricted because the design of our experiments excluded niche dimensions that may have a striking influence in more complex settings. From theoretical evidence, coexistence between IGP predators is only possible if the weaker IGP predator is the stronger competitor for food (Holt & Polis, 1997). Basically, that is the case with *P. persimilis* and *N. californicus* sharing the common prey spider mites (Schausberger & Walzer, 2001). Thus, in a greenhouse crop system, further important niche dimensions (fluctuating environmental conditions, availability of alternative

food, possibility for intra- and interplant dispersal) may allow coexistence of the specialist *P. persimilis* and the generalist *N. californicus*, albeit local displacement may occur.

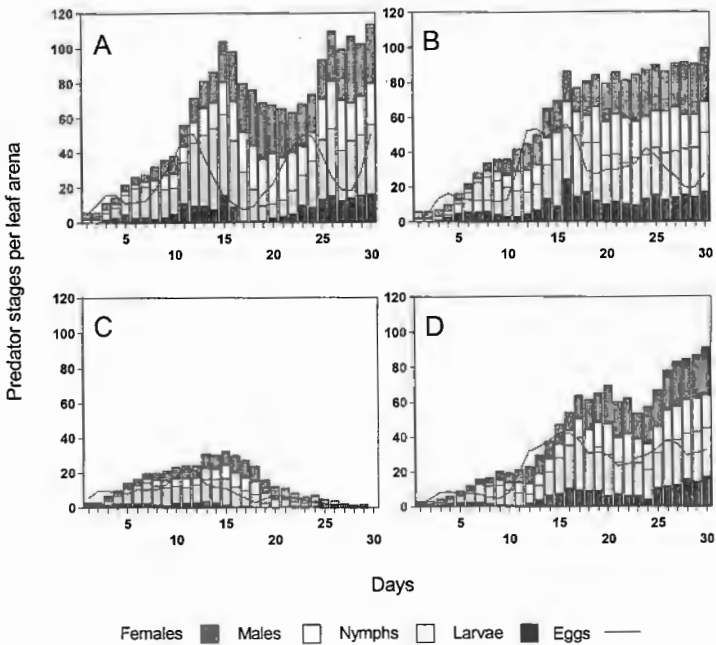


Figure 1. Population development of *P. persimilis* (A,C) and *N. californicus* (B,D) reared singly (A,B) and in combination (C,D) with prey, *T. urticae*, added in regular intervals.

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Effect of various release schedules of *Eretmocerus mundus* on the control of *Bemisia tabaci* in organic greenhouse peppers, in Israel – preliminary results

Phyllis Weintraub¹, Nurit Sapira², Elad Chiel³, Shimon Steinberg³

¹ARO, Gilat Research Center, D.N. Negev, 85280, Israel, E-mail: phyllisw@netvision.net.il;

²Research and Development, Sapir Center, D.N. Arava, 86825, Israel; ³BioBee Biological Systems, Sde Eliyahu, 10180, Israel

Abstract: Trials were carried out in two varieties of sweet pepper grown in walk-in tunnels covered with insect-proof screening in the Arava Valley, Israel. Adult *Bemisia tabaci* (Grennadius) were released in all tunnels and allowed to establish for 2 weeks before release of *Eretmocerus mundus* Mercet. The total number of *E. mundus* released was the same for all tunnels, however, the release schedules were varied: 2/m² once a week for 4 weeks; 4/m² once every 2 weeks, twice; and 8/m² one time. Results were monitored by counting parasitized *B. tabaci* on leaves and by monitoring adult whiteflies with yellow sticky traps. The most efficacious release schedule appeared, from these preliminary results, to be 2 *E. mundus*/m² per week for 4 weeks. *Encarsia* spp. invaded the tunnels.

Key words: *Eretmocerus mundus*, *Bemisia tabaci*, *Encarsia* spp., pepper, organic greenhouse

Introduction

In Israel, about 45 hectares of organic pepper are grown in screen- or greenhouses. Of that, 17 hectares are grown in the Arava Valley, south-east Israel. The amount of organic hectareage has been increasing by about 10% per year for the last few years. As the area under organic production increases, so does the relative importance of various pest species.

Bemisia tabaci (Gennadius) is a serious polyphagous pest of many agronomic and ornamental crops in all continents. It causes economic damage through feeding, excretion of honeydew and, indirectly, through transmission of plant viruses. Although known to occur in the Mediterranean region since the late 1800's, first records of severe infestations and damage are from the mid-1970's (Gerling, 1996). Whitefly management primarily depends on application of insecticides; however, in organic agroecosystems, the number of organic insecticides is extremely limited and are less efficacious than their conventional counterparts. This, combined with *B. tabaci*'s notorious ability to develop resistance to insecticides, has encouraged the development of integrated and biological control methods.

While a number of parasitoids of *B. tabaci* are known to occur worldwide (Gerling, 1986; Kirk & Lacey, 1996), two genera are known to occur naturally in the Mediterranean region, *Eretmocerus* (represented solely by *mundus*) and four *Encarsia* spp. (Gerling, 1996). *Eretmocerus mundus* is a solitary ecto-endoparasitoid of whitefly nymphs (Rose *et al.*, 1995). Eggs are deposited under the nymph, hatch, and then penetrate the host as first instar larvae. In the course of its development, the entire whitefly nymph is consumed by *E. mundus*. Parasitized whitefly nymphs are easily identified: in early stages the mycetomes of the whitefly nymph move ventrally and become misshapen; in later stages the pupa of the *E. mundus* can be easily observed. *Encarsia* spp., on the other hand, has a "J" shape in the larval stages, and later stages excrete reddish to brownish myconium.

All stages of the whitefly are subject to parasitism by *E. mundus*, but studies have shown that parasitism is significantly higher in the second instars, and lowest in the fourth (Foltyn & Gerling, 1985; Jones & Greenberg, 1998). The highest rate of parasitoid survival was also from second instar whitefly nymphs. Using second instar nymphs as host, Jones *et al.* (1999) found that whitefly mortality decreased as the density of *E. mundus* increased; 1 *E. mundus*/100 nymphs killed/parasitized about 19 nymphs, while at a density of 15 *E. mundus*/100 nymphs each female killed/parasitized approximately 5 nymphs. There is an initiative, supported by the European Union and joined by institutions in Israel, France and Spain, to mass produce and release *E. mundus* for the control of *B. tabaci*. The objective of this study was to determine the optimum release schedule of *E. mundus* for control of *B. tabaci* in organically grown sweet peppers.

Materials and methods

Experimental site and plants

Trials took place at the Yair Research Station in the Arava Valley, Israel. Sixteen walk-in tunnels, each 6 x 13 m, were covered with 50 mesh insect-proof screening; entrances were covered with two layers of screening. Two varieties of sweet pepper seedlings, Nibla (yellow) and Parker (red), were planted in each tunnel on 2 September 2001. Seedlings were planted in double rows in three beds. Nibla was planted in the northern half and Parker in the southern half of each tunnel; the varieties were separated by 70 cm. There were a total of 180 plants per tunnel. The soil was pre-treated with 1.5 m³/100 m² compost and organic mineral additives one month prior to planting. Irrigation and fertilization was according to standard agricultural practices in the area. Powdery mildew (*Leveillula taurica*) was treated with sulfur.

Parasitoid and host cultures

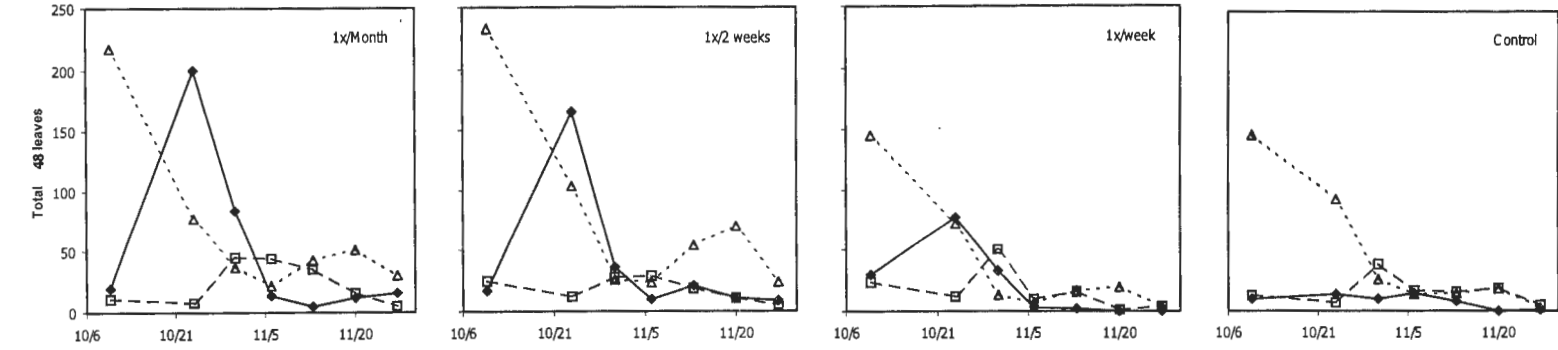
The *E. mundus* used in this study was supplied by BioBee Biological Systems, Sde Eliyahu, Israel. The *E. mundus* colony was started from specimens originally collected in the Arava valley in 1999, and since then reared in greenhouses on various host plants. Twenty thousand *B. tabaci* were released in each tunnel on 25 September 2001. Two weeks later, *E. mundus* were released. Release schedules were calculated so that each tunnel would receive the same total number of parasitoids over the course of the trial. Parasitoids were released from 4 vials evenly distributed over the length of the tunnels. Release schedules were as follows: 2 *E. mundus*/m² once a week for 4 weeks; 4/m² once every 2 weeks, twice; and 8/m² one time. There were four replicates of each release and control; tunnels were randomly assigned. The initial date for all releases was 10 October 2001.

Monitoring

Whitefly adults were monitored with two yellow sticky traps per tunnel that were renewed weekly. Parasitoids were monitored once a week by taking 6 leaves, which upon quick inspection seemed to have the largest number of whitefly nymphs, from each cultivar from each tunnel; 24 leaves/treatment/cultivar.

Statistics

Data were analyzed by 2-way ANOVA and means were separated by Tukey-Kramer analysis, $\alpha = 0.05$.



303 Figure 1. Total number of non-parasitized *B. tabaci* (open triangle), *E. mundus* (closed diamond) parasitized *B. tabaci* and *Encarsia* spp. (open squares) parasitized *B. tabaci* in two sweet pepper varieties at different release schedules of *E. mundus*. Total number of *E. mundus* released in all tunnels was 8/m².

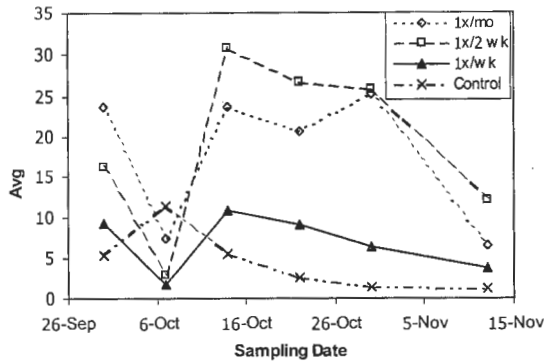


Figure 2. Yellow sticky trap catches of adult whiteflies at different release schedules of *E. mundus*.

Results and discussion

Examination of pepper leaves revealed that, besides the *E. mundus* that were released, two species of *Encarsia* (*E. lutea* and another undetermined species) also parasitized the whiteflies. After 2-way analysis of variance (treatment versus pepper variety) it was determined that there was no significant difference ($P = 0.69$) between the numbers of parasitized whiteflies on either cultivar. Results of monitoring whitefly nymphs on both cultivar leaves are shown in Fig. 1. Immediately before the release of *E. mundus*, leaf samples were taken; 2 *E. mundus* and 2 *Encarsia* spp. were found. After the initial releases, *E. mundus* populations increased in all tunnels until 24 October, then declined, as did whitefly populations. However, *Encarsia* spp. invaded all tunnels and, in some cases, became the predominant whitefly parasitoid. Results of yellow sticky trap catches are shown in Fig. 2. The lowest whitefly density was found in tunnels where *E. mundus* were released once a week.

Throughout the entire trial, the greatest density of whiteflies was found in the tunnels where the release schedule was 8 *E. mundus*/m², one time. The lowest whitefly densities, besides the control, were in the tunnels in which the release schedule was 2/m² once a week for 4 weeks. The impact of the *Encarsia* spp. was not determined.

These preliminary results will not only serve as a guide for future trials, but raise questions as well. Is the invasion of *Encarsia* spp. a seasonal event, limited only to the autumn? Was the decline in *E. mundus* related to the coming of winter and colder weather? Should the release schedule of 2 *E. mundus*/m² be extended for a longer period of time? The trials reported herein will be modified and repeated in the coming seasons.

Acknowledgements

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Biological parameters of *Orius* spp. for control of thrips in Japan

Eizi Yano¹, Kazuya Nagai², Kazuhiro Watanabe³, Kaori Yara⁴

¹National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan, E-mail: yano@affrc.go.jp; ²Okayama Prefectural Agricultural Experiment Station, San'yo-cho, Okayama 709-0801, Japan; ³Yamagata Horticultural Experiment Station, Sagae, Yamagata 991-0043, Japan; ⁴National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604, Japan

Abstract: Recent studies about life history and predation of Japanese indigenous *Orius* spp. are reviewed in relation to their mass rearing and evaluation of their potential for biological control of thrips. *O. sauteri* has been studied in detail. The minimum amount of *Ephestia kuehniella* eggs to rear one individual of *O. sauteri* from egg to adult emergence and for oviposition and adult survival was estimated as fundamental information for economic rearing. Life history studies of *O. sauteri* suggest that it cannot control thrips effectively under low temperature or short photoperiod. Another species, *O. strigicollis*, which shows low diapause incidence, is preferred for commercial use.

Key words: *Orius* spp., life history, predation, mass rearing, thrips, biological control

Introduction

Thrips palmi Karny and *Frankliniella occidentalis* (Pergande) are serious greenhouse pests that were accidentally introduced into Japan recently. Since both species are difficult to control with insecticides, biological control with *Orius* spp. seems an attractive alternative. Seven *Orius* species have been reported as being indigenous to Japan (Yasunaga, 1993, 1997). Biological studies are essential for developing their mass rearing techniques and evaluating their potential for biological control. Biology of one of them, *Orius sauteri* (Poppius) has been studied in detail.

The development of mass rearing techniques is needed to commercialize applications of *O. sauteri* in greenhouses, because *O. sauteri* should be released into greenhouses at the proper times. Since *Orius* spp. can feed on the eggs of lepidopterous insects in the field, many species have been successfully reared on eggs of *Ephestia kuehniella* Zeller. For developing economic mass rearing, it is important to estimate the minimum amount of diet to rear one individual from egg to adult emergence and for adult oviposition and survival.

Studies about life history, predation and reproductive diapause of indigenous *Orius* spp. have made rapid progress in Japan. These studies provide useful information for evaluating their potential for biological control.

The objective of this article is to review the recent studies about biological parameters of *Orius* spp. in Japan for future development of biological control of thrips with them.

Development, longevity and reproduction of *O. sauteri*

Effect of temperature and diets on development, longevity and reproduction of O. sauteri

The development, longevity and reproduction of *O. sauteri* reared on *T. palmi* larvae were studied at different temperature conditions and L16:D8 in the laboratory. Thermal constant

(K) and developmental zero (T_0) for eggs and nymphs were calculated at 62.1 day-degrees and 11.1°C and 180.8 day-degrees and 10.3°C, respectively. Egg mortality rate was always 7.1% or less. Nymphal mortality rates were 21.7 – 24.0% at 20, 25 and 30°C, but 48.6% at 15°C. Female longevity was greatest at 15°C and shortest at 30°C. Female lifetime fecundity reached a maximum at 25°C. The intrinsic rate of natural increase per day (r_m /day) was highest at 30°C (table 1).

Table 1. Development, longevity and reproduction of *O. sauteri* at different temperatures (Nagai & Yano, 1999).

Temperature (°C)	Developmental time (days) ^a		Female longevity (days) ^a	Lifetime fecundity ^a	r_m /day
	Egg	Nymph			
15	13.7±0.2a	40.9±0.6a	35.8±12.9a	12.2± 5.3a	0.0135
20	7.5±0.2b	18.9±0.3b	19.6± 7.1b	51.3± 7.2b	0.0763
25	4.5±0.1c	11.5±0.2c	20.3± 1.7b	74.5±10.7b	0.128
30	3.2±0.1d	9.5±0.2d	9.0± 0.5c	52.8± 5.4b	0.166

^aMean ± S.E. Means followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey-Kramer HSD test or Scheffé test).

O. sauteri was reared on *E. kuehniella* eggs and life history parameters were measured at 25°C and L16:D8. Developmental time during the nymphal stages was 13.8 days. Total fecundity and female longevity were 103.9 and 27.9 days, respectively. The intrinsic rate of natural increase per day (r_m /day) was 0.115. Table 2 summarizes the comparison of life history parameters of *O. sauteri* fed on *T. palmi* larvae and *E. kuehniella* eggs. *E. kuehniella* eggs can be considered a good diet for mass rearing of *O. sauteri*.

Table 2. Development, longevity and reproduction of *O. sauteri* fed on *T. palmi* larvae and *E. kuehniella* eggs at 25°C and L16:D8 (Nagai & Yano, 1999; Yano *et al.*, 2002).

Diets	Nymphal developmental time (days)	Female longevity (days)	Lifetime fecundity	r_m /day
<i>T. palmi</i> larvae	11.5±0.2	20.3±1.7	74.5±10.7	0.128
<i>E. kuehniella</i> eggs	13.8±0.2	27.9±1.1	103.9±19.3	0.115

The minimum amount of the diet for rearing individuals

The minimum number of *E. kuehniella* eggs for rearing *O. sauteri* individuals was measured at 25°C and L16:D8. To study the effect of the amount of diet on the nymphal development and survival, individual nymphs were supplied with 5, 10, 30 and 60 *E. kuehniella* eggs every four days after hatching until adult emergence. The effect of the amount of diet on adult reproduction and longevity was studied for ten days after the adult emergence. A pair of adults was supplied with 10, 20, 40 and 100 *E. kuehniella* eggs every four days. An individual requires at least 30 eggs for nymphal development and survival and 20 eggs for adult oviposition and survival per four days (table 3 and 4).

Table 3. Development and survival during nymphal stages and body weight of emerged adults of *O. sauteri* reared on different numbers of *E. kuehniella* eggs (Yano *et al.*, 2002).

Number of <i>E. kuehniella</i> eggs supplied / 4 days	Developmental time ^a (days)		% Survival ^b	Body weight (mg) ^a	
	Female	Male		Female	Male
5	18.3±0.6a	17.6±0.8a	41.9	177± 3a	156±13a
10	15.2±0.5b	14.3±0.3b	63.3	240± 8b	182± 6ab
30	13.7±0.1c	13.8±0.5b	76.7	278±10c	200± 4b
60	13.6±0.3c	13.3±0.3b	82.8	286± 6c	198± 8b

^aMean ± S.E. Means followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey's test). ^bSurvival rates are significantly different ($p < 0.05$, $\chi^2 = 4.52$).

Table 4. Survival and fecundity of adults of *O. sauteri* in ten days after emergence (mean±S.E.) reared on different numbers of *E. kuehniella* eggs (Yano *et al.*, 2002).

Number of <i>E. kuehniella</i> eggs supplied / 4 days / pair	% Survival		Number of eggs laid in ten days / female
	Female	Male	
10	93	13	12.5±1.4a
20	93	60	23.4±2.4b
40	87	87	48.9±4.4c
100	100	100	54.2±3.4c

Means followed by the same letter in the same column are not significantly different at $p = 0.05$ (Tukey's test). Female survival rates are not significantly different ($p > 0.05$, $\chi^2 = 0.128$). Male survival rates are significantly different ($p < 0.05$, $\chi^2 = 9.808$).

Predation by *Orius sauteri* on *Thrips palmi*

Functional responses of different stages of *O. sauteri* to densities of *T. palmi* were studied at different temperatures. Most stages of *O. sauteri* exhibited Holling's Type-II response. The number of *T. palmi* consumed by *O. sauteri* increased with increasing temperature and the stage of *O. sauteri* nymphs. *O. sauteri* late fifth stage nymphs consumed a much smaller number of *T. palmi* larvae than early fifth stage nymphs. *O. sauteri* adult females consumed a slightly smaller number of *T. palmi* larvae than early fifth stage nymphs. *O. sauteri* adult males consumed a smaller number of *T. palmi* larvae than adult females. *O. sauteri* nymphs killed more *T. palmi* larvae than *T. palmi* adults. However, there was not much difference between the two stages of *T. palmi* killed by *O. sauteri* adult females. Selective predation experiments on *O. sauteri* on different stages of *T. palmi* revealed that *O. sauteri* young nymphs preferred *T. palmi* larvae to adult thrips (Nagai & Yano, 2000).

Biological control of thrips with *Orius* spp. in Japan

Many biological studies have been accumulated about *O. sauteri*. Low reproduction and predation rate under low temperature condition mean ineffectiveness of this species in winter. Another important factor in considering the effectiveness of *Orius* spp. as biological control agents is induction of reproductive diapause. Use of non-diapause strains or species is

desirable for year-round use of natural enemies. *O. sauteri* exhibits reproductive diapause under short photoperiod (Kohno, 1997, 1998). *O. minutus* shows almost the same reproductive diapause as *O. sauteri* (Kohno, 1997). *O. tantillus*, which is found only in the southwestern islands, has no reproductive diapause (Nakashima & Hirose, 1997) but the reproductive rate is low. *O. strigicollis*, which is mainly distributed in western Japan, is thought to show lower diapause incidence than *O. sauteri*. *O. sauteri* and *O. strigicollis* have been registered as biotic pesticides in 1998 and 2001, respectively, in Japan. *O. strigicollis* is preferred for commercial use to *O. sauteri* because of its character of reproductive diapause and ease of mass production. *O. strigicollis* is widely used for controlling *T. palmi* on eggplants and *T. palmi* and *F. occidentalis* on sweet peppers in Kochi Prefecture in the southwestern part of Japan. More biological studies are necessary for evaluating potential of *O. strigicollis* as a biological control agent in future.

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The Publication Commission:

Dr. Horst Bathon
Federal Biological Research Center
for Agriculture and Forestry (BBA)
Institute for Biological Control
Heinrichstrasse 243
D-64287 Darmstadt (Germany)
Tel. +49 6151 407-225, Fax ++49-6151-407290
e-mail: h.bathon.biocontrol.bba@t-online.de

Prof. Dr. Luc Tirry
University of Gent
Laboratory of Agrozoology
Department of Crop Protection
Coupure Links 653
B-9000 Gent (Belgium)
Tel. +32 9 2646152, Fax ++32-9-2646239
e-mail: luc.tirry@rug.ac.be

Address General Secretariat IOBC/WPRS:

INRA – Centre de Recherches de Dijon
Laboratoire de Recherches sur la Flore Pathogène dans le Sol
17, Rue Sully – BV 1540
F-21034 Dijon Cedex
France

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