IOBC / WPRS

Working group "Integrated Control in Protected Crops, Temperate Climate"

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Preface

This bulletin contains the proceedings of the triennial meeting of the IOBC/wprs Working Group “Integrated Control in Protected Crops, Temperate Climate” (the 14th full meeting) held in Sutton Scotney, United Kingdom, 18-22 September, 2011.

The bulletin contains 46 contributions authored by 110 people from ca. 16 countries on numerous aspects of biological and integrated pest management in protected crops. Among the topical themes of the 14th triennial meeting were invasive pests and their IPM in new areas of distribution, direct and indirect influence of the external environment on IPM in greenhouses, and pesticide issues and biological control in the framework of the sustainability directive 2009/128/EC. In addition to these themes, IPM and biological control of several challenging pests were addressed by the authors. The student competition attracted two papers.

The local organization of the meeting was excellently handled by Phil Walker (Phil Walker Consultancy), Jude Bennison (ADAS Boxworth, UK) and Rob Jacobson (Rob Jacobson Consultancy Ltd.), in collaboration with the Association of Applied Biologists represented by Carol Millman and Bernadette Lawson. I thank the team for their hard work and enthusiasm aimed at a scientifically and socially successful meeting. Organizing an IOBC meeting is done on a voluntary basis, which makes the team’s work ever more valuable considering the time pressures and workloads everyone faces nowadays.

I express my gratitude also to another team that I had the pleasure of collaborating with when editing the bulletin: the scientific committee of the Working Group consisting of Gerben Messelink, Juliette Pijnakker (Wageningen UR Greenhouse Horticulture, The Netherlands) and Tom Pope (ADAS Boxworth, UK). As a team, we determined and applied the criteria for the acceptability of the contributions to the bulletin. The criteria were the same as those used in evaluating the student competition papers: study design and rigour; originality; significance and appropriateness to the WG’s mission (“to promote the research, development, implementation, and training of Integrated Pest Management systems in protected crops, as well as promoting cooperation between scientists, advisors and producers working in this field”); and organization and clarity of the paper.

The reviewing process of the contributions cannot be as strict as a proper scientific review process, since the bulletin is meant for rapid distribution of new knowledge and interim results. However, by applying the above criteria several requests were made to the authors regarding, particularly, the organization and clarity of the papers and the use of at least minimal statistical treatment of data, when appropriate. We hope the light reviewing process ensures good scientific quality of the contributions published in the bulletin.

Irene Vänninen, Working Group Convenor
4th July, 2011
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Field results of a sachet release system using the predator


Ian Baxter¹, Audun Midthassel¹, Ward Stepman¹, Robert Fryer¹, Fernando Puerto Garcia¹, Jennifer Lewis¹, Phil Walker², Jan Hulshof³

¹Certis Europe B.V., Newbury House, Court Lodge Farm, Hinxhill, Ashford, Kent, TN25 5NR, United Kingdom, ²Phil Walker Consultancy Acorn Cottage, Chapel Lane, West Wittering, Chichester, West Sussex. PO20 8QG United Kingdom, ³Biotus Oy, Särkeläntie 6, 30100 Forssa, Finland.

Abstract: Using sachets as a delivery system for the predatory mite *Amblyseius swirskii* into a crop is popular with growers as the inoculation of predators is slower and provides an element of flexibility over the timing of the introduction. The sachet itself is a complicated micro-environment, providing refuge and food for *A. swirskii*, generally in the form of a factitious host mite, *Carpoglyphus lactis*, which itself is sustained by materials within the sachet. The sachet should be able to maintain a breeding population of *A. swirskii*, whilst releasing predators at the appropriate rate and duration. This paper describes four field trials undertaken in different climates to compare the *C. lactis* prey mite sachet system with that of an alternative prey mite, *Suidasia medanensis*. It was found that *C. lactis* released predators more rapidly than the *S. medanensis* system in the first seven-days. However, the release profile of the *S. medanensis* sachets demonstrated a more sustained presentation of *A. swirskii* into the crop during subsequent weeks. The implications of these different release profiles for the end-users are discussed.

Key words: *Amblyseius swirskii*, *Suidasia*, sachets, IPM

Introduction

The polyphagous predator, *Amblyseius swirskii* is a frequently utilised biological control agent commercially available throughout much of the world where modern and intensive horticulture is prevalent. Under normal commercial practices *A. swirskii* are inoculated into the crop either by placement of small quantities of a carrier medium containing predators (sprinkler tubes) or via a slow release over a period of weeks of mites from a sachet (Sampson, 1998). The medium used to deliver the *A. swirskii* both in sprinklers and sachets generally consists of several important elements: the predator, a prey mite (to sustain the predator) and food for the prey mite. This paper is concerned with the sachet method of predator introduction.

Until recently the majority of commercial *A. swirskii* sachet delivery systems have incorporated the prey mite *Carpoglyphus lactis* (Linnaeus). These factitious hosts fulfil an important function of providing an adequate food source for *A. swirskii*, whilst not creating an unfavourable micro-environment for the predators within the sachet itself. Throughout the duration of sachet deployment the *A. swirskii* will feed on *C. lactis* and the sachet will function as an incubator sustaining several complete generations of *A. swirskii* before expiring. The micro-environment within the sachet must be sufficiently nutritious and generally favourable for the *A. swirskii* so as to allow both predators and factitious prey to be sustained beyond a single generation. *C. lactis* would appear to fulfil these essential qualities
of balance, whereas many other mite species apparently do not (BCP Certis, unpublished data, Biotus Oy, unpublished data).

This paper describes field results of sachet releases of *A. swirskii* produced and sustained in the sachet delivery system on an alternative factitious host, *Suidasia medanensis*. The implications of differences observed in the release-rate profile of the *C. lactis* and *S. medanensis* systems are discussed with respect to the end-users strategy and needs.

**Material and methods**

**Test material**

1. *S. medanensis* based *A. swirskii* sachets were obtained from BCP Certis (BCP Certis, Newbury House, Hinxhill, Kent, UK).
2. *C. lactis* based *A. swirskii* sachets were obtained from the locally appointed Koppert distributor (Koppert B.V., 2650 AD Berkel en Rodenrijs, The Netherlands) in each of the countries in which the field trials were undertaken.

**Trial locations**

To test the different delivery systems across a range of climates, the release of predators from sachets was evaluated in Finland, UK, The Netherlands and Spain. At each site, a commercial glasshouse considered as ‘typical’ for the region, was used producing either cucumbers (Finland, UK, NL) or peppers (Spain).

**Initial assessment of predator numbers**

Immediately prior to the initiation of the experiment, five individual sachets were selected at random and the contents were placed into a 150ml white plastic pot and gently mixed together. Using a 0.1ml metal scoop, 10 sub-samples of the media were evaluated for the presence of *A. swirskii* and the numbers of mobile stages were recorded. The mean of the 10 samples was calculated and, having determined the average volume of a single sachet, the mean of the 0.1ml samples multiplied up accordingly to give the total number of predators per sachet.

**Placement of sachets into the crop**

Sachets were placed in the crop using their integral hooks on the foliage themselves. They were positioned such that they were not placed near pathways or at a point which, otherwise, would constitute being atypical of the glasshouse as a whole.

**Determining predator release rates**

Upon study initiation, a single sachet of either the *S. medanensis* or *C. lactis* system was placed onto a white glueboard (ca. 20 x 20cm). The sachet was positioned such that the hook formed a pivot so that the small mite exit holes were elevated off the glueboard. This positioning was achieved by placing the entire glueboard-sachet assembly into a 5l cylindrical plastic container, which had been placed on its side and was situated at the base of the crop so as to be in approximate identical abiotic conditions to that of commercially deployed sachets. There were three replicates of each treatment.

After 7-days, the glueboard was inspected under a binocular microscope and the number of *A. swirskii* to have exited the sachet was determined. The glueboard was replaced and a sachet randomly selected from one of those which was hung in the crop earlier and placed onto the board as described above. This procedure was repeated every seven days until the sachets were expired.
Results and discussion

The initial counts of predators are shown in Table 1. The release profiles of the sachets are presented in Figure 1 (a, b, c, d).

In three out of the four trials (UK, NL, Spain), the sachets based on the C. lactis prey mite contained a higher initial count of A. swirskii than the S. medanensis system. It is assumed that these differences reflect use of alternative processes and procedures from the respective manufacturers rather than a fundamental difference in how A. swirskii develops within the sachet. However, it would seem logical that by increasing the initial quantity of A. swirskii into the sachet then, upon deployment into the field, this sachet will present predators into the crop at a faster rate than a sachet containing fewer predators. In all four trials, predators were emerging at a faster rate during the first week from the C. lactis based system (C.B.S) over the S. medanensis based system (S.B.S). This includes the trial undertaken in Finland (where initial predator counts were lower, see table 1) where during the first week of deployment, the C.B.S presented an average of 15.5(±0.86SE) A. swirskii day$^{-1}$, whereas S.B.S was 9.9 (±1.28 S.E.) A. swirskii day$^{-1}$. It may be considered from these trials that S.B.S results in a lower initial output of A. swirskii from the sachets than the C.B.S.

At week two, in three out of the four trials (Finland, NL and Spain) the release profile of the two systems changed considerably with the S.B.S equalling or exceeding the average daily predator release of the C.B.S. This pattern continues (see fig. 1) throughout the remaining weeks of the trial where the daily S.B.S predator output is higher than C.B.S. It is concluded from these trials that S.B.S sachets produce a more sustained output of predators over the 5-6 week trial period than the C.B.S.

In terms of implications for the grower, the differences of the release profiles of these sachets may have some important advantages and disadvantages. For instance, where pest is already present in the crop and a rapid presence of predators is required, one may conclude that the C.B.S represents more certainty that predators will be quickly positioned in the crop. In contrast, where sachets have been introduced where pest and pollen availability is very low, then having a more consistent inoculation of A. swirskii over a sustained period of weeks may be considered advantageous. A slower-releasing sachet may provide the grower with increased flexibility over the precise timing of the introduction of the sachet into the crop, or allow for a greater window of opportunity when deployed alongside less IPM compatible treatments. Further work is needed to investigate the relationship between deployment rate and control results.

Table 1. The initial loading of Amblyseuis swirskii predatory mites in a trial comparing the output of two different prey-mite systems.

<table>
<thead>
<tr>
<th></th>
<th>Number of A. swirskii at trial initiation in each country</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Finland</td>
</tr>
<tr>
<td>C.B.S*</td>
<td>280</td>
</tr>
<tr>
<td>S.B.S*</td>
<td>317</td>
</tr>
</tbody>
</table>

*C.B.S = Carpoglyphus Based System; S.B.S = Suidasia Based System
Figure 1(a,b,c,d). The release of *Amblyseius swirskii* from either a *Carpoglyphus lactis* based sachet system (C.B.S.) or a *Suidasia medanensis* (S.B.S) sachet. N=3, error bars denote standard error. Figs. a, b and c denote trials data from commercial cucumber crops, whilst Fig. d is from peppers.

References

Reducing pesticide emission from greenhouses: a joint agenda setting

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Abstract: Despite a high degree of IPM in the Netherlands, pesticides used in greenhouse horticulture are exceeding environmental quality standards for surface water. In this paper the joint fact-finding agenda setting by stakeholders are described. An overview is given of the emission routes in hydroponic growing systems and emission reducing measures and remaining questions are discussed.

Key words: IPM, discharge, hydroponic growing systems, reuse, joint fact-finding

Introduction

In the Netherlands in greenhouses IPM is common practice, be it at a higher level in fruit vegetables than in ornamentals. The main driving forces are retail demands for residue-free vegetables, resistance development to pesticides, and societal and governmental pressure towards sustainable production. In the last decade the Dutch government aimed at making crop protection more sustainable. By 2010 the environmental burden due to pesticides had to be reduced by 95% compared to 1998. This resulted in stimulation of the development of biological control and IPM, by funding research and knowledge transfer projects, like Farming with Future (De Buck & Beerling, 2005).

In 2005 an underestimated problem was revealed by Teunissen (2005): the water quality in greenhouse horticulture areas was still below the environmental quality standard for surface water. This was due to pesticides, despite the high degree of IPM and reduction in pesticide use. This led to a shift in focus of Farming with Future from IPM toward water quality. In this paper our analysis of the problem and subsequent approach to solve it are described.

Analysis of the problem

In the Netherlands 75% of the greenhouse crops are grown in soilless, or hydroponic, growing systems. In hydroponics, nutrient solutions recirculate with the possibility of a 100% closed water and nutrient cycle. These systems are in theory better equipped to minimize emissions of nutrients and pesticides than soil-grown crops, where leaching of nutrients and pesticides to ground and surface water are difficult to prevent. Nevertheless, the emissions of pesticides from hydroponic systems are significant, as was stated by Teunissen (2005).

Joint fact finding

The first step the project Farming with Future took was discussing the problem with the growers’ organization, water boards, agrochemical industry, and supply and advice organizations. In subsequent multi-stakeholder meetings the technique of joint fact-finding was applied. The search for common interests in situations like these helps to focus on solutions instead of disagreements. But also the interests of the individual stakeholder should be acknowledged, since these will motivate him or her to come into action. In our case, it is in
the interest of the agrochemical industry, supply organizations and growers to prevent pesticides being banned and retain the societal ‘licence to operate’. But it is in the interest of water boards to have a better water quality. Instead of disagreeing about whether pesticides should be banned, the stakeholders are now focussing on their common interest and join forces to lower the levels of pesticides in surface waters to meet the quality standards.

The stakeholders agreed on an agenda with the following actions: 1) Raise the awareness among growers and advisors, to make them receptive for advises to reduce the emissions. At the same time, find out 2) what the emission routes of the pesticides to the surface waters are, and 3) how the emission via these routes can be reduced.

Raising the awareness

For hydroponic cultures reuse is the prevailing standard, but drain water is easily discarded when there is only the slightest doubt about the quality for cultivation. Growers tend to avoid risks, especially since costs and other consequences are still low. Due to a joint effort of stakeholders the awareness of upcoming consequences is now growing.

Water boards initiated studies focusing on greenhouses (i.e. Kruger, 2008; Tolman, 2010) and communicated to growers their water quality figures. The growers’ organization together with the other stakeholders started to communicate the necessity to reach a ‘practically zero emission greenhouse for water by the year of 2027’, as was agreed upon with the water boards and the national government (Glami Agreement 2006-2010, succeeded by Platform Sustainable Greenhouse Horticulture and its sustainability agenda 2011-2015). This was a result of the obligation to implement the EU Water Framework Directive 2000/60/EC.

In the meantime the government realised that the registration procedure for pesticides was based on wrong assumptions. The current procedure uses a fixed emission percentage of 0.1% of the applied pesticides, which appeared to be a 2 to 50 fold underestimation of the actual emissions (Vermeulen et al., 2010). Already in 2013 the Dutch registration procedure will be adapted to these insights. When the emission does not decrease significantly, this is likely to result in the banning of pesticides and less new ones to be registered. The stakeholders communicate also these new insights and consequences.

These new findings appear to be an impulse for growers to accelerate the reduction of pesticide emission via water flows. But it is essential to involve advisers in the development of emission reducing strategies, since many growers are influenced by their adviser who generally is more conservative and risk-avoiding.

Emission routes

In hydroponics the emission of pesticides to surface water is assumed to follow the water flows (Figure 1) and can therefore be used to determine the significance of the emission routes (Vermeulen et al., 2010). In general, the largest contribution is discharge of recirculation water directly to the surface waters or indirectly via the sewage. The amount discharged however may vary from almost 0 to more than 3000 m³ha⁻¹year⁻¹, with large variations between, but also within crops (Van Paasen & Welles, 2010). The pesticides detected in drain and discharged water are not only applied via drip irrigation, but also via spray and space treatments (Kruger, 2008).

Discharge of filter water is only recently recognised as the second significant emission route; daily cleaning of the filters may add up to a yearly discharge of approximately 450 m³ filter
water per ha. Some minor emission routes of pesticides to the environment are leakages in the water system (but this is considered not to reach surface water), and overspill of drain water silos, or rainwater basins after first flush (this is the compulsory collection of the first two mm of rain after 48h of dryness).

![Diagram of water flows and pesticides emission routes in a hydroponic growing system.](image)

**Figure 1.** Water flows (•) and pesticides emission routes (○) in a hydroponic growing system.

Emission of pesticides to the air will occur during spray and space applications (drift), but also afterwards due to volatilisation. Part of it will end up via the cover in the condensation water. When condensation water is not reused, this flow is very significant (estimated at ca. 1000m$^3$ per ha per year; Vermeulen *et al.*, 2010), but reuse is obligatory and common practice in the Netherlands.

**Emission reducing measures**

The size of the emission routes implies that most gain is in preventing discharge. One of the main reasons for discharge is the accumulation of sodium (Na) in the recirculation water, as certain levels of Na damage to the crops. The supply water and fertilizers should therefore be low in Na, and growers are advised to have sufficient storage for rainwater (a minimum of 500m$^3$ per ha is obliged). The additional water needed in dry periods is preferably reversed osmosis water (filtered ground water), since surface and tap water are both higher in Na.

The occurrence of root zone diseases or viruses is also a reason to stop reusing the nutrient solution. The recirculating water can be disinfected effectively by heat treatment or UV radiation. Fear for spreading a disease is generally not justified, on the condition that enough attention is paid to maintenance and adequate capacity of the disinfector.

Discharge also occurs unintentionally due to system failures or damage like leakage. For example, failure of the disinfector may cause significant emission (20-40m$^3$ha$^{-1}$day$^{-1}$). This can be prevented with sufficient storage of drain water and rapid repair. Also mismanagement may cause unintentional discharge. Suboptimal water en nutrient management may result in small amounts of daily discharge, or flooding of drain silos. Also discharge of filter water, the second largest emission flow, falls in the category of preventable emissions. Awareness of the amount of discarded water and nutrients already stimulates growers to reuse this water.
Another approach in reducing the emission of pesticides is the optimisation of the application itself. Especially with drip applications there are several measures that help not only to increase the effectiveness of the product, but also decrease its emission. The timing of the application should be such that maximum uptake by the plant is ensured. Furthermore, the water should be reused at least 3 weeks in the summer or 6 weeks in wintertime before any discharge. This is particularly important for slow degrading pesticides.

*Further steps in reducing emission*

The decision to discharge is repeatedly not based on solid Na figures, but on the grower’s experience and feelings about the ‘state of the crop’. Recently it is demonstrated in the laboratory that growth hampering in rose is caused by the accumulation of a not-yet identified organic substance, which can be broken down with advanced oxidation (UV-activated $\text{H}_2\text{O}_2$; Van Os et al., 2010). This is now subject for further study in rose and other crops.

Starting from the assumption that not always all discharge can be prevented, also an ‘end of pipe’ solution should be available. For this purpose several techniques are being evaluated in cooperation with the waste and drinking water industries. Because of the urgency (2013!) of reducing the pesticide emission, this receives special attention. In addition, purification techniques for additional reuse of water and nutrients are being developed.

*Conclusions*

Pesticide related challenges in greenhouse horticulture have much in common with the challenges in waste management, for which a management tool called *The Ladder of Lansink* was developed by the Dutch Government in 1980. The hierarchical steps in this ladder are: 1) prevention, 2) reuse (of products), 4) recycling (of materials), 5) incineration (with energy production) and 6) landfilling as the last option. In analogy with this, a ‘pesticide emission ladder’ then could be: 1) prevention: prevent the need for pesticides and use alternatives, 2) reuse: prevent emission of pesticides by maximising reuse of water, 3) optimise use: apply pesticides with minimal emission, 4) purification: eliminate pesticides from discharged water.

In order to reduce the emission of pesticides it is necessary to battle on all these fronts. In the past the alternatives for pesticides received most, if not all attention. From the perspective of sustainability and the ‘pesticide emission ladder’ this is sensible. But in the meantime, as long as growers consider pesticides indispensable and use them, also considerable attention should be paid to the next steps of the ladder. This is in particular important for meeting the short term goals of reducing pesticide emission, but also from the perspective of sustainable water use.

*References*


Biotype, origin and insecticide resistance of *Bemisia tabaci* interceptions in the UK: Implications for IPM

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Abstract: Limited information is available with respect to *Bemisia tabaci* biotypes entering the UK and whether insecticide resistance within outbreak populations occurs. Using a PCR-based TaqMan assay, historic *B. tabaci* interceptions were analysed, of which 57% were determined to be Q-type and 26% B-type. A number of very recent interceptions were exclusively Q-type. Phylogenetic analysis indicated that plant origin is a good indicator of the source populations of *B. tabaci* for some countries/regions but not for others. A recently established field strain (Q-type) was shown to be highly resistant to imidacloprid, acetamiprid and pymetrozine but no tolerance to flonicamid was seen. The findings indicate that *B. tabaci* entering the UK are mostly Q-types that may exhibit high levels of resistance to insecticides commonly used for their control and, because of this, IPM/biological strategies must be developed that remove overreliance on the chemical eradication of this insect.

Key words: whitefly, resistance, biotyping, phylogenetics

Introduction

*Bemisia tabaci* (Gennadius) is not established in the UK although it is found on a relatively frequent basis with typically 20-40 outbreaks/interceptions annually (Cuthbertson et al., 2011). It has been acknowledged for many years that *B. tabaci* exists as several discrete biotypes exhibiting different biological and behavioural characteristics (De Barro et al., 2011). These differences mean that knowledge of biotype is important when developing and implementing control/eradication strategies for this pest. The Q biotype has been demonstrated to develop resistance to insecticides rapidly and, given the importance of determining biotype with respect to controlling *B. tabaci*, it is somewhat of an oversight that this fundamental knowledge is absent for the UK. The present work sought to determine the biotypes of historic interceptions that had been kept at Fera, as well as that of more recently captured insects. The resistance status of a recently intercepted strain was also determined against four commonly used insecticides.

Material and methods

*Insects*

*Bemisia tabaci* used in resistance testing were derived from two laboratory cultures. One culture (“Lab” strain) had been held >20 years whilst the second (“Com” strain) derived from a recent outbreak at a commercial glasshouse in the UK. Insects were cultured under quarantine conditions on poinsettia (*Euphorbia pulcherrima* c.v. Lilo Pink) plants at 23 ± 1°C following the method of Cuthbertson et al. (2005). Historic interceptions (n=68) that had been collected approximately 8-10 years previously were held at -80°C. All insects had been identified and subjected to DNA extraction prior to storage. More recent samples captured from imported plant material in 2010/11 (n=3) were similarly extracted and stored.
**Biotyping and “geotyping”**

The biotype of the two strains held at Fera, 68 historic samples and three recent interceptions was determined using the TaqMan method of Jones et al (2008). Phylogenetic analysis of the historic interceptions was undertaken to ascertain whether the stated source of the insects (i.e. where the infested plant material had originated) was the likely source of the insects, or whether the whitefly had infested the plants during transportation to the UK. A region of the mitochondrial cytochrome oxidase I (mtCOI) gene was amplified using the polymerase chain reaction (PCR) following similar methods to those described by Frohlich et al. (1999), and sequenced. These sequences were aligned using the CLUSTALW algorithm with B. tabaci mtCOI sequences available in GenBank from whitefly deriving from known geographic regions. Phylogenetic analysis was undertaken using MEGA 5.

**Insecticide resistance**

The susceptibility of the Lab and Com B. tabaci strains to four commonly used insecticides was examined. Insects were exposed to leaf discs (40 mm diam.) treated with a range of aqueous dilutions of imidacloprid (Intercept WG), acetamiprid (Gazelle WG), pymetrozine (Chess WG) and flonicamid (Teppeki) using a leaf dip method (Cuthbertson et al. 2009). Treated leaves were placed in 50mm diam. Petri dishes and 10 adult females were introduced into each. A minimum of 4 replicates were setup for each dose and the dishes were subsequently held at 23 ± 1°C. Survival was monitored for 96 hours and LD₅₀ values calculated using Probit analysis.

**Results**

The Lab strain was determined to be the B biotype whilst the recently acquired Com strain was shown to be Q-type. Of 68 historic interceptions examined, a positive analysis was obtained for ca. 84%. Of these, 57.4% of the insects were Q-biotypes whilst 26.4 % were B-types. The remainder (16.2%) did not produce a positive result for either B- or Q-type and must be assumed to be other biotypes (Table 1).

**Table 1. The biotypes of historic UK B. tabaci interceptions**

<table>
<thead>
<tr>
<th>Geographic origin</th>
<th>B-type</th>
<th>Q-type</th>
<th>Other biotypes</th>
<th>Reliability of phylogenetic analysis for determining source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portugal</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>Good</td>
</tr>
<tr>
<td>Spain</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>Good</td>
</tr>
<tr>
<td>Israel</td>
<td>1</td>
<td>16</td>
<td>2</td>
<td>Good</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>Poor</td>
</tr>
<tr>
<td>Denmark</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>Poor</td>
</tr>
<tr>
<td>Africa</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>Good</td>
</tr>
<tr>
<td>Unknown</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>Goodᵇ</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18 (26.4%)</td>
<td>39 (57.4%)</td>
<td>11 (16.2%)</td>
<td></td>
</tr>
</tbody>
</table>

ᵃThe source region as suggested by the origin of the plant material. ᵇLikely geographic source could be inferred for many of the insects of unknown origin.
Phylogenetic analysis was undertaken for a number of the historic interceptions. Comparisons with sequences from \textit{B. tabaci} of known geographic origin showed that the stated sources of the infested plants was a good indicator of where the insects had derived from in most cases (Table 1). However, as might be expected, plants imported from Denmark and the Netherlands were infested with insects originally deriving from a number of different populations and, as a result, phylogenetics only provided information with respect to where these countries were potentially receiving \textit{B. tabaci} from. It was possible for the likely origin of whiteflies for which no information was available to be inferred in many cases (Fig. 1).

![Figure 1](image-url)  
\textbf{Figure 1.} Phylogenetic trees were generated using the Neighbour-Joining method of MEGA 5 to examine the relatedness of \textit{B. tabaci} interceptions (underlined) to whitefly of known geographic populations (those with GenBank accession numbers). The example shown here is a bootstrap consensus tree inferred from 1000 replicates with partitions reproduced in <30\% of replicates collapsed.

Comparisons of the susceptibility of the Lab (B-type) and Com (Q-type) strains to four widely used insecticides showed that considerable resistance (>30\%) was present in the latter insects to three of the four compounds evaluated (Table 2). However, no resistance was observed to flonicamid in the Com strain, and conversely, these insects showed a significantly lower LD\textsubscript{50} than the Lab strain.
Table 2. The estimated doses of insecticides required to kill 50% of *B. tabaci* of the Lab (susceptible) and Com (field) strains.

<table>
<thead>
<tr>
<th></th>
<th>LD$_{50}$ (95% CI) Lab strain</th>
<th>LD$_{50}$ (95% CI) Com strain</th>
<th>Resistance factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>0.69 (0.4-1.1)</td>
<td>22.96 (11.7-97.65)</td>
<td>32.85</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>0.59 (0.4-0.9)</td>
<td>18.81 (13.3-33.39)</td>
<td>31.94</td>
</tr>
<tr>
<td>Pymetrozine</td>
<td>1.17$^b$</td>
<td>41.50 (20.50-1801.8)</td>
<td>35.62</td>
</tr>
<tr>
<td>Flonicamid</td>
<td>1.46 (0.95-4.12)</td>
<td>0.67 (0.43-0.91)</td>
<td>0.46</td>
</tr>
</tbody>
</table>

$^a$Dose is expressed as proportion/multiple of field rate, $^b$ limits could not be calculated.

**Discussion**

The results of the biotyping experiments showed that the majority of historic UK *B. tabaci* interceptions were the Q-biotype. Three recent interceptions were also Q-type and the findings would indicate that most *B. tabaci* now entering the UK are probably this biotype. The levels of resistance observed in the Com strain were sufficiently high to probably render three of the compounds tested ineffective against *B. tabaci*, should similar levels of resistance occur in outbreaks in the UK. As has been seen previously, cross resistance to neonicotinoids and pymetrozine was observed. Interestingly, the Lab strain, which has no known exposure to insecticides, was significantly less susceptible to flonicamid than insects of the Com strain.

The phylogenetic analysis demonstrated that the origin of plant material is a reliable indicator of where *B. tabaci* originate in many cases. However, plants from the Netherlands and Denmark were seen to be infested with whiteflies from a variety of geographic populations. From the present work, it is clear that Q-biotype *B. tabaci* are regularly entering the UK and there is the potential that many outbreak populations may be resistant to one or more of the insecticides that can be used against them. As such, IPM strategies must be designed to remove reliance on insecticide-based control strategies and should take into account that the likely target will increasing be the Q-biotype. These issues that are currently being addressed by ongoing research at Fera.

**References**


The potential use of flowering alyssum as a ‘banker’ plant to support the establishment of *Orius laevigatus* in everbearer strawberry for improved biological control of western flower thrips

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**Abstract:** Western flower thrips (WFT), *Frankliniella occidentalis* has recently become a serious pest of everbearer strawberry in the UK due to increasing problems with resistance to spinosad. Biological control of WFT with *Neoseiulus (Amblyseius) cucumeris* on everbearers is currently unreliable on farms with high WFT population densities. *Orius laevigatus* has good potential for use in combination with *N. cucumeris*, but is expensive to release and slow to establish on the crop, particularly when strawberry flowers are scarce. In a pilot experiment, flowering alyssum, *Lobularia maritima* proved to be a good host plant for *O. laevigatus*. Once established on the alyssum, *O. laevigatus* quickly dispersed to and established on flowering everbearer plants and rapidly reduced numbers of WFT. Alyssum has a long flowering period and has the potential for use as a combined ‘trap’ plant for WFT and ‘banker’ plant to support *O. laevigatus* populations in everbearer strawberry for improved biological control within an IPM programme.

**Key words:** everbearer strawberry, western flower thrips, *Frankliniella occidentalis*, biological control, *Orius laevigatus*, banker plants, alyssum, *Lobularia maritima*

**Introduction**

Western flower thrips (WFT), *Frankliniella occidentalis* has recently become a serious pest of everbearer strawberry in the UK due to increasing problems with resistance to spinosad. Although biological control of WFT with *Neoseiulus cucumeris* on glasshouse-grown strawberries is successful (Sampson 2008), control by this predator is not reliable on tunnel-grown everbearers, particularly on farms with high WFT populations. UK everbearers are mostly grown in ‘Spanish’ tunnels and have a long flowering and fruiting season which favours WFT. The pest overwinters in everbearer fields and high numbers can develop early in the season on second year crops. On some farms, WFT damage to everbearer fruit has been so severe following failure of spinosad to control the pest that total crop losses have occurred. *Orius laevigatus* has good potential to supplement control by *N. cucumeris* on strawberry (Boullenger *et al.* 2008) but is expensive and slow to establish, particularly when flowers are scarce. The pilot experiment described here tested candidate flowering ‘banker’ plants for *O. laevigatus*, to help the predators to establish and to support their populations in everbearer strawberry crops for improved biological control of WFT.

**Material and methods**

**Phase 1: Selection of potential banker plant**

An ideal banker plant for *O. laevigatus* would be an easily grown, inexpensive, non-invasive, compact plant that flowers prolifically and continuously from April onwards and one that the predator can both feed and breed on. Four candidate flowering plant species were selected:
1. 60-day strawberry cv. Elsanta
2. Lobularia maritima (Alyssum), cv. Clear Crystal
3. Impatiens (‘busy Lizzie’)
4. Tagetes erecta (‘African marigold’)

The strawberry cv. Elsanta was included at the request of growers, as although it flowers for only a few weeks, it does so at a predictable time after planting and could potentially be planted sequentially as a banker plant. L. maritima has been recorded as a natural early-flowering host plant for O. laevigatus in Spain (Alomar et al 2006). Impatiens is cheap, widely available and flowers continuously over a long period. T. erecta has been recorded as a potential banker plant for Orius insidiosus in greenhouse roses in Brazil (Bueno et al 2009).

The young plants were obtained as plugs from propagators and were grown in 3-litre pots in a research glasshouse until flowering. The flowering plants were set up in a randomised block design on 10 June 2010, with six replicate plots per plant species and three replicate plants (of the same species) per plot. Four O. laevigatus adults (two females and two males) were released to each plant. The glasshouse was unheated with natural daylength, venting at 19°C night, 25°C day. Assessments were made just before O. laevigatus release and one, three and four weeks later. On each date, numbers of O. laevigatus adults and nymphs and WFT adults and larvae per plant were assessed in situ by tapping each plant over a white tray, then returning all insects to each plant. Any strawberry fruit were examined in situ for O. laevigatus nymphs, particularly between the receptacle and the calyx. Numbers of flowers or flower heads per plant were recorded. The data were subjected to Analysis of Variance.

Phase 2: Test O. laevigatus dispersal from banker plants to everbearer strawberry plants

Everbearer strawberry plants cv. Eve’s Delight were potted into 3-litre pots and grown in a research polythene tunnel until they began to flower. Following completion of Phase 1, 12 flowering alyssum plants with similar numbers of O. laevigatus and 36 strawberry plants with similar numbers of flowers were placed in a research glasshouse. Twelve replicate plots were set up, each with one central alyssum plant and three strawberry plants. There were six replicate plots for each of two treatments: strawberry plants touching the alyssum plant so that O. laevigatus nymphs could walk between plants and strawberry plants placed 15cm away from the central alyssum plant. The plots were spaced 0.5m apart. Assessments were made two, 14 and 26 days after the experiment was set up. On each date, numbers of O. laevigatus adults and nymphs and WFT adults and larvae per alyssum and strawberry plant and numbers of flowers and fruit were assessed in situ as in Phase 1. Ripe fruit were removed after each assessment to simulate commercial harvest. The data were subjected to Analysis of Variance.

Results and discussion

Phase 1: Selection of potential banker plant

O. laevigatus produced nymphs on all the flowering plant species, but mean numbers of nymphs per plant were significantly higher on both alyssum and strawberry than on Impatiens and Tagetes, 21 and 26 days after adult release (Table 1). Numbers of nymphs were consistently higher on alyssum than on strawberry on both dates. On the same dates, total numbers of O. laevigatus (adults plus nymphs) were significantly higher on alyssum than on all other plant species (Figure 1). On all dates, there were significantly more open flower heads per alyssum plant than open flowers per plant on the other species and this is likely to have led to better establishment of O. laevigatus. Alyssum was selected for Phase 2 of the experiment.
Table 1. Mean numbers of *O. laevigatus* adults (+ nymphs) per plant 0, 4, 21 and 26 days after adult release (different letters indicate significant differences between plants *P*<0.05).

<table>
<thead>
<tr>
<th>Date</th>
<th>Days after <em>Orius</em> release</th>
<th>Mean numbers of <em>O. laevigatus</em> adults (+ nymphs) per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Strawberry</td>
</tr>
<tr>
<td>10 June</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14 June</td>
<td>4</td>
<td>0.3 (0)</td>
</tr>
<tr>
<td>1 July</td>
<td>21</td>
<td>0.5 (2.3ab)</td>
</tr>
<tr>
<td>5 July</td>
<td>26</td>
<td>0.5 (1.2ab)</td>
</tr>
</tbody>
</table>

Figure 1. Mean total numbers of *O. laevigatus* (adults + nymphs) per plant 26 days after adult release. * significantly different than other plant species, *P*<0.05.

Phase 2: Test *O. laevigatus* dispersal from banker plants to everbearer plants

On each assessment date, numbers of *O. laevigatus* (adults plus nymphs) were statistically similar on strawberry plants touching and not touching the alyssum plants, thus the data from strawberry plants in both positions were combined. This result indicated that alyssum plants do not need to be planted immediately adjacent to strawberry plants if used as ‘banker plants’.

When growing the potential banker plants on to the flowering stage for Phase 1, all had unintentionally become infested with WFT. The WFT had spread from an additional potential banker plant species (*Bacopa*) that was discarded when WFT were found in the flowers. However, the presence of WFT on the alyssum plants used in Phase 2 allowed numbers of both WFT and *O. laevigatus* to be recorded.

On day 2, means of 1.1 *O. laevigatus* and 8.4 WFT per strawberry plant were recorded, indicating that both thrips and predators had quickly dispersed from the alyssum to the flowering strawberry plants (Figure 2). On day 14, means of 11.6 *O. laevigatus* and 1.5 WFT per strawberry plant were recorded. Most of these *O. laevigatus* were nymphs, indicating that they had bred on the strawberry plants. By this time, green and white strawberry fruit had developed, on which *O. laevigatus* nymphs were easily seen. The day 14 results indicate that during population increase, the *O. laevigatus* had reduced the numbers of WFT. On day 26, means of only 1.1 *O. laevigatus* and 0.1 WFT per strawberry plant were recorded. By this time the strawberry plants had very few flowers and most of the fruit had ripened and been picked.
On days 2, 14 and 26, mean numbers of *O. laevigatus* were maintained at around six per alyssum plant (Figure 2). Mean numbers of WFT were reduced from 9.4 per plant on day 2 to 2.7 and 4.6 per plant on days 14 and 26, respectively. The results indicated that whereas *O. laevigatus* numbers fluctuated on the strawberry plants due to flushes of flowers and fruit, stable populations were maintained on the flowering alyssum. It was concluded that alyssum cv. Clear Crystal has potential for use as a combined ‘trap’ plant for WFT and ‘banker plant’ to support *O. laevigatus* populations in everbearer strawberry for improved biological control of WFT. The results of this pilot experiment will be validated in a commercial crop in 2011.

![Graph](image)

**Figure 2.** Mean numbers of WFT (adults + larvae) and *O. laevigatus* (adults + nymphs) per strawberry and alyssum plant on days 2, 14 and 26 after Phase 2 experiment set-up.

**Acknowledgements**

Thanks to Defra, HDC and other Horticulture LINK partners for funding this research as part of project HL01107, Syngenta Bioline Ltd. for supplying *O. laevigatus*, both Syngenta Bioline and BCP Certis for technical discussions and Ball Colegrave Ltd. and Bordon Hill Nurseries Ltd. for supplying alyssum cv. Clear Crystal seeds and plants, respectively.

**References**


The use and exchange of biological control agents worldwide

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Abstract: The Convention on Biological Diversity, signed in 1992, promotes the equitable and respectful sharing of access and benefits to genetic resources. A primary goal is to protect genetic resources that potentially have commercial value for biomedical and agricultural applications. Parties to the Convention on biological diversity have recently agreed in Nagoya, Japan, to adopt an international Access and Benefit-Sharing (ABS) regime. In the mean time, several countries have restricted the access to their biological resources. Research on biological diversity, discovery and exportation of new biological control agents are now on hold in some countries. Three years ago, IOBC Global put together a Commission on Biological Control and Access and Benefit-Sharing to provide scientific advice to oversee the design and implementation of an ABS regime that ensures practical and effective arrangements for the collection and use of biological control agents (Cock et al., 2010). During this conference I will review the work of the Commission and present recommendations on how biological control should be managed by governmental and non-governmental organizations, as well as the biological control industry, within the future ABS regime.

References

Developing a biologically-based IPM program for western flower thrips, *Frankliniella occidentalis*, in greenhouse floriculture

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Abstract: Few conventional insecticides registered in Canada today effectively control western flower thrips. Consequently, biological control agents are increasingly used. Recommendations and procedures developed for these natural enemies in vegetable crops though, do not translate directly to ornamentals. Furthermore, as tolerance for cosmetic damage is extremely low, a single biocontrol agent (the pesticide paradigm) rarely provides satisfactory levels of control. Strategic selection and use of several natural enemies together, within a bio-based IPM program, can provide an effective solution. Here, we report on trials on interactions between different natural enemies; by taking an integrated approach to their deployment, we are aiming to achieve maximum efficacy in the most cost-effective manner.

Key words: western flower thrips, biological control, microbial control, integration, compatibility

Introduction

Western flower thrips (WFT) is a pest of global significance, impacting many economically-important crops (Kirk & Terry 2003). Due to its high reproductive rate and cryptic habits, repeated applications of active compounds are frequently required to achieve control. WFT is now resistant to many classes of insecticide (Jensen 2000). Few effective products are registered in Canada, prompting a major shift in growers’ approach to thrips management and biological controls are increasingly used. Unfortunately, natural enemies have frequently been seen as simple ‘replacements’ for chemicals, with resulting dissatisfaction over comparative performance and reliance is still placed on pesticides to provide the desired levels of control. More strategic integration of biologicals is needed, requiring a critical evaluation of interactions (positive and negative) between beneficial species and an analysis of performance to ensure that pest control is achieved in the most cost-effective manner.

Several natural enemies (e.g., entomopathogens, predatory mites and insects) are commercially available to manage WFT larvae on foliage and flowers, and pupating thrips in the growing substrate (Ebssa et al. 2001, Jacobson et al. 2001, Berndt et al. 2004, Ansari et al. 2007, Bosco et al. 2008, Buitenhuis & Shipp 2005).

To ensure efficient integration of the different biocontrol agents, it is essential to consider their basic compatibility, to assess potentially beneficial or antagonistic interactions, and the cost-benefits that may result from using more than one natural enemy. Here we report on research to adapt, improve and integrate above- and below-ground WFT biocontrol strategies for ornamentals as part of a broader initiative to promote the resilience and uptake of bio-based IPM strategies by the Canadian floriculture industry.
Material and methods

1. Integration of microbial biocontrol agents
The trial had two primary objectives: i) to determine effects of application method on *Steinernema feltiae* efficacy against WFT; and ii) to assess the relative efficacy of two microbial control agents (*S. feltiae* and *Beauveria bassiana*) when used alone and in combination. *Steinernema feltiae* (Nemasys®) were obtained from Becker-Underwood, Inc. (Ames, IA, USA). BotaniGard®-WP (containing 4.4 x 10^10 cfu/g *B. bassiana* strain GHA) was obtained from Bioworks Inc. (Victor, NY, USA).

Unrooted chrysanthemum cuttings (var. Chesapeake) were directly planted in standard 6” pots in Sunshine® Mix #1 (Sun Gro Horticulture Canada Ltd., AB, Canada), four plants/pot. Two weeks after potting, blocks of 50 pots were treated as follows: a. untreated control; b. an initial soil drench of 50 million nematodes/100m² followed by 8, weekly foliar sprays at 50 million nematodes/200m²; c. weekly spray-drench (sprench) (total 9 treatments) at 50 million nematodes/200m²; d. weekly sprays of BotaniGard (weeks 1-3) at 1.2g/l 0.02% Slither® (Loveland Chemical Industries, Greeley, CO, USA) with a fourth spray at week 5; e. weekly nematode sprench (as per treatment ‘c’) followed 24h later with a BotaniGard spray (as per treatment ‘d’). Sprays were applied using an electric backpack sprayer (Dramm BP-4, 140 psi, Dramm Integrated Plant Health) fitted with a dual fan nozzle (Yamaha D-5); sprenches were applied using the same sprayer at 85 psi and fitted with an adjustable cone nozzle.

Thrips assessment
Every 2 weeks, thrips populations were assessed using a ‘plant washing’ technique. After 10-weeks, 5 pots were selected at random from each block and 20 leaves from each pot (total 100 leaves per treatment) were examined for the presence/absence of thrips feeding scars. Plant damage was analyzed with ANOVA using Tukey’s test to compare across treatments.

2. Compatibility of fungi with a soil predator
Small dish assays were used to evaluate effects of two fungal biopesticides (Met-52™ Granular Bioinsecticide, Novozymes Biologicals Inc., Salem, VA, USA; 9.0 x 10^8 cfu/g *Metarhizium anisopliae* Strain F52; and BotaniGard® 22 WP) on the rove beetle, *Atheta coriaria*. *A. coriaria* were obtained from Koppert Canada. Met-52 is labelled for use as a soil treatment so *Atheta* would be directly exposed to the fungus, whereas overspray or run-off from a foliar application of BotaniGard may also contact the predator.

In all experiments, *A. coriaria* adults were used in within three days of receipt. Suspensions containing 5x10^5 and 5x10^7 conidia/ml 0.01% Triton X-100 were prepared from each fungal product. In total, five treatments were included in the assays: a. deionized water (control); b. Met 52 10^5; c. Met 52 10^7; d. BotaniGard 10^5; e. BotaniGard 10^7.

Sterile tight-fit Petri dishes (50mm Ø, PALL Corporation, MI, USA) were lined with filter papers (Whatman™ #1, 55mm Ø). For each treatment (*n* = 15 dishes), the filter paper was inoculated with 0.3ml of suspension or water. One adult *A. coriaria* and ten late 2nd instar WFT were introduced into each dish through a hole in the lid, which was closed with a foam ear plug. Dishes were held in a growth chamber (16L:8D h, 20 ± 1°C) for 48 h. *A. coriaria* were then transferred to clean dishes containing a new filter paper moistened with 0.3ml deionized water. Every 48h thereafter for 12 days (14 days total), *A. coriaria* were fed ten late 2nd instar WFT. Dead beetles were surface sterilized with 70% ethanol, rinsed 2x in sterile 0.01 % Triton and incubated at 25°C to confirm death due to fungal infection. The whole experiment was replicated three times. *A. coriaria* mortality data were pooled and analyzed using a Chi square test. Individuals that died in the first 48 h were excluded from the analysis.
Results and discussion

Plants were naturally-infested with WFT; pre-treatment infestation levels were ca. 6 larvae/pot, replicating the status of a commercial crop in a similar stage of production. Thrips increased over time in all treatments, but numbers were significantly lower on plants treated with nematodes and/or fungi than the control ($F_{6,41} = 8.642, P < 0.001$); however, differences among treatments were not significant (data not shown). All plants showed thrips feeding damage; however, there were significant differences among treatments ($F_{6,69} = 4.077, P = 0.002$) (Figure 1). Most damage was observed in the control; 42% of the leaves showed feeding scars whereas only 21% of the leaves were scarred in the nematode sprench + Botanigard treatment. Although differences were not significant, the nematode sprench appeared to be more effective than the foliar spray.

Superior protection was obtained using the combined vs individual treatments. The microbials used provide control in contrasting environments, i.e., thrips pupae in the growing medium (nematodes) and larvae/adults on the foliage (fungi). BotaniGard would primarily regulate feeding stages consequently reducing leaf damage. As plants grow, the leaf canopy expands and may impede movement of nematodes to the soil following a sprench application with a resulting impact of efficacy. Plant growth stage should be considered when applying any soil-targeted treatment and techniques adopted to ensure efficient deposition onto the medium. In addition, thrips pupation behavior changes as plants develop, affecting the relative efficacy of controls (Buitenhuys & Shipp 2005, 2008; Steiner et al. 2010). Strategic use of complementary control agents, which may alter according to crop growth stage, should thus be considered for optimal effects (Ebssa et al. 2006).

![Figure 1. Mean + SE number of chrysanthemum leaves (out of 20) per plant showing thrips feeding damage after treatment with Steinernema feltiae (sprench or foliar spray and/or Botanigard.](image)

_Atheta coriaria_ mortality was higher in the fungus treatments than the control (Table 1). However, when analyzed separately, mortality of beetles exposed to Met 52 ($10^5$) was not significantly different from the control ($p = 0.089$). _Atheta_ mortality was positively correlated with the test concentration used but differences were not significant ($p = 0.21$ for Met $52 \times 10^5$ vs $10^7$, $p = 0.66$ for BotaniGard $10^5$ vs $10^7$). These data indicate that both mycoinsecticides are relatively safe to adult beetles and may be used concurrently.
Table 1. Mortality of *Atheta coriaria* adults exposed to two concentrations of Met 52™ and BotaniGard®. Data pooled from three replications and analyzed with $\chi^2$ test ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Met 52 $10^5$</th>
<th>Met 52 $10^7$</th>
<th>BotaniGard $10^5$</th>
<th>BotaniGard $10^7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survived</td>
<td>83.33 %</td>
<td>67.44 %</td>
<td>48.89 %</td>
<td>66.67 %</td>
<td>58.14 %</td>
</tr>
<tr>
<td>Dead</td>
<td>16.66 %</td>
<td>32.56 %</td>
<td>51.11 %</td>
<td>33.33 %</td>
<td>41.86 %</td>
</tr>
<tr>
<td>Total $n$</td>
<td>42</td>
<td>43</td>
<td>45</td>
<td>42</td>
<td>43</td>
</tr>
</tbody>
</table>

$\chi^2$ test $p = 0.015$

References


Does foliar trichome density affect walking activity and speed of *Aphidius colemani*, and its rate of parasitism of *Aphis gossypii* on chrysanthemum?

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**Abstract:** The parasitoid *Aphidius colemani* is one of the major biological control agents of *Aphis gossypii* and plays an important role in the regulation of aphid populations on chrysanthemum under protected cultivation. The high density of foliar trichomes of the aphid resistant chrysanthemum cultivar White Reagan (WR) reduces the survivorship and fecundity of *A. gossypii*. The objective of this study was to evaluate the influence of chrysanthemum foliar trichome densities on the walking speed, walking activity and rate of parasitism of *A. colemani* on *A. gossypii*. Leaf discs of the aphid susceptible cultivar Yellow Snowdon (YS) or the resistant cultivar WR were put in Petri dishes to evaluate the walking activity and walking speed of *A. colemani* in the absence of hosts. The parasitism of the 2nd and 3rd instar nymphs of *A. gossypii*, reared on the two cultivars, was evaluated by counting the number of hosts containing parasitoid larvae. Parasitoid walking activity (= % time active of total time on leaf) was higher on WR (64%) than on YS (47%). No significant differences were observed in the walking speed of and the rate of parasitism by *A. colemani* on *A. gossypii* on the two cultivars, so in this case the characteristic of aphid resistance of WR may positively influence the reduction of the aphid population by *A. colemani*.

**Key words:** *Aphis gossypii*, host-plant resistance, host-searching activity, tritrophic interaction

**Introduction**

Chrysanthemum (*Dendranthema grandiflora* Tzvelev) is one of the important ornamentals grown and marketed worldwide. *Aphis gossypii* Glover is considered a major pest of this ornamental under greenhouses conditions (Bueno *et al*., 2003, Bueno, 2005). *Aphidius colemani* Viereck is one of the effective biological control agents of *A. gossypii* in greenhouses (Carnevale *et al*., 2003).

The difference in aphid resistance levels observed between the chrysanthemum cultivars Yellow Snowdon (YS) and White Reagan (WR) is supposed to be related to the density of glandular trichomes present on their leaves (11.3 ± 8.74 and 16.6 ± 10.63 trichomes/mm² of leaf for YS and WR, respectively) (Soglia *et al*., 2002). Deleterious effects on survival (Soglia *et al*., 2002) and reproductive capacity (Soglia *et al*., 2003, 2005) of the aphid *A. gossypii* were observed on WR, the cultivar with the highest density of trichomes. The development and mortality of the parasitoids *A. colemani* and *L. testaceipes* was not affected when exposed to aphids maintained on the cultivar WR (Soglia *et al*., 2006). However, it is as yet unknown what the effect is of a high hair density on the searching behavior and the rate of parasitism of these parasitoids.
This study aimed to evaluate the influence of two chrysanthemum cultivars with different densities of leaf trichomes on the walking activity and speed and on the rate of parasitism of *A. colemani* by *A. gossypii*. Also, we wanted to see if the combined effect of host-plant resistance and parasitism on the cultivar with the highest number of hairs resulted in a larger reduction of the aphid population or not.

**Material and methods**

**Insect rearing**

*A. gossypii* were collected on the two chrysanthemum cultivars and reared on seedlings of the same cultivars under greenhouse conditions. Aphid nymphs of 2nd and 3rd instars were used in the experiments. *A. colemani* was reared on colonies of *Schizaphis graminum* Rondani on sorghum leaves (*Sorghum bicolor* L., cultivar BR 303) in an acclimatized room at 23 ± 2°C, RH 80 ± 10% and a photophase of 12h.

**Walking activity and speed of *A. colemani***

The walking activity of *A. colemani* (= % time active of total time on leaf) was evaluated in Petri dishes (5cm in diameter) containing leaf discs (4cm diameter) of one of the chrysanthemum cultivars. The leaf discs were maintained with the abaxial side up on a layer 1% agar/water. The Petri dishes were sealed and the amount of time that the parasitoids walked was observed during 5-minute periods. We observed 30 parasitoid females on each chrysanthemum cultivar.

The walking speed of *A. colemani* (= length of walking path per unit of time) was determined in the area of the main vein of the leaves of the two chrysanthemum cultivars. The leaves were maintained in Petri dishes (15cm diameter) as described to walking activity. The shape and size of the leaves were standardized; all were 15cm long. We measured the time spent by a parasitoid female when walking the distance from the basal end to the apex of the chrysanthemum leaf. We observed 20 female parasitoids on each chrysanthemum cultivar.

The experiments were carried out in an acclimatized room at 23 ± 2°C, and in a completely randomized design with two treatments (YS and WR cultivars). Data were subjected to analysis of variance and means were compared by an F test at 5% probability.

**Rate of parasitism of *A. gossypii* by *A. colemani***

The rate of parasitism of *A. gossypii* by *A. colemani* was evaluated by using a plastic container with a volume of 1 liter. Each container contained 30 nymphs of *A. gossypii* on a leaf of one of chrysanthemum cultivars. An *A. colemani* female was released in the center of the container and kept in the arena for 1 hour. After this period, the female parasitoid was removed and the leaf with the nymphs of *A. gossypii* was transferred to a Petri dish (15cm diameter) sealed with plastic film, containing a solution of agar/water 1% and kept in a climatic chamber at 25 ± 1°C, RH 70 ± 10% and a photophase of 12h. Three days after exposure to the parasitoids, the nymphs of *A. gossypii* were dissected to assess the percentage of aphids with larvae of *A. colemani*.

This experiment was conducted in a randomized design, using two treatments and 30 repetitions for each cultivar. Data were subjected to analysis of variance (ANOVA) and means were compared by an F test at 5% probability.
Results and discussion

The walking activity of \( A. \ colemani \) differed between the two chrysanthemum cultivars (\( F = 4.65, p = 0.035, df = 1 \)). On the aphid resistant cultivar WR, walking activity was significantly higher than on the susceptible cultivar YS (Table 1).

Table 1. Means (± SD) of walking activity and walking speed, and the rate of parasitism (%) of \( A. \ colemani \) on nymphs of 2nd and 3rd instars of \( A. \ gossypii \) on two chrysanthemum cultivars with different densities of trichomes.

<table>
<thead>
<tr>
<th>( \text{Aphidius colemani} )</th>
<th>Chrysanthemum cultivars</th>
<th>Yellow Snowdon</th>
<th>White Reagan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking activity (%)</td>
<td></td>
<td>47.3 ± 29.74 a*</td>
<td>64.0 ± 30.30 b</td>
</tr>
<tr>
<td>Walking speed (mm/s)</td>
<td></td>
<td>1.7 ± 1.06 a</td>
<td>1.6 ± 0.96 a</td>
</tr>
<tr>
<td>% Parasitism</td>
<td></td>
<td>37.8±26.56 a</td>
<td>43.9±22.46 a</td>
</tr>
</tbody>
</table>

*Means followed by the same letter on the same line are not significantly different according to the F test at 5% probability.

No significant difference (\( F = 0.05, p = 0.828, df = 1 \)) was observed for walking speed of \( A. \ colemani \) on the two chrysanthemum cultivars (Table 1). Sütt erlin & van Lenteren (1997) also found that the walking activity of the parasitoid \( Encarsia formosa \) on gerbera cultivars was not influenced by different types and densities of hairs. However, van Lenteren & de Ponti (1991) showed that different densities of trichomes present on the leaf surface of cucumber plants \( Cucumis sativus \) L. influenced the walking speed of \( E. \ formosa \): the higher the hair density, the slower the walking speed. The same authors showed that different hair densities on tomato and sweet pepper cultivars did not influence walking speed. They concluded that the long hairs on cucumber influenced the walking speed of \( E. \ formosa \), whereas the short hairs on, for example, tomato did not influence the parasitoid’s walking speed.

The percentage of parasitism of \( A. \ gossypii \) by \( A. \ colemani \) was not significantly different on the two chrysanthemum cultivars (\( F = 0.52, p = 0.477, df = 1 \)) (Table 1).

This study shows no negative influence of the more hairy, aphid resistant cultivar WR on the walking speed and the rate of parasitism of \( A. \ colemani \) on nymphs of \( A. \ gossypii \). Thus, the combined use of the parasitoid \( A. \ colemani \) as a biological control agent and the aphid resistant chrysanthemum cultivar WR may have a positive effect in reducing the population of \( A. \ gossypii \).

Acknowledgements

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References


Can trichome density explain the differences in behaviour and performance of *Amblyseius swirskii* on greenhouse ornamentals?

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Abstract: Biological control in ornamental crops is challenging due to the wide diversity of crops and cultivars. In this study, we tested the hypothesis that trichome density on different host plants influences the behaviour (walking speed and prey finding) and performance (predation and oviposition capacity) of the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). Tests were done on leaf disks of ornamental plant species differing in trichome density (rose, chrysanthemum and gerbera) and compared to a smooth surface (plastic). Thrips (*Frankliniella occidentalis* Pergande) (Thysanoptera: Thripidae) were used as prey. Behaviour and performance of *A. swirskii* were influenced by plant species. Up to a certain density of trichomes, trichome number had a negative effect. Walking speed was highest on plastic, followed by rose. No differences were found between chrysanthemum and gerbera. Proportion of time spent walking was the same on all leaf species. Predation of thrips was highest on gerbera and least on rose. Predation rates on chrysanthemum and plastic were intermediate. In contrast, no differences in oviposition rate were found among plant species. According to these results, release rates of *A. swirskii* may need to be adjusted depending on the crop in which it is used.

**Key words**: *Amblyseius swirskii*, trichome density, behaviour, performance, biological control

Introduction

Biological control in ornamental crops is challenging due to the wide diversity of crops and cultivars. In some crops, pest control seems to be more easily achieved than in others. Some of the differences in efficacy may be attributed to plant characteristics. For example, foliar pubescence, glandular trichomes, waxy leaf surface, leaf toughness, and plant architecture, can impede or facilitate natural enemy movement and thus, significantly influence encounter rate with hosts or prey (Cortesero et al., 2000; Price et al., 1980). Host plant species has been shown to influence predation or parasitism rate and functional response of biological control agents (e.g. van Lenteren et al., 1995; Skirvin & Fenlon, 2001; Madadi et al., 2007; Saber & Rasmy, 2010).

The predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is increasingly used in ornamental greenhouse crops in Canada for thrips control. Growers report differences in the efficacy of *A. swirskii* on various crops. We hypothesized that *A. swirskii* efficacy could be related to leaf characteristics such as foliar pubescence, either through altered host finding behaviour and/or the availability of trichomes as preferred oviposition sites for phytoseiid predatory mites (Roda et al., 2001). Most studies on phytoseiid mites focus on the effect of trichomes as domatia (e.g. Romero & Benson, 2005; Loughner et al., 2010), but detailed studies quantifying the walking behaviour of biological control agents on leaf surfaces with different trichome densities have mainly been done with parasitoids (e.g. van Lenteren et al., 1995; Sütterlin & van Lenteren, 1997) and spider mite
predators on vegetable crops (Krips et al., 1999; Rott & Ponsonby, 2000). In this study we tested the effect of trichome density of different greenhouse ornamental plants on the behaviour and performance of A. swirskii.

Material and methods

**Predatory mite and prey rearing**
Cohorts of A. swirskii females (<24h) and first instar thrips were reared according to methods described in Buitenhuis et al. (2010).

**Behavioural observations**
Searching behaviour was recorded in the presence of prey on leaf disks (mature leaves) of plant species with different numbers of trichomes: gerbera (Gerbera jamesoni var. Festival), chrysanthemum (D. grandiflora var. Chesapeake) and rose (Rosa kordesii var. William Baffin). A disk of transparent plastic served as a substrate with no trichomes. The number of trichomes per cm$^2$ was calculated for each plant species based on counts made on five randomly chosen 25mm$^2$ areas of leaf tissue. For each observation, one adult female A. swirskii (1-2 days old, starved for 24h) was transferred onto a 2.5cm diam leaf disk held on a water saturated sponge in a 60 ml Solo cup. The mite was left undisturbed for 5 min, to minimize effects on behaviour due to the transfer. One first instar thrips was placed in the arena after 5 min using a wet paint brush. The arena was filmed with a digital video camera for 10 min. The arena was illuminated by two microscope cold light sources. The experiment was carried out at 21 ± 1°C. Each predatory mite was used only once, and a new leaf disk was used for every observation. Each trial was replicated six to eight times per substrate type. Every video recording was analyzed for walking distance. Time spent walking, stopping and feeding, time of first prey contact were measured using Etholog 2.2.5 (Ottoni, 2000). Average walking speed, and walking activity (% time walking) of A. swirskii on the different substrates was calculated.

**Predation and oviposition rate**
Experiments to determine A. swirskii predation and oviposition rate were carried out on the same plant species and cultivars using leaf disks with similar trichome densities as in the previous trials. Adult female A. swirskii (1-2 days old) were starved individually for 24h. Leaf squares (1.5x1.5cm) of each plant species were placed, upside down, on a water-saturated sponge in a 60ml Solo cup. Fifteen first instar thrips were added to the disc, followed by one starved A. swirskii female. The cup was closed with fine mesh (100 µm) screening held in place by a plastic lid with a 2cm diam. opening. Cups were placed in a controlled environmental chamber (25°C ± 1, RH 70% ± 10, L:D 16:8). After 24h, number of thrips consumed and A. swirskii eggs present were counted. The trial was replicated 18-21 times for each plant species.

Results and discussion

**Behavioural observations**
Rose had the least number of trichomes, chrysanthemum and gerbera were similar (table 1). Plant species influenced the walking behaviour of A. swirskii (table 1). Walking speed decreased with an increase in number of trichomes per cm$^2$ up to a certain density: A. swirskii
walked fastest on plastic, followed by rose. There were no significant differences in walking speed between chrysanthemum and gerbera. Proportion of time spent walking was the same on leaf disks of all plant species, no differences in time spent grooming or resting were observed. There were no differences among plant species in time taken for a predator to find the prey. This may be due to the small number of replicates in which prey was encountered (plastic 8, rose 6, chrysanthemum 3, gerbera 2, out of 14 replicates).

Table 1. Number of trichomes per cm$^2$ and *Amblyseius swirskii* behaviour on different substrates in the presence of prey – *Frankliniella occidentalis*. Values within columns followed by same letter are not significantly different ($\alpha < 0.05$, ANOVA).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Trichomes/cm$^2$</th>
<th>Walking speed (mm/sec)</th>
<th>% time walking</th>
<th>Time to find prey (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>None</td>
<td>1.44 ± 0.24 c</td>
<td>64.2 ± 10.3a</td>
<td>262.8 ± 78.2a</td>
</tr>
<tr>
<td>Rose</td>
<td>90 ± 28 a</td>
<td>0.68 ± 0.11 b</td>
<td>48.6 ± 8.8a</td>
<td>152.1 ± 25.7a</td>
</tr>
<tr>
<td>Chrysanthemum</td>
<td>205 ± 9 b</td>
<td>0.41 ± 0.14 a</td>
<td>57.8 ± 11.4a</td>
<td>266.6 ± 133.1a</td>
</tr>
<tr>
<td>Gerbera</td>
<td>222 ± 8 b</td>
<td>0.31 ± 0.05 a</td>
<td>50.3 ± 7.8a</td>
<td>60.2 ± 33.8a</td>
</tr>
</tbody>
</table>

**Predation and oviposition rate**

Trichome densities were comparable to the previous trials. There were significant differences among plant species in predation rate (table 2). Predation of thrips was highest on gerbera and least on rose. Predation rates on chrysanthemum and plastic were intermediate. Contrary to expectations, no differences in oviposition rate were found among plant species (table 2).

Table 2. Number of trichomes per cm$^2$ and *Amblyseius swirskii* daily predation rate and oviposition rate on different substrates in the presence of prey – *Frankliniella occidentalis*. Values within columns followed by same letter are not significantly different ($\alpha < 0.05$, ANOVA)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Trichomes/cm$^2$</th>
<th>Predation rate</th>
<th>Oviposition rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>None</td>
<td>6.00 ± 0.47 ab</td>
<td>0.38 ± 0.13 a</td>
</tr>
<tr>
<td>Rose</td>
<td>92 ± 17 a</td>
<td>4.62 ± 0.47 b</td>
<td>0.71 ± 0.16 a</td>
</tr>
<tr>
<td>Chrysanthemum</td>
<td>443 ± 58 b</td>
<td>5.85 ± 0.42 ab</td>
<td>0.65 ± 0.13 a</td>
</tr>
<tr>
<td>Gerbera</td>
<td>316 ± 12 b</td>
<td>6.75 ± 0.46 a</td>
<td>0.85 ± 0.20 a</td>
</tr>
</tbody>
</table>

**Conclusions**

Although the results of this study indicate that trichome density has an effect on both host finding behaviour and predation rate of *A. swirskii*, it is likely that other factors such as trichome length and shape (e.g. gerbera has long woolly trichomes, chrysanthemum short stiff ones) and surface waxiness can account for some of the observed differences. In addition, other plant effects (architecture and secondary metabolites) and the influence of crop management practices on *A. swirskii* efficacy should be studied. However, this study has yielded valuable information on the behaviour and performance of *A. swirskii* on different greenhouse ornamental crops which can be used to adjust recommendations on the use of this predator and improve the efficacy.
Acknowledgements

Thanks to Sébastien Rocheleau, Erik Glemser and Rebecca Eerkes for technical assistance. Funding was obtained through an Agriculture Adaptation Council – Canada Ontario Research and Development (CORD) IV Grant (Project No. 9006) to Flowers Canada (Ontario) and Agriculture and Agri-Food Canada Matching Investment Initiative; and Agriculture and Agri-Food Canada under the Growing Forward Program.

References


Evaluation of *Trichogramma brassicae* for the control of carnation tortrix moth and light brown apple moth in protected nursery stock

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**Abstract:** Initial experiments using insect cages to contain host and parasitoid showed that *Trichogramma brassicae* (as Tricholine from Syngenta Bioline Ltd.) were able to successfully parasitise egg masses of carnation tortrix moth (*Cacoecimorpha pronubana*), providing that the eggs were young and pale green when parasitoids were active. Egg masses turned black about 10 days after being parasitised. Mature egg masses, which were yellower in colour, were not parasitised. Tests in the laboratory showed that *Trichogramma* adults emerged from parasitised eggs on cards between 9 and 13 days (mean 11 days) after delivery. This time interval needs to be taken into account when planning introductions of this beneficial. A further trial on *Chaenomeles* plants in a polythene tunnel on a commercial nursery, which were naturally infested with light brown apple moth (*Epiphyas postvittana*), showed that weekly introductions of the parasitoid at 20 per m² between mid June and mid September gave good control over this period, although two applications of Dipel DF (*Bacillus thuringiensis*) were also needed to ensure complete control.

**Key words:** Carnation tortrix, *Cacoecimorpha pronubana*, light brown apple moth, *Epiphyas postvittana*, hardy nursery stock, *Trichogramma brassicae*

**Introduction**

In the UK, a high percentage of hardy nursery stock is grown under protection, in polythene tunnels or glasshouses, in order to increase the speed of growth and thus reduce the time taken to reach the saleable stage. However, the increased temperature in these environments has favoured a range of pests, including light brown apple moth (LBAM) and carnation tortrix. Female moths become active in spring and lay egg masses containing between 15 and 50 eggs on dorsal leaf surfaces. The egg masses are pale green when first laid, and are difficult for growers to monitor as they are well camouflaged against the leaves. Larvae hatch in about 7-10 days, depending on temperatures, and immediately disperse by crawling or ‘ballooning’ on silken threads. The larvae of both species infest the growing points of a wide range of plants such as *Daphne*, *Chaenomeles*, *Forsythia*, *Potentilla*, *Choisy* and *Ceanothus*, reducing plant vigour and quality. As they grow, larvae spin leaves or shoots together and are thus protected from insecticide sprays, making chemical control difficult. Growers often need to spray five or six times during the summer to ensure adequate control, and although some IPM compatible products are available, such as Dipel DF (*Bacillus thuringiensis*), no other biological control agents for LBAM or carnation tortrix have been used. There is a need for an effective parasitoid for these pests and the work described here tested the efficacy of the egg parasitoid *Trichogramma brassicae*. 
Material and methods

Initial cage tests 2008
Liner plants of Chaenomeles each with an egg mass of carnation tortrix moth were selected from a naturally infested crop at Wyevale Nurseries, Hereford, UK, and the number of eggs counted and the stage of development noted. Plants were placed singly in replicated, ventilated insect cages in a polythene tunnel at the nursery and a single card of the Trichogramma brassicae product Tricholine, (containing 50 parasitized eggs of the flour moth Ephestia kuehniella) was hung on each plant. Plants were monitored at intervals until egg hatch occurred. The number of hatched carnation tortrix moth eggs, the number that turned black and the time taken for parasitoids to emerge were recorded. In addition, samples of Tricholine cards were retained in the laboratory and the time taken for parasitoid emergence recorded. This process was repeated on five occasions between late June and mid August.

Full scale trial 2009
Following the successful cage tests, a further trial was undertaken the following year in a polythene tunnel of ca. 500m$^2$, containing a wide range of hardy nursery stock in liner pots, which were naturally infested with LBAM. Trays of Chaenomeles liner pots (190 plants in total) were placed down the middle of the tunnel, and all assessments were carried out on these plants. Trichogramma brassicae were used as the product Tricholine, supplied by Syngenta Bioline. Cards containing black parasitised eggs of the flour moth were placed evenly within the tunnel at approximately one card per 10m$^2$, every week from 17 June until 26 August (10 introductions), at a mean rate of 20 parasitoids per m$^2$ per week. Each delivery of cards was held in the laboratory until the first wasps emerged, and then immediately placed in the tunnel. This procedure was necessary because the previous cage tests had shown that the parasitoids took between nine and 13 days to emerge. During this extended period, overhead watering in the tunnel would make the cards wet, reducing quality and effectiveness of the parasitoids.

At weekly intervals, each of the Chaenomeles plants was visually examined, and the leaves checked for LBAM egg masses. Egg masses were recorded as being in one of the following categories: emerged, which were silver in colour and the emergence hole could be seen using a hand lens; viable, these were pale green, darker green, or pale yellow in colour depending on age, and varied in number from 15 to more than 50; and parasitized, which had turned black. Emerged egg masses remained on the leaves for many weeks, but eventually disintegrated and were not visible.

Results and discussion

Table 1 shows the results of parasitoid incubation in 2008.

The mean time between delivery and emergence of T. brassicae was 11 days (at room temperature, which varied between 15 and 21°C). The parasitoids were delivered in a diapause state (Sopp, pers. comm.) which is why emergence times were extended.

A total of five experiments were carried out during the cage tests in 2008. The results from one experiment are shown in Table 2 below. The experiment was set up on 15 July, when the parasitoids were first emerging, and assessed on 25 July.
Table 1. *T. brassiace* emergence times in the laboratory

<table>
<thead>
<tr>
<th>Tricholine batch number</th>
<th>Date arrived</th>
<th>Date hatch commenced</th>
<th>Time to first emergence (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25 June</td>
<td>7 July</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>2 July</td>
<td>15 July</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>8 July</td>
<td>21 July</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>18 July</td>
<td>27 July</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>19 August</td>
<td>28 August</td>
<td>9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2. Results of an experiment with *T. brassicae* and carnation tortrix in insect cages in a polythene tunnel.

<table>
<thead>
<tr>
<th>Plant/cage number</th>
<th>Total eggs in egg mass</th>
<th>Stage at start of experiment</th>
<th>Number of black parasitized eggs</th>
<th>Number of hatched eggs</th>
<th>% parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>Pale green</td>
<td>26</td>
<td>10</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>Pale green</td>
<td>28</td>
<td>26</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>Darker green</td>
<td>0</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>Pale green</td>
<td>12</td>
<td>9</td>
<td>57</td>
</tr>
<tr>
<td>5</td>
<td>66</td>
<td>Darker green/pale yellow</td>
<td>0</td>
<td>66</td>
<td>0</td>
</tr>
</tbody>
</table>

These results, and those from later experiments, indicated that only newly laid egg masses, which were still pale green, were suitable for oviposition by the parasitoid. As egg masses matured, they turned a darker green and then pale yellow, and the head of the larva could be seen as a dark spot inside the egg. Emergence of the new generation of *T. brassiace* occurred between nine and 10 days during these experiments. This compares with a mean figure of nine days quoted by Knutson (1997), but is clearly dependent on temperature.

Figure 1 below shows the results of the full scale trial in 2009, when *T. brassicae* were introduced weekly in a polytunnel at 20 per m$^2$ per week between 17 June and 26 August.

The first black egg masses were seen on 2 July, about two weeks after the first introduction of Tricholine cards. Percentage parasitism at this assessment was only 7%, but by the following week on 8 July, parasitism had increased to over 90%. Parasitism remained at over 70% until 12 August, when levels dropped to over 50%. The biopesticide Dipel DF (*Bacillus thuringiensis*) was applied on 18 July and again on 25 July in the trial, to reduce the numbers of larvae as some plant damage was still occurring. This product acts as a stomach poison when larvae consume treated foliage, but has no adverse effect on parasitoids such as *T. brassicae*.

Considering that the *Chaenomeles* plants assessed in the full scale trial were evenly spaced within a 500m$^2$ tunnel, and were surrounded by liners of other nursery stock species, the searching ability of *Trichogramma* parasitoids is clearly excellent. Within the polythene tunnel, the air was relatively still and no doubt this benefited the flying ability of the parasitoid. Knutson (1997) summarized the biology of *Trichogramma* and its use in classical biological control programmes. He states that the parasitoid locates egg masses by homing in on the pheromones and chemical signals left behind when the tortrix moth oviposits. Tiny wing scales may be left behind and these may also act as chemical and visual cues for the parasitoid. (Nordlund & Beevers, 1981).
Parasitic wasps in the family Trichogrammatidae are very small, ranging in size from 0.2 to 1.1 mm in length (Knutson, 1997). Despite their small size, they can be effective outdoors, and have been used in biological control programmes for several lepidopteran pests, including codling moth in apples (Hassan, 2006). Thus there is clearly potential for their use in IPM programmes outdoors for nursery stock in the future. Despite the high mean percentage parasitism of LBAM achieved in the 2009 trial, supplementary sprays were still needed. However, at the release rate used (20 per m$^2$ per week), the cost was similar to that of a full chemical programme. Growers in the UK are aiming to increase their use of biological controls wherever possible, and so the findings of this project will be of immediate practical value to nursery stock growers.

Acknowledgements

This work was sponsored by the Horticultural Development Company (HDC), to whom we are grateful. Thanks are also due to the great support given by Wyevale nurseries, Hereford, UK, particularly Steve Reed and Steve Price. Technical support from the project co-ordinators Dr Paul Sopp and Mr John Adlam is also gratefully acknowledged.

References

Dispersal of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) in greenhouse pepper for biological control of European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae)

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Abstract: The European corn borer (ECB), *Ostrinia nubilalis* Hübner, is an economic pest of greenhouse sweet pepper in Ontario and no adequate biological controls currently exist for this pest. The objective of this study was to investigate the potential of an egg parasitoid, *Trichogramma ostriniae* Pang et Chen, as a biocontrol agent for ECB. Results of this study indicate that *T. ostriniae* can disperse to the upper canopy of greenhouse peppers. Although the number of infested fruits obtained from treated plots was not significantly less than that in the control plots, further work with release rates and strategies to prolong the period of emergence and longevity of the parasitoids are needed to determine if such factors can significantly reduce damage by ECB in greenhouse peppers.

Key words: *Ostrinia nubilalis*, *Trichogramma ostriniae*, dispersal, greenhouse pepper

Introduction

The European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is an important pest of greenhouse pepper in southern Ontario. Females lay eggs in a mass of 5 to 50 eggs on the undersides of foliage. Shortly after hatching, the newly emerged larva locates a fruit and bores into it under the calyx (Hagerman, 1997). The short time spent by the larva on the foliage combined with its habit of remaining inside the fruit for most of its life provide this stage with much protection and make it very difficult to manage. There are currently no satisfactory biological controls for this pest in greenhouse pepper in Ontario.

Research in the United States indicate that the egg parasitoid (*Trichogramma ostriniae* Peng et Chang (Hymenoptera: Trichogrammatidae), originally from China, has much potential as a biological control agent against ECB (Hassan & Guo, 1991; Hoffmann *et al.* 1995) and can effectively disperse and locate ECB egg masses in corn fields (Wright *et al.*, 2001). However, the height and leaf surface area of a greenhouse pepper crop are very different from those of a corn crop, and the effects of such factors on the dispersal and searching abilities of *T. ostriniae* are unknown. The objective of this trial was to determine whether *T. ostriniae* could disperse to the upper canopy of greenhouse pepper crops which are on average 4 to 5m high during the summer months when ECB is prevalent. It is assumed that egg masses of ECB are deposited on leaves anywhere from mid- to upper canopy where developing fruits are located.

Material and methods

This trial was done in a 6 ha commercial greenhouse pepper operation. The cultivar, Natanja, an orange coloured pepper, was planted in the greenhouse on January 15, 2010. The trial occupied six bays or houses. Each house measured 62m x 11.4m or 707m² and was
considered as one plot. Three houses were treated with *T. ostriniae*, and three others, untreated, were used as controls. Treatments alternated with controls, and each treatment and control were separated by an entire house which served as a buffer. Each house consisted of seven walkways, and each walkway had a row of 172 plants on either side of it. Control plots were treated according to the grower’s regular management practices for ECB which included applications of Confirm® (tebufenozide). The treatment plots were treated similarly to the control plots except for weekly releases of *T. ostriniae*.

**Releases of *T. ostriniae***

1. **Open cards placed on plants**

A total of 12 weekly releases of *T. ostriniae* pupae on open cards were made beginning June 11, 2010, and ending August 25, 2010. These cards are described as open because the pupae were exposed on the cards. The number of *T. ostriniae* released in each of the treated plots (707m$^2$) was 160,000 (80 cards of 2,000 pupae) or approximately 2.26 million per ha. The number of release points in each plot totalled 80 using 16 release points in each of the innermost five rows. No parasitoids were placed in the two outermost rows. Release points in each row were spaced 3.7m apart and alternated between the two plant rows of each walkway such that each plant row along the walkway held eight release points. At each release point, a card with approximately 2000 pupae of *T. ostriniae* was hung on the plant about 30cm from the growing point of the selected plants.

2. **Closed cards placed on growing media**

Just one release of *T. ostriniae* in closed cards was made on 22 July, 2010, in one house that was located in a section separate from where the replicated treatments were located. Cards used in this section are described as closed because the pupae were enclosed in double-layered cards and adult emergence was facilitated by small openings at the sides. Approximately 86,000 pupae in a total of 24 cards, were distributed evenly in the house in 24 locations and placed on the growing media slab. The rate used was 1.21 million per ha, about half that used in the replicated treatment.

**Assessment of parasitism**

To assess parasitism, 20 cards with sentinel eggs of *Ephestia kuehniella* were placed in 2 inner rows of the replicated treated plots. Five locations were used in each row, and 2 cards were placed at each location. Spacing between sentinel egg locations along the row was about 11 m. Within each row, 5 cards of sentinel eggs were placed at the base of the plant, about 30 cm from the lowermost leaf, and 5 in the upper canopy, about 30cm from the growing point. Cards were collected about 7 days after being placed in the greenhouse. Dates for placement and collection of sentinel eggs were as follows:

1$^{st}$ placement of sentinel eggs - 17 June 2010, collected 23 June 2010

**Assessment of dispersal**

For the replicated treated plots, a total of 18 yellow sticky cards were used in each plot to assess dispersal of the parasitoids within the canopy. Cards were placed out on two dates about two weeks apart, and were collected on 23 June and 6 July, approximately 7 days after placement in the canopy. Six sticky cards were placed in rows 1, 4, and 7. Cards were placed at three locations along each row, two cards per location, with about 18m between locations. At each location, one card was placed about 30cm from the growing point, and the other,
about 30cm above the lowest leaves. The total number of adult *T. ostriniae* caught on the traps was counted for both dates. For the unreplicated treatment where the closed cards were used, 18 sticky cards were also used and were collected on 13 August, about 3 wk after placement in a pattern similar to that used in the replicated plots.

**Impact on Fruit Damage by ECB**

To assess the impact of *T. ostriniae* on ECB populations, the number of fruits from only the mid-row of treated and control plots was recorded weekly for the duration of the trial. Fruits were collected twice per week from the replicated plots and examined for presence of ECB or its damage symptoms. The total number of fruits believed to be damaged by ECB for each treatment was added at the end of the trial.

**Statistical Analysis**

An analysis of variance (PROC GLM (SAS Ver. 9.13)) was done on untransformed data to detect differences between treated and untreated plots for numbers of *T. ostriniae* caught between sticky trap locations, and for numbers of fruit collected from treated and untreated plots were significant at \( P < 0.05 \). Subsequently, Fischer’s Least Significant Difference (FLSD) tests were used to determine differences between sticky trap locations for number of adult *T. ostriniae* caught, and for numbers of fruit damaged by ECB.

**Results and discussion**

**Assessment of parasitism**

Absence of an average of 60-75% of the eggs from all the cards collected on 23 and 30 June prevented assessment of percentage parasitism. It is not clear what caused the loss or removal of the eggs. Perhaps feeding by either predators released for biological control of other pests, or by naturally occurring ones, resulted in removal of the *Ephestia* eggs from the sentinel cards.

**Assessment of dispersal**

The number of adult *T. ostriniae* caught on sticky cards located in the upper canopy was significantly greater than that caught on cards located at the base of the plants \( (P < 0.0001) \). Where open *T. ostriniae* cards were used and placed on the plant, the average number of *T. ostriniae* caught per card located close to the growing point was 13.1, whereas the average number caught at the base of the canopy was 3.6. This result is not unexpected given that the parasitoids were placed close to the upper canopy in these plots. However, in the non-replicated, closed card treatment in which all *T. ostriniae* were placed on the slabs which lay on the floor, the average number of *T. ostriniae* caught per card in the upper canopy was similarly high at 11.0 per card, and that caught in the lower canopy was just 0.8. Although the closed card treatment was non-replicated, we believe that the size of the plot \( (707m^2) \) was sufficiently large to give a good indication of the ability of the parasitoids, released from the bottom of the crop, to disperse to the top of the canopy.

**Impact on Fruit Damage by ECB**

Although differences in the average number of fruits with ECB feeding damage from treated and control plots were not statistically significant \( (P > 0.05) \), the total number of ECB-damaged fruits from control plots was 195, whereas that from the *T. ostriniae* treated plots was somewhat less at 164. Possibly, the difference was not more marked because of reduced
parasitism resulting from loss of pupae from the open cards due to the same factors that contributed to loss of the sentinel eggs mentioned above.

**Conclusions**
This trial suggests that *T. ostriniae* can disperse efficiently to the upper canopy of greenhouse peppers, even when released from the base of the crop. This is significant because eggs of the ECB are likely laid on the younger, developing leaves in the upper canopy. Although the number of fruits obtained from treated plots was not significantly less than that in the control plots, it is possible that natural predation could have been a mortality factor contributing to lowered numbers of parasitoids in the crop. We believe that further trials should incorporate different release rates, strategies to prolong the period of emergence and longevity of the parasitoids, and the closed card system which provides greater protection to the parasitoids.

**Acknowledgements**

We gratefully thank Dave Delellis and Blake Fischer of Del Sol Greenhouses Inc., Kingsville, Ontario for their participation in this study. We also acknowledge that the *T. ostriniae* used for this trial were supplied by Anatis Bioprotection Inc., Quebec.

**References**

Progress towards biological control of *Bactericera cockerelli* in covered crops in New Zealand

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**Abstract:** *Bactericera cockerelli* is a North American pest species known in New Zealand as the tomato/potato psyllid (TPP). First reported in New Zealand in 2006, it has now become a major pest on both greenhouse and outdoor solanaceous crops in New Zealand. Effective biological control agents are urgently needed to increase and improve control options for growers. Searches conducted within New Zealand have identified a number of psyllid predators that are potential biocontrol agents for TPP. In addition, in 2009 a North American parasitoid, *Tamarixia triozae*, was imported into quarantine facilities at Plant & Food Research, Auckland, for assessment as a biological control agent for TPP.

**Key words:** Tomato/potato psyllid, parasitoids, *Tamarixia triozae*, predators, *Drepanacra binocular*

**Introduction**

The tomato/potato psyllid (*Bactericera cockerelli* (Sulk) Hemiptera: Triozidae) (TPP) is a North American pest first reported in New Zealand in 2006 (Teulon et al., 2009). TPP has now become a major pest on both greenhouse and outdoor solanaceous crops in New Zealand (Teulon et al., 2009). TPP is a challenging pest to control as it damages plants directly and also vectors the bacterial pathogen ‘*Candidatus Liberibacter solanacearum*’ (Liefting et al., 2009), the causal agent of zebra chip disease (Sengoda et al., 2010). The presence of TPP in New Zealand (NZ) has disrupted existing integrated pest management programmes, and necessitated an increase in insecticide use on vulnerable crops (especially tomatoes and potatoes). Effective biological control agents (BCAs) are urgently required to increase and improve grower options in solanaceous crops. Since 2006 Plant & Food Research (formerly Crop & Food Research) has worked with the NZ horticultural industries to identify potential BCAs, both within NZ and overseas. The initial research is summarised in this paper.

**Material and methods**

**Locating and assessing potential biological control agents within New Zealand**

The natural enemies of NZ’s native psyllids represent a pool of potential biocontrol agents for TPP, but there have been few systematic surveys for psyllid predators and parasitoids. To address this research gap, a survey was carried out on the natural enemies of a common native psyllid species, *Trioza vitreoradiata*, which feeds on native *Pittosporum* shrubs and trees. *Pittosporum crassifolium* trees were searched at 10 locations around Auckland between December 2006 and January 2007. At each location 20 fresh shoots with psyllid infestations were first sampled for predators by visually scanning the shoots and then removing the shoots for closer inspection in the laboratory. In addition, five shoots at each location were collected to see if any psyllid parasitoids emerged. Six common predators were collected and...
maintained in the laboratory for use in no choice feeding tests (Table 1). In each test, a single predatory larva was confined to a small pot containing 20 to 30 late instar TPP nymphs (mainly 4th instars) on a capsicum leaf, with the cut end of the stem in a vial of water to keep the leaf alive. The number of surviving TPP nymphs was recorded at 24h and additional nymphs provided to ensure a constant food supply. TPP numbers were recorded again at 48h at the end of the trial. Similar tests were also carried out using *T. vitreoradiata* nymphs on *P. crassifolium* leaves. For each predatory species, five replicate tests were conducted using TPP and five using *T. vitreoradiata*. Trial results for each host species were analyzed separately using ANOVA.

The three most promising natural enemies, *Adalia bipunctata* (two spot ladybird), *Harmonia conformis* (large spotted ladybird) and *Drepanacra binocular* (hook tipped lacewing), were then used in a small scale cage trial in a glasshouse to investigate their ability to control TPP infestations on capsicum and tomato plants. For each predatory species two cages (70 x 70 x 70cm) were set up containing a tomato plant in one and a capsicum plant in the other. Plants were infested with high numbers of TPP. In each cage six to eight adults of the predatory species were released, and the number of predator eggs, larvae and adults counted and recorded daily. Psyllid numbers in the cages were scored daily, using a 1 (very low) to 5 (very high) scale for psyllid eggs, larvae and adults. When totalled, a score of 15 would therefore indicate a plant saturated with psyllids, while a score of 3 would indicate a very light infestation and limited food supply for the predators.

**Selecting, importing and testing potential biological control agents from beyond New Zealand**

A literature review was carried out on the overseas natural enemies (in commercial production) that could be useful for covered crops in NZ (Workman & Davidson, 2007). The review concluded that four overseas species had potential to provide effective biological control of TPP: a mirid (*Dicyphus hesperus*), two green lacewings (*Chrysoperla carnea* and *C. rufilabris*) and a parasitoid (*Tamarixia triozae*). Of these, *T. triozae* was selected as the most promising overseas BCA for TPP and was imported from Mexico into NZ quarantine in 2009. *Tamaraxia triozae* (Burks) (Hymenoptera: Eulophidae) occurs naturally in Central and North America, where it attacks psyllid species from a number of families (La Salle, 1994). Female *T. triozae* typically oviposit a single egg on the ventral surface of the host and the larva develops externally beneath the body of the host.

Host specificity testing of *T. triozae* is currently underway to ascertain the impact that the parasitoid is likely to have on NZ’s native psyllid fauna. Preliminary results from the first round of host specificity tests undertaken in quarantine conditions (at 22°C, in 16 h light cycle) are reported here. The first round of host specificity testing involved no choice oviposition tests using four common native psyllid species (Table 2). For each no choice test two small cages (vented “cookie jars”) were set up with three female *T. triozae* (1 to 10 days old, possibly mated) per cage. In one cage the parasitoids were offered c. 50 nymphs (4th and 5th instars) of a native psyllid species on leaves of its host plant. In the other cage the parasitoids were offered 50 TPP nymphs (4th and 5th instars) on a capsicum leaf. The cut end of the stems were placed in vials of water to keep the leaves alive. Both cages were left undisturbed for 48 h, then psyllid nymphs were examined under a binocular microscope. Each nymph was inverted and the number of *T. triozae* eggs were counted.
Results and discussion

Locating and assessing potential biological control agents within New Zealand

A range of parasitoids and predators were found to attack *T. vitreoradiata*, including one parasitoid in the Eulophidae family. The eulophid species belongs to the *Tamarixia* genus, and is probably an undescribed species, of unknown origin (John LaSalle, J. Berry, pers. comm.). It may be a recent arrival, as the first NZ record is from Auckland in 1997. Since 2009 this species has been referred to as *Tamarixia* “finz” (standing for “found in NZ”) to distinguish it from *T. triozae* imported into quarantine in Auckland that year. *Tamarixia* “finz” was widespread (detected at 6/10 sites) and was sometimes the most abundant natural enemy on *T. vitreoradiata*. Six predatory insects were also found to be commonly associated with *T. vitreoradiata* in Auckland (Table 1). Laboratory tests indicated that all the predators would feed on TPP in no choice situations (Table 1) and that some populations of the *T. “finz”* parasitized TPP in no choice situations (data not presented). In the small scale trial, the three predatory species tested (*A. bipunctata*, *H. conformis* and *D. binocular*) reduced the number of psyllid scores on capsicum from high (initial score of 9) to moderate (scores of 6 to 4) within eight days. However, only the *D. binocular* appeared to have potential to become a BCA on tomato, as the two ladybird species trialled avoided going onto the tomato plants.

Table 1: Consumption rates for predatory insects in no choice laboratory tests (*n = 5*), offered either the tomato/potato psyllid (*Bactericera cockerelli*) or a native psyllid (*Trioza vitreoradiata*). Species that share a letter within a column did not have significantly different consumption rates on that host (*P > 0.05*).

<table>
<thead>
<tr>
<th>Predator species</th>
<th>Common name</th>
<th>Mean no. psyllid nymphs eaten per predator per 48 hrs (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>B. cockerelli</em></td>
</tr>
<tr>
<td><em>Sejanus albisignata</em></td>
<td>(a predatory mirid)</td>
<td>2.8 (0.5) a</td>
</tr>
<tr>
<td><em>Micromus tasmaniae</em></td>
<td>brown lacewing</td>
<td>7.4 (0.9) a</td>
</tr>
<tr>
<td><em>Halmus chalybeus</em></td>
<td>steely blue ladybird</td>
<td>8.6 (1.7) a</td>
</tr>
<tr>
<td><em>Adalia bipunctata</em></td>
<td>two spot ladybird</td>
<td>9.8 (2.4) a</td>
</tr>
<tr>
<td><em>Harmonia conformis</em></td>
<td>large spotted ladybird</td>
<td>11.8 (4.6) ab</td>
</tr>
<tr>
<td><em>Drepanacra binocular</em></td>
<td>hook tipped lacewing</td>
<td>21.6 (2.6) b</td>
</tr>
</tbody>
</table>

Host specificity testing of *Tamarixia triozae*

In the no choice tests, *T. triozae* oviposited on only one native psyllid species (*Trioza curta*) out of the four that it was offered (Table 2). Egglaying on *T. curta* was low (a total of 26 *T. curta* nymphs were parasitised compared with 237 TPP nymphs). No *T. triozae* adults emerged from parasitized *T. curta* nymphs, whereas *T. triozae* adults emerged from 44% of the parasitized TPP nymphs. The poor emergence results suggest that *T. triozae* would not be able to maintain itself over time in situations where *T. curta* was the only host available (Van Driesche and Murray, 2004).

Conclusions

A range of potential BCAs for TPP has now been identified within New Zealand. In addition to the seven species discussed in this paper, further laboratory work has confirmed that sheet web
spiders (Linyphiidae), eleven spotted ladybird (Coccinella undecimpunctata), damsel bugs (Nabis kingergii) and small hoverflies (Melanostoma fasciatum) will also feed on TPP in no choice tests (MacDonald et al., 2010, G. Walker, unpub. data). Ongoing field work indicates that in northern New Zealand lacewings and hoverflies rapidly establish in unsprayed potato crops and may provide some level of TPP control in the early growing season (G. Walker, unpub. data). More work is required however to establish which of the potential BCAs are suitable for use within covered crops. If permission is gained to release T. triozae into New Zealand, this parasitoid will further expand control options in both field and covered crops.

Table 2. Summary of no choice tests carried out to assess the oviposition response of Tamarixia triozae to four native New Zealand psyllid species. In treatment cages (T) T. triozae adult females were offered native psyllid nymphs. In control cages (C) T. triozae adult females were offered the target pest, TPP nymphs. All parasitized nymphs were observed for 21 days to determine if T. triozae adults emerged.

<table>
<thead>
<tr>
<th>Plant host</th>
<th>Psyllid species</th>
<th>No. of tests</th>
<th>T. triozae eggs?</th>
<th>T. triozae adults?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pittosporum crassifolium</td>
<td>Trioza vitreoradiata</td>
<td>15</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Metrosideros excelsa</td>
<td>Trioza curta</td>
<td>15</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Dodonaea viscosa</td>
<td>Psylla dodonaeae</td>
<td>15</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Sophora microphylla</td>
<td>Psylla apicalis</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Acknowledgements

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References


Integrated Pest Management solutions for the control of
*Polyphagotarsonemus latus* in ornamentals: from trial to practice

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**Abstract:** The broad mite *Polyphagotarsonemus latus* is a major pest of ornamental crops, with outbreaks causing serious economic damage. Due to the disappearance of broad spectrum pesticides, control of broad mites proves to be a serious challenge for growers. Recent research focus on biological control of this pest showed promising results that could be implemented in an Integrated Pest Management (IPM) approach. In this paper we describe results of trials testing the biological efficacy of 2 complementary control strategies: biological and physical. In field trials on the sensitive ornamental plant Azalea, we show the strength of both independent strategies and suggest how these could be alternated to control broad mites throughout the production cycle.

**Key words:** broad mite, pest control, predatory mites, heat treatment, IPM, Azalea

**Introduction**

The Begonia mite *P. latus* (Acari: Tarsonemidae) is a major polyphagous pest of a wide range of food crops (Gerson 1992) and ornamental plants (Zang 2003) in greenhouses. Mobile stages of the mite give typical feeding damage to plant leaves and buds, caused by putative toxins injected during feeding. Damage symptoms generally indicate high population numbers that prove difficult for control. Tolerance for damage is necessarily low in ornamental crops, as plants become unsellable when showing even minor symptoms. *P. latus* is a relevant threat for economic damage. Dispersal from an infested plant to the entire crop happens through either contact, air currents or phoresy (i.e. hitchhiking on other organisms; Soroker et al. 2003). However, propagation through cuttings prove to be a high risk factor for maintaining and dispersing broad mites throughout the crops.

The loss of broad spectrum acaricides has made broad mite control a serious challenge throughout Europe. Recent efficacy studies on broad mites have therefore focussed on either biological control (e.g. Van Maanen et al. 2010), natural or selective chemicals (e.g. Gobin and Bangels 2008; David et al. 2009) or a combination of both in an Integrated Pest Management (IPM) approach (e.g. Audenaert et al. 2009).

This paper gives the results of efficacy trials for control of the broad mite *P. latus* on ornamental plants. We tested a number of biological and physical control strategies in a field setup on Azalea. Both control strategies provided good control and could be used complementary in an IPM strategy at different stages in the crop production cycle.

**Material and methods**

**Biological control**

To test the efficacy of predatory mites to control broad mites, we performed two randomized block design semi-field trials with 4 replicates in a greenhouse; one in spring (March-June) and one in fall (mid September-December). Efficacy of commercially available *A. swirskii,*
A. andersonii, N. californicus and N. barkeri were compared through release on plots of 25-150 Azalea plants. Large plots were on separate growing tables, small plots were separated by a water barrier on submerged growing tables. Predatory mites were released using two techniques: broad scattering from a container at a dose rate of ±20 predatory mites per m², once every 2 weeks; and natural dispersal from commercial mite bags at a dose rate of 1 bag (estimated contents ±500mites; based on regular counts of batches) per m², renewed once every 4 weeks. Broad mites were counted under microscope after extraction of shoots in 70% alcohol. Numbers of broad mites were compared with ANOVA and post hoc tests.

In a single experiment, unreplicated experiment at field scale, the number of broad mites were assessed in either untreated plots, plots treated with Vertimec 18EC (0,5ml/l; 10l/are) and plots in which A. swirskii was released (20 predatory mites per m²). In this trial, the broad mites of entire Azalea indica plants were sampled with a Berlese trap.

Physical control
To test a solution for control during the propagation fase, a test with heat tratment of cuttings of Azalea indica was performed. Preliminary research showed that 46°C was the minimal temperature for efficacy against broad mites. In this article, the results of a trial to determine the relationship between exposure time, efficacy on broad mites and plant safety. Five cuttings per treatment were soaked in water of 46°C for 6, 9, 15 and 30 minutes, and then planted. Succesful rooting and growth were then evaluated and compared to cuttings from an untreated control.

Results and discussion

Biological control
Plots treated with predatory mites had significantly lower numbers of broad mites in both spring (ANOVA SS=182.13; df=8; p=0.038) and fall trials (ANOVA SS=469; df=3; p=0.001). A. swirskii showed perfect control control of P. latus in spring applications, independent of the application method (Figure 1). Both application methods of A. swirskii differed significantly from control in a Fisher LSD posthoc test (both p=0.01), but except from A;andersoni broad application not from the other mites. In the fall trial, a Tukey post hoc test showed all predatory mites to differ significantly (P<0.03) from control, but not from each other. Ongoing trials (in 2011) confirm that A. swirskii gives the best options to prevent leaf damage. Earlier laboratory trials (Van Maanen et al. 2010) showed that A.swirskii feeding on a broad mite diet had sufficient oviposition capacity to control broad mites in a 1:10 predator:prey ratio. In our trial, we used much lower ratios (estimated ratio in summer: bags 1:30; broad 1:750; in winter: broad 1:250), but still maintained good control through repetitive applications (bags 2 weekly and broad 4 weekly). This is promising as high predator to prey ratios often prove to costly for practical applications. Neoseiulus californicus showed very good efficacy in spring, but this was not maintained in the fall trial. Control by N. barkeri was good, but more variable between replicates, while control by A. andersoni was poor. In this experiment, we did not look at long term establishment of a predatory mite population, long term trials are ongoing.

The comparison of a chemical treatment (Vertimec EC) and a predatory mite treatment (A. swirskii) and untreated control showed similar efficacy for both chemical and biological treatments. Untreated plants showed 881 broad mites per plant whereas Vertimec treated plants had only 3 broad mites and swirskii treated plants had 48.
Spring application  \hspace{5cm} \text{fall application}

Figure 1: Numbers of broad mites counted after repetitive release of various species of predatory mites in two trials. The spring trial (assessed 10 June) compared application in bags and a broad scattering vs control. In the fall trial (assessed 15 December) only scattering was used. Error bars indicate standard deviations. Letters indicate significant differences.

**Physical control**

Treatment of cuttings by submersal in warm water (46°C) showed good control from 6 minutes onwards. At this temperature cuttings survive a submersal of up to 15 minutes (Figure 2). Only after 30 minutes treatments, 1 out of 5 cuttings did not show root formation and growth. Hot water treatment thus proves to be a good method to prevent transfer of broad mites from old plants to new plants.

Figure 2: Number of broad mites on cuttings after different periods (minutes) of submersal in water of 46°C, as well as percentage survival of cuttings, i.e. cuttings that grow into plants.
Conclusion
This research aims at integrating various control techniques to control broad mites in ornamental plants. As both complementary strategies – biological and physical control – show promising results, growers have good options for control of this major pest beyond mere chemical control. The optimal positioning of each control strategy in a growth cycle will however be challenging, and remains to be determined for different ornamental plants.

Even when recent trial results for integrated control of broad mites are highly promising, growers of ornamental plants are still reluctant to rely too much on the use of natural enemies, as they fear even the slightest damage to leaves or flowers. Our results, as well as currently ongoing “on-farm” large scale trials confirm that damage can be avoided with an optimized IPM strategy (unpublished data). Visits to trials at a farm scale combined with good guidance in control strategy will help the mentality change needed to spread IPM practices to more growers.

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References


Combined use of a mulch layer and the soil-dwelling predatory mite *Macrocheles robustulus* (Berlese) enhance the biological control of sciarids in potted plants

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Abstract: Soil-dwelling predatory mites are important predators of sciarid flies (*Bradysia* spp.). The predatory mite *Macrocheles robustulus*, has been commercially available since 2010. The effectiveness of this predator in the control of sciarid flies has, however, not yet been tested. In this study we compare the effectiveness of *M. robustulus* and the frequently used *Hypoaspis aculeifer* in controlling sciarids in potted chrysanthemum under greenhouse conditions. We also evaluate the potential of using a mulch layer to improve establishment, population increase and performance of the predators. Both predators had a significant impact on sciarid densities, with a reduction of 97.1% by *M. robustulus* and 87.1% by *H. aculeifer*. When the predators were introduced in combination with a mulch layer of Biotop®, predator densities increased by a factor 3.1 for *M. robustulus* and 11 for *H. aculeifer*. The increase of predatory mite density was associated with an increase in the density of astigmatid mites, on which the predators were reared and that were introduced simultaneously with the predators. Sciarid density was reduced by 99.5%, when *M. robustulus* was introduced together with the Biotop® mulch layer, significantly lower compared to treatments with *H. aculeifer* with or without Biotop®. These results demonstrate that *M. robustulus* is an effective predator of *Bradysia* spp. and that in combination with Biotop® it provides better control than the frequently used *H. aculeifer*.

Key words: *Bradysia* spp., Sciaridae, *Macrocheles robustulus*, *Hypoaspis aculeifer*, mulch layer, Chrysanthemum, biological control.

Introduction

Sciarid flies are important pest in crops world-wide. They cause damage in mushrooms, greenhouse flowers and various vegetable crops (Castilho *et al.*, 2009; Chambers *et al.*, 1993; Oetting & Braman, 2004; Kim *et al.*, 2004). These pests were traditionally controlled by pesticides (Bartlett & Keil, 1997). However, pesticide resistance has made control progressively more difficult (Bartlett & Keil, 1997). In the past decades several biological control agents have been tested including nematodes (*Steinernema feltiae* Filipjev), *S. carpocapsae* (Weiser), pathogens (*Bacillus thuringiensis* Berlinger subsp. *israelensis*-H14), predatory beetles (*Atheta coriaria* Kraatz), and predatory mites (Chambers *et al.*, 1993; Kim *et al.*, 2004; Oetting & Braman, 2004).

The soil-dwelling predatory mites, *Hypoaspis aculeifer* (Canestrini) and *Hypoaspis miles* (*Stratiolaelaps scimitus*) (Berlese) (Acari: Laelapidae) are frequently used to control sciarid flies in greenhouses. Since 2010, *Macrocheles robustulus* (Berlese) (Acari: Macrochelidae), another species of soil-dwelling predatory mite, has become commercially available. An earlier study has shown that *M. robustulus* may be an effective predator of pupae of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Messelin & van Holstein-Saj, 2008). However, the effectiveness of this predator against other pests has not yet been tested.

Although soil predatory mites are widely used, effective control often requires repeated introductions, due to poor survival and population increase of the predators in the soil,
especially before pest densities increase. In preliminary studies we have found that by adding a mulch layer of Biotop®, a product derived from potato skin which is used for weed control, the density of *M. robustulus* increased by a factor 2.5- 4.5, which was associated with an increase in density of astigmatid mites. Hence, Biotop® may improve population increase of predatory mites even before pest infestation of the crop, thereby increasing the effectiveness of biological control and reducing the cost of introductions. In this study we compare the effectiveness of *M. robustulus* and *H. aculeifer* in controlling sciarids (*Bradysia* spp.) and thrips (*F. occidentalis*) in potted chrysanthemum and test if control can be improved by adding a mulch layer of Biotop®.

Material and methods

**Insect rearing**

*Hypoaspis aculeifer* was reared on *Tyrophagus putrescentiae* (Schrank), and *M. robustulus* was reared on *Carpoglyphus lactis* (L.). Both astigmatid prey mites were reared on bran. Both predatory and astigmatid mites were kindly provided by Koppert Biological Systems (Berkel & Rodenrijs, The Netherlands).

**Experiment set-up**

Chrysanthemum cuttings (cv. Pink Time Omega) were planted in pots (5l/27.5cm diameter) filled with clean potting soil. Three cuttings were planted per pot. The pots were placed in a greenhouse compartment (mean temperature: 22°C, relative humidity: 69%) and were infected by naturally occurring sciarids (*Bradysia* spp.). One week later, the pots were randomly assigned to different treatment groups. The experiment had a randomized block design, with four replicates of the following treatments: (1) Control, (2) *M. robustulus*, (3) *M. robustulus* + Biotop®, (4) *H. aculeifer*, (5) *H. aculeifer* + Biotop®.

In pots that were assigned to a mulch layer treatment, a layer of 0.5cm of Biotop® was added on top of the potting soil. One week after Biotop® application, the pots were placed in cages (1 x 2 x 2m) made of fine gauze in the same greenhouse compartment. In the centre of each cage, which represented an experimental unit, 9 pots with chrysanthemum and 900 predatory mites were distributed together with their respective prey mite (see insect rearing). Prey mite release quantity was calculated to be sufficient to support the predators for 1 week, according to rearing data (biomass per cage: *C. lactis* = 0.55g; *T. putrescentiae* = 0.35g). *Frankliniella occidentalis* was introduced at densities of 27 adults per cage in two consecutive weeks after the introduction of the predators.

The experiment started at the beginning of September 2010 and continued for 10 weeks. On the third week the light regime was changed from 13L:11D to 11L:13D to trigger flowering. From the third week on, yellow sticky cards (Horiver®) were replaced weekly to monitor numbers of adult sciarids and thrips. On the third, sixth and ninth week soil samples were taken to assess numbers of astigmatid and predatory mites. For each cage, five samples of 100ml potting soil were taken from randomly selected pots and combined to form one 500ml sample. In treatments with a mulch layer, the samples included both the mulch layer and the soil underneath it (approximately 30% mulch per sample). Insects and mites were extracted from the soil samples by heat using Tullgren funnels. They were collected in 70% ethanol, filtrated over paper and identified under a stereo microscope. A repeated measures ANOVA was performed on Log (x+1) transformed numbers of sciarids, *F. occidentalis*, predatory mites and astigmatid mites (Genstat 13.3). Differences among treatments were tested at the 5% level using Fisher’s LSD (Least Significant Difference) method.
Results and discussion

On the third week of the experiment average density of sciarids in the control treatment was 37 adults per cage. Sciarid density peaked on the 7th week with 733 adults per cage and average density throughout the experiment was 284 adults per cage per week (Figure 1).

Both predatory mites reduced densities of sciarids significantly (Figure 1). When the predators were introduced without Biotop®, sciarid density was lower in the M. robustulus treatment than in the H. aculeifer treatment: a reduction of 97.1% vs. 87.1% respectively in comparison to the control (Figure 1). The difference between the predators was, however, not significant. When M. robustulus was introduced with Biotop®, sciarids were practically eradicated (99.5% lower than in controls; Figure 1) and their density was significantly lower than in the H. aculeifer treatments with or without Biotop®.

![Figure 1: Densities of sciarids and predatory mites. Shown are average (± s.e) numbers of adult Bradysia spp. caught on yellow sticky cards per cage per week (grey bars) and the average number of predatory mites extracted from soil samples taken 3, 6 and 9 weeks after the start of the experiment (500ml soil per cage; white bars). Within each series, bars marked with different letters differed significantly (P < 0.05).](image)

The addition of Biotop® resulted in a significant increase in the densities of predatory mites (Figure 1) and astigmatid mites (data not shown). The increase was stronger in the H. aculeifer treatment. It is likely that the astigmatid mites fed on fungi that grow on the Biotop® mulch layer, which resulted in a higher prey density and in a population increase of the predators.

The fact that M. robustulus effectively controlled sciarids despite the high density of astigmatid mites indicates that sciarids may be the preferred prey of this predator. Higher...
H. aculeifer densities in combination with Biotop® did not result in improved control of sciarids. This may be due to the higher density of astigmatid mites in the H. aculeifer treatment and/or due to a preference of H. aculeifer to astigmatid mites as prey. These hypotheses were, however, not tested in the present study.

Both M. robustulus and H. aculeifer are known to prey on pupae of F. occidentalis in the soil and to reduce thrips densities in potted chrysanthemum (Messelink & van Holstein-Saj, 2008). However, in this study the density of thrips as similar in all treatments, and averaged 50 adults per cage per week (data not shown). The predators had no effect on thrips densities, even in the Biotop® treatment where predator densities were relatively high. This indicates that the predators may prefer sciarids and astigmatid mites as prey. Prey preference of these predators will be the subject of a forthcoming study.

This study demonstrates that M. robustulus is at least as effective as H. aculeifer in controlling Bradysia spp. Moreover, introduction of M. robustulus together with Biotop® improves control as compared to the commonly used H. aculeifer, resulting in near eradication of this pest. By supporting higher populations of the predatory mites, Biotop® may reduce the required introduction frequency and thus reduce the cost of biological pest control. This novel introduction strategy will be further developed in future work.

Acknowledgements

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References

Control of whitefly (*Trialeurodes vaporariorum* (Westwood)) and thrips (*Thrips tabaci* Lindeman) with the predatory Phytoseiid mite *Typhlodromips montdorensis* (Schicha) on cucumber plants

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**Abstract:** In a commercial semi-field trial in England, the predatory mite *Typhlodromips montdorensis* (Schicha) gave 97% control of *Trialeurodes vaporariorum* (Westwood) and 100% control of *Thrips tabaci* Lindeman larvae. Mites were applied to the plants in a preliminary commercial formulation in proprietary paper sachets. From a single release point mites spread from the top to the bottom of plants in large numbers, and the presence of all life stages demonstrates that they successfully established and bred on the plants. The peak mite population recorded on a single plant was 584 mites, of which 22% were eggs, 42% adults and 36% juveniles. Mites were present and easily visible amongst adult whiteflies in the heads of the plants.

**Key words:** *Trialeurodes vaporariorum*, *Typhlodromips montdorensis*, cucumbers, integrated control, whitefly, thrips

**Introduction**

The Phytoseiid mite *Typhlodromips montdorensis* Schicha was collected and researched in Australia during a project to identify native predators of *Frankliniella occidentalis* (Steiner *et al.*, 2003). It showed considerable promise as a predator of thrips in commercial crops (Steiner & Goodwin, 2002) and was commercially available on a small scale. However, difficulties in commercial production, and the widespread availability of *Neoseiulus cucumeris* on a large scale at relatively low cost, and in effective delivery systems, reduced interest in the commercial development of *A. montdorensis* as a product for thrips control. At the same time, developing regulation of invertebrate biological control agents increased the difficulties of releasing non-native organisms onto the market.

Studies of the thermal biology of *T. montdorensis* (Hatherly *et al.*, 2003) provided the means to argue that the potential for establishment outside greenhouse environments in temperate climates was negligible, and paved the way for the issue of permits for release of this mite in the UK and the Netherlands. More recent developments in production methodology now permit large scale mass production of *T. montdorensis* on a factitious host, and the use of a controlled release sachet system for delivery of the predator onto crops. These changes have revived interest in the commercial use of this predatory mite. Here, we report the results of a single, replicated semi-field trial for control of whitefly and thrips in cucumber.

Chant and McMurtry (2007) moved this mite to the genus *Transeius*. Here we retain the nomenclature of Moraes *et al.* (2004) for the purposes of continuity.
Material and methods

Glasshouse semi-field trial
A 25m x 19m glasshouse was divided into 16 plots, subdivided into four blocks of four plots. Each plot contained 20 cucumber plants (c.v. Cratos®, Enza Zaden, a powdery mildew susceptible variety) which were transplanted into 80 litre bags of coir fibre (Sinclair “Goldengrow” 100% coir) three weeks after sowing. Treatments were assigned according to a randomized block design, so that there were eight untreated plots and eight plots treated with T. montdorensis.

Adult whitefly were collected from a commercial colony, and released throughout the trial area to produce an infestation of around 10 adult whitefly per plant.

Thrips were not deliberately introduced, but were present on the plants at the start of the trial.

Predatory mites were machine-packed into sachets according to a provisional commercial specification and placed on to the crop the same day. One sachet was attached to each plant. The number and distribution of adult whitefly were assessed prior to mite release. Plants were paired according to the initial numbers of whitefly present, and five similarly infested plants were selected from each plot for subsequent assessments.

Visual assessments
A visual assessment method was employed to monitor the presence of whitefly, thrips and mites on cucumber leaves during the trial. Leaves were turned over and insects and mites were counted using a hand lens. This method was selected as previous work by the authors had shown a strong correlation between data generated by examination of destructively sampled leaves under a binocular microscope, and that generated by more rapid visual assessments of leaves in situ on the plants. Leaves for assessment were selected at a set height above the base of the plant. At the start of the trial, this equated to the top of the plant, but rapid plant growth resulted in the leaves selected being progressively further from the growing point during the course of the trial. The sampling thus followed the development of a single cohort of whitefly.

Destructive sampling
At the end of the trial, two cucumber plants were destructively sampled in their entirety to assess the number of whitefly, thrips and mites present on plants within each treatment. Samples were removed to the laboratory and counted underneath the binocular microscope. Leaf area was also assessed for those leaves removed to the laboratory.

Persistence of mites
Following the end of the formal trial, heating to the glasshouse was turned off, and temperatures declined sharply. The numbers of mites on leaves 5, 6, and 7 of the plants were monitored over the next five weeks, to provide a preliminary indication of their ability to persist in crops grown at lower temperatures, such as late season protected crops of cucumbers, peppers and aubergines grown in passive greenhouses in the Mediterranean.

Environment
Environmental data within the glasshouse was collected using data loggers located on the floor of the glasshouse, and additionally from an aspirated screen located centrally. The latter formed part of the environmental control system of the glasshouse.
During the principal period of the trial the mean temperature was 18.5°C, with a temperature range of 7.3-44.6°C and a mean RH of 76%. During the period in which we assessed mite persistence, the mean temperature was 9.8°C, with a temperature range of 1.9-24.8°C and a mean RH of 57%.

**Results and discussion**

**Glasshouse semi-field trials**

The population of whitefly was reduced by 97% in plots treated with *T. montdorensis* (Figure 1). Although data were collected from a single level of the plant, the reduction in whitefly numbers occurred over entire plots, from top to bottom of the plant. Observation also showed that adult and juvenile predatory mites were present on leaves occupied by adult whitefly. This confirmed that *T. montdorensis* is able to emerge from sachets and travel up the plant stem to predate upon whitefly inhabiting the upper reaches of the plant.

Although the majority of predation appears to be on whitefly eggs and early larval instars, the population of later instar larvae continued to decline throughout the trial. Third and fourth instar larvae, which were clearly dead because they were empty of contents, were present on leaves in treated plots. Fourth instar whitefly larvae that were still alive showed lateral black marks indicative of haemolymph leakage and were observed being attended by adult *T. montdorensis* in separate trials.

Although thrips populations were not high, they correspond well to those reported from field trials in the Mediterranean during the same period. At the end of the trial, plants in the control plots had an average of 20 thrips larvae and 5 thrips adults per plant, whereas there were no thrips larvae and less than 1 thrips adult per plant in the treated plots. This result provides strong evidence for the ability of *T. montdorensis* to control thrips (Figure 2).

**Destructive sampling**

Destructive sampling confirmed that mites were present all over the plant despite having been released from a single point at the base of the plant. The peak mite numbers recorded on a single plant were 584, of which 22% were eggs, 42% adults and 36% juveniles (Figure 3). The distribution of age classes on different parts of the plant suggests that *T. montdorensis* laid eggs as they moved up the plant and consumed early whitefly stages on the leaves as they went. The greatest number of mite eggs was located just below the plant head, which corresponds closely with the peak density of mobile *T. montdorensis* and with the presence of whitefly adults and eggs.
There were significantly more adult whiteflies in the control plots compared to the *T. montdorensis* plots at the end of the trial, despite a uniform distribution of adults at the beginning.

Sampling of cucumber plants after the glasshouse heating was switched off revealed a rapid initial decline in numbers. Nevertheless, small numbers of *T. montdorensis* were still present 5 weeks later, despite a decline of the average temperature to 9.8°C and a recorded minimum of 1.9°C. Hatherly *et al.* (2003) report a lower developmental threshold of 10.3°C, and show that 100% mortality occurred within 14 days in UK winter field trials. It would seem that even brief periods of higher temperature in protected environments permit longer survival.

![Number of mites](image)

**Figure 3.** Number and age distribution of *Typhlodromips montdorensis* on cucumber plants. Leaf 1 is at the top of the plant; Leaf 21 is at the base of the plant.

**References**


Biological control of greenhouse whitefly on roses with phytoseiid mites

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Abstract: The predatory mites *Amblyseius swirskii*, *Amblydromalus limonicus*, *Transeius montdorensis* and *Euseius ovalis* were evaluated as biological control agent of *Trialeurodes vaporariorum* on roses. When *A. swirskii*, *A. limonicus* and *E. ovalis* were released on separate plants in the same cage, *E. ovalis* increased to higher population levels than *A. swirskii* and *A. limonicus* but was not able to control the whiteflies. When *A. swirskii*, *A. limonicus* and *T. montdorensis* were released in separate cages, *A. limonicus* achieved a better control of whitefly populations than the other two predatory mites. In both cage trials only *A. limonicus* successfully controlled *T. vaporariorum*.

Key words: *Amblyseius swirskii*, *Amblydromalus limonicus*, *Transeius montdorensis*, *Euseius ovalis*, *Trialeurodes vaporariorum*, roses

Introduction

Greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) is an important pest in greenhouse roses in the Netherlands. The generalist phytoseiid predatory mite *Amblyseius swirskii* (Athias-Henriot) is used against thrips and whiteflies in IPM-roses. This predatory mite is effective against whitefly in roses when the mites are introduced repeatedly in high quantities. Typical release strategies in cut rose production include releases of 50 to 100 *A. swirskii* per m$^2$ every 1 to 2 weeks.

*Amblydromalus limonicus* (Garman & McGregor) and *Euseius ovalis* (Evans) are known predators of whiteflies (Swirski & Dorzia, 1968; Borah & Rai, 1989). In laboratory studies we have observed that *Transeius montdorensis* (Schicha), a predator of thrips (Steiner et al., 2003) also feeds on eggs and nymphs of *T. vaporariorum*.

In this study the performance of these predatory mite species were compared with *A. swirskii* as biological control agents of *T. vaporariorum* on roses.

Material and methods

*Euseius ovalis, Amblyseius swirskii and Amblydromalus limonicus*

The first experiment was carried out in 2 cages (2.5x0.9x2m) in an experimental greenhouse in the period April-May 2009. Three rose plants (var. Illios) free of chemical residue were placed in each cage. The plants were placed in small containers with water and soap as a barrier to prevent contamination and did not touch each other or the screen of the cage. No synthetic pesticides were applied during the experiment. Each cage contained one replicate of each treatment, i.e. *E. ovalis*, *A. swirskii* and *A. limonicus*. The mean temperature in the cages was 22.8°C; the mean relative humidity was 60.5%. The plants were infested with 100 adult *T. vaporariorum* per cage on April 9th and again 16 and 23 days later. On April 14th, 26 young...
female predatory mites were released on the plants. At the same time a small amount of cattail (Typha latifolia) pollen was sprinkled over the plants.

To assess the population of predatory mites and whitefly 15 leaflets were randomly picked from each plant every week for 5 weeks starting 11 days after predatory mite release. The density of whiteflies (eggs and juveniles) and phytoseiid mites (all stages) were counted under a stereo-microscope in the laboratory.

**Transeius montdorensis, Amblyseius swirskii and Amblydromalus limonicus**

The second experiment was carried out in 12 cages (1.5x0.9x2m) in a greenhouse in the period February-May 2011. Three rose plants (var. Avalanche) were placed in each cage. The only chemical residue on the plants was from a Baycor Flow (a.i. bitertanol) treatment 1 day before predatory mite release. Phytoseiulus persimilis Athias-Henriot was released against spider mites in all cages. The cages were randomly assigned with 4 treatments and 3 replications, (1) T. montdorensis, (2) A. swirskii, (3) A. limonicus and (4) untreated control. The plants were infested with 100 adult T. vaporariorum per cage on February 11th, 100 adults again 7 days later and 50 adults 14 days later. Thirty young female predatory mites were released on each plant on February 15th. At the same time 0.25 g cattail pollen per cage was sprinkled over the plants. The mean temperature in the cages during the experiment was 22.0°C; the mean relative humidity was 61.3%.

In this experiment 30 leaflets were randomly picked from each cage at weekly intervals for 13 weeks starting 6 days after the release of predatory mites. The density of whiteflies (eggs and juveniles), thrips (larvae and adults), spider mites (motiles) and phytoseiid mites (all stages) was counted under a stereo-microscope in the laboratory.

**Statistics**

Cumulative mite and whitefly days were calculated according to:

$$\sum_{i} \left( \frac{x_i + x_{i+1}}{2} \cdot t \right)$$

where $x_i$ is the number of mites or whiteflies at sampling date $i$, $x_{i+1}$ is the number of mites or whiteflies at sampling date $i+1$ and $t$ is the number of days between the sampling dates (Park & Lee, 2005) and analyzed by ANOVA using Minitab. Mean separation was performed with Fisher LSD test.

**Results and discussion**

**Euseius ovalis, Amblyseius swirskii and Amblydromalus limonicus**

In the first cage trial E. ovalis was the most abundant predator and build up higher populations than A. swirskii or A. limonicus. (Fig. 1, Tab. 1) However E. ovalis was not able to control the whiteflies sufficiently whereas A. limonicus did. The number of cumulative whitefly days was much lower on the plants with A. limonicus than on the plants with the other predatory mites (Tab. 1). Our results are in agreement with Pijnakker et al. (2005) who mentioned that E. ovalis can establish in high numbers on roses in presence of whitefly.

**Transeius montdorensis, Amblyseius swirskii and Amblydromalus limonicus**

In the second cage trial A. limonicus established well on the plants and most successfully controlled the whiteflies. The T. montdorensis population remained low and the A. swirskii population remained in-between the other two phytoseiid species. Both A. swirskii and T. montdorensis reduced the whitefly population significantly compared to the control (Fig. 2, Tab. 2).
Figure 1. Population fluctuations (± s.e.) of *Trialeurodes vaporariorum* and 3 predatory mite species on leaves in the first experiment.

Table 1. Cumulative whitefly and phytoseiid days in cage trial 1

<table>
<thead>
<tr>
<th>Cumulative mite / insect days ± s.e.</th>
<th><em>Euseius ovalis</em></th>
<th><em>Amblyseius swirskii</em></th>
<th><em>Amblydromalus limonicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. vaporariorum</em></td>
<td>731.0 ± 51.6a*</td>
<td>1135.8 ± 176.7a</td>
<td>111.2 ± 19.9b</td>
</tr>
<tr>
<td>Phytoseiidae</td>
<td>67.3 ± 0.0a</td>
<td>6.5 ± 1.6b</td>
<td>24.5 ± 8.5b</td>
</tr>
</tbody>
</table>

*values within rows followed by the same letters are not significantly different (Fisher test, P<0.05)*
Figure 2. Population fluctuations (± s.e.) of *Trialeurodes vaporariorum* and 3 predatory mite species on leaves in the second experiment.

Table 2. Cumulative whitefly and phytoseiid days in cage trial 2

<table>
<thead>
<tr>
<th>Cumulative mite / insect days ± s.e.</th>
<th>Treatments</th>
<th>Transeius montdorensis</th>
<th>Amblyseius swirskii</th>
<th>Amblydromalus limonicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5857 ± 1144a*</td>
<td>2911 ± 217b</td>
<td>2625 ± 295b</td>
<td>194 ± 44c</td>
</tr>
<tr>
<td><em>T. vaporariorum</em></td>
<td>0.0 ± 0.0a</td>
<td>8.8 ± 2.1a</td>
<td>30.0 ± 3.0b</td>
<td>64.5 ± 6.0c</td>
</tr>
<tr>
<td>Phytoseiidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*values within rows followed by the same letters are not significantly different (Fisher test, *P*<0.05)

*T. urticae* was present in all cages on a low level and was controlled well by *P. persimilis*. Numbers of thrips increased in the control and *T. montdorensis* cages during the experiment while it remained low in the *A. swirskii* and *A. limonicus* cages.
Based on the results of both trials, *A. limonicus* is a promising biological control agent for whitefly in roses and has the potential to improve biological control in commercial rose production. *A. limonicus* like *A. swirskii* and *T. montdorensis*, is a generalist predatory mite that also feeds on thrips and spider mites (van Houten et al., 2008). Currently, we are investigating if *A. limonicus* can also improve thrips control in roses. The second trial does not indicate that *T. montdorensis* can improve whitefly control compared to *A. swirskii*.

**References**


Biological control of thrips and whitefly on strawberries with *Amblydromalus limonicus* and *Amblyseius swirskii*

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**Abstract:** The performance of the predatory mite *Amblydromalus limonicus* was compared to *Amblyseius swirskii* in the control of thrips (*Frankliniella occidentalis*) and whiteflies (*Trialeurodes vaporariorum*) on greenhouse-grown strawberries. In addition, it was investigated if the provision of pollen as supplementary food improves the performance of *A. limonicus*. Both predatory mite species significantly reduced whitefly and thrips densities compared to the untreated control. Whitefly control was significantly better with *A. limonicus* than with *A. swirskii* whereas the differences in thrips control were less pronounced. The addition of pollen had a positive effect on the *A. limonicus* population but did not improve pest control.

**Key words:** *Amblydromalus limonicus*, *Amblyseius swirskii*, *Frankliniella occidentalis*, *Trialeurodes vaporariorum*, strawberries

**Introduction**

Western flower thrips, *Frankliniella occidentalis* (Pergande) and greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) are important pests in protected strawberry cultivation. The predatory mites *Neoseiulus cucumeris* (Oudemans) (against thrips) and *Amblyseius swirskii* (Athias-Henriot) (against thrips and whiteflies) are commonly released to control these pests. However, thrips control with *N. cucumeris* is not consistent and optimal growing temperatures for strawberries in greenhouses and tunnels are sometimes too low for *A. swirskii*. *Amblydromalus limonicus* (Garman & McGregor) feeding on *F. occidentalis* and *T. vaporariorum* (Van Houten *et al.* 2008).

In this study the performance of *A. limonicus* as a biological control agent of thrips and whiteflies on strawberries was compared to *A. swirskii* in cage experiments. In addition, the effect of adding pollen as an additional food source on the performance of *A. limonicus* was investigated.

**Material and methods**

The experiments were carried out in 24 cages (3x1x1m); 12 for *T. vaporariorum* and 12 for *F. occidentalis*. The cages were arranged in a completely randomized block design with 4 treatments and 3 replications in unheated greenhouses at Koppert Biological Systems. Twenty potted strawberry plants (var. Elsanta) were placed in a line in each cage on March 26th, 2010. The treatments were (1) *A. limonicus*, (2) *A. limonicus* and pollen, (3) *A. swirskii* and (4) untreated control (no predatory mites released). The plants for the whitefly experiment were infested 3 times with 100 adult *T. vaporariorum* per cage on April 26th, May 3rd and May 10th. In the thrips experiment, 30 adult *F. occidentalis* females and an unknown number of males
were released 4 times on April 26th, May 3rd, May 10th and May 17th. All plants were flowering at the first release day. Three days after the first pest release, 500 predatory mites were released per cage by sprinkling the medium in which they were reared over the plants. In treatment 2 where A. limonicus was released, 2 gram of cattail (Typha latifolia) pollen was sprinkled over the plants at the same day and again 2, 4 and 6 weeks later.

To evaluate the population development of pests and predatory mites 25 leaflets were picked from each cage at weekly intervals for 8 weeks starting 1 week after predatory mite release. In the thrips experiment, 20 flowers were also picked as long as the plants were flowering. In the whitefly experiment, the number of immature whitefly stages and predatory mites (motile stages and eggs) were counted directly on the leaves with a stereo-microscope. Plant parts picked from the thrips experiments were washed with hot water and soap over a 90µm sieve. The number of thrips larvae and adults and motile predatory mites was counted on the sieve with a stereo microscope.

The mean temperature in the cages during the experiment was 19.1°C and the mean RH was 74.0%.

Statistics
Cumulative mite and insect days were calculated according to:
\[
\sum_{i=1}^{n} \left[ \frac{x_i + x_{i+1}}{2} \right] \cdot t
\]
where \(x_i\) is the number of mites or insects at sampling date \(i\), \(x_{i+1}\) is the number of mites or insects at sampling date \(i+1\) and \(t\) is the number of days between the sampling dates (Park & Lee, 2005) and analyzed by analysis of variance (ANOVA) using Minitab. Mean separation was performed with Tukey test.

Results and discussion

Whitefly experiment
The whitefly population remained consistently lower in all plots where predatory mites were released than in the untreated control (Fig. 1). The whitefly population decreased faster in both A. limonicus treatments than in the A. swirskii treatment. In both A. limonicus treatments the whitefly density decreased to about 1 per leaflet 5 weeks after predatory mite release and remained at this level until the end of the trial. The number of cumulative whitefly days was highest in the control, followed by the A. swirskii treatment and both A. limonicus treatments and the A. limonicus population was higher than the A. swirskii population. The pollen application had a positive effect on the A. limonicus population but did not improve whitefly control (Fig. 1, Table 1). There was a slight contamination with predatory mites in the untreated control but this did not significantly influence the results.

Thrips experiment
The A. limonicus population on the leaves was, as in the whitefly experiment, consistently higher than the A. swirskii population. Adding pollen also had a positive effect on the A. limonicus population. All predatory mite species controlled the thrips but A. limonicus reduced the thrips population faster than A. swirskii (Fig. 2, Table 1). Also, in the flowers both predatory mite species controlled F. occidentalis successfully (Fig. 3). The number of cumulative phytoseiid days in the flowers was significantly higher than in the untreated control and the number of cumulative thrips days in the flowers significantly lower but there was no difference between the three different predatory mite treatments (Table 1).
Figure 1. Population fluctuations (± s.e.) of *T. vaporariorum* and predatory mites on strawberry leaves in the whitefly experiment.

Table 1. Mean cumulative mite and insect days ± s.e. in the thrips and whitefly experiment

<table>
<thead>
<tr>
<th>Cumulative mite/insect days ± s.e</th>
<th>Control</th>
<th>Amblyseius swirskii</th>
<th>Amblydromalus limonicus</th>
<th>Amblydromalus limonicus + pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whitefly experiment</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>T. vaporariorum</em></td>
<td>499.1 ± 20.4a*</td>
<td>263.4 ± 13.7b</td>
<td>186.9 ± 11.1c</td>
<td>147.9 ± 15.7c</td>
</tr>
<tr>
<td>Phytoseiidae</td>
<td>11.4 ± 2.8a</td>
<td>42.3 ± 4.7a</td>
<td>91.3 ± 12.8b</td>
<td>170.6 ± 3.7c</td>
</tr>
<tr>
<td><strong>Thrips experiment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. occidentalis</em> (leaves)</td>
<td>4.5 ± 0.2a</td>
<td>1.2 ± 0.3b</td>
<td>0.6 ± 0.1bc</td>
<td>0.2 ± 0.1c</td>
</tr>
<tr>
<td>Phytoseiidae (leaves)</td>
<td>1.9 ± 0.1a</td>
<td>15.6 ± 1.6b</td>
<td>21.9 ± 2.6b</td>
<td>42.0 ± 3.5c</td>
</tr>
<tr>
<td><em>F. occidentalis</em> (flowers)</td>
<td>10.8 ± 1.8a</td>
<td>5.6 ± 0.9b</td>
<td>4.5 ± 1.2b</td>
<td>2.8 ± 0.3b</td>
</tr>
<tr>
<td>Phytoseiidae (flowers)</td>
<td>0.0 ± 0.0a</td>
<td>4.3 ± 0.5b</td>
<td>3.5 ± 0.6b</td>
<td>5.2 ± 0.7b</td>
</tr>
</tbody>
</table>

*values within rows followed by the same letters are not significantly different (Tukey test, *P*<0.05)
Figure 2. Population fluctuations (± s.e.) of *F. occidentalis* and predatory mites on strawberry leaves in the thrips experiment.

Figure 3. Population fluctuations (± s.e.) of *F. occidentalis* and predatory mites in strawberry flowers in the thrips experiment.
Our results indicate that *A. limonicus* is a promising biocontrol agent for whiteflies and thrips in strawberries. It built up higher populations than *A. swirskii* on strawberry leaves and performed better than *A. swirskii* on whiteflies and equally well on thrips. The temperature in our experiment was higher than it is usually in protected strawberry cultivation systems. As *A. limonicus* develops at 13°C in the laboratory and *A. swirskii* does not perform well below 18°C it might be a good alternative to *A. swirskii* in strawberry greenhouses.

References


**Tuta absoluta** Meyrick (Lepidoptera, Gelechiidae), a new pest in Montenegro

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**Abstract:** In the past few years *Tuta absoluta* has spread rapidly through Mediterranean countries. *Tuta absoluta* is considered to be one of the most important lepidopterous pests on tomato. The main area of tomato production in Montenegro is in the south of the country, where crops are typically grown in greenhouses. In order to detect the arrival of this pest, pheromone traps were set up in greenhouses, at four locations, at the beginning of July 2010. The first captured moths were found in the middle of July in one locality at the coast. In the period from the last week of July to the first week of August, large galleries in tomato leaves, green and ripe fruits were detected at all four monitored locations, as well as at some other sites. The same symptoms as those on tomato were detected on leaves of *Solanum nigrum*, which as a weed, is usually present around and inside greenhouses. At one location, during the end of August, symptoms were detected on aubergine leaves which were grown outdoors. From symptoms on infested plants, morphological features of larval instars, pupae and adult moths, the presence of *Tuta absoluta*, as a new pest in Montenegro, was confirmed.

**Key words:** *Tuta absoluta*, tomato, greenhouses, detection

**Introduction**

*Tuta absoluta* was originally described as *Phthorimaea absoluta* (Meyrick, 1917). The genus was subsequently changed to *Gnorimoschema* (1962) and *Scrobipalpula* (1964). The species was later placed in a genus *Scrobipalpuloides*. The correct name of the species is now *Tuta absoluta* (Povolny, 1994) (EPPO, 2005). The species originated from South America and was first detected in Europe (Spain) in 2006 (EPPO, 2008a). Recently, *Tuta absoluta* has been considered to be a serious threat to tomato production in the whole of the Mediterranean region (www.tutaabsoluta.com). *Tuta absoluta* is a pest of tomatoes and causes damage by mining the leaves and burrowing into fruit. Tomato plants can be attacked at any developmental stage, from seedlings to mature plants (EPPO, 2005). As well as tomato, *T. absoluta* is also reported to attack potato (*Solanum tuberosum* L.), aubergine (*Solanum melongena*), other solanaceous species and common beans (*Phaseolus vulgaris* L.) (www.tutaabsoluta.com).

Tomato production in greenhouses is an important crop in southern Montenegro. In 2010, *T. absoluta* was detected in the main areas of tomato production, where it was recorded damaging crops and proved difficult to control. This has meant that *T. absoluta* is now considered to be a potentially limiting factor to tomato production in Montenegro.

**Material and methods**

Pheromone traps Csalomon® were set up in the main tomato production areas in Montenegro. Four locations were chosen for traps to be set up: two in greenhouses in Zeta and Bjelopavlići, both in the vicinity of the city of Podgorica, and two in greenhouses at the
Montenegrin coast in Ulcinj and Radanovići. Traps were set up at the beginning of July and checked at intervals of 10 days. At each location, after the first moths had been captured, all potential host plants grown in greenhouses and outdoors were assessed for symptoms associated with attack by this pest. Observations were also made at other locations, where pheromone traps had not been set up. Inspections of fresh tomato fruits from markets were also done in these four places.

Results and discussion

In 2010, the first captured moths in pheromone traps were detected in the middle of July in Radanovići. Although large numbers of moths (200) were captured, no symptoms of attack were found in tomato crops. Soon after the first detection of moths at Radanovići, moths were captured in pheromone traps from all the other locations. Numbers of moths captured during the period of observation are presented in Table 1.

Table 1. Number of captured moths in tomato crops in 2010 (Radanovići*, Ulcinj, Zeta and Bjelopavlići)

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>No. of capt. moths</th>
<th>Date</th>
<th>Location</th>
<th>No. of capt. moths</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 July</td>
<td>Radanovići</td>
<td>200</td>
<td>16 July</td>
<td>Zeta</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ulcinj</td>
<td>Not observ.</td>
<td></td>
<td>Bjelopavlići</td>
<td>Not observ.</td>
</tr>
<tr>
<td>26 July</td>
<td>Radanovići</td>
<td>170</td>
<td>27 July</td>
<td>Zeta</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Ulcinj</td>
<td>108</td>
<td></td>
<td>Bjelopavlići</td>
<td>23</td>
</tr>
<tr>
<td>6 August</td>
<td>Radanovići</td>
<td>145</td>
<td>7 August</td>
<td>Zeta</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Ulcinj</td>
<td>123</td>
<td></td>
<td>Bjelopavlići</td>
<td>44</td>
</tr>
<tr>
<td>16 August</td>
<td>Radanovići</td>
<td>98</td>
<td>17 August</td>
<td>Zeta</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Ulcinj</td>
<td>88</td>
<td></td>
<td>Bjelopavlići</td>
<td>47</td>
</tr>
<tr>
<td>27 August</td>
<td>Radanovići</td>
<td>134</td>
<td>28 August</td>
<td>Zeta</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Ulcinj</td>
<td>128</td>
<td></td>
<td>Bjelopavlići</td>
<td>49</td>
</tr>
<tr>
<td>8 Sept.</td>
<td>Radanovići</td>
<td>101</td>
<td>9 Sept.</td>
<td>Zeta</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Ulcinj</td>
<td>94</td>
<td></td>
<td>Bjelopavlići</td>
<td>55</td>
</tr>
</tbody>
</table>

* Only in location Radanovići tomato and aubergine are grown together

In the period from the end of July to the first week of August, the presence of mines on tomato leaves was detected in all locations in which traps were set up, as well in some other greenhouses in the area of Ulcinj, Bar, Tivat, Zeta and Bjelopavlići. Mines from infested plants were typical of those caused by larvae of *T. absoluta*. Mines with the same in appearance as those found on tomato were also recorded on leaves of *Solanum nigrum* (Solanaceae). This weed is usually present around and inside greenhouses. In addition, visual inspection of infested tomato fruits indicated attack caused by *Tuta absoluta*.

In Radanovići, where the first moths were captured in 2010, and where tomato and aubergine are grown together, the first symptoms of attack were noticed on aubergine leaves at the end of July, but not in tomato leaves. Infested tomato leaves were not detected until in August.
In addition to visual observations of damaged plants in greenhouses, infested samples of leaves and fruits were analyzed in the laboratory. Based on morphological features of larval instars, pupae and adult moths which were reared from infested leaves, as well those captured on pheromone traps, *T. absoluta* was confirmed as a new pest in Montenegro.

The presence of *T. absoluta* attacking tomato crops grown in greenhouses in the northwestern part of the Montenegrin coast, in the area of Herceg Novi, was detected at the beginning of September. At the same time the sporadic presence of mines was detected outdoors, on aubergine leaves. According to tomato producers, damage thought to be caused by *T. absoluta* was noticed at the beginning of August, but that chemical treatment (with spinosad and imidacloprid had no effect. Due to these control failures tomato crops had to be destroyed prematurely. The latest that *T. absoluta* was detected was in the second half of September in the peninsula area of Luštica, where it was found on tomato grown outdoors and on *Solanum nigrum*.

At the end of September severe infestation of young tomato plants (up to 15cm tall) was detected in one greenhouse in area of the Podgorica (Danilovgrad). All of the 2000 plants were found to be infested with *T. absoluta* and as a result the plants had to be destroyed.

Infested fresh tomato fruits were also found on a fruit market in the city of Podgorica in the second half of September and during October.

According to our survey in 2010, *T. absoluta* was present in greenhouses in all tomato producing areas in southern part of Montenegro, while outdoors only crops in coastal areas were affected. Infestations were more severe in greenhouses than outdoors, and on tomato crops than other crops.

Concerning that in all observed locations temperature conditions was similar, variation in the detection results between locations and the higher number of captured moths in Ulcinj and Radanovići could be due to *T. absoluta* having been introduced in late summer or beginning of autumn in 2009. In those locations this was the period when tomato production was close to be finished and therefore symptoms of eventual attack may have remained undetected.

After the first detection in Spain in 2006 (EPPO, 2008a) *T. absoluta* rapidly spread in Mediterranean countries: Algeria (EPPO, 2008b), Morocco (EPPO, 2008c), France (EPPO 2009a), Italy (EPPO 2009b), Tunisia (EPPO 2009d), Albania (EPPO 2009f), Portugal (EPPO, 2009g), Malta (EPPO 2009h). The pest is also detected in Slovenia (Knapič & Marolt, 2009, cit. Ostrauskas & Ivinskis, 2010), Netherlands (EPPO 2009c), United Kingdom (EPPO, 2009e) and Switzerland (EPPO, 2009i). The latest NPPO reports indicate presence of the pest in Bulgaria (EPPO, 2010a), Hungary (EPPO, 2010e), Germany (EPPO, 2010c), Cyprus (EPPO, 2010b), Turkey (EPPO, 2010g), Israel (EPPO, 2010d), Greece (Roditakis *et al.*, 2010), and Lithuania (Ostrauskas & Ivinskis, 2010).

It is not clear how *T. absoluta* was introduced or has spread in Montenegro because symptoms of infestations appeared at more or less the same time in different locations. However, it is possible that *T. absoluta* was introduced more than once with imported fruits from surrounding countries where this pest was already established: Bosnia and Herzegovina (Ostojić, 2010; Đurić & Hrnčić, 2010), Croatia (oral communication 2010), Italy (EPPO 2009b), Albania (EPPO 2009f) and Kosovo (EPPO 2010f).

Acknowledgements

This study would not have been possible without the support of Phytosanitary Directorate of Montenegro, which provided the pheromone traps. We thank our technicians Milorad Rašević and Branislav Vučković for the help in the field and laboratory work and tomato producers that allowed us to sample infested plants.
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Hyperparasitoids: A threat to IPM of aphids on sweet pepper?

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Abstract: This paper provides a brief introduction to hyperparasitism and explores the concept at various trophic levels. Studies in commercial pepper crops in the UK during 2010 are described in which seven different species of hyperparasitoid were detected. In addition, observations of intraguild predation are reported, which could also impact on the control of aphids by primary parasitoids. Finally, the paper considers how a thorough understanding of hyperparasitoid foraging behavior could enable us to interrupt the process and thereby reduce the commercial impact of hyperparasitism.

Key words: sweet pepper, IPM, aphids, parasitoids, hyperparasitoids, intraguild predation

Hyperparasitism

Hyperparasitoids are secondary insect parasitoids that develop at the expense of primary parasitoids, thereby representing a highly evolved trophic level. This paper will focus on the hymenoptera that attack parasitoids of aphids with particular reference to pests of sweet peppers. Much of the general information has been sourced from three key review papers; Sullivan, 1987; Sullivan, 1988; Sullivan & Volkl, 1999. Sullivan (1987) divides aphid hyperparasitoids into two categories based on adult ovipositional and larval feeding behaviours:

1. Endophagous: The female deposits her egg inside the primary parasitoid larva while it is still developing inside the live aphid but before the aphid is mummified. The egg does not hatch until after the mummy is formed and then the hyperparasitic larva feeds on the primary larval host. This category includes hyperparasitoid species of the genera Alloxysta, Lytoxysta, Phaenoglyphis, and Tetrastichus.

2. Ectophagous: The female deposits her egg on the surface of the primary parasitic larva after the aphid is killed and mummified. The hyperparasitic larva feeds externally on the primary host while both are still inside the mummy. This category includes hyperparasitoid species of the genera Asaphes, Dendrocerus, Pachyneuron and Coruna.

At the next higher trophic level, aphid hyperparasitoids attack each other. Although difficult to prove in the field, it has been demonstrated in the laboratory that both intraspecific tertiary parasitism (autohyperparasitism) and interspecific tertiary hyperparasitism (allohyperparasitism) can occur (Bennett & Sullivan, 1978; Sullivan, 1972). Success in the competition between hyperparasitic larvae depends on the developmental stage of the hyperparasitoid larva already inside the mummy at the time of oviposition by the second hyperparasitoid. One of the best known examples of tertiary parasitism is seen in a food chain in the alfalfa agroecosystem where Acyrthosiphon pisum is the herbivore and Aphidius smithi, Alloxysta victrix and Asaphes californicus are the primary, secondary and tertiary parasitoids respectively (van der Bosch et al., 1982).

Host specificity has received most attention at the level of primary parasitoids because it was thought that hyperparasitoids tended towards polyphagy (Vinson & Iwantsch, 1980; van der Bosch, 1981). In Europe, the genera Dendrocerus (6 species.), Asaphes (2 species),
**Pachyneuron** (4 species), **Coruna** (1 species) and **Euneura** (2 species) comprise together 15 species of ectohyperparasitoids of aphids. Of these, 11 species have a broad host range and attack various aphidid genera and species, independent of the aphid host. Only **Pachyneuron gibbiscuta** and **Euneura laeviuscula** are believed to be host specific. Endohyperparasitic species are much more numerous with more than 50 described species within the genera **Alloxysta**. There are only a few species which attack a broad range of unrelated aphid and primary parasitoid hosts; eg **Alloxysta victrix** and **Phaenaglyphis villosa**. The vast majority of the species seem to be host specific, attacking either a single specific aphid host independent of the primary parasitoid or a single primary parasitoid genus independent of the aphid host.

Field studies with hyperparasitoids raised the hypothesis that females of primary parasitoids tend to emigrate from an area populated by hyperparasitoids. This has been supported by laboratory experiments with the specialised primary parasitoid, **Aphidius uzbekistanicus**, and the hyperparasitoid, **Alloxysta victrix** (Hollier, et al. 1994; Petersen et al. 2000). Boenisch (1995, unpublished thesis) showed that *D. carpenteri* also evokes an escape reaction in *A. uzbekistanicus*.

More recent studies have investigated how floral diversity impacts on parasitoid and hyperparasitoid development. Araj et al. (2006) demonstrated that the longevity of both parasitoids and their associated hyperparasitoids were enhanced by the presence of flowering plants. However, if the hyperparasitoids benefit more from the flowers than the primary parasitoids, then biocontrol of the aphid pest could be compromised. This could be the case in pepper crops.

**Observations from commercial pepper crops in the UK**

Prior to the 2010 season, we had very little information about levels of hyperparasitism in aphid populations in commercial pepper crops in the UK. We knew that they were present because typical emergence holes had been seen in mummified aphids but we had no information about the species involved or the extent of their activities.

Batches of up to 180 mummified aphids were collected from several commercial pepper crops at two sites between 12 June and 20 September 2010. On each occasion, the mummies were placed in ventilated Petri dishes, incubated at room temperature and the emerging wasps were sorted into genera before being identified by a specialist taxonomist.

Samples of intact *Myzus persicae* / *Aphidius* spp. mummies were collected from three different areas of organic crops in Somerset, UK. There was a large variation (18%-46%) in the proportion of mummies from which any wasps emerged. It is possible that some of the failures may have been attacked by *Orius* spp. or *Aphidoletes aphidomyza* (see below) or died of natural causes. Hyperparasitism varied from 8% to 63% but didn’t always increase as the season progressed. A total of five species were found; *Dendrocerus carpenteri*, *D. aphidium*, *D. laticeps*, *Asaphes vulgaris* and *Pachyneuron aphidis*.

Samples were also collected from two very different situations in conventional crops in Essex, UK. The first was from parasitoid open rearing units (ORUs) which consisted of *Aphelinus abdominalis* on *Sitobion avenae* on barley plants. The second was *Aphidius* spp. on *Myzus persicae* within the crop. The sample collected from the ORUs in July 2010 had a very high percentage of adult wasp emergence (i.e. 93%). Of these, 28% were *A. abdominalis* and 72% were hyperparasitoids including 10% *Dendrocerus* spp., 36% *Alloxysta brevis* and 26% *A. brachyptera*. Only 27% of the mummies collected from the conventional pepper crop in September 2010 yielded live adult wasps. Of these, 21% were *Aphidius* spp. and 79% were hyperparasitoids. The latter included two genera; *Dendrocerus* spp. (47%) and at least two species of *Asaphes* spp. (totalling 32%).
Some interactions between biocontrol agents in pepper crops

In addition to hyperparasitism, we also observed intraguild predation (IGP) on mummified aphids by *Aphidoletes aphidimyza* and *Orius* spp. in the monitored crops.

Enkegaard *et al.* (2005) investigated IGP between *A. aphidimyza* and *Aphidius colemani* in 24 laboratory experiments. They found that *A. aphidimyza* larvae readily killed parasitised but not yet mummified *Aphis gossypii* when offered alone and in combination with unparasitised aphids. We observed and captured images of *A. aphidimyza* larvae feeding on *Myzus persicae / Aphidius* spp. mummies on pepper plants in commercial crops.

There are also records of predatory bugs feeding on mummified aphids; for example Meyling *et al.* (2002) reported that the predatory bug, *Anthocoris nemorum*, preyed readily on immature *A. colemani* contained within *M. persicae* mummies. We have observed and captured images of *Orius* spp. larvae feeding on *Myzus persicae / Aphidius* spp. mummies on pepper plants in commercial crops.

Can we reduce the impact of hyperparasitoids?

A thorough understanding of hyperparasitoid foraging behavior could enable us to implement measures to interrupt the process and thereby reduce the commercial impact of hyperparasitism. There is limited information available on the cues involved in host location. Aphid honeydew may represent an unspecific cue providing information of the presence of aphids as first step in locating the primary parasitoids (Grasswitz *et al.* 1998). The presence of honeydew influences foraging behavior and residence times in some species (Buitenhuiss *et al.*, 2004 & 2005). Attraction to host plant volatiles could provide the foraging female with additional reliable information (Sing & Srivastava, 1987). Thereafter, the female would have to acquire information about the presence of parasitised individuals and this would appear to be achieved by contact (Sing & Srivastava, 1988; Buitenhuiss *et al.*, 2005). One species, *Asaphes vulgaris*, is known to use kairomones arising from the silky cocoon of aphidiid primary parasitoids for host finding (Christiansen-Weniger, 1994). Chow & Mackauer (1999) investigated host marking behaviour in *D. carpenteri* and concluded that the aphid mummy is marked with a contact pheromone after oviposition and this deters other females of the same species. If the correct semiochemicals were identified, it is possible that they could be incorporated into traps to divert hyperparasitoids away from their hosts.

Our work in commercial crops has continued into 2011 with more detailed sequential sampling of mummified aphids in an attempt to determine which species are operating at the secondary and tertiary trophic levels. In addition, in conjunction with colleagues at Rothamsted Research, we are exploring the cues involved in host location with a view to developing methods of managing hyperparasitoids.

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A potential role for entomopathogenic nematodes within IPM of *Tuta absoluta* (Meyrick) on organic tomato crops

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**Abstract:** A series of four trials evaluated the potential of *Steinernema feltiae* and *S. carpocapsae* to control *Tuta absoluta* larvae in tomato leaves. In an initial ‘proof of concept’, high rates of 10 million nematodes/litre were found to be as effective as spinosad. The second trial demonstrated that 80% mortality could be achieved with 1 million *S. feltiae*/litre at a cost comparable to the standard chemical insecticide. The third and fourth trials evaluated the lower application rate on a large scale in a 1.17ha tomato crop using the nursery’s own robotic spray equipment. This provided 40-50% mortality. *Steinernema feltiae* could make an important contribution to the overall IPM programme by slowing down the population growth of *T. absoluta* while the primary biological control agents (*Nesidiocoris tenuis* or *Macrolophus* spp.) become established. This could be particularly important in organic tomato crops where there are very few effective alternatives.

**Key words:** tomato, *Tuta absoluta*, *Steinernema feltiae*, *Macrolophus caliginosus*, IPM

**Introduction**

*Tuta absoluta* (Meyrick) was first intercepted in the UK on Spanish imports in March 2009 (Korycinska & Moran, 2009) and there soon followed an outbreak in a commercial crop (Fera, 2009). The British Tomato Growers’ Association and the Horticultural Development Company (HDC) immediately initiated a programme of research to develop control measures that could be used within the existing IPM programme. The studies focused on organic tomato crops because the options for interventions with chemical sprays were extremely limited.

*Macrolophus caliginosus* (Wagner) is now established on many UK tomato nurseries and currently offers the best option for primary biological control of *T. absoluta*. However, populations of *M. caliginosus* are slow to establish in tomato crops and it was considered necessary to have a second line of defence (SLoD) to support the predator. While the insecticide, spinosad, is approved for organic crops and could fulfil the SLoD role, reliance on a single chemical is unlikely to provide a long-term solution. Therefore, other IPM compatible options were sought.

Previous experience of the control of *Liriomyza* leafminers with entomopathogenic nematodes (Jacobson, 1997) indicated that they could provide an alternative SLoD for use against *T. absoluta* larvae. In the present series of experiments, we first investigated the potential of *Steinernema feltiae* (Filipjev) and *S. carpocapsae* (Weiser) with relatively high application rates in a ‘proof of concept’ experiment. We then established a more cost-effective application rate and tested it on a large-scale in a commercial crop using the
nursery’s own robotic sprayer. All trials were undertaken in organic tomato crops at Horticilha, Cilha Queimada, Alcochete, Portugal.

Material and methods

**Proof of concept trial (February 2010)**

Sprays were applied to infested leaflets (cv. Dimple) both on the plant (Part 1) and removed from the plant in trays (Part 2). There were four treatments; i) untreated control, ii) positive control comprising spinosad (as Spintor 480SC) at 25ml per 100 litre water, iii) *S. feltiae* (as Nemasys®) diluted to 10m infective juveniles (IJ’s)/litre, and iv) *S. carpocapsae* (as NemasysC®) diluted to 10m/litre. Treatments were applied at dusk to extend drying time and thus allow the nematodes more time to find entry holes to mines. Sprays were applied to the point of run off with a manual Matabi pneumatic sprayer fitted with a standard lance/nozzle.

**Part 1** - For each treatment, 40 leaflets, each containing a medium sized *T. absoluta* larva, were selected and clearly marked. Leaflets were sprayed, collected when dry the following morning, kept in culture boxes with tissue paper refuges at 21+/-2°C and examined daily until larvae were dead or pupated. Cadavers were dissected to determine the presence of nematodes. A sub-sample of cadavers was retained to monitor the nematodes’ development.

**Part 2** – 160 leaflets, each containing medium sized *T. absoluta* larva, were divided into sets of 40 and each set placed in a plastic tray. One treatment was applied to each tray using the Matabi pneumatic sprayer. Thereafter, the leaflets were treated as in Part 1.

**To determine cost effective application rates (March 2011)**

The overall approach was the same as the ‘proof of concept’ trial but based on tomato cv. Sunstream. On this occasion, there were seven treatments in Part 1 and five treatments in Part 2; *i.e.* untreated control, *S. feltiae* diluted to 10m (Part 1 only), 3m and 1m/litre, and *S. carpocapsae* diluted to 10m (Part 1 only), 3m and 1m/litre.

**Commercial crop scale evaluations (March and April 2011)**

There were two consecutive trials in a mature 1.17 ha organic tomato crop (cv. Sunstream). *Steinernema feltiae* was applied at dusk at 1m/litre using a Berg self-propelled, robotic sprayer. The unit carried two vertical booms, one spraying to the left of the sprayer, the other to the right. As most of the active *T. absoluta* mines were in the lower two thirds of the canopy, the booms were set up to target this area of the crop. The sprayer was calibrated to spray to the point of run off on both sides of the leaves delivering c. 2,500 litres/ha. There were six monitored plots within the treated area. Within each plot, 40 infested leaflets were selected and subsequently treated the same as in the previous trials. There was an additional untreated plot in the first trial but not in the second due to the risk of substantial crop damage.

For the most part statistical analysis is informal, and results are presented in graphical form. End-point analysis (days 7, 8 and 10) is used for all the trials, though the cumulative mortality is indicated in the plots.

**Results and discussion**

The results of the ‘proof of concept’ trial are shown in Figure 1. There was very little mortality of *T. absoluta* in the untreated controls, while those treated with spinosad died within six days of the spray application. The first dead *T. absoluta* larvae were seen in all
Steinernema treatments two days post-application and all were dead by day 6. Although there was no formal replication in this trial the results from the two separate trials were very similar with the ‘in tray’ trial being temporally advanced by 1-2 days relative to the crop trial. Dead larvae dissected on day 4 contained nematodes at a range of development stages with the most advanced being adult females. By day 8, there was evidence of offspring within female nematodes and these had been released into the cadaver by day 10. These results were broadly consistent with the parallel findings of Batalla-Carrera et al. (2010).

Figure 1. Mortality of Tuta absoluta larvae after treatment with spinosad, Steinernema feltiae or S. carpocapsae to infested leaves either in a) the crop or b) contained in trays.

The results of the trial to determine a more cost-effective application rate are shown in Figure 2. A probit analysis on the S. feltiae data was inappropriate as mortality exceeded 80% on all concentrations, whereas it produced a LD$_{50}$ for S. carpocapsae, but 50% at around a dose of 2m IJ/l is ineffectual. Based on these results, the treatment with S. feltiae diluted to 1m/litre was carried forward to the next stage. The cost of 1m/litre at 2,500 litres/ha was ca. 350 Euro/ha, which was comparable to the cost of spinosad.

Figure 2. The effect of different concentrations of Steinernema feltiae or S. carpocapsae infective juveniles (IJ’s) applied to tomato leaves in a) the crop or b) contained in trays on the percentage mortality of Tuta absoluta larvae 7 days after treatment.
The results of both crop-scale trials are shown in Figure 3. A standard error is presented for each mean (six replicates). In trial 1, very little natural mortality was observed in the untreated controls. Dead *T. absoluta* larvae were seen in the *S. feltiae* treatments three days post-treatment. By day 10, mortality in the plots ranged from 15% to 71% (overall mean 49.8%). At least one nematode was found in each dead *T. absoluta* larvae, thus confirming the cause of death. One plot showed considerably reduced mortality because some nozzles were temporarily blocked. If data from that plot are excluded, the overall mortality rises to 56%.

![Figure 3](image)

**Figure 3.** The mean percentage mortality (± 1 S.E.) of *Tuta absoluta* larvae in infested leaf samples taken after crop wide applications of *Steinernema feltiae* using a robotic sprayer.

The results of the second crop-scale trial (Figure 3) were similar to trial 1 but overall mortality was lower (range 32% to 53%; mean 40.3%). The environmental conditions for the second crop-scale trial were less favourable than the first trial for nematode activity on the leaf surface; the mean night temperature of 20.6°C and relative humidity of 74% being 3.1°C higher and 9% lower respectively. As a consequence, the spray dried on the leaves more rapidly, restricting the time available for nematodes to find an entrance hole.

In warm climates such as Portugal, it seems most probable that nematode treatments will be restricted to the period from mid-autumn to early spring. However, there should be more opportunities to use such treatments throughout the cooler UK growing season.

While the level of control with nematodes can probably be further improved by fine tuning the spray technique and by incorporating wetters/spreaders to improve leaf coverage, it seems unlikely that nematodes will become the only control measure against *T. absoluta*. Nonetheless, we believe that they can contribute to an overall IPM programme by slowing down *T. absoluta* population growth while the primary biological control agents (*Nesidiocoris tenuis* and/or *Macrolophus* spp.) become established in the crop. Their use in the first half of the growing season will also allow the limited number of allowed spinosad treatments to be held back until later in the season when fruit damage becomes a more serious issue.

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References


**Aphidoletes aphidimyza** oviposition behaviour when multiple aphid pests are present in the greenhouse

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**Abstract:** The generalist aphid predator *Aphidoletes aphidimyza* was investigated for oviposition behaviour on the pest aphids *Myzus persicae* and *Aulacorthum solani* in greenhouse trials. Oviposition was significantly lower on plants with *A. solani* than with *M. persicae*. *Myzus persicae* were concentrated at the growing points of plants while *A. solani* predominately colonized lower leaves, indicating that lower leaves may be unattractive or undetected oviposition sites for *A. aphidimyza*. Thus, this natural enemy may not provide effective control of *A. solani* in the greenhouse, especially in the presence of other, more accessible aphid species. Future tests will determine the extent of this effect on aphid biological control programs.

**Key words:** foxglove aphid, green peach aphid, *Aphidoletes aphidimyza*, oviposition behaviour

**Introduction**

*Aphidius colemani* is likely the most popular natural enemy used for control of aphids in the greenhouse, yet this wasp is primarily efficacious for small-bodied aphid species (specifically, green peach aphid (*M. persicae*) and melon aphid (*A. gossypii*)) and does not control larger species such as foxglove aphid (*Aulacorthum solani*). *Aphidius ervi* is available for control of large-bodied aphids, but is not considered a reliable natural enemy for small species, and is also 4-5x the price of *A. colemani*, making it cost prohibitive to many growers. Unfortunately, simultaneous outbreaks of multiple aphid species such as *A. solani* and *M. persicae* (currently the top 2 aphid pests in northeastern US floriculture greenhouses) (van Driesche et al. 2008) can be a common occurrence in operations growing multiple crops, and biocontrol programs are complicated by the lack of a single effective control agent.

The predatory midge *Aphidoletes aphidimyza* could potentially fill this niche; the larvae are generalist aphid predators, and application costs are comparable to those of *A. colemani*. Markkula and Tittanen (1982) found that the addition of a single application of *A. aphidimyza* (at a rate of 1 pupa per 3 aphids) provided better control of *M. persicae* in sweet pepper than 6 applications of mevinphos (an organophosphate insecticide). Although numerous lab studies have been done on the suitability of various aphids for the mass rearing of *A. aphidimyza* (e.g. Markkula and Tittanen 1976; Kuo-Sell 1989), no work has been done to determine *A. aphidimyza* oviposition preference for different pest aphid species in the greenhouse. Understanding the oviposition behaviour of *A. aphidimyza* under these conditions is a key step in determining its potential effectiveness against multiple aphid species outbreaks.
Material and methods

**Insect rearing**
*A. solani* and *M. persicae* were collected from ornamental plants in a garden centre in Ithaca, NY in 2009. Species were reared separately in screened cages in a growth chamber (21 ± 1°C, 16:8 L:D, RH = 20-30%) on pansy (*Viola × wittrockiana* Gams., var. Majestic Giant).

**Experimental Set Up**
Greenhouse trials (repeated across 2 greenhouse compartments) were conducted in mid-June, 2010. Adult aphids (*M. persicae* or *A. solani*) were placed in the centre of 6 week old pansy plants and allowed to naturally distribute. Treatments included i) *M. persicae* at low density (2 adults/plant), ii) *M. persicae* at high density (8 adults/plant), iii) *A. solani* at low density (3 adults/plant), iv) *A. solani* at high density (16 adults/plant) and vi) control (no aphids). Aphid densities were chosen to provide similar whole-plant population densities of each species after ca. 1wk of reproduction. After 6d, the treatment pansies were randomized among un-infested plants in the greenhouse (1 plant/treatment/bench; 27 plants/bench total; 4 benches/compartment). 80 emerged *A. aphidimyza* adults were then released in the compartments (at sunset; ventilation fans were turned off to promote settling of midges in the crop). The number of *A. aphidimyza* adults released (4 adults/m^2^) was 2x the current recommended high-release rate. The average temperature in the greenhouse compartments was 22.4°C (range: 15-32°C); no supplemental lighting was used.

After 2 nights of oviposition, whole pansy plants were destructively sampled and the number of aphids and *A. aphidimyza* eggs per leaf were counted. The position of each leaf on the plant was recorded to determine if there were differences in location between the two aphid species. Specifically, pansies were divided into 4 strata: bottom, middle or top leaves (based on height from the soil surface, i.e. bottom leaves = ca. 0-2cm from the soil surface, middle leaves = ca. 2-4cm, and top leaves = ca. 4-6cm) and the centre growing point (meristem) of plant.

**Statistics**
For all data, a log-log transformation was used to better meet the assumptions of variance (+1 was added to all y values to eliminate zeros). A mixed model (Proc Mixed, SAS, version 9.13) (SAS Institute 2003) showed no effect of greenhouse compartment, so data from both compartments were combined. At the leaf level, leaves without aphids were omitted from analyses. Effects of aphid density, species, and plant strata (where appropriate) on the number of *A. aphidimyza* eggs were analyzed using an ANOVA on transformed data (Proc Mixed, SAS); untransformed means and standard errors are reported. The effect of aphid density on *A. aphidimyza* egg deposition was analyzed using a linear regression.

**Results and discussion**
*Aphidoletes aphidimyza* oviposition on pest aphids
At the time of data collection, total densities of the two aphid species were similar across both greenhouse compartments (700 for *A. solani*, 886 for *M. persicae*). At the whole plant level (Fig. 1), aphid density as well as species significantly affected *A. aphidimyza* oviposition (*F*(1,26) =20.3, *P*=0.0001 and *F*(1,26) =8.6, *P*=0.007, respectively); there was no density by species interaction (*F*(1,26) =0.07, *P*=0.7972). The mean number of *A. aphidimyza* eggs laid on *M. persicae* infested plants was significantly higher at 48.6 ± 6.6 eggs per plant, versus 3.4 ± 1.29 on *A. solani* plants (*t*(216) = 7.0, *P*<0.0001). On a per leaf basis, aphid density, species and
plant strata all significantly affected A. aphidimyza oviposition ($F_{(1,216)} = 58.8$, $P<0.0001$, $F_{(1,216)} = 31.2$, $P<0.001$ and $F_{(3,216)} = 4.4$, $P=0.005$ respectively). There was also a significant strata by species interaction ($F_{(3,216)} = 10.58$, $P<0.0001$) and a three-way interaction of species, density and strata ($F_{(6,216)} = 18.9$, $P<0.0001$), but no density by species interaction ($F_{(1,216)} = 2.26$, $P=0.1346$). The mean number of A. aphidimyza eggs laid per aphid-infested leaf was $6.0 \pm 1.54$ for M. persicae, which was statistically higher than for A. solani (at $0.5 \pm 0.18$ eggs per leaf) ($t_{(222)} = 8.8$, $P<0.0001$).

![Figure 1. Number of aphids (all stages) of M. persicae or A. solani versus number of Aphidoletes aphidimyza eggs per plant (untransformed data shown). Coefficients of determination ($R^2$) for regressions on log-log transformed data are 0.42 for M. persicae and 0.40 for A. solani.]

**Distribution of aphids on plants**

A. solani most often colonized bottom leaves of plants (with 56% of the population found on this stratum), while M. persicae mostly colonized the growing point (centres) of pansies (67% of the population) (Fig. 2). Interestingly, A. aphidimyza oviposition did not vary accordingly with preferred location of the aphid species. Midge laid the highest proportion of eggs in plant centres regardless of species (85.5% for A. solani, 87.9% for M. persicae), and virtually no eggs were found on bottom leaves (even if densely infested with A. solani). Thus, lower leaves may represent a less-preferable oviposition site for the midges to access, or aphids may be harder to detect on these leaves.

This research demonstrates that, along with density and species, location of an aphid pest on a plant also significantly influences egg laying of Aphidoletes aphidimyza. Because of this, A. aphidimyza may be less likely to control A. solani on ornamental plants, especially in the presence of the more easily accessible M. persicae. However, preferred location of various aphid species is likely affected by plant stage and may vary significantly with different species of ornamental plants. Furthermore, larvae of A. aphidimyza may be able to move between strata of individual plants to pursue aphids. Thus, further research into how oviposition preference/aphid location affects efficacy of A. aphidimyza in the greenhouse is needed and is currently underway.
Figure 2. Percent of the total aphid population (across 2 greenhouse compartments) found in each plant stratum for A. solani (black bars) and M. persicae (grey bars). Plant strata consisted of bottom leaves (B), middle leaves (M), top leaves (T), and plant centres (C).

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References


Light quality influences trap catches of *Frankliniella occidentalis* (Pergande) and *Trialeurodes vaporariorum* (Westwood)

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Abstract: The effect of different light environments on trap catches of *Frankliniella occidentalis* and *Trialeurodes vaporariorum* was investigated in a commercial greenhouse rose production unit during late autumn. Two top light treatments were used: 1) High pressure sodium lamps (HPSLs) and 2) HPSLs and light emitting diodes (LEDs) with 20% blue and 80% red light. More thrips and fewer whiteflies were caught on yellow sticky traps, and more thrips were found in the flowers, in areas where LEDs were used in addition to HPSLs compared to areas where only HPSLs were used. No effect of the light treatments was found on the population level of *Amblyseius swirskii*, but a lower ratio of predatory mites to thrips was found on the plants where LEDs were used. The results suggest that using blue and red LEDs as interlighting, or otherwise supplementary to HPSLs, will change thrips and whitefly spatial distribution in the rose crop, and that natural enemy release rates probably need to be adjusted accordingly.

Key words: *Amblyseius swirskii*, *Frankliniella occidentalis*, light emitting diodes, light quality, high pressure sodium lamps, phototaxis, roses, sticky traps, *Trialeurodes vaporariorum*

Introduction

Extensive research and development is currently invested in designing light emitting diode based lamps (LEDs) for commercial greenhouse plant production in order to optimize plant production, and to save energy (Johansen et al., 2011 and references therein). LEDs with blue and red wavelengths are now available on the market. Use of these lamps enriches the greenhouse light environment in the blue and red part of the light spectrum, particularly during the night or on cloudy days, as well as deep into the canopy where light penetration from the sun- and high pressure sodium lamps (HPSLs) is reduced.

The changed wavelength composition can influence the performance of *Frankliniella occidentalis* and *Trialeurodes vaporariorum* in the crop. Violet-blue and green-yellow light play an important role in their phototactic behaviour, the type and strength of the responses depending on the species. Little is known about the effect of red light on these species, but there is some evidence for red light being less attractive than other colours (*F. occidentalis*), or ignored or inhibitory (*T. vaporariorum*) (Johansen et al., 2011 and references therein).

The aim of our study was to investigate if LEDs with blue and red light influence trap catches and population levels of *F. occidentalis* and *T. vaporariorum* in cut roses when they are used in combination with HPSLs.
Material and methods

The experiment took place in two separate greenhouses with roses (GH1, variety ‘Ingrid Alexandra’ and GH2, variety ‘Passion’) located at the south-western coast of Norway (59.0°N 5.7°E), from 17/9 to 10/12 2009. The natural day-length in this period decreased from 12 hrs 42 min to 6 hrs 20 min. In both greenhouses, supplemental light was applied for 20 hrs per day with HPSLs (600 W SONT, 150 W/m² installed, 150µmol m⁻² s⁻¹ PAR). The climatic set points were 18-19°C (ventilation at 23.0-23.5°C), and 79% relative humidity (RH). *Frankliniella occidentalis* was established at a moderate level in both greenhouses, and were controlled by *Amblyseius swirskii* (0.5-1.0 bags/m² reapplied every 5th week). In GH2, there was a low population of *T. vaporariorum*, which were controlled by *Encarsia formosa* and banker plants with *Macrolophus caliginosus*. Carbonkick Assimilator and Carbonkick Booster were sprayed weekly to suppress the spider mites and rose powdery mildew.

In each greenhouse, six experimental plots consisting of 12 rose plants in two rows were established (randomly distributed). Three of the plots were illuminated with HPSLs (150µmol m⁻² s⁻¹), and the other three plots were illuminated with a combination of HPSLs (150µmol m⁻² s⁻¹) and LEDs emitting 20% blue (400-500nm) and 80% red (620-630nm) light (50-60µmol m⁻² s⁻¹). Five yellow sticky traps were placed 20cm above the top of the canopy in each plot. Each week, the traps were replaced and adult *F. occidentalis* and *T. vaporariorum* were counted. At the same time *F. occidentalis* and *A. swirskii* were counted in five randomly chosen rose flowers per plot (destructive sampling, counting used a binocular microscope). The first counting was done immediately before the LED-lamps were switched on (17/9). Temperature and RH within the plant canopy in the plots were continuously measured during the experimental period.

Results and discussion

The thrips population was similar in both greenhouses and light treatments prior to the experiment (Figure 1). One week after the LEDs were switched on, and throughout the rest of the experimental period, significantly more thrips were found on the sticky traps in the plots illuminated with LEDs + HPSLs than in the plots illuminated with only HPSLs in both greenhouses (GLM; GH1: $F = 18.18$, $p = 0.012$; GH2: $F = 13.22$, $p = 0.022$). There was no difference in climate between the light treatments or greenhouses during the experimental period (temperature: $21 ± 2^\circ$C, RH: $75 ± 6%$ (mean ± SD).

The blue LED light is likely to have been important in increasing trap catches. Blue (400-490nm) is amongst the most attractive colours to *F. occidentalis*, whereas the attraction to red is low (Matteson & Terry, 1992). Blue LEDs (465 nm peak emission) have been found to enhance catches of *F. occidentalis* on blue sticky traps (Chen *et al.*, 2004). Here blue LEDs were found to enhance catches of *F. occidentalis* also on yellow sticky traps. Whether the increased attraction found in our experiment was caused directly by the emitted light from the LEDs, or by changed reflectance from the traps or plants, was not investigated.

The light intensity was higher in the plots with LEDs than in the plots with only HPSLs. Matteson & Terry (1992) found that attraction of *F. occidentalis* to blue and violet sticky traps was correlated with light intensity, but not their attraction to yellow sticky traps. The light intensity in both light treatments in our experiment, however, was higher than the optimum light intensity of (4000-6000lux) found for *F. occidentalis* take off in flight chambers (Liang *et al.*, 2010). Still higher light intensities may actually decrease flight activity.
Figure 1. Number of adult *Frankliniella occidentalis* caught on yellow sticky traps in areas with different light environment in a commercial rose greenhouse. LED = light emitting diodes, HPSL = high pressure sodium lamps. GH1 and GH2 denote greenhouse 1 and 2.

More *F. occidentalis* was also found in the rose flowers in the LEDs + HPSLs plots in both greenhouses, than in the plots with only HPSLs, however a significant difference was only found in GH1 (GLM: $F = 15.15, p = 0.018$). *Amblyseius swirskii* were well established in the flowers in GH2 at the beginning of the experimental period, but after spraying with spinosad (5/11) in order to control caterpillars, they almost disappeared and did not re-establish. No effects of the light treatments were seen on their population level, but there were fewer predatory mites per thrips in the flowers in the plots with LEDs than in the plots with only HPSLs. In GH1, *A. swirskii* were only found in very low numbers throughout the experimental period, and no conclusion about the effect of light could be drawn.

*Trialeurodes vaporariorum* was only found in GH2. The population level was very low in the beginning of the experimental period, and no difference could then be seen between the light treatments. From mid-october, when the whitefly population increased, more whiteflies were found on the sticky traps in the plots illuminated with only HPSLs compared to the plots with LEDs + HPSLs (GLM: $F = 7.54, p = 0.052$). Green-yellow light (500-600nm) is attractive to *T. vaporariorum* (e.g. Vaishampayan *et al*., 1975a; Coombe, 1981, 1982; Affeldt *et al*., 1983; Webb *et al*., 1985), and stimulates landing (e.g. Coombe, 1982). These behavioural responses correspond roughly to the primary peak of the spectral sensitivity curves of the whitefly’s eyes (Mellor *et al*., 1997). Blue-violet light (400-490nm) are found to be repellent to *T. vaporariorum* (Affeldt *et al*., 1983; Webb *et al*., 1985), and red light (610-700nm) is either inhibitory or neutral (Vaishampayan *et al*., 1975a). These colour responses may explain the preference of the whiteflies for sticky traps illuminated by only HPSLs, which emit most of the energy in the yellow-red range (550-650nm) and very little blue light, indicated in our experiment.
Our experiment suggests that using blue and red LEDs as interlighting, or otherwise as a supplement to HPSLs, might change the spatial distribution of adult thrips and possibly whiteflies in the rose canopy according to their preferred wavelengths. The effects will probably vary with the time of the year. In our experiment, the artificial light was the sole light source for about 7-14 hrs each day. In summer, however, the natural light will be a much larger part of the greenhouse light environment in terms of irradiance and photoperiod, and the effects of the LEDs might be less pronounced. How behaviour and fitness will be affected if blue and red LEDs are the sole supplemental light source in the greenhouse remains to be seen. Both these aspect should be further studied.

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References

Responses of the greenhouse whitefly to elevated CO₂ on tomato

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Abstract: The effect of continuous exposure to 400, 800 and 1200ppm CO₂ on growth parameters of tomato seedlings and on fecundity and longevity of Trialeurodes vaporariorum (Westwood) was studied in growth chambers. Root/shoot ratio of tomato decreased with increasing CO₂ concentration, but was not affected by insect feeding. Stem length was significantly affected by both elevated CO₂ and insect feeding, but not by their interaction which means whiteflies did not substantially modify the response of their host plants to elevated CO₂. C/N-ratio of leaves increased with increasing CO₂ level. CO₂ treatment significantly reduced total number of eggs laid per female but not the female longevity. Whitefly fecundity thus decreased together with the significantly increasing C/N ratio, which may indicate diminishing plant quality for greenhouse whiteflies by the elevated CO₂.

Key words: Trialeurodes vaporariorum, Aleyrodidae, tomato, elevated carbon dioxide, greenhouse

Introduction

A new cooling system and air humidity control for greenhouses maintains the temperature within an optimum range without vent-based ventilation most of the time, which, in turn, allows continuously high CO₂ concentration in the greenhouse (Särkkä et al., 2008). Elevated CO₂ improves crop yields due to higher rate of photosynthesis and water-use efficiency (Jablonski et al., 2002). Särkkä et al. (2008) demonstrated that the new cooling system coupled with a higher CO₂ concentration lead to a decrease in N content in upper leaves of tomatoes, which are preferred by whiteflies. Tripp et al. (1992) observed that whitefly numbers decreased with CO₂ enhancement and suggested that whitefly development was limited by the reduced N content in the foliage.

Materials and methods

Tomato plants (Tomato cv. ‘Espero’) were grown in 3.5l pots from seeds in three chambers (16 plants per chamber) with CO₂ concentrations of 400, 800, or 1200ppm. Day/night temperature was 24/21°C, photoperiod L16:D8, and photosynthetically active photon flux 180-230µmol m⁻² s⁻¹. Fertigation (mixture of Vihannes-Superex, Mg(NO₃)₂, and Ca(NO₃)₂ + HNO₃) was given daily. In each chamber, 10 plants were infested with whiteflies, and six plants served as controls without insects. To avoid pseudo replication, treatments were rotated weekly between and within the chambers. The experiment lasted nine weeks from sowing.

Whiteflies were reared in cages at 26±2°C, at L16:D8. For measuring fecundity, small confinement cages made of gelatine capsules were attached to the abaxial leaf surface by double sided adhesive tape that had a hole of the size of the capsule in the middle. One female + one male/capsule were introduced to two leaves per plant (for further details see Koivisto 2010). The insects were transferred to new leaves and capsules every four days. Eggs were
counted and left to develop into immatures. Female longevity was calculated assuming that an individual died the day it was found dead in the capsule. At the end of the experiment, a two-leaf sample was taken from every plant for C/N analysis. Fresh weight of all leaves, stems, and washed roots per plant, and the length and width of stems was measured. Samples were dried at +60°C for one week to measure dry-weight. The biomass results were analyzed by GLM by using CO$_2$ and insects as fixed factors and plant damage as a covariate. Fecundity and female longevity was analyzed by GLM, using CO$_2$ as a fixed factor and plants nested in CO$_2$ and capsule nested in plant as random factors. Insects that died accidentally or escaped as well as plants that died prematurely were omitted from the data.

Results and discussion

Biomass measurements
Stem length was significantly affected by both elevated CO$_2$ and insect feeding, but not by their interaction (Fig. 1). Root/shoot ratio was significantly decreased by elevated CO$_2$, but not by insect feeding (Fig. 2). Irrespective of insect presence, then, plants were allocating more to shoot growth with increasing CO$_2$ levels. C/N ratio in upper leaves increased significantly with increasing CO$_2$, reflecting a decrease in the nutritional quality of leaves for the insects (Fig. 3). Nitrogen is an important limiting resource for phytophagous insects, and therefore amount of N affects insect performance (Awmack & Leather, 2002). Specific leaf area (leaf area divided by leaf dry weight cm$^2$/g) was significantly decreased by elevated CO$_2$ (data not shown): however, it first declined at 800ppm and then slightly increased at 1200ppm but not to the same level as at 400ppm indicating thicker leaves at elevated CO$_2$.

![Stem length](image)

**Figure 1.** Stem length (mean ± std. error in cm), at CO$_2$ concentrations of 400, 800 and 1200ppm, in the presence and absence of whiteflies (controls n=5-6, whitefly damaged n=9-10).

Greenhouse whitefly fecundity
Elevated CO$_2$ did not significantly reduce the longevity of females (max. 20 days at 1200ppm) (data not shown), but it significantly reduced the total lifetime egg number per female (Fig. 4). For phloem feeders, N is a limiting resource due to its relatively low
concentration in phloem sap (Bi et al., 2003). Whitefly fecundity was decreased by the elevated CO$_2$ in accordance with the increasing C/N ratio, which is in line with previous results by Tripp et al. (1992). This presents a possibility that whitefly infestations could be reduced in (semi-)closed greenhouses through modification of the plant growth and N content by elevated CO$_2$. Nevertheless it is not known yet what the interactive effects of enhanced CO$_2$ and N fertilization will be if the latter can be substantially enhanced in combination with elevated CO$_2$. Factors that influence N content will also have an impact on several other factors of plant biochemistry and anatomy. Hughes & Bazzaz (2001) stated that simultaneous measurements of both insect and plant responses to elevated CO$_2$ are crucial to predict the extent of changes in insect herbivory with increasing CO$_2$ level. Our measurements showed that whiteflies did not substantially modify the response of their host plants to elevated CO$_2$.

![Figure 2. Root/shoot ratio (mean ± std. error in g) at CO$_2$ concentrations of 400, 800 and 1200ppm, in the presence and absence of whiteflies. For controls n=5-6, whitefly damaged n=9-10.](image)

![Figure 3. C/N ratio (mean ± std. error in %) at CO$_2$ concentrations of 400, 800 and 1200ppm, in the presence and absence of whiteflies. For controls n=4-6, whitefly infested n=8.](image)
Figure 4. Mean number of eggs per female whitefly at CO₂ concentrations of 400ppm (n=16), 800ppm (n=15) and 1200ppm (n=13).

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References


Effectiveness of pesticides and potential for biological control of the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Europe

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Abstract: Since 2006 the South American leaf miner *Tuta absoluta* occurs in tomato crops in Europe. In the winter season tomatoes from Mediterranean countries are being packed in packing stations in The Netherlands. *Tuta absoluta* is frequently monitored by means of pheromone traps in the packing stations. In order to safeguard the current practise of biological control of pests and pollination by bumble bees in tomato we investigated the effectiveness of pesticides and the possible occurrence of indigenous natural enemies. A rearing of *T. absoluta* was set up in 2009 in cages in a well confined greenhouse. Pesticides, which are currently applied against caterpillars or leaf miners were tested on the leaf miner *T. absoluta*. Particularly diflubendiamide (Fame), abamectin (Vertimec), spinosad (Tracer) and emamectin benzoate (Proclaim) were effective against caterpillars of *T. absoluta*. In 2010 a field survey was carried out in order to find natural enemies of *T. absoluta* in The Netherlands. Young leaf miners were parasitized at least by two ectoparasitoids, *Elachertus inunctus* and *Pnigalio soemius* (Hymenoptera: Eulophidae). *Dicyphus errans* and *Heterotoma* sp. (Heteroptera: Miridae) and an unidentified mason wasp (Hymenoptera: Eumenidae) were observed to attacking *T. absoluta* larvae, with *D. errans* being the most common species.

Key words: *Tuta absoluta*, *Elachertus inunctus*, *Pnigalio soemius*, *Dicyphus errans*, parasitoids, predators, integrated control

Introduction

The leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) originates from South-America. The moth is a serious pest on several Solanaceae, but favours tomato. A review of the biology is given by Desneux *et al.* (2010): the average development from egg to adult stage is 39.8 days at 19.7°C and a female may lay up to 260 eggs. The lifespan varies between 10 and 15 days for females and 6-7 days for males. The temperature thresholds for the development of eggs, larvae and pupae are 6.9; 7.6 and 9.2°C. Several authors report tolerance to pesticides, for instance in Argentina *T. absoluta* has become tolerant to deltamethrin and abamectin (Lietti *et al*., 2005). Griepink (1996) has revealed the sexpheromone of *T. absoluta*, which is very useful for monitoring the pest. Observation while rearing *T. absoluta* shows that oviposition takes place on both sides of the leaves. Initially the eggs are shiny and less than 0.5 mm in diameter. The caterpillars mine the leaves, but may also mine the stems and even the fruit. Occasionally, caterpillars of *T. absoluta* leave the mine and start a new mine on an other leaf. Pupation takes usually place within the mine, the stem or attached to the plant.

*Tuta absoluta* appeared in Spain in 2006 for the first time. In February 2009 it appeared also in France, Morocco and Algeria, and spread very quickly to the east. By September 2009 it appeared in Libya, Greece and even Turkey (Desneux *et al*., 2010; van der Straten *et al*., 2011). In The Netherlands the moths are found during the winter in packing stations, where tomatoes from Mediterranean countries are being packed before sale. *Tuta absoluta* is
transported together with imported tomatoes, vine tomatoes in particular, to packing stations. Frequently, the moths are monitored by pheromone traps in packing stations and in proximate tomato greenhouses. Dutch growers consider this pest as a threat for the current practice of biological control and pollination with bumble bees, if frequent spraying against this pest would be necessary. Therefore growers asked for advice to prevent the risks.

In this study we present some possibilities for future IPM strategies in The Netherlands. We selected and evaluated a range of pesticides and we collected natural enemies of \textit{T. absoluta} in The Netherlands in order to assess the possibilities of biological control with indigenous species.

\textbf{Material and methods}

\textit{Insect rearing}
The Dutch authorities decided not to ask for a Q-status for \textit{Tuta absoluta}. Although rearing \textit{T. absoluta} was not necessary under quarantaine conditions, all possible precautions were taken to prevent escapes from the rearing. \textit{Tuta absoluta} was reared in cages of 0.7 x 0.7 x 1m in a well confined greenhouse compartment equipped with fine meshed screens in the ventilators. The compartment was only accessible via two empty rooms with one tomato plant each to attract possible escaping female moths. The rearing was started with material originating from Spain and Italy in 2009. Tomato plants were regularly added to the rearing and old plants were removed when moths had stopped emerging.

\textit{Tests of pesticides}
Pesticides that are currently being applied against caterpillars or leaf miners in The Netherlands were selected (Table 1). The effect of these pesticides was tested on caterpillars of \textit{Tuta absoluta}.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|}
\hline
Active ingredient & Trade name & Dosage/100l & Number of tests \\
\hline
lufenuron & Match & 150ml & 2 \\
methoxyfenozide & Runner & 40ml & 2 \\
indoxacarb & Steward & 12.5g & 4 \\
abamectin & Vertimec & 50ml & 4 \\
cyromazine & Trigard & 100ml & 2 \\
spinosad & Tracer & 20ml & 3 \\
flubendiamide & Fame + Addit & 25ml + 125ml & 3 \\
adjuvant, emulsified vegetable oil & Addit & 125ml & 1 \\
emamectin benzoate & Proclaim & 75g & 2 \\
teflubenzuron & Nomolt & 100ml & 1 \\
\textit{Bacillus thuringiensis} & Xen Tari & 100g & 1 \\
\textit{Bacillus thuringiensis} & Turex & 50g & 1 \\
water & & & 5 \\
\hline
\end{tabular}
\caption{Pesticides tested against the leaf miner \textit{Tuta absoluta}.}
\end{table}
The tests were carried out on 5 young tomato plants/treatment. These plants were offered to *T. absoluta* for oviposition and when mines with caterpillars (ca. 40-100/plant) were present the different pesticides were applied by means of hand spraying equipment (Birchmeier) until run-off (100ml/treatment). Five tests were carried out in total, sometimes with a different choice of pesticides. In each test the pesticides were applied once. After a week the leaves were inspected under a microscope and the number of living and dead caterpillars was determined.

**Collection of natural enemies**

Tomato plants infested with *T. absoluta* were placed in the nature reserve of Kinderdijk, The Netherlands in the period March-September 2010. Once a week a new plant was taken to the nature reserve and an older plant was taken back to our institute before the leaf miners pupated. The reserve is well-known for the occurrence of high densities of other microlepidoptera and their associated parasitic wasps. During the field exposure period, the plants were monitored and the presence and behaviour of natural enemies was observed.

**Results and discussion**

**Tests of pesticides**

The tests showed that a single application of flubendiamide, spinosad, emamectin benzoate and abamectin were effective against caterpillars of *T. absoluta*.

![Figure 1. Mortality % of *Tuta absoluta* caterpillars one week after treatment with pesticides.](image)

Application of *Bacillus thuringiensis* (Bt) formulations resulted in low mortality rates, which is in contrast with the finding of Gonzalez-Cabrera *et al.* (2011). The low mortality rate as a result of Bt application may be explained by the fact that Bt affects caterpillars only when ingested. Because the leaf miners feed mostly within leaves and leave them only occasionally, ingestion rate of Bt may be low. Possibly, mortality rates would have been higher, have we waited for a longer period between application and scoring of mortality rates.

**Collection of natural enemies**

Classical biological control with natural enemies from the origin of the pest is certainly an excellent option, but searching for natural enemies indigenous in Europe is also a possibility. Previously, European parasitoids showed to be able to control *Liriomyza trifolii* and *Liriomyza huidobrensis* (Diptera: Agromyzidae), leaf mining flies from North and South America. European members of the family Gelechiidae or other microlepidoptera may also have natural enemies that attack *T. absoluta* effectively.
On the tomato plants placed in the nature reserve in Kinderdijk, a mason wasp (Hymenoptera: Eumenidae) and the bug *Heterotoma* sp. (Heteroptera: Miridae) were found occasionally. The bug *Dicyphus errans* (Heteroptera: Miridae) was found frequently on the tomato plants with *T. absoluta* and this relative of *Macrolophus pygmaeus* seems an interesting indigenous predator. It would be worthwhile to investigate if releases of *Dicyphus errans* along with *Macrolophus pygmaeus* would further improve biological pest control in greenhouse tomatoes and maybe also in other crops. Other predators in southern Europe listed by Desneux *et al.* (2010) are *Nabis pseudoferus, Nesidiocoris tenuis, Macrolophus pygmaeus* and *Dicyphus marroccanus*.

Parasitic wasps were also observed searching and probing on the mines on tomato plants from June onwards and two species were successfully reared from *T. absoluta* caterpillars until the adult stage. The wasps were identified as *Elachertus inunctus* Nees and *Pnigalio soemius* (Walker) (Hymenoptera: Eulophidae). Both species are ectoparasitoids and their larvae developed inside the mines on the young *T. absoluta* caterpillars.

Within a short period of time we have found natural enemies of *T. absoluta* in The Netherlands. Further searching will most likely yield more species in The Netherlands. In southern Europe other parasitoids have been found on *T. absoluta: Hemiptarsenus zilahisebessi, Necremnus tidius, Necremnus artynes, Braconidae sp., Diadegma ledicola, Trichogramma achaeeae, Trichogramma* sp. (Desneux *et al.*, 2010).

We conclude that effective pesticides are available if necessary. The discovery of several European natural enemies on *T. absoluta* is promising for the development of biological control against this pest with indigenous natural enemies.

**Acknowledgements**

The projects on *Tuta absoluta* in 2009 and 2010 were funded by the Product Board for Horticulture (Productschap Tuinbouw). We are grateful for the identification of *Elachertus inunctus* Nees and *Pnigalio soemius* (Walker) by Christer Hansson, Lund University, Sweden. We also thank Gerben Messelink, Juliette Pijnakker and Amir Grosman for their remarks on the draft of this paper.

**References**


Tuta absoluta egg predation by Orius insidiosus

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Abstract: The objective of this work was to determine the predation capacity of Orius insidiosus (Hemiptera: Anthocoridae) on eggs of Tuta absoluta under laboratory conditions. Males and females of O. insidiosus were exposed individually to different egg densities of T. absoluta (10, 20, 40 and 60 eggs). Leaflets of tomato plants containing eggs of T. absoluta were kept inside a Petri dish (9cm diameter) together with the predator for 24h. The maximum number of eggs eaten by males and females of O. insidiosus was 19.1±0.24 and 32±0.33, respectively. At densities of 10 and 20 T. absoluta eggs, consumption was similar for males and females. At densities of 40 and 60 eggs, consumption of eggs by females was significantly higher than that of males. These data indicate that O. insidiosus might be a potential biological control agent of T. absoluta.

Key words: predator, biological control, Anthocoridae, tomato

Introduction

The tomato leaf miner Tuta absoluta originates from South America but is now an extremely devastating pest in tomato crops in South America, Europe and Africa north of the Sahel (Desneux et al., 2010). This pest can cause both yield losses up to 100% and decrease of fruit quality in open field and in greenhouse crops (Filho et al., 2000).

The application of chemical products against this pest has shown limited efficacy and resulted in the appearance of resistant populations to a number of active ingredients (Siqueira et al., 2001). Thus, strategies like biological control may provide more reliable management methods than chemical control.

Currently, in Brazil, biological control of T. absoluta is primarily through releases of Trichogramma pretiosum (Hym.: Trichogrammatidae). However, this parasitoid does not yet provide reliable protection against the pest (Parra & Zucchi, 2004), so there is a need for collection and identification of new natural enemies.

Today, little is known about the potential of predators to control T. absoluta. In Spain, predators of the families Miridae and Nabidae are being evaluated as potential biological control agents of this pest (Cabello et al., 2009; Mollá et al., 2009; Nannini, 2009; Urbaneja et al., 2009). A survey conducted in tomato crops in Brazil has reported, among others, Orius sp. as a natural enemy of T. absoluta (Bacci et al., 2008, Medeiros et al., 2009). However, there are as yet no studies which evaluated the possibility of using these anthocorids as biological control agents of T. absoluta.

The objective of this study was to determine the predation capacity of O. insidiosus when offered eggs of T. absoluta under laboratory conditions, and to determine if this predator has potential for biological control of T. absoluta.
Material and methods

Insect rearing
Nymphs and adults of *O. insidiosus* were collected on *Amaranthus* spp. plants in the field and they were reared in the laboratory according to the method described by Bueno et al. (2006). The tomato leaf miner *T. absoluta* was reared on potted tomato plants kept in an acrylic cage (150x150x180cm) maintained inside a greenhouse. Pupae of *T. absoluta* were collected from the stock culture and kept individually in glass tubes, which were sealed with PVC until adult emergence. One female and one male *T. absoluta* were released in small plastic cages (200ml) containing a small drop of honey as food and a leaflet of tomato plants *Lycopersicon esculentum* cv. Santa Clara as an oviposition substrate. In order to prevent the leaflet from wilting, the stem was kept inside an eppendorf tube with distilled water. These tubes were sealed with plasticine. The leaflets were changed every day and the eggs of *T. absoluta* were counted. The eggs were used in the test to determine the predation capacity of *O. insidiosus*.

Predation tests
Males and females of *O. insidiosus* up to 3-days-old were collected from the stock culture and kept as group in a Petri dish (5cm diameter) without access to food for 24h before the tests were performed. Water was provided by a small piece of moistened cotton during the 24h period. Males and females of *O. insidiosus* were exposed individually to 4 egg densities: 10, 20, 40 and 60 eggs/leaflet. Individual predators were released in a Petri dish (9cm diameter) containing the leaflet with the eggs. During the tests, the predators were kept in controlled environment chambers at 25±1°C, RH 70±10% and 12h photophase. After a 24h period, the number of eggs consumed by the predators was determined. The experiments were replicated 10 times for each egg density offered to the predators.

To evaluate the effect of different egg densities on the predation capacity of males and females of *O. insidiosus*, the data of egg predation were analysed by a two-way analysis of variance (ANOVA) and the means were compared by the Tukey test (*P* ≤ 0.05).

Results and discussion

The daily egg consumption of *O. insidiosus* males and females increased with the quantity of *T. absoluta* eggs offered (*F* = 42.44; *df* = 6.26; *P* < 0.0001) (Figure 1). The maximum egg consumption was observed at the two highest eggs densities: the males consumed on average 19.1±0.24 eggs and the females 32.0±0.33 eggs. The females of *O. insidiosus* consumed significantly higher number of eggs than the males at the densities of 40 and 60 eggs (*F* = 28.635; *df* = 4.76; *P* < 0.0001) (Figure 1). However, at the densities of 10 and 20 *T. absoluta* eggs, the number of eggs consumed was not significantly different for both sexes of the predator, although males seemed to consume fewer eggs.

According to Mendes et al. (2002), lepidopteran eggs represent good quality food for *Orius* spp., but the number of prey consumed varies with the prey species and its developmental stage. Adults of *O. insidiosus* when fed lepidopteran eggs were able to consume daily 8.88 of *Spodoptera frugiperda* (Noctuidae) (Isenhour et al., 1990) and 2.1 eggs *Ostrinia nubilalis* (Crambidae) (Musser & Shelton, 2003) which is considerably lower than what we found for *T. absoluta*. 
Figure 1. Number of eggs of *Tuta absoluta* consumed (X ± EP) by males and females of *O. insidiosus*. Same small and capital letters on the bars indicates no significant differences according to the sex and the densities, respectively (*P* < 0.05).

Also, the size of the prey influences the consumption rate of the predators (Urbaneja *et al.*, 2009). Eggs of noctuid Lepidoptera are on average 0.5mm in diameter (Nurindah *et al.*, 1999) and eggs of *O. nubilalis* are approximately 0.75mm in diameter (Cordeiro *et al.*, 2008). Adults of *O. insidiosus* when fed smaller prey, such as *Anagasta kuehniella* (Pyralidae) whose eggs are approximately 0.3mm in diameter (Klomp & Teerink, 1978), consumed 15.7 eggs per day (Calixto *et al.*, unpublished data). The eggs of *T. absoluta*, have a smaller diameter (0.22mm (EPPO, 2006)) than these other species, and were, not surprisingly, consumed in greater numbers by *O. insidiosus* adults. The results show that adults of *O. insidiosus* were able to find, prey upon and consume the eggs of *T. absoluta* in large numbers. However further studies should be conducted to evaluate the real potential of this natural enemy to control the pest *T. absoluta* under commercial greenhouse conditions.

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Effects of supplemental pollen and fibers on canopy abundance of *Amblyseius swirskii*

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**Abstract:** *Amblyseius swirskii* (Athias-Henriot) will quickly leave the foliage of plants that lack both leaf hairs (leaf trichomes) and a supplemental food source (pollen). Many floral crops lack leaf trichomes. Applying artificial leaf hairs (low densities of tiny fibers) and/or pollen to the canopy of plants lacking these resources enhances phytoseiid persistence and egg production.

**Key words:** *Frankliniella occidentalis*, phytoseiid mites, biological control, habitat and resource enhancement

**Introduction**

Predaceous phytoseiid mites, *Amblyseius swirskii* (Athias-Henriot) and *Neoseiulus cucumeris* (Oudemans) can provide effective control of *Frankliniella occidentalis* (Pergande). However, frequent inundative releases are usually needed, and control can be inconsistent. Recent advances have revealed that these predators remain longer, live longer, and oviposit more on plants with leaf hairs and pollen. Because many important greenhouse crops (garden impatiens, New Guinea impatiens, begonias, some vegetable transplants and herbs, etc.) lack leaf hairs, adding a tiny amount of fibers and pollen to the foliage along with the mites should substantially increase their effectiveness and reliability.

The mechanisms governing phytoseiid response to leaf hairs (non-glandular trichomes) and pollen in plant habitats are well understood. The presence of leaf hairs (Loughner et al., 2008, 2009; Walter, 1996; Romero and Benson, 2005) and availability of pollen (Nomikou et al., 2009; Arthurs et al. 2009) strongly and consistently increase the retention, survival and reproduction of many phytoseiids. Loughner et al., (2009) conclude that a behavioral response to the presence or absence of leaf trichomes drives phytoseiid densities. In the absence of leaf hairs, these predators disperse rapidly (within 72 hours) and simply do not establish on glabrous plants. Although there is the potential of thrips to benefit from pollen resources (Hulshof et al., 2003), *A. swirskii* substantially reduces thrips populations (van Rijn et al., 2002; Arthurs et al., 2009; Messelink, 2006). A light level of pollen (200-300 grains per leaf) is sufficient to increase phytoseiid oviposition and rate of immature development (McMurtry and Scriven, 1966) and should increase phytoseiid retention and reproduction without excessively benefiting thrips populations.

Our objectives were to evaluate: a) the effect of adding foliar pollen and trichome-mimic fibers on *A. swirskii* abundance; b) relative *A. swirskii* egg production on diets of various candidate pollens; c) the effect of various types of candidate trichome-mimic fibers on *A. swirskii* abundance.
Material and methods

General methods. Amblyseius swirskii were maintained in lab culture on grape and bean leaves provisioned with Tetranynchus urticae Koch. Colonies and experimental plants were maintained in controlled environment chambers at 23°C, 70% RH and a 16:8 h light:dark cycle. The number of A. swirskii was analyzed with a generalized linear model using a Poisson distribution and natural log link function with various treatments as categorical predictor variable (STATA, glm command).

a) Effect of extraneous pollen and trichome-mimic fibres on A. swirskii abundance.
Ten adult female A. swirskii were added to each of nine young bean or eight impatiens plants per treatment to which the following treatments had been applied: Bean treatments included patches of cotton fibers alone, field-collected cattail pollen alone, both cotton fibers and pollen, or nothing. Impatiens treatments included finely-chopped acrylic yarn fibre alone, cattail pollen alone, both acrylic yarn fibres and pollen, or nothing. Pollen was dusted finely on the leaves with a pollen duster. Cotton patches were applied by hand, and acrylic yarn fibres were applied with a flour sifter. Numbers of adult and immature mites, including eggs, were counted on each plant after 72 h for the beans and 7 d for the impatiens.

b) Relative A. swirskii egg production on diets of various pollens.
A single mated adult female A. swirskii was confined on a bean leaf disc to which one of the following pollen species had been added: ragweed, grass, corn, plum, cherry, apple, or cattail collected in 2010 or in 2008. No pollen was applied to the controls. Except for the cattail pollens, all pollens were purchased from commercial sources. Ten to 29 females were used per pollen treatment. After 24h each mite was transferred to a new leaf disc with fresh pollen. Data from the first 24h was discarded to avoid influence of the previous diet. Eggs laid per female between 24 and 48h were used.

c) Effect of various types of trichome-mimic fibres on A. swirskii abundance.
Ten adult female A. swirskii were released onto individual bean seedlings onto which various trichome mimic materials had been hand applied in a 6cm² patch on the top surface of leaves. Fibre treatments included cotton patches, jute, paper pulp, or celluflo (a cellulose-based material used in liquid filtration). Five replicates were used per treatment. The total number of mites per plant was recorded after 48h.

Results and discussion

a) Effect of extraneous pollen and trichome-mimic fibres on A. swirskii abundance.
Both pollen and fibres are important to A. swirskii (Fig. 1); on both bean and impatiens, more mites were recovered on plants having both pollen and trichome mimic fibres (Fig. 1A: cotton patch coefficient = 1.373, z = 9.26, P>|z|<0.001; pollen coefficient = 0.477, z = 3.89, P>|z|<0.001; Fig. 1B: acrylic yarn coefficient = 0.831, z = 7.66, P>|z|<0.001; pollen coefficient = 0.976, z = 8.73, P>|z|<0.001). The addition of trichomes, in particular, would almost certainly enhance biocontrol, particularly on glabrous plants.
Figure 1. Mean *Amblyseius swirskii* on A) bean with cotton patches and/or pollen and B) impatiens with acrylic yarn fiber trichome mimics added to plants using a flour sifter and/or pollen.

**b) Relative *A. swirskii* egg production on diets of various pollens.**
Oviposition by *A. swirskii* is significantly increased when several types of pollen are provided to adult females (Fig. 2). Apple, cherry, and plum pollens were promising and are available in quantities suitable for applying in orchards. Corn, grass, and ragweed pollens are only available in very expensive, small quantities used for allergy testing. Further tests are underway to evaluate the effect of supplemental pollen on thrips populations in the presence of *A. swirskii*.

**c) Effect of various types of trichome-mimic fibres on *A. swirskii* abundance.**
When our standard cotton patches and the other materials were hand applied in 6cm² patches on the leaf tops, the total number of *A. swirskii* was similar for paper pulp compared to cotton and lower for jute, celluflo, and the pollen only control compared to the cotton (Fig 3). When the trichome mimic materials were further tested by sifting all materials directly onto plants, none of the materials applied with pollen improved phytoseiid numbers over pollen alone. Clearly, application method influenced the effectiveness of mimic materials. Because none of the materials performed exceptionally well as measured by a combination of *A. swirskii* response and our ability to apply the material, we tested an acrylic yarn. It adhered to bean leaves and could be applied using a flour sifter after some initial fine chopping. Patches of this acrylic yarn were equivalent to patches of cotton when trichome mimics were gently pressed onto leaves by themselves or with pollen (Fig. 3).

Application of supplemental pollen and fibers has potential to enhance biocontrol of thrips, particularly on glabrous plants, by increasing retention of mites in the canopy and increased reproduction. Increased mite retention and reproduction could allow release rates and/or frequency to be reduced, both lowering cost and increasing effectiveness of biocontrol.
Figure 2. Mean (+/- SEM) eggs oviposited by one adult female *Amblyseius swirskii* on a bean leaf disc treated with pollen (n = 10 to 29 individuals per treatment).

Figure 3. Mean (+/- SEM) *Amblyseius swirskii* on leaves 48h after 10 adult females were released on bean seedlings with various trichome mimic materials applied in a patch on the leaf top (n=5).

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**References**


**Aphidius gifuensis**: a promising parasitoid for biological control of two important aphid species in sweet pepper

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**Abstract:** The parasitoid *Aphidius gifuensis* is able to parasitize both the green peach aphid *Myzus persicae* and the foxglove aphid *Aulacorthum solani* in sweet pepper. In a greenhouse experiment we showed that rates of parasitism on green peach aphids alone were equal to the commonly used *Aphidius colemani*, but lower than with *Aphidius matricariae*. Foxglove aphids were suppressed very effectively by *A. gifuensis*. In contrast, *A. matricariae* was not able to parasitize this aphid. When the two aphid species were offered simultaneously, *A. gifuensis* suppressed both aphid species, whereas the presence of foxglove aphids had a negative effect on the control of green peach aphids by *A. matricariae*. We conclude that *A. gifuensis* is a promising candidate for biological control of both foxglove aphids and green peach aphids in sweet pepper, especially when these aphids occur together.

**Key words:** biological control, *Myzus persicae*, *Aulacorthum solani*, *Aphidius colemani*, *Aphidius matricariae*, *Aphidius ervi*

**Introduction**

Aphids can be very destructive pest species in sweet pepper crops. The most important species is the green peach aphid *Myzus persicae* (Sulzer). Especially the red phenotype is notorious for its fast reproduction and tendency to colonize the flowers and young leaves. This behaviour directly results in reduction in plant growth and fruit production. Moreover, the honeydew these aphids secrete, contaminates leaves and fruits which consequently facilitates growth of sooty mould. A second important species is the foxglove aphid *Aulacorthum solani* Kaltenbach. This aphid typically induces strong plant responses such as yellow necrotic spots and leaf deformation, which can occur even when aphid densities are low. At higher densities, aphid induced damage can result in leaf drop.

Braconid wasps of the genus *Aphidius* are the most important parasitoids of aphids. The aphiidiids that are currently used for biological control of green peach aphids in greenhouses are *Aphidius colemani* Viereck and *Aphidius matricariae* Haliday. However, these wasps are not effective against the larger foxglove aphids. The most frequently used parasitoid against this aphid species is currently *Aphidius ervi* Haliday. This parasitoid also parasitizes green peach aphids, but the number of offspring is often low because of strong disruptive behaviour in aphid colonies. Surveys of aphid parasitoids in North America and Europe indicate that the currently used parasitoids are also the most abundant parasitoids of green peach aphids and foxglove aphids in natural ecosystems (Acheampong *et al*., 2007; Kavallieratos *et al*., 2010). However, a recent survey of Asian parasitoids showed that *Aphidius gifuensis* Ashmead is an important parasitoid of both green peach aphids and foxglove aphids (Takada, 2002). Here we evaluate the efficacy of this parasitoid as a biological control agent of these two aphid species in comparison with the currently used parasitoids. Moreover, we study host preference of *A. gifuensis* and *A. matricariae* for either of the two aphid species in the laboratory.
Material and methods

Greenhouse experiment
We conducted a greenhouse experiment in spring-summer to evaluate the effects of *A. gifuensis* on green peach aphids (red phenotype) and foxglove aphids separately and when present together in a sweet pepper crop. Sweet pepper plants, cv Ferrari, were planted in 10 litre pots with peat in a greenhouse compartment (144m²). An experimental unit consisted of 4 potted sweet pepper plants enclosed in a walk-in-cage (1 x 2 x 2m) made of fine gauze. A total of 28 cages was used. Each plant was grown according to a three-stems-per-plant system, resulting in 12 sweet pepper stems per cage. The experiment had a randomized block design with four replicates of the following treatments: (1) *M. persicae + A. gifuensis*, (2) *A. solani + A. gifuensis*, (3) both aphids + *A. gifuensis* (4) *M. persicae + A. matricariae*, (5) *A. solani + A. matricariae*, (6) both aphids + *A. matricariae* and (7) *M. persicae + A. colemani*. The parasitoid *A. gifuensis* was reared on *M. persicae* on sweet pepper and *A. colemani* on *Aphis gossypii* Glover on cucumber (standard product). Both parasitoids were supplied by Koppert Biological Systems (Berkel & Rodenrijs, The Netherlands). *Aphidius matricariae* was reared on *M. persicae* and supplied by Viridaxis (Gilly, Belgium). The plants were infested with aphids when the crop was ca. 1m high at densities of 5 aphids of mixed age per stem (15 aphids per plant). In the mixed aphid treatments, two plants were infested with *M. persicae* and two with *A. solani*. Female parasitoids of 1 day old, mated and fed with honey, were released two weeks later in densities of 30 aphids per cage. The average aphid densities at that time were ca. 900/cage for *M. persicae*, 700/cage for *A. solani* and ca. 800 for the mixed cages. Thus, the parasitoid:aphid ratios were 1:30, 1: 23 and 1:26, respectively. Densities of aphids and parasitized aphids (mummies) were monitored during 4 consecutive weeks by weekly counting the total number of aphids and mummies on 24 randomly selected leaves per cage: 12 leaves in the upper part of the plants and 12 leaves in the lower plant parts. Mummy counts were cumulative, because mummies that already emerged were not separated from closed mummies. The average temperature and relative humidity during the experiment was 23.3°C and 77 % respectively. For statistical analyses, a repeated measures ANOVA was performed on Log (x+1) transformed numbers of aphids, and arcsin transformed rates of parasitism. Differences among treatments were tested at the 5% level using Fisher’s LSD (Least Significant Difference) method.

Host preference
Host preference of *A. gifuensis* and *A. matricariae* for either of the two aphid species was studied in the laboratory, in order to understand parasitoid behaviour in a greenhouse crop with two aphid species. The experiment was conducted in plastic boxes of 5cm high and a diameter of 6cm with a sweet pepper leaf disc that was embedded upside-down in water agar (1% agar). Each leaf disc was infested with 10 third instars of each aphid species, thus 20 aphids in total, which were placed randomly on the discs. One naïve mated female parasitoid was added to each box and was observed for 10 min. The number of wasp-aphid encounters (contact with antenna) and attacks (insertion of ovipositor into the aphid) was recorded. Observations were replicated with 20 individuals per parasitoid species. Parasitoids were removed after 10 minutes and the boxes were closed with a lid with insect gauze and placed upside down in a climate chamber at 25°C and 70% RH. The number of mummies per aphid species was counted after 1 week.

Results and discussion
In the greenhouse experiment, suppression of green peach aphids alone by *A. gifuensis* was equal to *A. colemani*, but *A. matricariae* had significantly higher rates of parasitism (F_{6,62} =
Populations of *A. solani* alone were controlled very effectively by *A. gifuensis*, resulting in up to 90% parasitism in the fourth week, whereas *A. matricariae* did not parasitize *A. solani* at all (Fig. 1). In the mixed aphid treatments, *A. gifuensis* suppressed both aphid species (Fig. 2). This was confirmed in the laboratory experiment, which showed that *A. gifuensis* parasitizes both aphid species when offered together (*7.5(± 0.61 se) and 5.5 (± 0.77 se) attacks/female on *M. persicae* and *A. solani*, respectively).

Rates of parasitism in the mixed aphid treatment with *A. matricariae* were significantly lower than in the treatment with *M. persicae* alone. Because *A. matricariae* does not reproduce in *A. solani*, all mummies in the mixed aphid treatment belonged to parasitized aphids of *M. persicae*, representing 45% of parasitism of this aphid species in the mixed treatment. This is much lower than in the treatment with *M. persicae* alone (95%) and suggests that the presence of *A. solani* has a negative effect on the control of *M. persicae* by *A. matricariae*. This may be explained by the behaviour of *A. matricariae* in the presence of both aphid species. The laboratory experiment showed that *A. matricariae* does not have a strong preference for either of the two aphid species when offered together (5.7(±0.63 se) and 4.7 (±0.59 se) attacks/female on *M. persicae* and *A. solani* respectively). However, the parasitoids reproduced successfully only in *M. persicae*. Thus the unsuitable host *A. solani* may distract *A. matricariae* from *M. persicae* when both aphid species are present. Such indirect positive effects of an unsuitable host on a suitable host were previously shown for other parasitoids-aphid systems (Meisner *et al*., 2007), but detrimental effects for biological control were not yet demonstrated.

In practice, sweet pepper crops are often infested earlier in the season by green peach aphids than by foxglove aphids. Our results suggest that a population increase of *A. gifuensis* on green peach aphids might be effective to suppress later infestations of foxglove aphids. A rapid response to this aphid might be useful, because damage already occurs at low aphid densities. Also other studies report that the presence of one pest species can enhance biological control of another pest species when the two pests share a natural enemy (Harmon & Andow, 2004; Messelink *et al*., 2008). We conclude that *A. gifuensis* is a promising candidate for biological control of both foxglove aphids and green peach aphids in sweet pepper, especially when these aphids occur together. In further studies we will evaluate the searching abilities of this parasitoid and assess effects of temperature on aphid attack rates.
Figure 2. Population dynamics of the aphids *M. persicae* and *A. solani* when present alone or together in a cage on sweet pepper plants with the parasitoids *A. gifuensis*. Shown are average (± s.e.m.) aphid densities per 24 leaves.

Acknowledgements

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References

Generalist predatory bugs control aphids in sweet pepper

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Abstract: Biological control of aphids is often focused on releases of specialized natural enemies. Here, we evaluate the effects of inoculative releases of the generalist predatory bugs \textit{Orius laevigatus}, \textit{Orius majusculus} and \textit{Macrolophus pygmaeus} on green peach aphids and western flower thrips in a greenhouse grown sweet pepper crop. We found that compared to the two \textit{Orius} species, \textit{M. pygmaeus} was by far the best predator for controlling aphids. Several releases of aphids did not result in an establishment of this pest in the compartments with \textit{M. pygmaeus}, whereas aphids attained high densities in the \textit{O. laevigatus} or \textit{O. majusculus} treatments, causing serious crop damage. Thrips were controlled by all predators, but compartments with \textit{M. pygmaeus} initially showed some thrips damage on the fruits. Currently, \textit{Orius laevigatus} is the predator used most in inoculative releases in sweet pepper in Europe, but our data suggests that it might be better to use \textit{M. pygmaeus} instead or in addition to \textit{O. laevigatus}, when control of both thrips and aphids is required.

Key words: biological control, \textit{Myzus persicae}, \textit{Frankliniella occidentalis}, \textit{Orius majusculus}, \textit{Orius laevigatus}, \textit{Macrolophus pygmaeus}

Introduction

Biological control in sweet pepper is one of the success stories of the greenhouse industry since decades (Ramakers, 2004). This success is mainly based on inoculative releases of anthocorid predatory bugs and generalist phytoseiid predatory mites, which successfully control thrips, broad mites and whiteflies. One of the last obstacles for a completely pesticide-free cropping system are aphids. Biological control of these pests in sweet pepper is difficult and expensive, as effective control requires repeated releases of natural enemies (Bloemhard \& Ramakers, 2008). So far, aphid control strategies are mainly based on frequent releases of specialized aphid parasitoids and the predatory midge \textit{Aphidoletes aphidimyza} (Rondani) (Ramakers, 1989; Blümel, 2004). Additionally, growers release chrysopid, syrphid or coccinellid predators to suppress high aphid densities. However, none of these natural enemies is able to establish in a crop without aphids. Biological control of aphids might be greatly improved by generalist predators that are able to establish in a sweet pepper crop prior to aphid infestations, because this can result in rapid responses to new aphid infestations and prevent establishment of aphids. The same principle applies for thrips control in sweet pepper. In Europe, the most efficient and widely-used predatory bug for control of thrips is \textit{Orius laevigatus} (Fieber), which is released inoculatively in sweet pepper crops (Dissevelt \textit{et al.}, 1995). However, this predator might be less effective for aphid control than other generalist predatory bugs (Alvarado \textit{et al.}, 1997). In this study we test the feasibility of controlling both aphids and thrips by an inoculative introduction of generalist predators. We compare the effectiveness of \textit{O. laevigatus}, \textit{Orius majusculus} (Reuter) and the mirid bug \textit{Macrolophus pygmaeus} Rambur (also known as \textit{Macrolophus caliginosus}).
Material and methods

The 3 species of generalist predatory bugs were evaluated in 6 greenhouse compartments, 24m$^2$ each, at the research facility of Wageningen UR Greenhouse Horticulture. The windows of these compartments were equipped with insect gauze to prevent contamination with organisms from outside the greenhouse. Sweet pepper plants cv Ferrari were planted in the beginning of April 2010, 4 rows of 9 plants per compartment. Plants were grown according to standard cultivation methods on rockwool slabs with drip irrigation for water and nutrients supply. The predators *O. laevigatus* and *M. pygmaeus* were supplied by Koppert Biological Systems and *O. majusculus* was supplied by Biobest NV. Each predator treatment was released in two compartments and each compartment was divided in two fields of 18 plants each. The predatory bugs were released twice at densities of 100 adults (sex ratio 50%) per field (5.5 individuals/plant) 2 and 4 weeks after planting, when the plants had started to flower. Plants were infested 4 times with the red phenotype of green peach aphids *Myzus persicae* (Sulzer), and twice with western flower thrips, *Frankliniella occidentalis* (Pergande), starting 1 week after the last predator releases. Thrips were introduced at densities of 2 adult females per plant in two consecutive weeks. Aphid introductions started at the same time, but in 4 consecutive weeks at densities of 2, 2, 18 and 60 nymphs per plant. Densities of pests and predators were assessed weekly for 6 weeks, starting 3 weeks after the first pest releases. In each field, 10 flowers and 20 leaves were randomly chosen and all pests and predators present were counted. Sweet pepper fruits were harvested as soon as they became red. The number of peppers severely contaminated by aphid honeydew and/or visible thrips damage (silvery spots) was recorded per field during the entire experiment. The average temperature and relative humidity in the greenhouse compartments were 23°C and 70%, respectively.

Differences in population dynamics of pests among the treatments were analysed using a linear mixed effects model with the log(aphids densities +1) as dependent variable, and time and compartment as random factors to correct for repeated measures and pseudoreplication within compartments. Fruit yield was analysed with a standard ANOVA with the log-transformed total number of fruits per field and arcsin-transformed fraction of contaminated fruit. Differences among treatments were tested at the 5% level using Fisher’s LSD (Least Significant Difference) method.

Results and discussion

Our results show that *M. pygmaeus* is a better predator for controlling aphids in sweet pepper than the two *Orius* species (Fig. 1). Differences among all treatments were significant ($F_{2,3} = 196.29; P<0.001$). Several releases of aphids did not result in an establishment of aphids in the compartments with *M. pygmaeus*, whereas aphids increased to high densities in the treatments with *O. laevigatus* or *O. majusculus*, causing serious damage to the fruits (Table 1). Thrips were controlled by all predators, but compartments with *M. pygmaeus* showed initially some silver damage on the fruits (Table 1). Thrips larvae were hardly found on the leaves, but some thrips adults were found in the flowers in the first weeks after pest releases. These results are consistent with an earlier study, which showed that the anthocorid bugs *O. majusculus* and *O. laevigatus* are more effective thrips predators than *M. pygmaeus* (Montserrat et al., 2000). The excellent results with *M. pygmaeus* in controlling aphids can be explained by the high densities attained by this predator in the first weeks after pest infestations: average densities were 2.9 ($\pm 0.29$ s.e.m.) individuals per flower, which was twice as high as for *O. laevigatus* ($1.3 \pm 0.23$ s.e.m.). The pollen and nectar provided in the flowers are apparently good food...
sources for *M. pygmaeus*. Earlier studies showed that sweet pepper leaves are unsuitable for reproduction of this predator, but that *M. persicae* is a highly suitable prey for this predator (Perdikes et al., 2004). *Orius majusculus* is a typical leaf-dwelling predator and was never found in the flowers. The cryptical behaviour of this predator makes it difficult to monitor the densities on plants. However, aphid densities in the presence of this predator were significantly lower than in the treatment with *O. laevigatus* (Fig. 1).

Figure 1. Population dynamics of the green peach aphid *M. persicae* in a sweet pepper crop with either *M. pygmaeus*, *O. majusculus* or *O. laevigatus*. Shown are average (± s.e.m.) densities per leaf. Different letters indicate significant differences among treatments through time (p < 0.05).

<table>
<thead>
<tr>
<th>Predator</th>
<th>% fruits with aphid honeydew (± s.e.m.)</th>
<th>% fruits with thrips damage (s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. laevigatus</em></td>
<td>87.3 (1.1) a</td>
<td>0.0 (0.0) b</td>
</tr>
<tr>
<td><em>O. majusculus</em></td>
<td>73.3 (5.6) b</td>
<td>1.6 (0.6) b</td>
</tr>
<tr>
<td><em>M. pygmaeus</em></td>
<td>1.1 (0.8) c</td>
<td>28.2 (2.0) a</td>
</tr>
</tbody>
</table>

*Orius laevigatus* is currently the main predatory bug for thrips control in sweet pepper in Europe. Although *M. pygmaeus* is less effective as a thrips predator, our data clearly demonstrate that it is better to release *M. pygmaeus* for controlling both thrips and aphids.

In practice, releases of *O. laevigatus* may be desirable to quickly control infestations of adult thrips, especially when TSWV infections are present. However, it needs to be clear whether releases of *M. pygmaeus* with *O. laevigatus* has a negative impact on pest control through intraguild predation or competition (in the flowers) between these two predatory bugs. It is known that *O. majusculus* can prey on the nymphal stages of *M. pygmaeus* (Jakobsen et al., 2004), thus intraguild predation might be expected for *O. laevigatus* as well.
If such predator interference affects biological control negatively, a re-design of the biological control system in sweet pepper might be considered in order to improve multiple pest control, including aphids. Strategies based on *M. pygmaeus* alone might need additional releases of effective predatory mites for thrips control, such as *Amblyseius swirskii* (Messelink *et al.*, 2005). These predatory mites may in turn disrupt the control of aphids by hyperpredation on aphidophagous gall midges (Messelink *et al.*, 2011), but these midges might be redundant when *M. pygmaeus* controls aphids. Future research needs to clarify whether *M. pygmaeus* alone or in combination with other natural enemies is sufficiently effective for controlling thrips, aphids and other important pest species, such as caterpillars or whiteflies.

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**References**


Participatory development of integrated management strategies for pest insects in cucumber

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Abstract: The pest complex in cucumber includes several serious pests such as spider mite, thrips and the tarnished plant bug. In Sweden, the European tarnished plant bug, Lygus rugulipennis Poppius (Heteroptera: Miridae), is one of the most common pest within the genus and has been recorded attacking a wide range of cultivated crops including cucumber. The management of tarnished plant bugs in greenhouse relies on the use of the systemic compound Confidor WG 70 (imidacloprid). However, according to new EU directives, integrated pest management must be applied throughout the EU from 2014. This means that the use of chemical pesticides must be clearly justified, that the risks of leaching and occupational health problems must be dealt with and that new pest control methods must be developed.

The alternatives being developed to the present day chemical control methods include treatment with the entomopathogenic fungus Beauveria bassiana (Balsamo) Vuillemin, which in trials has demonstrated the potential to control the tarnished plant bug. Another strategy for dealing with the pest is to use a so-called trap crop. This is a crop grown to attract pests and thus protect the main crop from attack. Sunflower and white mustard have been shown to be very attractive to L. rugulipennis compared with cucumber. Behaviour studies have been able to identify plant scents and aggregation and sexual pheromones to which both L. rugulipennis males and females react and are attracted. A combination of trap crops that attract the pest insect and biological control with B. bassiana has the potential to become a useful alternative to today’s chemical control.

The current project will concentrate on the European tarnished plant bug, and only investigate the incidence of the other pests in time and space. The incidence of L. rugulipennis and thrips in the greenhouses will be measured throughout the growing season and results will be used in discussions with growers in the development of integrated plant protection strategies. The pathogenicity of B. bassiana against nymphs and adults of L. rugulipennis will be evaluated in laboratory studies and the most efficacious concentrations will be further evaluated in greenhouse trials and used in inoculation of traps. Pheromone traps pre-treated with conidia of B. bassiana for auto-dissemination of pathogen among the tarnished plant bug population and the potential for using bumblebees as a vector for carrying B. bassiana to trap plants will also be investigated. Potential trap plants for L. rugulipennis will be investigated in the laboratory and greenhouse and the most attractive will be selected and used to concentrate L. rugulipennis to it and then a selected chemical or alternative control measures applied.
Development of genetic control in the tomato leafminer, *Tuta absoluta*

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Abstract: Since the arrival in 2006 of a new tomato pest moth, the tomato leafminer (*Tuta absoluta*), European tomato growers have suffered significant losses. Furthermore, restrictions to their choice of pesticides have made control of this moth extremely difficult. Pesticide-free control methods, including natural predators and pheromone, are increasingly sought-after. A new chemical-free pest control method is being developed in *T. absoluta*, with the aim of providing another control option available to growers. This technique, called RIDL, is a variant of the sterile insect technique, in which a pest insect is mass-reared, sterilised by irradiation, and mass-released over an area infested by their wild counterparts. RIDL improves on this concept by avoiding the requirement for irradiation, so insects are likely to be more competitive in the field. Improved insect performance translates to improved efficiency of pest control. Avoidance of irradiation also means that the scale and location of a control programme is not restricted to those that would justify investment in a costly irradiation facility. This extends to protected crops, which would benefit from this more flexible technology. SIT and RIDL work best in settings where immigration and emigration of a pest is low. Protected cultivation, especially in greenhouses, inherently provides this by restricting movement of the pest. Here, we describe RIDL and how we plan to apply it to *T. absoluta* and other pests, with particular reference to protected crops.

Key words: RIDL, sterile insect technique, *Tuta absoluta*

Introduction

The tomato pest moth, *Tuta absoluta*, has historically been confined to South America, where it is a serious pest of tomato. In 2006 it was discovered in Spain (Urbaneja *et al.*, 2007). Since then it has spread throughout Europe and the Mediterranean basin, and there are reports that it has now reached the Middle East (Abdul Razzak *et al.*, 2010; Seplyarsky *et al.*, 2010). Since its arrival, it has caused devastating losses to tomato growers, both in protected and outdoor cultivation. Damage is caused by larvae mining the foliage and, to a lesser extent, feeding on the fruit. In South America, reduction of pesticide efficacy and development of resistance has been reported (Lietti *et al.*, 2005; Salazar & Araya, 1997). With a high reproductive capacity and short generation cycle, *T. absoluta* is likely to develop resistance to insecticides in Europe. With insecticides being increasingly being withdrawn from the market and residue limits lowered (EU Parliament Directive 994) due to stringent EU regulations, and supermarkets’ desire for zero-residue produce, non-chemical control methods are highly attractive for this pest.
RIDL – a novel control method

The sterile insect technique (SIT) (Knipling, 1959), which controls pest populations by mass-releasing sterile insects to mate with their wild counterparts, is a species-specific and chemical-free pest control method. SIT has historically been successful against a number of agricultural pests. Due to a reliance on costly gamma radiation sources to induce sterility, SIT programmes require significant investment and are generally restricted to large-scale, area-wide control operations. Irradiation of insects – especially moths, which require a high dose to induce sterility – can reduce the quality, and therefore field performance, of released insects (Cayol et al., 1999).

A novel control method called Release of Insects Carrying a Dominant Lethal (RIDL®) (Thomas et al., 2000) works by the same principle as SIT, but avoids the need for the damaging irradiation step. Germline transformation technology, mediated by genetic elements called transposons, allows engineering of insect phenotypes, such as larval lethality. RIDL, initially developed in Drosophila melanogaster (Thomas et al., 2000) but now transferred to important pest species (Fu et al., 2007; Gong et al., 2005; Koukidou et al., 2008; Phuc et al., 2007), uses the tetracycline-repressible genetic system (Gossen et al., 1994; Gossen & Bujard, 2002) to repress lethal phenotypes. Supplying tetracycline or suitable analogues to the insect feed suppresses lethality and therefore permits rearing in a laboratory or mass-rearing facility. Withdrawing the dietary additive leads to induction of the lethal phenotype. In the case of a ‘bi-sex’ lethal RIDL strain, for example, the insects can be reared happily in the facility with tetracycline. After release in the wild, however, tetracycline is no longer available to their progeny and they die. Death of progeny has the same effect, in population terms, as parental sterility. This type of RIDL might, therefore, be considered a genetic equivalent of radiation-induced sterility, but without the costs to insect quality.

An SIT-type method also requires that released insects are marked, in order to distinguish them from wild insects in monitoring traps and thereby track their relative levels in the field. A key feature of RIDL strains is the inclusion of a visual and genetic marker, such as an expressed fluorescent protein. RIDL strains also provide in-built biological containment. Rearing a pest insect in large numbers carries the risk of accidental release into the surrounding area. As RIDL strains are reproductively non-viable outside artificial rearing (in the absence of tetracycline), this risk is greatly reduced.

Genetic sexing

For SIT, male-only sterile insect releases are generally considered to be more efficient than bi-sex releases. This is presumably because, for the latter, sterilised males may mate with their sterile female counterparts in transit to the field or after release, therefore distracting them from their intended task of mating with wild females. In addition, the females of many pest insects cause the pest damage: for example fruit flies damaging fruit when ovipositing and female mosquitoes transmitting disease when blood-feeding (males do not blood-feed). In the Mediterranean fruit fly (Ceratitis capitata), female-lethal genetic sexing strains have been generated by chromosomal translocation (Franz, 2005). In the most commonly used strains, male-only collections of pupae can be produced by heat-treating them as eggs. This technology is, however, labour-intensive to develop and not readily transferrable to other species. The strains are also genetically unstable and tend to revert to wild-type over time.

RIDL strains that confer female-specific lethality have been developed in the Mediterranean fruit fly (Fu et al., 2007), the Mexican fruit fly (Anastrepha ludens) (Koukidou et al., 2008) and strains of the dengue-transmitting mosquito, Aedes aegypti, have been developed that show tetracycline-repressible female flightlessness (Fu et al., 2010).
Transformation technology has facilitated development of these transgenic sexing strains, and similar phenotypes should be transferable into other species, including moth pests.

**RIDL in the field**
The cotton pest moth, pink bollworm, has been controlled by SIT in southwestern USA for a number of years. We developed an engineered marker-only strain, called OX1138B, to provide a more reliable method of marking irradiated released moths.

To compare performance of OX1138B with the wild-type strain, called APHIS, currently used in the SIT programme, we co-released 1.1 million irradiation-sterilised moths of each strain over three cotton fields in Arizona over the course of a 2-month period. As measured by recapture on pheromone traps, the transgenic strain’s field performance – total recapture, persistence in the fields and dispersal ability – was comparable to that of APHIS.

In a 2010 experiment with the mosquito *Ae. aegypti* in the Cayman Islands, males of a bisex RIDL strain were released in a small town on Grand Cayman (Alphey, 2010). Monitoring of the wild population, by trapping adults and setting oviposition traps (to count eggs laid by wild females), showed that these RIDL releases were able to suppress the wild population by approximately 80% compared to non-treatment areas during the trial period. This proof of RIDL’s efficacy provides encouragement for its prospects of controlling agricultural pests, such as *T. absoluta*.

**Developing RIDL in Tuta absoluta**
The first step to developing RIDL strains is to generate transgenic strains by microinjection of plasmid into pre-blastoderm embryos. Transformation events, mediated by co-injected sources of transposase, are detected in the subsequent generation by screening for the transformation marker (e.g. fluorescent protein).

In our laboratory, pink bollworm and diamondback moth are readily transformed (unpublished). Prototype RIDL strains have been developed in these insects (data not shown). Diamondback moth can devastate brassicaceae in a very short space of time, often before applied chemicals are able to kill the larvae. Release of RIDL diamondback moth in protected brassica nurseries, which are common in Europe, could provide valuable chemical-free protection for these high-value crops.

We are currently developing these methods in *T. absoluta*, with the aim of later generating RIDL strains. In a tomato greenhouse setting, we anticipate that a female-lethal transgenic sexing strain would be most appropriate. When pest levels are low, for example at the start of the growing season, male-only releases of RIDL moths would be released in large numbers. When female adults eclose or enter the greenhouse, the waiting RIDL males would mate with them immediately, resulting in death of progeny. This approach would be highly compatible with control methods that target non-adult stages, such as natural predators and applied *Bt* toxin, so would be a good fit with current IPM practices.

**Summary**
With growers in Europe becoming increasingly squeezed financially, the damage caused by *T. absoluta* has been hugely damaging. Chemical control is being developed, but with a history of developing resistance to pesticides, their efficacy may not survive in the long-term unless IPM is increasingly adopted. As discussed, RIDL could form an important part of this approach. As RIDL is developed in *T. absoluta* over the coming few years, it is likely that more field demonstration of this technology will be conducted against a number of pests, easing potential obstacles for its use against this moth in its expanding range.
Acknowledgements

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An update on the use of biological control in greenhouse ornamental crops in Canada

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Abstract: The results of a recent survey of greenhouse ornamental growers in Canada show that 90% of commercial growers are currently using biological control. This is a significant increase from the 26% of growers who reported using biocontrol when previously surveyed in 2001. Reasons for the increased adoption of biocontrol are discussed.

Key words: greenhouse ornamentals, pest management, biological control, thrips

Introduction

Biological control in greenhouse ornamental production has been historically considered more difficult than in vegetable crops for a number of reasons such as a lower threshold for damage and presence of pests, a greater diversity of crops resulting in more complex control programs, intensive use of pesticides and quarantine restrictions on exported plant material (Parrella, 1990). However, a number of factors are driving change in growers’ perceptions of the relevance of biocontrol in ornamental crops, including the advent of new compatible pesticides, development of pesticide resistance, the loss of registered pesticides and the development of new biocontrol products (van Driesche & Heinz, 2004).

In Canada, first experimental steps in the use of biocontrol by greenhouse ornamental growers occurred in the early 1990s. A survey of Ontario growers in 1994 showed that 29% were using IPM practices (Murphy, unpublished), but biocontrol use was not reported. By the late 1990s, use of biocontrol against specific pests such as fungus gnats was becoming more common and a further survey of Canadian growers in 2001 reported that 26% of growers were using biocontrol specifically as part of IPM (Murphy et al., 2002).

Reducing the use of pesticides is a key objective of many industry groups and government organizations and biological control use can be a primary indicator of pesticide reduction in greenhouses. The Pest Management Centre of Agriculture and Agri-Food Canada promotes the reduced use of pesticides through the Pesticide Risk Reduction Program (PRRP), a joint program with the Pest Management Regulatory Agency of Health Canada. The key objective is to reduce the risks to the environment, non-target organisms and human health from pesticide use in agriculture. To achieve this objective, the PRRP works with grower groups, industry, provinces and researchers to identify gaps in pest management and opportunities for risk reduction and to develop and implement strategies to address them.

Flowers Canada Growers, the association representing greenhouse ornamental growers in Canada, in conjunction with provincial extension specialists, growers and regulatory bodies have developed a detailed pesticide risk reduction strategy to address key growers’ needs in a manner consistent with the PRRP.
Material and methods

As part of the above strategy, a survey of Canadian growers was proposed to determine their current pest management concerns and in particular the role that biological control plays in their pest management programs. Flowers Canada Growers represents 260 commercial growers in Ontario and has a further 70 members in British Columbia and three in the Atlantic provinces. These jurisdictions represent about 75% of Canadian production. A detailed survey was sent to all members in the above provinces requesting information on a number of subject matters related to pest management including:

- Major pest and disease priorities
- Pesticide issues including efficacy, resistance, registration of new products and compatibility with biocontrol
- Pesticide application technology
- Biological control
- Information and education

The focus of this paper is on the use of biological control, although where appropriate and relevant other information gained from the survey is noted.

Results and discussion

Forty growers (13% return) representing approximately 11% of the total area of greenhouse ornamental production in Canada, responded (Gates & Watson, 2010). A majority of growers (90%) responded affirmatively that they use biocontrol as part of their pest management programs. Murphy et al. (2002) observed that ornamental growers may use biocontrol only in a small area of production, in specific crops, in certain seasons only or for individual pests and as such it is difficult to quantify percentage use within individual greenhouses. While the above still holds true to some extent, it should be noted that current biocontrol usage at the individual grower level is more extensive than it was in 2002, covering a greater number of pests, a greater percentage of production area and more crops.

The number of growers using biocontrol is a dramatic increase from the 26% reported in 2001. Anecdotal evidence from biological control producers (David Neal, Koppert Canada, pers. comm.; Ronald Valentin, BioBest Canada, pers. comm.) working in the Niagara region of Ontario, notes that a rapid increase in biocontrol use was observed in 2007-8. This timing has some significance. In January 2006, the pesticide spinosad was registered for thrips control in Canada, the first new registration for control of western flower thrips (*Frankliniella occidentalis*) in more than 20 years. Initial control results were dramatic, but within 6-12 months of spinosad being available, growers were reporting control failures and by 2008, the lack of control was widespread. The rapid failure of this product in Canada is not without precedent, given that registration of new pesticides often lags several years behind the process in the USA. In the case of spinosad, it was registered in the USA in 1997 (John Sanderson, pers. comm.), nine years before being registered for greenhouse use in Canada. As a result pests such as thrips that have been documented entering Canada on vegetative cuttings (Romero, 2011), may have been exposed to new active ingredients for a number of years before Canadian growers have access to the same products.

It should be noted that Canada’s pesticide registration system is stringent and the market potential of the greenhouse ornamental industry is such that pesticide registrants can be reluctant to invest in the process. Current active ingredients registered for thrips control in
Canada include one pyrethroid and 5 organophosphates, demonstrating the paucity of control options. There is also a recent registration of Beauveria bassiana. Only one of the registered products, dichlorvos, is considered to be consistently effective against western flower thrips.

Canadian growers were placed in the position of having few, if any, effective pesticides for western flower thrips control. The decision for many growers was made out of necessity. Biological control already had a core group of adherents as evidenced by the 2001 survey (Murphy et al., 2002), but in a very short period of time, the number of growers seeking alternatives to pesticides for thrips control increased rapidly. Thrips had previously been considered the most difficult of the major pests to control either chemically or biologically (Murphy, 2002) and until 2007 was the major obstacle to increased adoption of biocontrol. Thereafter, however, its relevance to biocontrol reversed, and it became the primary driver for new growers starting up a biocontrol program.

The importance of thrips as the primary motivator for new biocontrol programs is borne out by the results of the grower survey. When asked why biocontrol was being used, 18 of 25 growers who mentioned specific pests, included thrips as one of their primary pest targets. A number of respondents noted that biocontrol provided better control of thrips than pesticides; and of 22 respondents who answered a question relating to inadequate control using pesticides, 14 specifically mentioned spinosad and thrips.

One of the benefits of this move to biocontrol has been the necessity for growers to ensure it is successful since there is no option of returning to a pesticide program. Growers have used and in some cases developed their own innovative strategies including ornamental peppers as banker plants for Orius (Valentin, 2011), trap crops to focus pest populations and act as a release point for predators, and extensive use of entomopathogenic nematodes. Discussions with biocontrol producers also led to the introduction of a new application method for Neoseiulus cucumeris, in a smaller slow release sachet than those that have been available for many years. The mini-sachets are available from three major biocontrol producers. They contain a starting population of between 100-250 predatory mites and a price of approximately C$0.10 each, about one third the cost of the larger sachets, allowing growers to place them in every hanging basket in their spring crops (which was the original targeted crop for this product). Hanging baskets can be a major source of thrips problems and have been a difficult crop (inaccessible, difficult to monitor and treat) in which to use biocontrol. This new distribution method for N. cucumeris has been widely adopted, with some growers even using it successfully for potted crops such as chrysanthemum and gerbera.

This is not to suggest that all growers have been successful, and issues with pesticide residues, inadequate introduction rates and quality control concerns have hampered successful biocontrol in a number of instances.

The move to biocontrol of thrips resulted in a ripple effect in which other pests had to be controlled biologically as well, since there is limited availability of pesticides compatible with the predatory mites used for thrips control. Of particular importance is the leafminer Liriomyza trifolii in chrysanthemum and gerbera. Registered active ingredients in Canada include permethrin, abamectin and cyromazine, none of which is currently offering effective control. A number of chrysanthemum growers in particular are using Diglyphus for control of leafminer, however, it is very sensitive to pesticides, even when used cautiously, and some growers have had difficulty in maintaining long term control of this pest.
Conclusions

Use of biological control in greenhouse ornamentals in Canada dates back to the early 1990s, but its acceptance as a legitimate pest management strategy did not occur until the late 1990s. In 2006-2007, the loss of effective control of thrips with the pesticide spinosad and the lack of alternative pesticide options, led to a rapid increase in the adoption of biocontrol. A survey of Canadian ornamental growers in 2010, showed that 90% of growers now use biocontrol with thrips being a key target pest.

References

The biology, life table and predation of \textit{Scolothrips longicornis} fed on \textit{Tetranychus urticae} eggs

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\textbf{Abstract:} Biology, life table parameters and predation rate of the predatory thrips, \textit{Scolothrips longicornis} Priesner fed on eggs of \textit{Tetranychus urticae} Koch on bean leaves were studied at 26±1°C, 60±10% RH and a photoperiod of 16L: 8D h. The following average parameters were obtained. Female longevity is 20.71 days, fecundity is 3.66 eggs/female/ day, egg mortality is 12%, pre-oviposition period is 1.65 days, oviposition period is 15.61 days, post-oviposition period is 3.90 days, total immature development time is 13.55 days and sex ratio is 67%. Life table parameters were estimated as net reproductive rate ($R_0$) 31.09, intrinsic rate of natural increase ($r_m$) 0.201 day$^{-1}$, finite rate of increase ($\lambda$) 1.22, mean generation time ($T$) 17.04 days and doubling time ($DT$) 3.44 days. Thus it is concluded that \textit{S. longicornis} can be considered as a valuable addition to the existing IPM methods for spider mites control.

\textbf{Key words:} biology, life table, \textit{Scolothrips longicornis}, \textit{Tetranychus urticae}

\textbf{Introduction}

Acarophagous thrips (Thysanoptera: Aeolothripidae, Thripidae) are important natural enemies with various degree of specialization on mites. All species of \textit{Scolothrips} appear to be specialized on spider mites (e.g, Gilstrap & Oatman, 1976). \textit{Scolothrips longicornis} Priesner occurs in the Middle East, India and North America (Priesner 1950, Gilstrap & Oatman, 1976, Pakyari \textit{et al.}, 2009). In Iran it is a common predator of spider mites in bean, cucumber and eggplant fields (Pakyari & Fathipour, 2009).

Detailed information on the biology of \textit{S. longicornis} in the literature is only provided by Sengonca & Weigand (1988) on some lifecycle aspects, and can therefore only be inferred from studies on other species of the genus, \textit{S. takahashii} (Gotoh \textit{et al.}, 2004) and \textit{S. sexmaculatus} (Coville & Allen, 1977, Gilstrap & Oatman 1976). To investigate the potential of \textit{S. longicornis} as a biocontrol agent against spider mites the present study examined the biology, life table and predation of \textit{S. longicornis} to assess its suitability for use in biological control programs against spider mites.

\textbf{Material and methods}

\textbf{Mite and thrips rearings}

The rearings of \textit{Tetranychus urticae} Koch and \textit{S. longicornis} were initiated using individuals collected from cucumber fields in the Varamin Tehran province. The mites were maintained on detached cucumber leaves placed upside down on a layer of wet cotton inside Petri dishes (Ø 150mm, lids with a Ø 30mm hole covered with fine nylon mesh). The dishes were kept in
a growth chamber (Binder KBWS 240, Germany) at 26±1°C, 60±10% RH and a photoperiod of 16:8 (L:D) h. For thrips, a cucumber leaf was placed in a Petri dish (Ø 180mm) and maintained in another growth chamber at conditions described above. Adults were transferred to a new mite-infested cucumber leaf every 2 days. The colonies were used for tests after a rearing period of 2 and 3 months for thrips and spider mites, respectively.

**Test arena**
Bean leaf discs (*Phaseolus vulgaris* L. cv. Sunray, grown under lab conditions without pesticides) with veins of 30mm in diameter served as the test arena. Each disc was placed upside down on a layer of wet cotton inside Petri dishes (Ø 60mm). The lids of the Petri dishes had a hole (Ø 15mm) covered with nylon mesh for ventilation.

**Survival and longevity**
To obtain eggs of standardized age, 20 *S. longicornis* mated females were placed at 26°C on bean leaf discs for 24h. Then females were removed and the leaf discs placed in a climate cabinet at 26°C, 60% RH and 16:8 L:D. Sixty eggs were assigned to experiment. Immature predators were transferred to fresh leaf discs every 2 or 3 days until pupation. Development and survival of immatures were recorded daily. Upon emergence of adults they were sexed. Females were placed individually together with a male on new leaf discs for two days, and observed daily. From the onset of reproduction, they were transferred daily to fresh leaf discs. Egg production was recorded daily until the female died, and longevity (from adult emergence to adult death) was determined. Age-specific survivorship (*l*ₜ) was recorded daily according to Carey (1993). Throughout the immature development and the lifespan of females, the thrips were fed daily with a surplus of ca. 100 eggs per day per thrips, this number having been determined in pre-tests. The number of prey items consumed was counted daily under stereomicroscope and replenished to the original number.

**Life table parameters**
Life tables were constructed on the basis of records of juvenile development and survival, adult sex-ratio, survivorship, and age-specific fecundity (*mₓ*: female progeny/female/day), and net reproductive rate (*R₀*), intrinsic rate of natural increase (*rₘ*), finite rate of increase (λ), mean generation time (*T*), and doubling time (*DT*) (Carey 1993) were calculated.

**Statistical analysis**
One-way analysis of variance (ANOVA; MINITAB INC. 2000), followed by multiple mean comparisons using the LSD test (*P* < 0.01) upon detection of significant differences, was used to analyze the data statistically for differences in development time, adult longevity and prey consumption between sexes and different stages.

**Results and discussion**
The immature development time of females and males were similar (Table 1), and near to that reported by Gotoh *et al.* (2004) for *S. takahashii* at 25°C under similar dietary conditions, but shorter at 26°C than that of *S. sexmaculatus* (15.7-16.1 days depending on the thrips, prey, and host plant species (Gilstrap & Oatman, 1976; Coville & Allen, 1977). The egg stage comprised 46% of the life time. The immature developmental periods were combined with the daily survival and oviposition rate (Fig. 1.). The sex ratio is ca. 70% females at 26°C, similar to values found at similar conditions for *S. takahashii* (Gotoh *et al.*, 2004) and *S. sexmaculatus* (Coville & Allen, 1977).
Table 1. Mean development time and adult longevity (± s.e) in days of *S. longicornis* fed on *Tetranychus urticae* eggs. Mean followed by the same letter in rows is not significantly different between sexes (LSD test, *P* < 0.01).

<table>
<thead>
<tr>
<th>Life stage</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>6.32±0.16a</td>
<td>6.02±0.22a</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar larvae</td>
<td>1.83±0.12a</td>
<td>1.87±0.17a</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar larvae</td>
<td>2.12±0.10a</td>
<td>2.33±0.19a</td>
</tr>
<tr>
<td>Prepupa</td>
<td>1.36±0.09a</td>
<td>1.20±0.11a</td>
</tr>
<tr>
<td>Pupa</td>
<td>1.94±0.10a</td>
<td>1.73±0.75a</td>
</tr>
<tr>
<td>Total immature</td>
<td>13.55±0.22a</td>
<td>13.13±0.09a</td>
</tr>
<tr>
<td>Pre-oviposition female</td>
<td>1.65±0.13</td>
<td>-</td>
</tr>
<tr>
<td>Oviposition female</td>
<td>15.61±0.47</td>
<td>-</td>
</tr>
<tr>
<td>Post-oviposition female</td>
<td>3.90±0.16</td>
<td>-</td>
</tr>
<tr>
<td>Adult longevity</td>
<td>20.71±0.57a</td>
<td>18.07±0.07b</td>
</tr>
<tr>
<td>Overall life stage</td>
<td>34.90±0.57a</td>
<td>31.14±0.48b</td>
</tr>
</tbody>
</table>

Figure 1. Adult female age-specific survival rate (*l*<sub>x</sub>) and age-specific fecundity of *Scolothrips longicornis* fed on *Tetranychus urticae* eggs.

The net reproductive rate (*R*<sub>0</sub>) was 31.09, intrinsic rate of natural increase (*r*<sub>m</sub>) 0.201/day, the finite rate of increase (*λ*) was 1.22, the mean generation time (*T*) was 17.04 days and the doubling time (*DT*) was 3.44 days. The oviposition period of 15.6 days amounted to 75% of female entire life time of 20.7 days (Table 1), with an average of 3.66±0.09 and 56.48±1.66 eggs produced per day and over the life-time of a female, respectively. Totally, 88% of eggs hatched and 67% of hatched eggs were female.

Compared with the *r*<sub>m</sub> value of acarophagous thrips species (0.155-0.202 day<sup>-1</sup> (Gilstrap & Oatman, 1976, Coville & Allen, 1977, Gotoh *et al*., 2004, this study) this suggests that acarophagous thrips are unlikely to provide consistent control of spider mites by their reproductive numerical response to the mites alone. However, the intrinsic rate of increase is not the only aspect that needs to be considered in evaluating predator’s control potential. Thus, *S. longicornis* may be able to compensate for its relatively low *r*<sub>m</sub> value by a large
consumption of mite eggs. At 25°C *T. urticae* lays a maximum of 4.4 eggs per day (Bounfour & Tanigoshi, 2001) but a female *S. longicornis* can daily consume an average of 24 eggs per day at 26°C implying that thrips populations will have the possibility to lower the higher \( r_m \) of *T. urticae*.

*Scolothrips longicornis* started consuming prey on the day after hatching from eggs. Females’ daily consumption was lowest in the pre- and post-oviposition periods and highest in the oviposition period (Table 2). Larvae in the 2\textsuperscript{nd} instar consumed more prey than those in the 1\textsuperscript{st} instar. The magnitude of daily consumption of spider mite eggs by immature thrips was similar to that found in other studies for other *Scolothrips* species at 25°C (Gilstrap & Oatman, 1976, Gerlach & Sengonca, 1986, Sengonca & Weigand, 1988). Average life-time egg consumption by females was ca. 30 eggs more over their life-time than that by males.

### Table 2. Daily and total (mean ± s.e) consumption of different life stages of *S. longicornis* fed on *Tetranychus urticae* eggs.

<table>
<thead>
<tr>
<th></th>
<th>1\textsuperscript{st} instar larvae</th>
<th>2\textsuperscript{nd} instar larvae</th>
<th>Pre-oviposition female</th>
<th>Oviposition female</th>
<th>Post-oviposition female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily consumption</td>
<td>10.08±0.02(^a)</td>
<td>11.45±0.19(^b)</td>
<td>4.07±0.18(^c)</td>
<td>16.24±0.16(^d)</td>
<td>3.39±0.13(^e)</td>
<td>20.66±0.29</td>
</tr>
<tr>
<td>(51)</td>
<td>(50)</td>
<td>(31)</td>
<td>(31)</td>
<td>(31)</td>
<td>(15)</td>
<td></td>
</tr>
<tr>
<td>Total consumption</td>
<td>32.84±0.41(^a)</td>
<td>30.62±0.39(^b)</td>
<td>6.65±0.67(^c)</td>
<td>254.48±7.26(^d)</td>
<td>12.61±0.75(^e)</td>
<td>243.8±1.56</td>
</tr>
<tr>
<td>(51)</td>
<td>(50)</td>
<td>(31)</td>
<td>(31)</td>
<td>(31)</td>
<td>(15)</td>
<td></td>
</tr>
</tbody>
</table>

*Means within the same row followed by the same letters (a, b) are not significantly different between 1\textsuperscript{st} and 2\textsuperscript{nd} instar larvae (LSD test, \( P < 0.01 \)).

**Means within the same row followed by the same letters (c, d, e) are not significantly different between the different female adult phases (LSD test, \( P < 0.01 \)).

Our study is a first step in evaluating *S. longicornis* as a biological control agent of *T. urticae*. More studies are needed to determine the effect of host plant varieties, prey mite stages and effect of environmental factors on the biology of *S. longicornis*.

### References


Biological control of tarsonemid mites in greenhouse grown gerberas

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Abstract: Several species of Phytoseiidae were evaluated as predators of Tarsonemus violae (Schaarschmidt) and the broad mite Polyphagotarsonemus latus (Banks) on gerbera plants in experimental and commercial greenhouses. Amblyseius cucumeris, A. swirskii, Typhlodromips montdorensis and A. andersoni appeared to possess the best abilities to control tarsonemids in this crop. These species displayed good survival and reduced mite densities at low levels directly after their releases. However, curative strategies did not effectively eliminate tarsonemids and the predators could not keep pests density below economic damage threshold year round without further releases.

Key words: pests, biocontrol, integrated pest management, predatory mites, Phytoseiidae, Tarsonemidae, gerbera, greenhouse

Introduction

The species Tarsonemus violae (Schaarschmidt) and Polyphagotarsonemus latus (Banks) are the main species of Tarsonemidae found in gerbera crops in greenhouses in the Netherlands. At request of Dutch growers and with funding of the Product Board for Horticulture, Wageningen UR Greenhouse Horticulture conducted experiments in the period 2009-2010 with 9 species of predatory mites on gerbera. In addition, integrated pest control strategies were tested in a commercial greenhouse with gerbera, including other occurring pests.

Material and methods

Greenhouse experiment 1

The experiment was conducted in an experimental greenhouse to assess the potential of nine Phytoseiid species to control tarsonemids. Forty gerbera plants (Gerbera jamesonii Hook, cv. Flipper) were obtained in June 2009 from a grower. The plants were heavily infested with two tarsonemid species Polyphagotarsonemus latus and Tarsonemus violae (Schaarschmidt) (about 100 tarsonemids per mature flower). The trial started in November 2009. The plants were transferred to an experimental greenhouse compartment (96m², temperature: 18-17°C, 85% relative humidity), equipped with an insect screen. Supplementary light (6,000lux) was applied for nine hours a day. Sulfur evaporators were switched off. The plants were placed on tables of 5m² with a drip irrigation system. They were standing on inverted saucers surrounded by water, to minimize movement of predators between plants.

At the start, the plants were stripped to 20 leaves and divided into four tarsonemid density groups after a pre-counting of one flower. The lowest number of tarsonemids was in block 1 (8 mites/flower) and the highest in block 4 (35 mites/flower). The experiment was set up in a randomized complete block design with 10 treatments in four replications. There were 10 plants per density group: one plant formed one plot. The treatments were: untreated, Amblyseius andersoni (Chant) (Aa), A. barkeri Hughes (Ab), A. californicus McGregor (Aca), A. cucumeris Oudemans (Acu), A. fallacis Garman (Af), Euseius ovalis (Evans) (Eo),
Typhlodromalus limonicus Garman & McGregor (Tl), Typhlodromips montdorensis (Schicha) (Tm) and A. swirskii Athias-Henriot (As).

The phytoseiids Aca, Tl, As and Aca were provided on bran or sawdust by Koppert, Aa and Tm in bran by Syngenta and Af on bean leaves infested with spider mites by Applied Bionomics. Ab was reared on Acarus farris and bran. Eo was reared in a greenhouse on castor bean plants Ricinus communis. In week 46, 100 predatory mites were introduced on each plant with some bran containing storage mites. Hundred predatory mites reared on pollen or spider mites (Tl, Eo and Af) were collected with a brush and transferred to the plant on a leaf with 0.5g of bran containing approximately 40,000 Acarus farris.

Forty-two days after predator release three young leaves per plant were sampled and examined under a stereo-microscope. Moreover two mature flowers were collected and washed with alcohol. In both leaf and flower samples, all the predatory and pest mites were counted. All nymphs and adult phytoseiids were placed in a Marc André medium on a slide for microscopic identification. Since the data were not normally distributed, a generalized linear model (GLM) was used for the analyses and the number of tarsonemids in the pre-counting was taken into account. Analyzing numbers of tarsonemids and predatory mites on the flowers, the negative binomial distribution appeared to fit best (Poisson distribution was inadequate). For pairwise comparisons of treatment means a likelihood ratio test was used.

**Greenhouse experiment 2**

This experiment was set up in 2010 with the four predatory mites species with the best potential in the first experiment. Hundred predators per plant were released in week six on gerbera plants cv. Whisper presenting a light infestation of tarsonemids (30 per plant). At the start of the experiment the plants had an average of 30 leaves. The assessment took place 4 weeks later. Since the infestation level was low, seven leaves and five flowers were examined per plant. The number of mites was analyzed with a GLM using a Poisson distribution.

**Experiment in a commercial gerbera greenhouse**

A commercial gerbera greenhouse was selected to verify the effect of releases of the predatory mites. Twenty four plots of 5m² were created in four rows of gerberas cv. Whisper.

Six treatments were tested in four replicates and compared to a control with no predators. The treatments consisted of six consecutive releases of 500 predatory mites per m² (83/plant) at one week interval starting in week 50 of 2009. A one meter wide path separated treatments between rows and an untreated buffer of 2m² within the row. The tested predatory mites were Aa, Ab, Aca, Acu, Tm and As. Releases of Acu, As, T m and Aa were repeated from week 35 until week 41 of 2010 at the same rate. Once a month (except October), the percentage of damaged flowers was assessed, and five flowers were randomly collected in each plot. In the laboratory, the flower samples were washed with alcohol and the number of predators and tarsonemids counted. Predators were mounted in slides for further identification.

**Results and discussion**

**Greenhouse experiment 1**

Six weeks after the release of the predators on plants with high tarsonemid infestation, the plants treated with As, Am, Aa and Acu had the lowest number of tarsonemids (Figure 1). The four predators reduced pest density, but did not eliminate the pest. We found two species of spontaneously occurring predatory mites on all the plants: Proctolaelaps sp. and Ameroseius sp. Leaf samples confirmed the results of flower samples. As and Acu were the most numerous species.
Figure 1. Pest densities six weeks after release in the first greenhouse experiment. For predator name abbreviations, see Material and methods for greenhouse experiment 1.

**Greenhouse experiment 2**
Four weeks after the introduction of the phytoseiids, the number of tarsonemids on the treated plants was significantly lower (0.7/flower) than in the untreated controls (30/flower). Leaf samples confirmed the results of flower samples (0.3/leaf in treated plants against 31/leaf in the untreated).

**Implementation in a commercial gerbera greenhouse**
Three and seven weeks after the last introduction of predatory mites we scored 60% less tarsonemids in plots treated with predatory mites than in the untreated ones (six per flower), regardless of the species of predator (Figure 2a). But after 12 weeks tarsonemid numbers were similar in all treatments and stayed low until December.

Figure 2. Density of tarsonemids (a) and damage level of flowers (b)

Injuries were found in average on 10% of the flowers year round, but reached 55% in week 20 and dropped to 2% from week 45 (Figure 2b). Injury level was similar in treated and control plots. The damage level in week 20 did not correspond to the high number of tarsonemids in the flowers in the same week nor six 6 weeks earlier. This level of damage can’t be explained by other pests (thrips, spider mites) either, as they were also present at low densities. In contrast with previous years, all flowers were marketable in the wintertime.
Predatory mite density was very low in all plots. As, Acu, Tm and the spontaneously occurring species Ameroseius sp. and Proctolaelaps sp. were able to survive on the gerbera crop for several generations (Figure 3). As was the most numerous predator and colonized most of the plots where other species were introduced. The presence of whitefly probably favoured this species. Ameroseius sp. and Proctolaelaps sp. were present in high numbers in the wintertime.

Repeated introductions of predatory mites were necessary in order to control the tarsonemid mites. Dutch gerbera growers will now experiment with releases of one of the four best species. Some will choose repeated inundative introductions against tarsonemids, but most will opt for sachets that they will introduce from September to December at early tarsonemid mites infestation. The choice of the predatory mite will probably depend on product price: Acu or As in combination with biocontrol of whitefly.

Introduction rates will depend on the tarsonemids infestation, the patience and the budget of the grower. Peña and Osborne (1996) found that a predator: prey ratio of Acu to P. latus of 1:5 to 1:15 significantly reduced mite density 14 days after predator release. In laboratory tests, Eo was not effective against the broad mite at a ratio of 1:150, but eliminated the pest on chili at predator: prey rates of 1:25, 1:50 and 1:100 after 9, 12 and 17 days, respectively, but could not overcome the pest (Hariyappa & Kulkarni, 1989).

References

Survey of tarsonemid mites in greenhouse grown gerberas in The Netherlands

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Abstract: Tarsonemus violae (Schaarschmidt) and the broad mite Polyphagotarsonemus latus (Banks) were found to be the main tarsonemid species in gerbera crops grown in greenhouses in The Netherlands after examination of material collected by growers. Tarsonemus violae has never been described before as a pest in the Netherlands. Injuries were supposed to be assigned to the cyclamen mite Phytodromus pallidus (Banks).

Key words: pests, biocontrol, integrated pest management, predatory mites, Phytoseiidae, tarsonemids, Tarsonemidae, gerbera, greenhouse

Introduction

The first biological control trials in gerbera were carried out in 1977 in The Netherlands (Linden, pers. com.). The first publication dates nevertheless from the mid-eighties (Bordas, 1989) with the introductions of the parasitoids Encarsia formosa Gahan against the greenhouse whitefly Trialeurodes vaporariorum Westwood and Diglyphus isaea (Walker) against the leaf miner Liriomyza trifolii (Burgess).

In spite of the high potential of the crop for biocontrol and the efforts of researchers, biocontrol companies and growers, implementation of Integrated Pest Management (IPM) in commercial greenhouses failed, mainly due to its lack of efficacy against whiteflies. For years IPM was limited by most Dutch gerbera growers to releases of Diglyphus isaea against leaf miners and the application of more of less selective pesticides against whiteflies, spider mites, caterpillars and powdery mildew.

IPM in gerbera regained popularity with the promotion campaign surrounding the predatory mite Amblyseius swirskii Athias-Henriot in 2005 and the availability of insecticide flonicamid in 2008. Diglyphus isaea continues to be the most successful application. It is released by most growers and doesn’t need to be re-introduced each year. Releaes of A. swirskii and the spider mite predator Phytoseiulus persimilis Athias-Henriot have now also become common practice and the gallmidge Feltiella acarisuga Vallot is occurring spontaneously. Even the biocontrol agents against whitefly Encarsia formosa and Delphastus catalinae have been making their come back since 2010 and growers are starting to limit their use of sulfur.

Unfortunately, since the reduction in the use of broad spectrum insecticides, problems with tarsonemids have been increasing. These tiny mites are barely visible with a magnifying glass. Their presence is often noticed too late when irreversible symptoms have already occurred. Diagnostic symptoms of infestation are leaf discoloration, downward curling of leaf margin, bronzing of leaves and growth deformity of flowers caused by the secretion of a toxin in the buds; the rays of injured flowers fail to develop. The damage diminishes marketability of flowers in the high quality category from 15 to 50%.
Tarsonemids are currently controlled with repeated sprayings of pesticides (fenbutatinoxide, abamectin, milbemectin, pyridaben). However chemical control is difficult since the coverage of pesticides on the whole plant is impossible to attain. Furthermore, injuries originate at the bud stage long before they are observed on the flowers and growers are often too late with the applications of pesticides. At the request of the Dutch growers and with funding from the Production Board for Horticulture, we started an inventory of species of tarsonemids in the Netherlands in crops grown under greenhouses. Additionally, the literature was reviewed for predatory mite species which are reported to prey on two tarsonemids species.

Material and methods

Survey of tarsonemids
An inventory of tarsonemids was carried out in 2008 and 2009 in 29 Dutch greenhouse grown gerbera crops. The samples were taken by crop protection advisors on damaged flowers and leaves and brought to researchers. The samples were washed with alcohol. In both leaf and flower samples, 60 tarsonemids per sample and all nymphs and adult phytoseiids were placed in a Marc André medium on a slide for microscopic identification. Tarsonemid mites were identified by a specialist taxonomist.

List of predatory mites
Reported predatory mites with potential as predators of *Polyphagotarsonemus latus* (Banks) and *Phytonemus pallidus* (Banks) were listed.

Results and discussion

Inventory of tarsonemids
The species *Tarsonemus violae* was found at 23 growers, *Polyphagotarsonemus latus* at 6 growers, *Tarsonemus fusarii* at 11 growers and *Tarsonemus ‘confusus’* at 1 grower. *Tarsonemus violae* and *P. latus* were observed in high numbers (up to 400 tarsonemids per flower). The two other species were found sporadically. *Tarsonemus fusarii* was always found in combination with *T. violae* or *P. latus*. Damages of tarsonemids were noticed in more than 30 different cultivars. The harmfullness of *Tarsonemus violae* has not been verified yet, but notable high numbers of mites were found in the damaged flowers.

Three species of predacious mites were observed in association with the tarsonemid mites: *Amblyseius barkeri* (after release or occurring naturally), *Amblyseius cucumeris* (after release) and *Amblyseius swirskii* (after release).

List of predatory mites
The potential of predatory mites as predators of tarsonemids has been reported for different crops by several authors (Table 1). There are several candidate biocontrol agents against tarsonemids.
Table 1. Species tested against the tarsonemids *Polyphagotarsonemus latus* and *Phytonemus pallidus* according to the literature.

<table>
<thead>
<tr>
<th>Tarsonemid Species</th>
<th>Predatory mites</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Polyphagotarsonemus latus</em></td>
<td><em>Agistemus floridanus</em></td>
<td>Ferla &amp; De Moraes, 2003</td>
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<tr>
<td></td>
<td><em>Amblyseius agrestis</em></td>
<td>Kolodochka &amp; Prutzenskaya, 1987</td>
</tr>
<tr>
<td></td>
<td><em>Amblyseius barkeri</em></td>
<td>Bonde, 1989; Fan &amp; Pettit, 1994</td>
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<tr>
<td></td>
<td><em>Amblyseius californicus</em></td>
<td>Castagnoli &amp; Falchini, 1993; Peña &amp; Obsorne, 1996; Jovicich et al., 2008 &amp; 2009</td>
</tr>
<tr>
<td></td>
<td><em>Amblyseius cucumeris</em></td>
<td>Weintraub et al., 2003; Li JiaMin Yang, 2003</td>
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<tr>
<td></td>
<td><em>Amblyseius delhiensis</em></td>
<td>Zaman Karimullah, 1987; Zaman, 1990</td>
</tr>
<tr>
<td></td>
<td><em>Amblyseius largoensis</em></td>
<td>Ochoa et al., 1991; Rodriguez &amp; Ramos, 2000 &amp; 2003</td>
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<td></td>
<td><em>Amblyseius longispinosus</em></td>
<td>Hariyappa &amp; Kulkarni, 1988</td>
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<td></td>
<td><em>Amblyseius nicholski</em></td>
<td>Wu, 1984</td>
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<td></td>
<td><em>Amblyseius swirskii</em></td>
<td>Hernandez-Suarez et al., 2006</td>
</tr>
<tr>
<td></td>
<td><em>Euseius concordis</em></td>
<td>McMurtry et al., 1984; Ferla &amp; De Moraes, 2003</td>
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<tr>
<td></td>
<td><em>Euseius hibisci</em></td>
<td>McMurtry et al., 1984</td>
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<tr>
<td></td>
<td><em>Euseius ovalis</em></td>
<td>Moutia, 1958; Hariyappa &amp; Kulkarni, 1989; Manjunatha et al., 2001; Karuppuchamy et al., 1994</td>
</tr>
<tr>
<td></td>
<td><em>Euseius stipulatus</em></td>
<td>Brown &amp; Jones, 1983; Badii &amp; McMurtry, 1984; McMurtry et al., 1984</td>
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<tr>
<td></td>
<td><em>Euseius victoriensis</em></td>
<td>Smith &amp; Papacek, 1985</td>
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<td></td>
<td><em>Iphiseius degenerans</em></td>
<td>McMurtry et al., 1984</td>
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<td></td>
<td><em>Proprioseiopsis aseus</em></td>
<td>Foufly, 1997</td>
</tr>
<tr>
<td></td>
<td><em>Phytoseiulus persimilis</em></td>
<td>Grange &amp; Leger, 1995</td>
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<td></td>
<td><em>Typhlodromalus laiae</em></td>
<td>Steiner et al., 2003</td>
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<tr>
<td></td>
<td><em>Typhlodromalus peregrinus</em></td>
<td>Peña, 1992</td>
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<tr>
<td></td>
<td><em>Typhlodromalus limonicus</em></td>
<td>McMurtry et al., 1984</td>
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<tr>
<td></td>
<td><em>Typhlodromips montdorensis</em></td>
<td>Steiner et al., 2003</td>
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<tr>
<td></td>
<td><em>Typhlodromus annectens</em></td>
<td>Badii &amp; McMurtry, 1984</td>
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<td></td>
<td><em>Typhlodromus occidentalis</em></td>
<td>McMurtry et al., 1984</td>
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<tr>
<td></td>
<td><em>Typhlodromus porresi</em></td>
<td>Badii &amp; McMurtry, 1984; McMurtry et al., 1984</td>
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<td></td>
<td><em>Typhlodromus rickeri</em></td>
<td>Badii &amp; McMurtry, 1984; McMurtry et al., 1984</td>
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<tr>
<td></td>
<td><em>Neoseiulus anonymus</em></td>
<td>Ferla &amp; De Moraes, 2003</td>
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<tr>
<td></td>
<td><em>Typhlodromus sp</em></td>
<td>Croft et al., 1998</td>
</tr>
<tr>
<td></td>
<td><em>Galendromus occidentalis</em></td>
<td>Croft et al., 1998</td>
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<td></td>
<td><em>Neoseiulus agrestis</em></td>
<td>Petrova, 2000</td>
</tr>
<tr>
<td></td>
<td><em>Neoseiulus bicaudus</em></td>
<td>Petrova, 2000</td>
</tr>
<tr>
<td></td>
<td><em>Neoseiulus californicus</em></td>
<td>Croft et al., 1998; Easterbrook et al., 2001; Fitzgerald &amp; Easterbrook, 2001</td>
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<tr>
<td></td>
<td><em>Neoseiulus cucumeris</em></td>
<td>Huffaker &amp; Kennett, 1953; Croft et al., 1998; Easterbrook et al., 2001; Fitzgerald &amp; Easterbrook, 2001, 2001; Petrova et al., 2000 &amp; 2002; Zeinalov, 2002; Tuovinen, 2002; Berglund, 2006; Berglund et al., 2007</td>
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<td></td>
<td><em>Neoseiulus fallacis</em></td>
<td>Croft et al., 1998</td>
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<td></td>
<td><em>Neoseiulus herbarius</em></td>
<td>Petrova, 2000</td>
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<tr>
<td></td>
<td><em>Neoseiulus reductus</em></td>
<td>Malov &amp; Tokunova, 1990; Radetskii &amp; Polyakova, 1991; Petrova et al., 2000</td>
</tr>
<tr>
<td></td>
<td><em>Typhlodromus pyri</em></td>
<td>Croft et al., 1998; Fitzgerald &amp; Easterbrook, 2001</td>
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<tr>
<td></td>
<td><em>Typhlodromus reticulatus</em></td>
<td>Huffaker &amp; Spitzer jr., 1951; Huffaker &amp; Kennett, 1953 &amp; 1956; Driesche &amp; van Hauschild, 1987</td>
</tr>
</tbody>
</table>
References


Side-effect testing of novel powdery mildew fungicides against biological control agents

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Abstract: There is increasing interest in using alternative fungicides, such as inorganic salts and plant extracts, including those from giant knotweed (Reynoutria sachalinensis), to control powdery mildew on protected herbs. However, it is important that these alternative fungicides are compatible with biological pest control, which is used in Integrated Pest Management (IPM) programmes by growers of protected herb crops.

This study tested mortality effects of a range of inorganic salts and an extract of giant knotweed on two biological control agents, Aphidius colemani and Neoseiulus cucumeris, which are used to control aphids and thrips on protected herb crops. The bioassays completed used worst case (Tier 1) scenarios where the biological control agent was either dipped or exposed to leaves sprayed with the fungicide.

Based on the IOBC classification of plant protection products for their side-effects on beneficial arthropods the inorganic salts and extract from giant knotweed were ‘non-toxic’ against N. cucumeris and A. colemani adults. Although the side-effects of these potential alternative controls for powdery mildew should be tested against the full range of biological control agents used in IPM programmes on protected herbs, these results indicate that they should be IPM compatible.

Key words: Powdery mildew, inorganic salt, giant knotweed, Aphidius colemani, Neoseiulus cucumeris, biological control, IPM

Introduction

Powdery mildew is common on protected herbs and if left untreated can severely reduce plant vigour and ultimately make plants unmarketable. Growers typically use conventional fungicides, such as sulphur, to control powdery mildew in herb crops. However, there is interest in using alternative fungicides, such as inorganic salts and plant extracts, including those from giant knotweed (Reynoutria sachalinensis), to control this disease. Conventional and alternative fungicides need to be compatible with the use of biological pest control, which is used in Integrated Pest Management (IPM) programmes by growers.

Understanding a product’s side-effects on biological control agents is required if it is to be successfully integrated into IPM programmes. Side-effect testing is structured to first test the worst case scenario (Tier 1) where the most vulnerable life-stage of a beneficial arthropod is brought into direct contact with the pesticide being tested. If a product is found to be harmful to the biological control agent in a Tier 1 test then more realistic semi-field (Tier 2) and field (Tier 3) testing should be completed.

The present study tested mortality effects of a range of inorganic salts and an extract from giant knotweed against adult Aphidius colemani and immature Neoseiulus cucumeris, which are used by herb growers to control aphid and thrips pests, respectively.
Material and methods

Insects and mites
Aphidius colemani and N. cucumeris were provided by BCP Certis (Ashford, UK). The A. colemani were provided as pupae within mummified aphids while the N. cucumeris were provided in bran carrier together with prey mites as a food source. The mummified aphids were placed into a ventilated Perspex cage in a controlled temperature (CT) room (21°C, 16 hours light: 8 hours dark). Adult parasitoids emerging from the mummified aphids were given a piece of cotton wool soaked in a 1:1 solution of honey and water as a food source.

Treatments
The treatments and controls in Table 1 were tested against A. colemani adults and immature N. cucumeris. Water and Decis (deltamethrin) were included as controls in the bioassays. Deltamethrin is known to be harmful to biological control agents and the rate selected has a Specific off-Label Approval (SOLA) for use on protected herbs in the UK.

Table 1. Treatments tested against Aphidius colemani and Neoseiulus cucumeris.

<table>
<thead>
<tr>
<th>Treatment No.</th>
<th>Product</th>
<th>Product rate</th>
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<tbody>
<tr>
<td>1</td>
<td>Water control</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Potassium bicarbonate</td>
<td>10g/l</td>
</tr>
<tr>
<td>3</td>
<td>Sodium silicate</td>
<td>10g/l</td>
</tr>
<tr>
<td>4</td>
<td>Sodium bicarbonate</td>
<td>10g/l</td>
</tr>
<tr>
<td>5</td>
<td>Potassium phosphate</td>
<td>10g/l</td>
</tr>
<tr>
<td>6</td>
<td>Ammonium dihydrogen phosphate</td>
<td>10g/l</td>
</tr>
<tr>
<td>7</td>
<td>Potassium dihydrogen phosphate</td>
<td>10g/l</td>
</tr>
<tr>
<td>8</td>
<td>Giant knotweed extract</td>
<td>2.5ml/l</td>
</tr>
<tr>
<td>9</td>
<td>Decis (deltamethrin)</td>
<td>500ml/600l</td>
</tr>
</tbody>
</table>

Aphidius colemani bioassays
The method used to expose A. colemani adults to the treatments was based on that described by Longley & Jepson (1997) for Aphidius rhopalosiphi. Treatments were applied to a broad bean leaf (Vicia faba cv. Sutton Dwarf) placed onto tissue paper and sprayed using a garden Mister. The upper and lower surfaces of the broad bean leaf were sprayed to run-off with the tissue paper soaking up excess liquid. Once treated the leaf was placed into the centre of a ventilated plastic Petri dish (90mm diam.). A piece of cotton wool soaked in a 1:1 solution of honey and water was also placed in the Petri dish as a food source for the parasitoids. Ten adult wasps (a mixture of females and males <48 hours old) were collected from the Perspex cage using a ‘pooter’ (aspirator). The group of ten wasps was transferred to the Petri dish containing the treated broad bean leaf. The Petri dish was then placed in a CT room (21°C, 16 hours light: 8 hours dark) and mortality checked after 24 hours. The sex of dead or live parasitoids was also recorded. Each treatment was replicated four times.

Neoseiulus cucumeris bioassay
The method used to expose N. cucumeris to the treatments was a simplified version of the method described by Dennehy et al. (1993) and by Steiner (pers. comm.) which these authors used to immerse Phytoseiulus persimilis protonymphs in small volumes of a test solution.
Petri dishes (60mm diam.) with tight fitting lids were prepared by first sticking a small piece of Blu-tack® in the centre of the base. Next a 16mm cork borer was used to cut discs from strawberry (Fragaria x ananassa cv. Elsanta) leaves that had not received any insecticide or acaricide applications prior to this experiment. A pin was pushed through the centre of each cut leaf disc before pushing the point of the pin into the Blu-tack®. Water was then poured into the Petri dish so that the leaf disc was held 0.5cm above the water surface. A granule of freeze dried pollen was then carefully placed onto each leaf disc as a food source.

A teaspoon-full of bran and mites was then taken from the container in which the mites were delivered and tipped into a coarse sieve. The mites and fine pieces of bran falling through the sieve were then tipped into a specimen tube containing 5ml of one of the treatments. The specimen tube was gently swirled for 5 sec. before tipping the contents onto tissue paper. The tissue paper rapidly absorbed the liquid and so avoided the risk of drowning the mites. Next a fine paintbrush and binocular microscope were used to carefully transfer five immature *N. cucumeris* mites to one of the leaf discs. The ventilated lid of the Petri dish was then replaced. The Petri dish was placed in a CT room (21°C, 16 hours light: 8 hours dark) and mortality checked after 24 hours. Each treatment was replicated up to 10 times and a minimum of five times.

**Results and discussion**

*Aphidius colemani* bioassays

Results from this bioassay showed levels of mortality within the range of 0 to 17.5% for the novel powdery mildew control products tested (Figure 1a). For the controls, no mortality was recorded for adult parasitoids exposed to water but when exposed to the deltamethrin mortality was 95%. The analysis by generalised linear modelling (GLM using Genstat 12; Payne, 2000) identified a highly significant treatment effect (*F* = 34.49, *P* < 0.001). Individual comparisons between the treatments were completed using the predicted means established through the GLM analysis and the confidence intervals calculated from the standard errors for each treatment mean. From individual comparisons between treatments, the mortality of parasitoids exposed to the water control was significantly lower than for sodium silicate, potassium phosphate, the giant knotweed extract and the deltamethrin control. All of the treatments tested had significantly lower levels of mortality than the deltamethrin control. Numbers of female and male *A. colemani* were not controlled for when this bioassay was set up but numbers were recorded at the end of the experiment. Females accounted for 76% of the total number of individuals tested while males accounted for 24% of the total. Mortality was similar for females (16%) and for males (17%) across all treatments. When the deltamethrin treatment was excluded, mortality was also similar for females (5%) and males (9%).

*Neoseiulus cucumeris* bioassay

Results from this bioassay showed levels of mortality of 5% or less for all treatments and controls tested with the exception of the deltamethrin treatment (Figure 1b). All immature mites dipped in the deltamethrin solution died almost immediately and so this treatment was excluded from the analysis. Results for the seven treatments and the water control were analysed by GLM. This analysis identified a highly significant treatment effect (*F* = 4.51, *P* < 0.001). Individual comparisons between the treatments were completed as previously described. Individual comparisons between treatments confirmed that potassium bicarbonate, sodium silicate, sodium bicarbonate and potassium phosphate did not differ significantly from the water control. Where significant differences did occur, for ammonium dihydrogen
phosphate, potassium dihydrogen phosphate and the giant knotweed extract, the proportions dead were actually lower than for the water control.

Based on the IOBC classification of plant protection products for their side-effects on beneficial arthropods the inorganic salts and giant knotweed extract would be classed as ‘non-toxic’ to *N. cucumeris* and *A. colemani*. Further side-effects testing of these products against the full range of biological control agents used in IPM programmes on protected herbs is required but the results presented here indicate that they should be IPM compatible.

![Bar chart](image)

Figure 1. Predicted proportions (%) of the total number of (a) *Aphidius colemani* adults and (b) immature *Neoseiulus cucumeris* killed after exposure to each treatment (different letters indicate significant differences between treatments).

**Acknowledgements**

This research project was funded by the Chemicals Regulations Directorate (CRD) on behalf of Defra. We thank BCP Certis for providing the *N. cucumeris* and *A. colemani* and Chris Dyer (ADAS) for help with the design and analysis of the experiments.

**References**


Spatial and temporal dynamics of *Frankliniella occidentalis* on protected ornamentals

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**Abstract:** Western flower thrips (WFT) *Frankliniella occidentalis* is a worldwide problem of various ornamentals and vegetables, especially under greenhouse. Here we present preliminary results of a study on spatial structure of a WFT population and its evolution over time on ornamentals under greenhouse. We used Spatial Analysis with Distances Indices (SADIE) methods to evaluate nonrandomness of the distribution and association of the distributions observed at different time. Spatial analysis of WFT population provided interesting information on the role of surrounding environment on the insect population inside the greenhouse.

**Key words:** *Frankliniella occidentalis*, spatial structure, SADIE

**Introduction**

The Western flower thrips (WFT) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is a worldwide problem of various ornamentals and vegetables, especially under greenhouse. It can cause direct (by feeding and egg-laying) as well as indirect (transmission of tospoviruses, such as TSWV and INSV) damage leading to a consistent reduction of commercial value of crops (Tommasini & Maini, 1995). Insecticide applications to control this pest can be unsatisfactory because of insecticide resistance (e.g., Bielza, 2008; Bielza *et al.*, 2009; Contreras *et al.*, 2010).

Herbivores and their natural enemies are influenced by the environment at the margin of the greenhouses (e.g., Landis *et al.*, 2000; Marshall & Moonen, 2002), but limited studies are available on the influence of off-greenhouse environment on thrips and their natural enemies presence in greenhouses (Bosco *et al.*, 2008; Atakan, 2010). The study of the spatial-temporal dynamics of thrips in greenhouse represents a basic aspect in understanding how external environment can influence their presence on protected crops.

Here we present preliminary results of a study on spatial structure of WFT population and its evolution over time on ornamentals under greenhouse. We used Spatial Analysis with Distances Indices (Perry & Dixon, 2002; Perry *et al.*, 1999) to evaluate spatial heterogeneity in thrips distribution within a greenhouse and to perform spatial association tests between thrips distributions at different times. Moreover we mapped local association of thrips to characterize factors involved in their spatial and temporal patterns.

**Material and methods**

Spatial and temporal patterns of WFT adults were investigated on ornamentals (i.e., *Delosperma* spp, *Lupinus* spp. and *Hypericum* spp.) cultivated in a commercial greenhouse in Veneto region, Italy, during 2010 growing season. Observations were performed in a glass greenhouse (90 x 135m; 12150m²), that during observation period was open in all sides. The
greenhouse is contiguous to a natural hedgerow (mainly *Celtis australis*, *Robinia pseudacacia*, *Sambucus nigra*) in proximity of the West side, while the East side faces other greenhouses. A vineyard is present on the North side, and a peach orchard on the South side. During observation period insecticides were applied to control thrips and other pests. Active ingredients used and application dates were recorded. WFT adult activity was measured using 15 x 15cm light blue and yellow sticky cards positioned into greenhouse in a regular grid of 30 x 10m. Sticky cards were fastened to 50cm tall plastic stakes. Cards initially were positioned 10cm above the canopy and were raised even with the top of the canopy as plants grew. Cards were collected and replaced at weekly intervals from 21 June (week 1) to 28 August (week 10). Flowering was noticed every week.

Data were analyzed using Spatial Analysis by Distance IndicEs (SADIE) to map the spatial distribution patterns of the thrips captured on traps. At each sampling point we assessed the local contribution to a group (cluster) of relatively high-density (patch) or to a group of zero or relatively small counts (gap). Tests of non-randomness based on the overall index of aggregation (Ia) and on the average indeces of patchiness ($v_i$) and gap ($v_j$) were performed ($\alpha = 0.05$) (Perry et al., 1999). Indices of local aggregation ($v_i, v_j$) were mapped in a two-dimensional map showing their spatial distribution (Winder et al., 2001). We also quantified the similarity between two patterns from different times, through the degree of spatial association between them (Perry & Dixon, 2002).

**Results and discussion**

The analysis evidenced a clear spatial pattern in WFT distribution within the greenhouse in four occasions (Table 1). WFT distribution was characterized by significant clustering into patches as well as gaps in the first two weeks (Table 1), and association test showed similarity between the two spatial structures (Table 2). In particular map of local aggregation indices evidenced that a patch was present along the southern side of the greenhouse (contiguous to a peach orchard), while gaps into thrips distribution were localized in the northern side. In week 3 the presence of the WFT was generalized in the greenhouse. Insecticides were applied weekly from week 3 until week 7 and determined a reduction in the thrips densities. However, nonrandomness in the WFT distribution was detected in week 8 (Table 1). The average index of patchiness indicated the presence of significant clustering into patch (Table 1). Map of local aggregation showed that in week 8 a patch was localized at the corner between the northern and east side, in correspondence of the door and close to the other greenhouses. Insecticides were applied in week 9. The presence of WFT was detected in week 10, and was characterized by a significant clustering into patches and into gaps (Table 1). Patch was localized in the area from the corner between the northern and western sides spreading along the northern side that is close to the vineyard. In the same week gaps in WFT distribution were found from the eastern side to the centre of the greenhouse. Similarities in the WFT distribution were observed from week 4 to week 7, and between weeks 6 and 9 or 7 and 9 (Table 2), when WFT densities were lowered by insecticide treatments. The spatial structure in week 10 was completely different from that found in week 1 (Table 2).
Table 1: Overall index of aggregation (Ia), mean cluster index ($v_i$) for patches and ($v_j$) for gaps, with associated probability ($P$) from randomization test, for WFT population. Bolded numbers indicate significant results at randomization test ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Week</th>
<th>Ia</th>
<th>$P_a$</th>
<th>mean $v_i$</th>
<th>$P$(mean $v_i$)</th>
<th>mean $v_j$</th>
<th>$P$(mean $v_j$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.017</td>
<td>0.0003</td>
<td>-2.011</td>
<td>0</td>
<td>1.902</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.665</td>
<td>0.0019</td>
<td>-1.495</td>
<td>0.0174</td>
<td>1.538</td>
<td>0.0047</td>
</tr>
<tr>
<td>3</td>
<td>0.718</td>
<td>0.9676</td>
<td>-0.678</td>
<td>0.9835</td>
<td>0.788</td>
<td>0.7595</td>
</tr>
<tr>
<td>4</td>
<td>0.877</td>
<td>0.7192</td>
<td>-0.999</td>
<td>0.4621</td>
<td>0.755</td>
<td>0.9335</td>
</tr>
<tr>
<td>5</td>
<td>1.179</td>
<td>0.174</td>
<td>-1.157</td>
<td>0.2015</td>
<td>1.01</td>
<td>0.4304</td>
</tr>
<tr>
<td>6</td>
<td>1.061</td>
<td>0.3278</td>
<td>-1.074</td>
<td>0.3205</td>
<td>1.116</td>
<td>0.2527</td>
</tr>
<tr>
<td>7</td>
<td>0.935</td>
<td>0.5873</td>
<td>-0.905</td>
<td>0.652</td>
<td>0.849</td>
<td>0.7821</td>
</tr>
<tr>
<td>8</td>
<td>1.478</td>
<td>0.0148</td>
<td>-1.19</td>
<td>0.3915</td>
<td>1.49</td>
<td>0.0155</td>
</tr>
<tr>
<td>9</td>
<td>0.974</td>
<td>0.5092</td>
<td>-1.034</td>
<td>0.373</td>
<td>0.938</td>
<td>0.5714</td>
</tr>
<tr>
<td>10</td>
<td>1.399</td>
<td>0.0354</td>
<td>-1.495</td>
<td>0.0153</td>
<td>1.175</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Table 2: Spatial association index (X) and associated probability ($P$) calculated for observation performed during investigation. Bolded indices indicate associations if $X > 0$ ($P < 0.025$) or dissociations if $X < 0$ ($P > 0.975$) (Perry and Dixon, 2002).

<table>
<thead>
<tr>
<th>Week</th>
<th>X</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.831 &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.27</td>
<td>0.127</td>
</tr>
<tr>
<td>3</td>
<td>0.51</td>
<td>0.031</td>
</tr>
<tr>
<td>4</td>
<td>0.589</td>
<td>0.022</td>
</tr>
<tr>
<td>5</td>
<td>0.762</td>
<td>0.002</td>
</tr>
<tr>
<td>6</td>
<td>0.41</td>
<td>0.021</td>
</tr>
<tr>
<td>7</td>
<td>0.152</td>
<td>0.238</td>
</tr>
<tr>
<td>8</td>
<td>0.235</td>
<td>0.129</td>
</tr>
<tr>
<td>9</td>
<td>0.549</td>
<td>0.045</td>
</tr>
</tbody>
</table>

The knowledge of information of pests’ spatial structure and its evolution over time could be used to improve monitoring of pests and identify periods or locations at risk (Coutts et al., 2004; Park et al., 2009; Poncet et al., 2010). The transient dynamics in spatial structure demonstrated here, can be a critical point in WFT management. Spatial analysis of WFT population provided interesting information on the role of surrounding environment on the insect population inside the greenhouse. At the beginning of the observations hot spots in WFT infestation were found in the area close to the peach orchard. Late in the season thrips densities were reduced by insecticide applications. However new hot spots originated but occurred from the other greenhouses probably vectored through cultural operation in week 32. Moreover the hedgerow and the vineyard appear to be source of thrips infestation. The presence of WFT appears not spatially stable during observation period and different thrips inflows were founded in the greenhouse.
Acknowledgements

We thank Gruppo Padana (Paese, Italy) for support during the study. This work was partially funded by Regione Veneto PSR - misura 124 “PROBIOSER” project.

References

Atakan, E. 2010: Influence of weedy field margins on abundance patterns of the predatory bugs *Orius* spp. and their prey, the western flower thrips (*Frankliniella occidentalis*), on faba bean. Phytoparasitica 38: 313-325.


An overview of invasive species on vegetables in greenhouses in southern part of Montenegro

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Abstract: In the last ten years vegetable production increased rapidly in greenhouses in southern part of Montenegro. It created conditions for introduction and appearance of new pests. The main vegetable production areas are surroundings of the city of Podgorica (Zeta and Bjelopavlići) and the Montenegrin seacoast.

In the period 2006 to 2010, surveys of pest status in greenhouses, resulted in detection of several new species: the broad mite Polyphagotarsonemus latus Banks in 2006 on peppers, the agromyzid leafminer Liriomyza bryoniae Klb. in 2006 on tomato, the western flower thrips Frankliniella occidentalis Pergande in 2008 on cucumber (Cucumis sativus), pepper (Capsicum annuum) and mangold (Beta vulgaris subsp. vulgaris var. vulgaris), and the carmine spidermite Tetranychus cinnabarinus Boisd. in 2009 on tomato. In addition to these new pests, the presence of some of the previously known pest was also confirmed in the survey: the cotton bollworm Helicoverpa armigera Hb. in pepper and tomato fruits, the European corn borer Ostrinia nubilalis Hb. in pepper fruits, and very polyphagous aphids (Myzus persicae Sulz., Aphis gossypii Glover and A. fabae Scop.), the greenhouse whitefly Trialeurodes vaporariorum West. and the two-spotted mite Tetranychus urticae Koch.

Key words: vegetables, greenhouses, new invasive pests

Introduction

Greenhouse production has great importance in Montenegro. In the last ten years vegetable production increased rapidly in its southern part. The predominantly grown greenhouse vegetable crops are tomato, pepper, cucumber, mangold and lettuce.

Until 2006 greenhouse vegetable producers in Montenegro were already familiar with certain insects and mites: aphids (Myzus persicae, Aphis gossypii and A. fabae), greenhouse whitefly Trialeurodes vaporariorum, the cotton bollworm Helicoverpa armigera, the European corn borer Ostrinia nubilalis, and the two-spotted spider mite Tetranychus urticae. Against those pests both sanitation practice and chemical control have been applied.

After the first detections of two new pests in 2006 – the broad mite Polyphagotarsonemus latus on pepper and the agromyzid leaf miner Liriomyza bryoniae on tomato and, considering the increasing importance of vegetable production, the Phytosanitary Directorate of the Montenegrin Ministry of Agriculture supported continuation of their monitoring and spreading as well the possible appearance of new (invasive) species of insect and mites with the aim to use the adequate and timely prevention and control measures.
**Material and methods**

Presence and pest status on vegetables (tomato, pepper and cucumber) in greenhouses were observed in the period 2006-2010. Monitoring included the main vegetable production areas in Montenegro (surroundings of the city of Podgorica and the Montenegrin seacoast).

Greenhouses were checked at three localities in the vicinity of Podgorica (city of Podgorica, Zeta and Bjelopavlići) with ten sites in total (city of Podgorica – two, Zeta – four and Bjelopavlići – four), and three localities along the seacoast (Ulcinj, Bar and Tivat) with five sites in total (Ulcinj – two, Bar – two and Tivat – one). One to eight greenhouses per locality with different crop species were inspected once or twice a month, starting from the end of May until the end of November. The size of inspected greenhouses was 500-1000m².

In all surveyed greenhouses, plants were visually inspected and random samples of 50 leaves were taken from each crop species. Determination of pests was done in the laboratory. In addition, in all localities, in each greenhouse, one yellow sticky trap 25x25cm (Terminator, Semenarna Ljubljana) and one blue sticky trap 25x10 cm (IVOG-System, Bug-Scan) were set up.

**Results and discussion**

In the period 2006 to 2010 surveys of pest status in greenhouses resulted in detection of several new species: *Polyphagotarsonemus latus* (Hrnčić & Radonjić, 2007), *Liriomyza bryoniae* (unpublished, 2007), *Frankliniella occidentalis* (Hrnčić et al., 2009) and *Tetranychus cinnabarinus* (unpublished, 2009) (Table 1).

After the first detection of *P. latus* on pepper in the city of Podgorica in September 2006, its presence was not detected in 2007 and 2008. Despite that the pest showed limited spreading, in 2009 symptoms of severe attack were noticed on pepper in two sites in locality Bjelopavlići (Hrnčić & Radonjić, 2010). During this survey pepper was detected as the only vegetable host. Although *P. latus* is highly polyphagous, pepper has been shown to be the most susceptible host (www.sardi.sa.gov.au, Fan & Petitt, 1998, Albajes et al., 1999, Angelini et al., 2003, Zhang, 2003).

Presence of *L. bryoniae* was officially confirmed in 2006 from infested tomato leaves. The adult flies were also found to be captured on yellow sticky traps. In the period 2007 to 2010 the pest spread widely and was confirmed from all inspected localities on tomato, cucumber and mangold. Visual inspection of sampled leaves showed higher infestations of cucumber and tomato than mangold. Our results are similar with those in Serbia (Dimić & Perić, 2003; Spasib, 2003).

After *F. occidentalis* was detected in June 2008 on cucumber leaves and flowers as well on blue sticky traps in locality Zeta, in the few next months it spread rapidly in localities of the city of Podgorica, Zeta and Bjelopavlići. Apart from cucumber, it was also found on pepper and mangold. In next two years the pest was detected in localities along the seacoast (Ulcinj, Bar and Tivat-Radanovići) on same host plants. At the end of July 2009, *F. occidentalis* was found outdoors in Ulcinj and Bar, in the flowers of weeds *Inula britanica*, *Picris hieracioides* and *Cichorium intybus*, near greenhouses (Hrnčić et al., 2009). *Frankliniella occidentalis* showed quick spreading and was noticed to be primarily dangerous for pepper, cucumber and mangold production.
Table 1. Newly established pests listed per locality, crop species, year(s) of detection and number of greenhouses where pest is found related to number of inspected greenhouses with certain host plant.

<table>
<thead>
<tr>
<th>Locality</th>
<th>No. of inspected sites</th>
<th>Crop species</th>
<th>Detected pests</th>
<th>Year of detection</th>
<th>No. of greenhouses with pest</th>
</tr>
</thead>
<tbody>
<tr>
<td>City of Podgorica</td>
<td>2</td>
<td>Tomato</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. latus</em></td>
<td>2006</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Pepper</td>
<td><em>T. cinnabarinus</em></td>
<td>2009-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. occidentalis</em></td>
<td>2008-2010</td>
<td>2/3</td>
</tr>
<tr>
<td>Zeta</td>
<td></td>
<td>Mangold</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. occidentalis</em></td>
<td>2008-2010</td>
<td>3/3</td>
</tr>
<tr>
<td>Bjelopavlići</td>
<td>4</td>
<td>Tomato</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>T. cinnabarinus</em></td>
<td>2009-2010</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pepper</td>
<td><em>P. latus</em></td>
<td>2009</td>
<td>2/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. occidentalis</em></td>
<td>2008-2010</td>
<td>3/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. occidentalis</em></td>
<td>2008-2010</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mangold</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
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<tr>
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<td></td>
<td><em>F. occidentalis</em></td>
<td>2008-2010</td>
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<tr>
<td>Ulcinj</td>
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<td>Tomato</td>
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<td>2007-2010</td>
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<td>Pepper</td>
<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
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<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
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<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
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<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
<td>2/2</td>
</tr>
<tr>
<td>Bar</td>
<td>2</td>
<td>Tomato</td>
<td><em>T. cinnabarinus</em></td>
<td>2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pepper</td>
<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cucumber</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>2/2</td>
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<td></td>
<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mangold</td>
<td><em>L. bryoniae</em></td>
<td>2007-2010</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
<td>2/2</td>
</tr>
<tr>
<td>Tivat-Radanovići</td>
<td>1</td>
<td>Tomato</td>
<td><em>L. bryoniae</em></td>
<td>2006-2010</td>
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<tr>
<td></td>
<td></td>
<td>Pepper</td>
<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
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</tr>
<tr>
<td></td>
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<td>Mangold</td>
<td><em>L. bryoniae</em></td>
<td>2006-2010</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. occidentalis</em></td>
<td>2009-2010</td>
<td>1/1</td>
</tr>
</tbody>
</table>
*Tetranychus cinnabarinus* was detected in the middle of July in 2009 on tomato, in locality Zeta, and at the beginning of October in locality Bjelopavlići. During 2010 it was confirmed from the same localities and host plant as in the previous year, and additionally found on tomato in locality Bar. The importance and serious potential threat for tomato production caused by *T. cinnabarinus* was reported by Kielkiewicz (1996) who stressed that in Polish greenhouses *T. urticae* was gradually replaced by *T. cinnabarinus*.

Apart from newly detected pests, during this survey some of previously known pests were confirmed from all inspected localities. Presence of lepidopterous species *Helicoverpa armigera* and *Ostrinia nubilalis* were detected from the middle of July until the end of September in pepper fruits and, additionally, *H. armigera* in tomato fruits, polyphagous aphids (*Myzus persicae*, *Aphis gossypii* and *A. fabae*), whitefly *Trialeurodes vaporariorum* and two-spotted mite *Tetranychus urticae* were detected on all inspected vegetable crops.

This survey showed that among newly detected vegetable pests, *F. occidentalis* and *L. bryoniae* had the most quick spreading and were detected from all inspected localities. *Frankliniella occidentalis* caused the most serious damages which affected cucumber and pepper flowers, pepper fruits, and cucumber and mangold leaves.

**Acknowledgements**

This study would not have been possible without the support of Phytosanitary Directorate of Montenegro. We thank our technicians Milorad Račević and Branislav Vučković for the help in field and laboratory work and vegetable producers that allowed us to sample infested plants.

**References**


www.sardi.sa.gov.au
Immersion treatments for imported poinsettia cuttings to control sweetpotato whitefly, *Bemisia tabaci* (Gennadius) biotype “B” in greenhouses

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**Abstract:** Hot water, insecticidal soap and horticultural oil immersion treatments were assessed for phytotoxicity and their efficacy in controlling sweetpotato whitefly (SPW) [*Bemisia tabaci* (Gennadius) biotype “B”] on poinsettia cuttings. The efficacy of the entomopathogenic fungus *Beauveria bassiana* also was examined. Acetamiprid was evaluated as a comparison.

Hot water provided 0-20% mortality of SPW life stages; insecticidal soap 93-100% mortality; horticultural oil 99-100% mortality; and *Beauveria bassiana* 3-30% mortality. Acetamiprid provided 5-63% mortality.

Various treatments identified in this study can be considered as moderate to highly efficacious, and have potential to be incorporated into a pest management program to control SPW on poinsettia cuttings, with minimal harm to plant material and reduced impact on biological control agents (BCA).

**Key words:** cuttings, *Bemisia tabaci* biotype B, hot water, insecticidal soap, horticultural oil, *Beauveria bassiana*, acetamiprid

**Introduction**

Vegetative cuttings are a potential pathway for global movement of insect pests. Inevitably, through local, national and international trade of cuttings, pests are introduced to greenhouses worldwide (Frey, 1993). This situation can result in introduction of cosmopolitan insect pests, insecticide resistant insect strains and/or invasive alien species. There also is a potential negative impact on biological control agents (BCAs) if insecticides were applied to stock plants prior to cuttings being taken or if they need to be applied to control incoming pests (Murphy, 2009).

Anecdotal evidence suggests that sweetpotato whiteflies (SPW) are introduced on imported poinsettia cuttings. Identifying efficacious treatments of cuttings would permit growers to establish insect pest-free cuttings or cuttings with substantially reduced pest levels.

The objectives of this study were to: 1) identify hot water, insecticidal soap and horticultural oil immersion treatments that were not phytotoxic to poinsettia; and 2) evaluate the above treatments as well as the entomopathogenic fungus *B. bassiana*, for their efficacy against SPW.

**Material and methods**

**Phytotoxicity study**

Poinsettia cuttings cultivar ‘Prestige’ were immersed in hot water at temperatures of 35-43°C for 5-60 minutes, and transferred to a cooling bath (21°C) immediately after for 1 min; or
immersed in insecticidal soap (Safers® Insecticidal Soap Concentrate – potassium salts of fatty acids 51%, Woodstream Canada Corp.) at concentrations of 5-40 ml/l for 1 min; or horticultural oil (Landscape® Oil – mineral oil 99%, Plant Products Co. Ltd.) at concentrations of 2.5-30ml/l for 1 min. For the oil treatments and higher concentrations of soap, additional treatments were done followed by a 1 min rinsing in 21°C water to remove any excess product. After immersion, cuttings were rooted in Oasis® strips under mist. Phytotoxicity was evaluated after 20 days based on survival and number of roots.

**Efficacy study**
A SPW colony was established and maintained in a growth chamber at 25-26°C, 50-60% RH and 14:10 h L:D. The whiteflies were reared on vegetative plants of poinsettia cultivar ‘Prestige’. Eggs, early nymphs or late nymphs were immersed in non-phytotoxic treatments of hot water, insecticidal soap or horticultural oil. In addition, registered label rates for foliar application of Botanigard® 22WP (0.6g/l) [B. bassiana (Balsamo) Vuillemin Strain GHA, Koppert Canada Ltd.] and acetamiprid (130mg/l) was tested using the same methodology. Mortality of eggs and nymphs was assessed 5 and 10 days, respectively, after immersion.

**Data analysis**
Treatments were classed as non-phytotoxic if survival ≥80% and root numbers were not significantly different to those of the control. Treatment efficacy was based on SPW mortality and classed as highly efficacious (> 80% mortality), moderately efficacious (50-80% mortality), or non-efficacious (< 50% mortality). Corrections for natural mortality were made using Abbott’s formula (Abbott, 1925). All data were subject to an ANOVA using PROC GLM (SAS Institute 2009) and means were separated using Tukey’s LSD multiple means comparison test (α = 0.05).

**Results and discussion**

**Phytotoxicity study**
Hot water treatments at 35°C for 15, 30 and 60 min, 39°C for 15 min, and 41°C for 5 min were non-phytotoxic to poinsettia cuttings (data not shown). Insecticidal soap treatments at 5, 10, 20 and 40ml/l without rinsing and 20 and 40ml/l followed by a 1 min rinsing in 21°C water were non-phytotoxic. Horticultural oil treatments at 2.5ml/l without rinsing and 5ml/l followed by a 1 min rinsing in 21°C water were non-phytotoxic. These treatments along with B. bassiana at 0.6g/l were used for the efficacy study on SPW.

**Efficacy study**
Hot water was non-efficacious in controlling SPW (Fig. 1). Insecticidal soap treatments (5 and 20ml/l no rinse), were highly efficacious against early and late nymphs; horticultural oil (2.5ml/l no rinse) was highly efficacious against early nymphs and horticultural oil (5ml/l plus water rinse) was highly efficacious against eggs, early nymphs and late nymphs (Fig. 2). Acetamiprid was moderately efficacious against SPW eggs, but not other life stages (Fig. 2). BotaniGard 22WP (0.6g/l) was non-efficacious against eggs, early or late nymphs (Fig. 3).
Figure 1. Corrected mean percent mortality (± SE) of the sweetpotato whitefly (SPW) after hot water immersion. Means with the same letter within a life stage are not significantly different (α=0.05, Tukey’s LSD).

Figure 2. Corrected mean percent mortality (± SE) of the sweetpotato whitefly (SPW) after immersion in insecticidal soap (I. soap), horticultural oil (H. oil) or acetamiprid. Means with the same letter within a life stage are not significantly different (α=0.05, Tukey’s LSD).
Ontario poinsettia growers have great interest in using BCAs to mitigate SPW infestations. Inevitably, whitefly populations on imported cuttings can reduce the chance of success of biological control programs (Murphy et al., 2008). In an attempt to start propagation with “clean” cuttings, the use of conventional insecticides to mitigate insect pest infestations can have a negative impact on BCAs. The use of effective reduced risk control products for cuttings prior to propagation can provide the solution for a clean and safe start. This study demonstrates the potential for insecticidal soap or horticultural oil immersion treatments to reduce SPW numbers on poinsettia cuttings. These strategies have the advantage of a quick contact kill, no likelihood of the development of resistance and no residues that would impact the subsequent use of BCA. In fact, the efficacy of BCAs could be enhanced by the reduced populations of SPW.

Acknowledgements

We thank the participation of greenhouse growers from the Niagara area of Ontario. Funding was provided by the Canadian Greenhouse Conference, Plant Products Co. Ltd., a MITACS Accelerate Internship sponsored by Flowers Canada Ontario to W. Romero and a NSERC Industrial Postgraduate Scholarship 1 sponsored by Flowers Canada Ontario to W. Romero.

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Murphy, G. D., Short, M., Cooper, A. M., Fast, M., & Neal, D. 2008: Biological control of whitefly in poinsettia in Ontario, Canada. IOBC/WPRS Bull. 32: 139-142.
Immersion treatments for imported chrysanthemum cuttings to control western flower thrips, *Frankliniella occidentalis* (Pergande) in greenhouses

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Abstract: In Ontario, preliminary assessments suggest that substantial numbers of western flower thrips (WFT) [*Frankliniella occidentalis* (Pergande)] are introduced on imported chrysanthemum cuttings. Because of the difficulty in detecting thrips at this stage of production and controlling them as needed, an alternative approach is to treat all cuttings before they enter the greenhouse. This study evaluates the strategy of immersing cuttings prior to propagation using various treatments considered as reduced risk.

Hot water, insecticidal soap and horticultural oil immersion treatments were assessed for phytotoxicity and their efficacy in controlling WFT. The efficacy of the entomopathogenic microbials *Beauveria bassiana* and *Steinernema feltiae* also was examined. Spinosad was evaluated as a comparison.

Hot water treatments provided 67-98% mortality of WFT life stages; horticultural oil 67-100% mortality; *B. bassiana* 93-95% mortality; and *S. feltiae* 53-63% mortality. Spinosad provided 27-58% control of larvae and adults.

Key words: cuttings, *Frankliniella occidentalis*, hot water, insecticidal soap, horticultural oil, *Beauveria bassiana*, *Steinernema feltiae*, spinosad

Introduction

Vegetative cuttings are a potential pathway for global movement of insect pests. Inevitably, through local, national and international trade of cuttings, these pests are introduced to greenhouses worldwide (Frey, 1993; Kirk & Terry, 2003). This situation can result in introduction of cosmopolitan insect pests, insecticide resistant strains and/or alien invasive species (Murphy, 2009). There also is a potential negative impact on biological control agents (BCAs) if insecticides need to be applied to control incoming pests. In addition, when cutting producers apply insecticides to stock plants, persistent residues may remain active against BCAs for a number of weeks (Murphy, 2009).

Preliminary studies in Ontario suggest that substantial numbers of WFT are introduced on imported chrysanthemum cuttings (Romero, 2011). Identifying efficacious treatments for cuttings would permit growers to establish pest-free (or substantially reduced pest levels) cuttings from the onset.

The objectives of this study were to: 1) identify hot water, insecticidal soap and horticultural oil immersion treatments that were not phytotoxic; and 2) evaluate the above treatments as well as the microbial products *B. bassiana* and *S. feltiae* for their efficacy against WFT.
Material and methods

Phytotoxicity study
Chrysanthemum cuttings cultivar ‘Sunny Shasta’ were immersed in hot water at temperatures of 35-43°C for 5-60 minutes, and transferred to a cooling bath (21°C) for 1 min; or immersed in various concentrations (5-40ml/l) of insecticidal soap (Safer’s® Insecticidal Soap Concentrate–potassium salts of fatty acids 51%, Woodstream Canada Corp.) for 1 min; or in various concentrations (5-30ml/l) of horticultural oil (Landscape® Oil –mineral oil 99%, Plant Products Co. Ltd.) for 1 min. After immersion, cuttings were rooted in Oasis® strips under mist. Phytotoxicity was evaluated 10 d after immersion based on cutting survival and number of roots.

Efficacy study
A WFT colony was established using thrips from infested imported cuttings, and was maintained in a growth chamber at 25-26°C, 50-60% RH and 12:12h L:D. Thrips were reared on potted plants of yellow flowering chrysanthemum cultivars ‘Golden Gate’ or ‘Chesapeake’. Larvae or adults of WFT were immersed in non-phytotoxic treatments of hot water, insecticidal soap or horticultural oil inside vented Petri dishes. In addition, registered label rates for foliar application of Botanigard® 22WP (1.2g/l) [B. bassiana (Balsamo) Vuillemin Strain GHA, Koppert Canada Ltd.], Steinernema-System® (1x10⁶ IJ/l) [S. feltiae (Filipjev), Biobest Canada Ltd.) or spinosad (50µl/l based on foliar application rates; Success® 480 SC, Dow AgroSciences) were tested using the same methodology. Mortality was assessed 24h after immersion, except for B. bassiana, which was assessed at 48h.

Data analysis
Phytotoxicity of treatments was evaluated based on cutting survival and number of roots. Treatments were classed as non-phytotoxic if survival ≥80% and root numbers were not significantly different to those of the control. Treatment efficacy was based on WFT mortality and classed as highly efficacious (> 80% mortality), moderately efficacious (50-80% mortality), or non-efficacious (< 50% mortality). Corrections for natural mortality were made using Abbott’s formula (Abbott 1925). All data were subject to an ANOVA using PROC GLM (SAS Institute 2009), means were separated using Tukey’s LSD multiple means comparison test at a significance level of α = 0.05.

Results and discussion

Phytotoxicity study
Hot water treatments at 35°C for 15, 30, 45 and 60 min, 39°C for 15 and 30 min, and 41°C for 5 and 15 min were non-phytotoxic to chrysanthemum cuttings (data not shown). Insecticidal soap treatments at 5, 10, 20 and 40ml/l and horticultural oil treatments at 5, 10, 20 and 30 also were non-phytotoxic (data not shown). These treatments along with the microbial products mentioned above were used for the efficacy study on WFT.

Efficacy study
Preliminary screening trials showed that immersion treatments in hot water below 39°C were non-efficacious in controlling WFT within a practical period of time. Hot water immersion at 39°C for 30 min and 41°C for 15 min were moderately to highly efficacious in controlling larvae and adults (Fig. 1). Hot water also reduced WFT egg hatch by 75% (data not shown).
Insecticidal soap at all concentrations and spinosad as an immersion treatment were non-efficacious against larvae and moderately efficacious against adults (Fig. 2). Horticultural oil at all concentrations was moderately to highly efficacious against larvae and adults (Fig 3.). BotaniGard 22WP was highly efficacious against larvae and adults (Fig. 4), but the 48h time frame required for efficacy is unlikely to be feasible. Steinernema-System was moderately efficacious against larvae and adults.

This study demonstrates the potential for hot water or mineral oil immersion treatments to reduce WFT numbers on imported chrysanthemum cuttings. These strategies have the advantage of a quick contact kill, no likelihood of the development of resistance and no residues that would impact the subsequent use of BCAs. In fact, the efficacy of BCAs could be enhanced by the reduced populations of WFT.

![Graph](image1.png)

**Figure 1.** Mortality (± SE) of western flower thrips (WFT) 24h after hot water immersion. Means with the same letter within a life stage are not significantly different (α=0.05, Tukey’s LSD).

![Graph](image2.png)

**Figure 2.** Mortality (± SE) of western flower thrips (WFT) 24h after immersion in insecticidal soap (I. soap) or spinosad. Means with the same letter within a life stage are not significantly different (α=0.05, Tukey’s LSD).
Figure 3. Mortality (± SE) of western flower thrips (WFT) 24h after immersion in horticultural oil (H. oil). Means with the same letter within life stage are not significantly different (α=0.05, Tukey’s LSD).

Figure 4. Corrected mean percent mortality (± SE) of western flower thrips (WFT) 48h after immersion in BotaniGard® 22WP or Steinernema-System®. Means followed by the same letter within life stage are not significantly different (α=0.05, Tukey’s LSD).

Acknowledgements

We thank the participation of several greenhouse growers from the Niagara area of Ontario. Funding for this research was provided by the Canadian Greenhouse Conference, Plant Products Co. Ltd., a MITACS Accelerate Internship sponsored by Flowers Canada Ontario (FCO) to W. Romero and a NSERC Industrial Postgraduate Scholarship 1 sponsored by FCO to W. Romero.
References

Comparing *Aphidius colemani* and *Aphidius matricariae* on *Myzus persicae* ssp. *nicotianae* in sweet pepper

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**Abstract:** The performance of the aphid parasitoids *Aphidius colemani* and *Aphidius matricariae* was compared on *Myzus persicae* ssp. *nicotianae*. The number of mummies produced per female was significantly higher at 15°C for *A. matricariae*. At 20, 25 and 30°C no differences were found. On sweet pepper plants, *A. matricariae* was significantly faster in controlling aphids compared to *A. colemani*.

**Key words:** biological control, *Myzus persicae nicotianae*, *Aphidius colemani*, *Aphidius matricariae*, sweet pepper

**Introduction**

The green peach aphid *Myzus persicae* Sulzer is one of the most common aphid pests in greenhouse sweet peppers in the temperate zone. Besides the green form, also a dark red form, *Myzus persicae* ssp. *nicotianae* Blackman, also known as the tobacco aphid, has recently become a serious pest. The taxonomic status of this aphid is still unclear. Blackman (1987) first described it as a separate species by comparing many morphological characters. However, other authors consider these forms as only one species with different phenotypes (Gillespie et al., 2010).

In most Dutch sweet pepper greenhouses both types of *M. persicae* appear. The green and sometimes light reddish *M. persicae* sensu stricto are mostly located in the lower parts of the plant. The typical dark red form of *M. persicae* ssp. *nicotianae* (*M. p. n.*) has the tendency to make more dense colonies in the top of the plant and in the flowers. Therefore it makes biocontrol with parasitoids difficult. Parasitic wasps seem to have problems with entering dense colonies. Until recently *Aphidius colemani* Viereck was the most preferred parasitoid used against *M. persicae*. However the choice for mass rearing this species was made in 1991 when this aphid species was less of a problem and the red form was not occurring at all. Main focus at that time was on the cotton aphid *Aphis gossypii* Glover in cucumber. Because *A. colemani* is more effective in controlling cotton aphid than the earlier used *Aphidius matricariae* Haliday (van Steenis, 1995), *A. colemani* was chosen to be mass reared as a biocontrol agent for both aphid species (van Schelt, 1994). With the increasing incidence of *Myzus* in sweet pepper we decided to evaluate both *A. colemani* and *A. matricariae* again on *M. p. n.* The fecundity at different temperatures was assessed, and the ability of the parasitoids to control *M. p. n.* populations on sweet pepper plants was compared in cage trials.
Material and methods

Fecundity test
The fecundity of *A. colemani* and *A. matricariae* was tested at different temperatures on leaf discs on agar following the procedure described by van Lenteren *et al.* (2003). Wasps were allowed to mate and fed with honey during 24 hours before testing. Individual female wasps were offered in total 80-100, 2nd and 3rd instar nymphs of *M. p. n.* After 24 hours wasps were removed and the trays further incubated at the test temperature. Every trial was carried out in 25 replicates. Test temperatures were 15, 20, 25 and 30°C at a RH of 70%. After 7 to 14 days, depending on the temperature, the total amount of mummies formed was counted. The results were compared using the Mann-Whitney test (*p* < 0.05).

Greenhouse cage experiment
We conducted a cage experiment in December 2010 to evaluate the effects of *A. colemani* and *A. matricariae* on *M. p. n.* in sweet pepper. Two sweet pepper plants (cv Spider), planted in 10 litre pots with peat, were put in a cage of 2 x 1 x 2m and comprised one treatment. The plants were approximately 1 meter high and started flowering at the start of the experiment. Each treatment was replicated 3 times. Per cage 250 *M. p. n.* were introduced on 8 spots in the upper part of the plants. After three days the aphids had multiplied to about 500 (all stages) per cage. In every cage 25 mated female wasps of maximum 24 hours age were released (ratio 1 female parasitoid: 20 aphids). The aphids and mummies (cumulative) of the parasitoid wasps were counted on 10 leaves per plant every week during six weeks.

*A. colemani* was reared on *A. gossypii* on cucumber by Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands). This strain was originally collected in a Dutch cucumber greenhouse. *A. matricariae* was reared on *M. persicae* and supplied by Viridaxis (Gilly, Belgium). The origin of this strain is Italy. The mean temperature during the trial was 20.4°C and the relative humidity 58%.

A repeated measures ANOVA was performed on Log (x+1) transformed numbers of aphids and mummies. Differences among treatments were tested at the 5% level using Fisher’s LSD (Least Significant Difference) method.

Results and discussion

Fecundity test
The number of mummies per female was significantly higher at 15°C for *A. matricariae* (*p* < 0.05). At the other temperatures, no significant differences were found. At 30°C both parasitoids produced only a few mummies with a delayed development and high mortality (Table 1). These data are partly in line with other published results. Zamani *et al.* (2007) tested Iranian strains of *A. colemani* and *A. matricariae* on *M. persicae* and *A. gossypii*. They concluded that *A. colemani* is more adapted to high temperatures with *A. gossypii* as host. However, on *M. persicae* no significant differences were found at all tested temperatures. Giri *et al.* (1982) tested an introduced American strain of *A. matricariae*. They found an optimum for most parameters at 21°C and a rapid decline above 24°C.

Greenhouse cage experiment
Aphid numbers were suppressed significantly faster by *A. matricariae* than by *A. colemani* (*p* < 0.01, Fig. 1). *A. matricariae* reduced the aphid population to almost zero within two weeks. *A. colemani* however took 5 to 6 weeks to suppress the population. This indicates that *A. matri-
cariae could control the aphids within one generation, while A. colemani needed 2 to 3 generations. Consequently the number of mummies per leaf was quite low with A. matricariae and rose to 100 per leaf for A. colemani at the end (Fig. 2). Though not presented in this paper this type of cage experiments were repeated several times with always the same type of population development: A “small hick-up” in aphid development with A. colemani and a quick decrease in aphid numbers with A. matricariae.

Table 1. Mean number of mummies per female wasp (± s.e.m.), tested individually at different temperatures.

<table>
<thead>
<tr>
<th></th>
<th>Mean number of mummies per female ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15°C</td>
</tr>
<tr>
<td>Aphidius matricariae</td>
<td>49.0 ± 3.2a</td>
</tr>
<tr>
<td>Aphidius colemani</td>
<td>37.3 ± 3.9b</td>
</tr>
</tbody>
</table>

Numbers in the same column followed by different letters are significantly different at P<0.05

Figure 1. Population dynamics of M. persicae nicotianae (± s.e.m.) in cages with sweet pepper plants with the parasitoids A. colemani or A. matricariae.

Figure 2. Population dynamics of the mummies of M. persicae nicotianae (± s.e.m.) in cages with sweet pepper plants with the parasitoids A. colemani or A. matricariae.
Conclusions
The better performance of *A. matricariae* on *M. p. n.* compared to *A. colemani* could not be explained by differences in fecundity at low temperatures, because the greenhouse trial was conducted at 20°C. Other characteristics of the aphid parasitoids, such as behaviour in dense aphid colonies, might play a role as well. One of the observations made was that *A. matricariae* seems to enter the colonies of *M. p. n.* more readily, especially in the flowers. However we have not been able to quantify this behaviour so far.

Because of the rapid impact of *A. matricariae*, the number of mummies in a commercial greenhouse can be maintained at a low level. This will diminish the chance of building up hyperparasite populations, which can severely disrupt the biocontrol of aphids at the end of spring. Therefore the use of *A. matricariae* is recommended to control *M. persicae* in pepper and other crops. The impact of high summer temperatures on the population of *A. matricariae* is still a concern. We are screening other parasitoids which may be more adapted to high temperatures.

References


Monitoring of western flower thrips under supplemental lighting conditions for greenhouse mini cucumbers

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Abstract: The natural infestation levels of Frankliniella occidentalis were monitored weekly using yellow sticky cards for greenhouse mini cucumbers grown under supplemental lighting using high pressure sodium lights (HPS) and light emitting diodes (LED) lights. The presence of LED lights alone increased the number of F. occidentalis collected. With respect to the LED treatments, blue was generally the most attractive.

Key words: supplemental lighting, high pressure sodium lights, light emitting diode lights, Frankliniella occidentalis

Introduction

In northern temperate regions, the production of greenhouse vegetables on a year-round basis is major priority among greenhouse growers in order to maintain market share through continuity of product supply. To achieve production during the winter months, supplemental lighting must be used to extend daylength and provide adequate lighting for plant growth and fruit maturation. To date, high-pressure sodium lights (HPS) have been the light source of choice. However, light emitting diodes (LED) are becoming increasingly popular due to their improved efficiency and the fact that individual wavelengths can be used. With respect to LEDs, red and blue wavelengths have been the most investigated by plant physiologists because of their influence on plant photosynthesis, plant growth and fruit development. However, little information is known about the impact of supplemental lighting on arthropod pests and beneficials (Vänninen et al., 2010; Johansen et al., 2011). Numerous studies have found western flower thrips (Frankliniella occidentalis) to be more attracted to low ultraviolet reflective white, blue and yellow traps compared to green, red, orange or black (Johansen et al., 2011). The present study examines the monitoring of F. occidentalis using yellow sticky cards for greenhouse mini cucumbers grown under supplemental lighting with HPS and LED lights.

Materials and methods

The trials were conducted in three greenhouse compartments (7.0 x 7.8m) at the Greenhouse and Processing Crops Research Centre, Harrow, Ontario from December 6, 2010 to April 15, 2011. The crop was mini greenhouse cucumbers (cv. Picowell) which were grown according to standard high wire production practices in raised gutter with rockwool as the growing medium. Two of the greenhouses were supplied with HPS above the crop canopy and the other greenhouse without HPS. Each greenhouse was divided into four quadrants corresponding to the LED treatments. The LED lights were located in the bottom third of the
crop canopy. The lighting treatments were: 2 greenhouses – HPS-No LED, HPS-White LED, HPS-Blue LED and HPS-Red LED and one greenhouse – No HPS-No LED and No HPS-Mixed blue and red LED (2 quadrants per treatment). The HPS/LED lights were operated between 0:00-18:00h, but both were turned off when the outside light level was >300W/m². In the greenhouse without HPS, the mixed LED lights operated for 20h per day regardless of the outside lighting conditions.

To monitor the naturally-occurring infestation of *F. occidentalis*, two yellow sticky cards (12.5 x 7.5cm) were suspended in the crop canopy in each quadrant. One trap was placed at the top of the canopy and the other trap in the bottom third (20cm above the LED light). The traps were replaced weekly and the number of *F. occidentalis* recorded. On 2 March, the HPS were permanently turned off due to the higher level of natural radiation and longer daylengths. The weekly counts from each treatment were analyzed using a repeated-measures analysis of variance (ANOVA) (SPSS Inc., 2004). All data were transformed log₁₀(x+1). For statistical testing, the weekly counts obtained from three consecutive weeks were consolidated into one group and used as replicates. Treatment means were compared using the Tukey test (α = 0.05).

Results and discussion

The pattern for natural infestation levels of *F. occidentalis* for the three greenhouses was similar and is illustrated in Figure 1 for one of the HPS greenhouses.

![Figure 1](image.png)

Figure 1. The number of *F. occidentalis* collected weekly on yellow sticky cards in greenhouse mini cucumbers grown under supplemental lighting (HPS and LED).
In general, the presence of LED lights increased the number of *F. occidentalis* collected on the sticky cards. This relationship was most evident with the sticky cards located near the LED lights. With respect to the treatment combinations, blue and white LED lights resulted in higher numbers of *F. occidentalis* on the cards with blue consistently the highest (Tables 1, 2, and 3).

Table 1. Sticky trap catches (Mean ± S.E.) of *F. occidentalis* among supplemental light treatments in two greenhouses from December 6, 2010 to March 2, 2011.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Greenhouse 1</th>
<th>Greenhouse 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top canopy</td>
<td>Mid canopy</td>
</tr>
<tr>
<td>HPS - No LED</td>
<td>3.1 ± 1.0 a</td>
<td>1.8 ± 0.6 a</td>
</tr>
<tr>
<td>HPS - LED Red</td>
<td>3.3 ± 0.8 a</td>
<td>1.0 ± 0.3 a</td>
</tr>
<tr>
<td>HPS - LED White</td>
<td>5.8 ± 0.9 b</td>
<td>6.4 ± 1.2 b</td>
</tr>
<tr>
<td>HPS - LED Blue</td>
<td>10.6 ± 2.9 b</td>
<td>3.8 ± 0.7 b</td>
</tr>
</tbody>
</table>

1 $F_3, 8 = 6.591, p = 0.015$ (Significant). 2 $F_3, 8 = 18.264, p = 0.001$ (Significant). 3 $F_3, 8 = 0.160$ (Not significant). 4 $F_3, 8 = 15.064, p = 0.001$ (Significant).

Table 2. Sticky trap catches (Mean ± S.E.) of *F. occidentalis* among supplemental light treatments in two greenhouses from March 2 to April 15, 2011.

<table>
<thead>
<tr>
<th>Treatment (No HPS)</th>
<th>Greenhouse 1</th>
<th>Greenhouse 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top canopy</td>
<td>Mid canopy</td>
</tr>
<tr>
<td>No LED</td>
<td>12.7 ± 2.0 a</td>
<td>8.8 ± 1.0 a</td>
</tr>
<tr>
<td>LED Red</td>
<td>10.7 ± 1.6 a</td>
<td>6.2 ± 1.0 a</td>
</tr>
<tr>
<td>LED White</td>
<td>19.8 ± 3.2 a</td>
<td>8.9 ± 1.9 a</td>
</tr>
<tr>
<td>LED Blue</td>
<td>69.0 ± 18.8 b</td>
<td>23.5 ± 8.4 b</td>
</tr>
</tbody>
</table>

1 $F_3, 8 = 24.910, p = 0.001$ (Significant). 2 $F_3, 8 = 4.999, p = 0.031$ (Significant). 3 $F_3, 8 = 3.974, p = 0.053$ (Not significant). 4 $F_3, 8 = 15.865, p = 0.001$ (Significant).

Table 3. Sticky trap catches (Mean ± S.E.) of *F. occidentalis* for two light treatments (LED and no LED) without any HPS from December 6, 2010 to April 15, 2011.

<table>
<thead>
<tr>
<th>Treatment (No HPS)</th>
<th>Greenhouse 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top canopy</td>
</tr>
<tr>
<td>No LED</td>
<td>60.9 ± 16.8</td>
</tr>
<tr>
<td>LED Red and Blue</td>
<td>61.8 ± 15.9</td>
</tr>
</tbody>
</table>

1 $F_1, 10 = 0.593, p = 0.459$ (Not significant). 2 $F_1, 10 = 65.593, p = 0.0001$ (Significant).

In commercial rose greenhouses, more *F. occidentalis* were collected on yellow sticky cards under LED lights with a combination of red and blue wavelengths compared to just HPS (Johansen & Smith-Eriksen, 2010). Electroretinogram studies for *F. occidentalis* have shown spectral peak responses in the ultraviolet range and one at 540nm (green-yellow).
(Matheson et al., 1992). Thus, *F. occidentalis* is not attracted to red wavelengths which agrees with the results found in the present study. The attractiveness of the yellow sticky cards may in part be due to the contrast of the yellow against a blue background (Vernon & Gillespie 1995). Chen et al. (2004) has shown that blue sticky cards equipped with blue LED lights collected more *F. occidentalis* than cards without LEDs.

In conclusion, as the use of supplemental lighting increases for greenhouse crop production, we also need to improve our knowledge of how pests and beneficials are affected by these new environmental conditions. In addition, the use of LED lights with or LED-equipped sticky cards with trap plants may be useful in increasing the efficiency of this control strategy.

References


Abstract: *Praon volucre* parasitizes several species of aphids in Brazil, mostly belonging to the tribe Macrosiphini. This study aimed to evaluate the quality of *Myzus persicae* as host for *P. volucre*. Experiments were conducted in a climatic chamber at 22±1°C, RH 70±10%, and a 12-h photophase. One 24h-old *P. volucre* female, mated and without previous oviposition experience was released into a Petri dish containing a *Nicandra* leaf disk (5cm diameter) on an agar/water solution (1%) and 20 second- and third-instar *M. persicae* nymphs during 90 minutes. The developmental time of the parasitoid was 16.8 days. Immature mortality was 36.5%, parasitism 57.5%, and emergence rate was 63.5%. The sex ratio expressed as fraction females was 0.37. The longevities of male and female were 16.3 and 20 days, respectively. The tibia length of parasitoids was on average 0.6mm (females) and 0.54mm (males). These results show that *M. persicae* is parasitized by *P. volucre*. However, the low sex ratio and the high immature mortality indicate that *M. persicae* is not a good quality host for *P. volucre*.

Key words: host-parasitoid relationship, developmental time, mortality, rate of parasitism, parasitoid tibia length

Introduction

Among the main Aphidiinae parasitoids species evaluated and selected for aphid biological control in Brazil, two species stand out: *Aphidius colemani* and *Lysiphlebus testaceipes* (Bueno, 2005). However, according to Sidney *et al.* (2010a) and Silva *et al.* (2009) the species *Praon volucre* shows potential to be used for biological control of aphids belonging to the tribe Macrosiphini, such as *Aulacorthum solani* and *Macrosiphum euphorbiae*. Starý *et al.* (2007) reported *P. volucre* developing in at least 10 different aphid species from Minas Gerais, a southeastern region in Brazil. One of the species parasitized was *M. persicae*.

Several characteristics of *P. volucre* have been studied in Brazil, like rate of parasitism and developmental time, quality of different aphid species for this parasitoid, storage of the parasitoid at low temperatures, its fertility life table and thermal requirements (De Conti *et al.*, 2008; Silva *et al.*, 2009; Sidney *et al.*, 2010b). However, the rate of parasitism, developmental time, mortality and sex ratio of this parasitoid where not yet determined with *M. persicae* as host.

This study aimed to evaluate the quality of *M. persicae* as host for *P. volucre*.

Material and methods

*M. persicae* populations were collected on *Nicandra* sp. plants in the field and taken to the laboratory. After identification, the aphids were transferred to Petri dishes (15cm diameter)
containing *Nicandra* sp. leaves in a 1% agar/water solution and maintained in a climatic chamber at 22±1°C, RH 70±10%, and a 12-h photophase. The *P. volucre* individuals used in the test were obtained from a stock colony maintained on *M. persicae* in the laboratory.

One 24-h-old *P. volucre* female, previously mated and without oviposition experience was released into a Petri dish (5cm diameter) containing a *Nicandra* sp. leaf disc on a 1% agar/water solution and 20 second- and third- instar *M. persicae* nymphs. The parasitoid female was allowed to remain in the Petri dish for 90 minutes; during this period the ovipositions were recorded. The dishes were then sealed with perforated PVC film, turned upside down and maintained in a climatic chamber until mummy formation. The aphids were periodically transferred to a new Petri dish containing fresh *Nicandra* sp. leaf discs as needed. Upon mummification, the aphids were put individually in glass vials (100mm × 8mm) until adult emergence. They were fed with honey and distilled water daily, deposited in the form of small droplets on the vial’s inner walls. The developmental time from oviposition to mummification and from oviposition to emergence, the emergence rate and the sex ratio were measured.

To measure the longevity of *P. volucre*, 15 males and 15 females were maintained individually in glass vials (100mm × 8mm) without hosts and fed with honey and water daily until they died. In order to determine parasitoid size, measurements of the right hind tibia of 15 male and 15 female *P. volucre* adults were made. The tibiae were removed and mounted under a coverslip on a slide with a droplet of 70% alcohol. The measurements were performed using an ocular micrometer under a microscope (100× magnification). The tibiae size data were analyzed by using an F Test at a P of 5%.

**Results and discussion**

The rate of parasitism of *M. persicae* by *P. volucre* was 57.5% (Table 1), and this value was similar to the value obtained by Silva *et al.* (2009) on *A. solani* (58.5%). However the rate we found was lower than De Conti *et al.* (2008) found with the hosts *M. euphorbiae* (87.5%) and *Uroleucon ambrosie* (74.9%). The successful development of parasitoid immatures to adults depends on host suitability. In host of good quality, the accumulation of reserves occurs throughout the parasitoid’s larval development, and positively influences adult biological characteristics, like longevity and fecundity (Boggs, 1981).

The developmental time of *P. volucre* from oviposition to mummification was 9.37 days, and from oviposition to emergence was 16.8 days (Table 1). Silva *et al.* (2009) found 7.7 and 15.8 days, respectively on *A. solani*, and Sigsgaard (2000) obtained 7.8 and 10.1 days on the aphid *Sitobion avenae*.

Emergence was 63.5% and immature mortality was 36.5% (Table 1). Reports by Starý (1989) and Silva *et al.* (2009) showed that under certain conditions, parasitoid larvae may not be able to complete their development and die before the mummy formation. Aragón *et al.* (2007) reported an emergence rate of 74.2% for *Praon* sp. on the aphid *M. euphorbiae*, and Silva *et al.* (2009) obtained 79.3% on *A. solani*.

The sex ratio was 0.37. The average longevity of *P. volucre* was 17.7 days, being 20 days for females and 16.3 days for males (Table 1). De Conti *et al.* (2008) obtained 18.4 days for *P. volucre* on *U. ambrosiae* and 19.9 days on *M. euphorbiae* as host. Silva *et al.* (2009) found 12.9 days, with 14.1 days for females and 11.8 days for males with *A. solani* as host.
Table 1. Biological parameters ($\bar{X} \pm SE$) of *Praon volucre* on the aphid *Myzus persicae* under controlled conditions (22±1°C, 70±10% RH and 12h photophase).

<table>
<thead>
<tr>
<th>Biological parameters of <em>Praon volucre</em> on <em>M. persicae</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitism (%)</td>
<td>57.5 ± 0.42</td>
</tr>
<tr>
<td>Development oviposition-mummy (days)</td>
<td>9.37 ± 0.08</td>
</tr>
<tr>
<td>Development oviposition-adult (days)</td>
<td>16.8 ± 0.11</td>
</tr>
<tr>
<td>Emergence (%)</td>
<td>63.5 ± 0.43</td>
</tr>
<tr>
<td>Sex ratio (fraction females)</td>
<td>0.37</td>
</tr>
<tr>
<td>Longevity males and females combined (days; n=30)</td>
<td>17.7 ± 0.24</td>
</tr>
<tr>
<td>Female longevity (days; n=15)</td>
<td>20 ± 0.18</td>
</tr>
<tr>
<td>Male longevity (days; n=15)</td>
<td>16.3 ± 0.25</td>
</tr>
<tr>
<td>Female hind tibia size (mm; n=15)</td>
<td>0.60 ± 0.05*</td>
</tr>
<tr>
<td>Male hind tibia size (mm; n=15)</td>
<td>0.54 ± 0.04*</td>
</tr>
</tbody>
</table>

*not significantly different by F test ($P = 0.1251$)

No significant difference was found for the size of male and female adult parasitoids adult size (Table 1). Aphidiinae females are normally larger than males, although both sexes have the same developmental time when reared on hosts of the same size (Sequeira & Mackauer, 1993; Mackauer *et al*., 1996). Thus, females grow faster and accumulate more resources per unit of time than males. De Conti *et al*. (2008) obtained tibia lengths of 0.71 and 0.78mm for *P. volucre* females and 0.62 and 0.67mm for males, when developing in the aphids *Uroleucon ambrosiae* and *M. euphorbiae*, respectively; these differences between male and female size were statistically significant. Like we found, Silva *et al*. (2009) did not find significant differences for adult size of the parasitoid *P. volucre* when developing in *A. solani* (0.67mm for females and 0.61mm for males).

These results show that *M. persicae* is parasitized by *P. volucre*. However, the low sex ratio and the high immature mortality indicate that *M. persicae* is not a good quality host for *P. volucre*.

Acknowledgements

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References


Change Laboratory for developing collective management strategies for an established and a potential alien pest species

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Abstract: Change Laboratory, a developmental work research methodology that stands at the crossroad of education, knowledge management and knowledge creation, is presented as a meta-tool for involving growers in transforming the innovation process of pest management. The tool was used in Finnish Ostrobothnia greenhouse cluster to develop collective management strategies against the greenhouse whitefly in the current conditions and in anticipation of the potential invasion of \textit{Bemisia tabaci}. We present how the whitefly problem and its ramifications were analysed and modelled in the Change Laboratory process, and introduce the initial solution concept that was developed in collaboration with growers, local advisors and researchers.

Key words: \textit{Trialeurodes vaporariorum}, \textit{Bemisia tabaci}, change laboratory, activity theory

Introduction

The Ostrobothnia greenhouse cluster with its nearly 400 nurseries produces the majority of tomatoes and cucumbers in Finland. The importance of the greenhouse whitefly \textit{Trialeurodes vaporariorum} in the area has accentuated with the increase of year-round cropping. New pests increasingly try to invade Finnish horticulture, and predicted climate change effects may favour their establishment (Lemmetty et al., 2011; Vänninen et al., 2011). With the potential invasion by \textit{B. tabaci} in mind, \textit{Trialeurodes vaporariorum} was chosen as a model pest to further the design of pre-emptive local pest management solutions that combine biocidal/ecological and sociological levels. Such solutions are envisaged to present new meta-tools that employ local specific tacit knowledge and collaboration between different actors and stakeholders. In 2009-10, a project constellation involving three levels of grower participation [\textit{sensu} Biggs & Farrington (1991)] in the innovation and research process was created: 1) management of whiteflies in conditions of new greenhouse technologies (conventional non-participatory research); 2) ecology of \textit{T. vaporariorum}, involving genetics, dispersal and biotic interactions (consultive); and 3) Change Laboratory (CL) as a tool of transforming pest management innovation processes (collaborative/collegial).

We see CL as a potential tool for empowering growers as developers of communal pest management practices to solve local problems, and as a tool of strengthening the interface of the knowledge systems of growers, advisors and scientists. We expect this to enhance participatory research with its acknowledged beneficial effects on the formulation of objectives and priorities of research projects, and improving human and social capital among participating individuals and communities (Carberry, 2001; Johnson et al., 2003). CL is firmly grounded in activity theory (Engeström et al., 1996; Engeström, 2007), but for simplicity’s sake we present the key concepts and principles of the method by applying them to the actual CL process run in village P in Ostrobothnia.
Methods: Change Laboratory method applied to the areawide whitefly problem

CL is an application of the developmental work research methodology and a strategic form of transformative intervention guided by a researcher-interventionist whose main task is to promote expansive learning (Engeström et al., 1996; Engeström, 2007). The cycle of expansive learning consists of six phases (Figure 1) that together cover the three stages of an innovation process sensu Lilja and Ashby (1999): design (stages 1-3 in Figure 1) testing (stage 4), and diffusion (stages 5-6). CL can be used for facilitating both intensive deep transformation and continuous incremental improvements. In the project, the CL was implemented in the spring of 2011, being composed of seven sessions of two hours each, plus two or three follow up sessions after the solution testing phase. In our case, of special interest was the interaction, or network relations (Engeström, 2005) between different activity systems: nurseries in P, and the advisory and research systems. The basic elements of an activity system are modelled with the activity system triangle. It models the interrelationships between subject (e.g. grower), tools, rules, division of labour, community and object which defines the very purpose of the system and serves as the motivation of the activity (Engeström, 1987). In the project, the purpose of the activity systems’ network is the communal pest management exemplified by whiteflies.

Figure 1. The phases of a change laboratory process (Engeström et al., 1996).

Gathering and interpreting mirror data

The first session is preceded by a preparatory stage that involves collecting mirror data which makes the practices and problems in the activity system visible. It serves as the first stimulus for the participants to see the challenging problems or disturbances by means of experimentally powerful examples, often video, interviews, and pictures, aiming to challenge the participants’ current convictions on the state of affairs. The following are examples of mirror data used in the CL process in P: interviews of growers and advisors, pictures showing problematic issues related to the dispersal and multiplication of the pest outdoors, graphs (e.g.
variation between companies in whitefly numbers to show differences in control efficacy). The model in the next paragraph formed one of the second stimuli to aid the participants in their thinking process and structuring the contradictions that called for solutions.

**Hypotheses-building and holistic modelling of the problem**

Mirror data reflects the problems and contradictions occurring in an activity system and serves as the first stimulus to analyse its problems (Engeström, 1987). The mirror data was collected as interviews of growers and other subjects, observations and monitoring data of whiteflies. Based on the mirror data, an initial model of problematic elements was built. The following four elements were determined to be problematic and in need of improvement: timing and release rates of biocontrol, immigration of whiteflies into greenhouses, monitoring practices, and removal and treatment practices of infested leaf material. Subsequently, employing root cause analysis and current reality tree techniques (Dogget, 2004), a more comprehensive model of the systemic problem was produced in the sessions. Such model presents an example of re-mediation, a process where the participants may change their mediators (e.g.: knowledge, theories) used to interpret problems and contradictions in their activity systems. The final model described the relationship of year-round and seasonal crops’ pest situations and the vicious circle that sustains the problems. Certain elements of the model such as the planting time of new year-round tomato crops are dictated by marked demands and cannot be changed. Such phenomena represent the basic dilemma and systemic contradiction: year-round crops ensure profitability of the industry, but sustain pest problems in the area, and new crops are planted exactly when they shouldn’t so it becomes difficult to keep them free of pests.

The second part of the model explored further reasons for too high population buildup of whiteflies in the crops: not understanding the value of regular monitoring of pests so that timing and release rates of biocontrol agents could be properly determined; and no action or damage thresholds to aid in decision-making in the local cropping conditions. Thus, there are contradictions (problems) with tools that the growers use: with insufficient tools, the shared object of the activity systems cannot be reached. Furthermore, information on whitefly numbers or biocontrol practices and their success is not systematically shared between companies. This is an example of a contradiction at the interface of activity systems.

**Results: potential outcomes from the CL intervention**

The main aim of a change laboratory is not to realize a predetermined objective or to solve an immediate visible problem, though these outcomes can be achieved. The main usefulness of CL method is in searching for the systemic causes of problems as inner contradictions in the system and to find a new way to overcome them. At the time of writing this, five sessions have been held, and the following solution concept emerged: 1) to implement a systematic monitoring system, and share information among growers so that further knowledge and thresholds could be constructed by research, 2) a webpage to share information, 3) a form of organization, a club, with meetings between the representatives of growers, advisors and researchers; and 4) a new division of labour between growers, advisors and researchers to support the generation of local solutions based on more participatory research on pest management. The remaining learning tasks are to test and evaluate the solutions package. The decision on the pest management club, for example, had not reached unanimous acceptance among all P’s growers when this article was in writing; therefore, it remains to be tested whether the six growers (key change agents) represent a sufficiently large germ cell, a qualitatively new form of the activity (Engeström, 1987), to result in truly discovering the
shared object and to prove the benefits of the new activity and thus facilitate its diffusion to the rest of the growers. Typically for an innovation diffusion process, the few pioneers adopt the new solution first, whereas the majority follows only after seeing how it works for the early adopters (Valente, 1998).

Already during the five sessions the key change agents produced several hypotheses for testing to improve the efficacy of biocontrol against whiteflies. Such encouraging signals suggest the process is proving its first benefits in creating collaborative problem-solving ties between growers, researchers and advisers. The remaining sessions, after the testing period in the summer of 2011, are expected to fine-tune the initial solution concept into a workable communal pest management strategy. If the pilot case in village P works out well, its implementation can be tried also in other villages of the region.

Acknowledgements

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Nesidiocoris tenuis as an invasive pest in Finnish tomato crops: attempt to eradicate the bugs with nicotine-based programmes

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Abstract: The effect of nicotine smoke treatment was tested against adult and nymphal stages of Nesidiocoris tenuis (Heteroptera: Miridae) in greenhouse conditions by exposing the bugs to the smoke in mesh bags and counting mortality 15 hours post-treatment. Nicotine was highly efficient against adult bugs, causing mortality of 91.4-97.7%. Nymphs were more resistant as it took longer for them to die, and due to the low number of nymphs available and higher control mortality than with adults the efficacy results were less conclusive for nymphs.

Key words: Miridae, tomato bug, pests, chemical control

Introduction

Nesidiocoris tenuis (Reuter) invaded two year-round tomato crops in the autumn of 2008 in villages A and B in the Finnish Ostrobothnia and caused substantial yield reduction after 4-5 months (cf. Arnó et al., 2010). This omnivorous bug is classified as both a pest and a beneficial agent in the CABI database (CABI, 2010). Of the several options of pathways of introduction that were considered the most likely was assumed to be contamination of Macrolophus bottles with N. tenuis. This could not be proven, however.

Subzero temperatures prevent the bugs from emigrating from greenhouses as the bugs do not tolerate cold (Hughes et al., 2010); this is likely the reason why the bugs are doing more harm than good in Finnish winter tomato crops (cf. Arnó et al., 2010; Castañé et al., 2011, in press). Eradication in winter by destroying the crops requires a demanding decision since tomato crops in Finland are most valuable exactly in winter. Since N. tenuis is not considered a dangerous quarantine pest in the EU or Finnish plant protection legislation, no reimbursement was available for growers should they have attempted eradication of the bugs by destroying the infested crops prematurely. Therefore in 2009, the bugs were kept in control using imidacloprid (IMI) drenching as the principal method until the yearly cropping break of two weeks in the first crop in village A in June. By this time, however, the bug was discovered in a second crop in village B. In October it was rediscovered in the first crop in village A as well as two new crops in village B – apparently the bugs had escaped outdoors in the summer and re-entered the new crops as soon as weather grew colder.

At this point, a decision was made to attempt eradication with chemicals in January 2010. Wide-spectrum insecticides (organophosphates, organochlorines, pyrethroids, phenylpyrazoles), either alone or in combination with neonicotinoids, are reported to have up to 100% efficacy against mirid plant bugs (e.g. Patel, 1980; Zhang et al., 2009; Ahirwar et al., 2010). These chemicals are used as sprays, however, so it is difficult to ensure good coverage in a dense crop. A fumigation treatment would ensure thorough exposure of the bugs to the chemical. On these grounds, a decision was made to apply a special permission to use...
nicotine smoke as the basis of the eradication programme. We could not find reliable information on the efficacy of nicotine smoke against *N. tenuis* or other mirid bugs in the literature. Mediterranean plant protection specialists who were consulted for chemical control of the bug had not tested nicotine, as their studies aim at the strategy of using chemicals for culling out the bugs, not eradication (Castañé *et al*., 2011, in press).

**Material and methods**

Bugs of undetermined age were collected from ‘Espero’ tomato plants in December 2010, and stored on ‘Espero’ seedlings for six days in plastic buckets closed with mesh lids. Additionally, bugs were collected from plants treated systemically with imidacloprid a week earlier to test if pre-exposure to it made the bugs more sensitive to nicotine smoke.

The bugs were aspirated from the plants in the buckets and put in 20 x 30cm mesh bags with a fresh tomato leaflet to feed upon. A total of 255 adults were exposed to nicotine, but their number per bag (see Table 1) was not equal due to difficulties in handling the bugs in greenhouse conditions. The number of nymphs in the buckets was smaller (91) and came to be highly variable between bags. The bags were hung on three levels in the crop: plant tops, middle of plants and at the base of the plant (floor level). The bags were hung in the crop so that their distance to the nicotine heaps to be smoked varied (see Figure 1 for experimental design). Temperature during the treatment was 18°C, and photoperiod L16:D8 hours.

![Figure 1. Placement of nicotine heaps (black rectangles) and mesh bags with bugs (1, 2, 3, C) in the 1.2ha greenhouse (one half shown). 1= bag in top of plant, 2=middle of plant, 3= at floor level, C=bags with bugs pre-exposed to systemic IMI. Grey areas show plant rows, white areas are isles between rows. For number of bags and bugs per bag, see Table 1.](image-url)

Untreated control bags were kept in a non-greenhouse room. Controls were not taken to the greenhouse as only uninfested compartments would have been available for this and we did not want to risk contaminating them with bugs. No separate controls were available for bugs pre-exposed to IMI before the smoke treatment.

Nicotine treatment with Nikotiini-kärytenauha (Berne Oy) (202.5g/kg nicotine, use rate 900g/1000m³) began at 9 pm. The vents were opened at 6 am the following morning. The bags were collected from the plants at noon, and the number of alive and dead bugs in them
was counted under the microscope. Mortality of the bugs in the treated bags was corrected for control mortality using the Abbot formula (Abbott, 1925). Statistical significance between the mortalities at different height levels in the crop was analysed with one way Anova.

An eradication programme involving sequential use of nicotine smoke and tau-fluvalinate (TFL) or systemic thiachloprid (TH) or IMI treatments was executed simultaneously in all eight nurseries in Jan-Feb 2010 where the bug had been detected since spring 2009: week 1: TFL or neonicotinoid – week 2: nicotine – week 4: nicotine, week 5: TFL or neonicotinoid, and week 6: nicotine. After the program, the crops were monitored weekly with yellow sticky traps (YST) (one trap per 250m²) until the end of the cropping cycle in June when greenhouses were emptied and cleaned. Monitoring continued in the new crops in August.

Results and discussion

Nicotine smoke was highly efficient against adult *N. tenuis*, with no differences between the plant height levels (ANOVA, F=1.20, p<0.3394). Bugs pre-exposed to IMI drenched plants displayed similar mortality to other treatments. They were, however, kept several days on tomato seedlings that did not contain IMI, thus we do not know what the result had been if the pre-exposure would have taken place immediately before the smoke treatment.

Table 1. Abbot-corrected mortality (%, mean ± S.E.) of *Nesidiocoris tenuis* adults placed at three height levels in the crop in mesh bags and of those that had fed on imidacloprid treated tomatoes before being collected from the plants. n = number of mesh bags per height level.

<table>
<thead>
<tr>
<th>Uncovered control</th>
<th>Tops of plants</th>
<th>Middle level of plants</th>
<th>Floor level</th>
<th>Pre-exposed to imidacloprid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>Tops of plants</td>
<td>Middle level of plants</td>
<td>Floor level</td>
<td>Pre-exposed to imidacloprid</td>
</tr>
<tr>
<td>8.9 ± 2.7 (n=5 with 6-19 bugs per bag)</td>
<td>97.6 ± 1.6 (n=7 with 2-19 bugs per bag)</td>
<td>96.2 ± 2.3 (n=7 with 8-17 bugs per bag)</td>
<td>91.4 ± 2.6 (n=3 with 7-18 bugs per bag)</td>
<td>97.7 ± 2.1 (n=6 with 8-15 bugs per bag)</td>
</tr>
</tbody>
</table>

*of which three were in the tops and three in the middle level of plants; these were combined as mortalities did not differ between these height levels for the pre-exposed bugs (t-test, p<0.187).*

Due to low number of bags with nymphs, usually only <5 nymphs per bag, and high control mortality (18.1 ± 11.7%, Abbot-corrected mean value ± S.E., n=3), results for nymphs are inconclusive. For these reasons, no attempt was made to analyse mortality differences between treatments. Nymphal mortality ranged from 0 to 68.4%, averaging 31.1% ± 6.8 (all plant height levels combined, n=12 bags, 77 nymphs), but this did not differ from control (t-test, p<0.3343). All nymphs in the treated bags were, however, in very weak condition: the only signs of life in those that lived were slight movements of antenna. The bugs were kept in the bags for another three days and their mortality rechecked. By that time the tomato leaflets were drying out which contributed to the increase of mortality. Even so, in control bags 37.5% of nymphs were alive upon rechecking, whereas in the treated bags all were dead (as were all adults, with 8 alive in control bags). This suggests nymphs are more resistant to nicotine than adults as it takes longer for the nymphs to die from nicotine, but this warrants further studies.

Null-trappings were observed in the treated crops and in other compartments of the same nurseries until middle of June when crops were removed and rooms cleaned. In October 2010, the bugs reappeared in very low numbers in two nurseries that had run the programme in village
B, and one nursery not previously infested. They reappeared also in village A in the same nursery company as before, but in a different greenhouse about 1 km away from the one where the chemical programme was executed. Some individuals most likely survived the programme with their densities below detection level – we know it is impossible to prove the absence of something particularly when the detection level of a species with the trap density used is unknown, as are trap efficiency and area of attraction (cf. Barclay & Humble, 2009). Clearly, a population below the detection level must have gone unnoticed in some of the treated crops up till May when year-round tomatoes are topped. Topped plants may slow down bug reproduction since the growing points of the plants that they prefer to feed upon (Arnó et al., 2010) are removed. The bugs may have escaped outdoors in the summer, and re-entered greenhouses when the weather cooled. On the other hand, a fifth infestation case late in the autumn of 2010 was discovered in a village 25 km away from A and B. This raises the question whether the bugs were reintroduced there via the initial pathway, although local dispersal between villages cannot be excluded despite the distance.

No further findings except the five nurseries have been made in the area or elsewhere in the country (Jari Poutanen, Finnish Food Safety Authority, pers. communic.). Work continues to deal with the pest either with legislative or technical means to limit its spread and, eventually, to eradicate this unwanted biodiversity enrichment from Finnish greenhouses.

Acknowledgements

We thank all the Mediterranean, Dutch and UK colleagues who provided information on chemical control, monitoring and biology of *N. tenuis*, and Veikko Rinne at the University of Turku for confirming the identification of the bugs.

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Evaluation of potential *Orius insidiosus* banker plants for western flower thrips biocontrol in ornamental crops

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Abstract: Successful use of biological control agents for control of western flower thrips (*Frankliniella occidentalis* (Pergande)) (WFT) has been documented in greenhouse vegetables, such as sweet peppers, but thus far has provided inconsistent results for ornamentals. The objective of this study was to identify an optimal banker plant (BP) species to improve the performance of the biological control agent – *Orius insidiosus* (Say), against WFT in greenhouse ornamental crops. Potential BP were placed into cages and exposed to 5 female (<1w) *O. insidiosus* for 48h; the number of eggs and oviposition location was recorded. Development and survival was recorded by placing (<24h) nymphs into cages with a cutting from a flowering BP plant species. Nymphs were observed until the adult stage was reached or the nymph died. Assessments of oviposition indicated that all plants were acceptable for *O. insidiosus* to reproduce. Based upon nymphal development and survival, castor bean and gerbera may be suitable BPs respectively; sunflower and marigold would not be acceptable BPs.

Key words: *Orius insidiosus*, biological control, banker plant, western flower thrips, greenhouse, floriculture

Introduction

Floriculture represents a large proportion of Canada’s greenhouse industry and revenue. In 2009, production of ornamentals accounted for 60% of total sales of greenhouse products (Statistics Canada, 2009). Pest management is a key priority for growers of ornamental crops as consumers demand an aesthetically pleasing product that is free of blemishes and pests. The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is categorized as a serious pest due to its broad host plant range and short life cycle and causes aesthetic feeding damage in addition to vectoring tospoviruses (Brødsgaard, 2004). Frequent applications of insecticides have ultimately resulted in the development of resistance in WFT populations to many chemical classes (Bielza, 2008). Biological control is consequently the best, if not the only, alternative method. The biological control agent, *Orius insidiosus* (Say), is currently not widely used for WFT biocontrol in greenhouse ornamentals as it is slow to establish and exert control. If introduced to an environment where there are no pollen producing plants and minimal pests, *O. insidiosus* are likely to emigrate from the crop or die (Skirvin et al., 2006). In order to control WFT in commercially grown ornamentals, establishing a banker plant (BP) system for *O. insidiosus* could provide a source of alternative food and a suitable location for oviposition. The objective of this study was to determine an optimal BP species for *O. insidiosus* to provide continuous rearing and release into ornamental crops. An *O. insidiosus* BP system may provide an alternative control method to insecticide use and may be more cost-efficient and consistent than currently used release strategies.
Material and methods

Insects and plants

Colonies of *O. insidiosus* were established using adults purchased from Biobest Canada Ltd. (Leamington, ON, Canada). Colonies were maintained at the Vineland Research and Innovation Centre (Vineland, ON, Canada) using kidney beans (*Phaseolus vulgaris* L.) and *Ephesia kuehniella* egg strips. A comparison of six plants will be made to the current standard, Black Pearl ornamental pepper. The pepper cultivar, Black Pearl, is not ideal as it requires 3 months to grow before it can be used as a BP and requires maintenance to continue flowering throughout the year. The potential BP species (Table 1) were chosen based upon a literature review of attractiveness to *Orius* and flowering characteristics. Plants were grown from seed (with the exception of Gerbera, which was purchased as a flowering plant) in greenhouses in Vineland, ON under conditions of 21±1°C, RH 70±5%, and 16:8h L:D.

Table 1. Plants evaluated in the study as a potential Banker Plant for *O. insidiosus*.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marigold, Lemon Gem</td>
<td><em>Tagetes patula</em></td>
<td>Veseys, Charlottetown, PE</td>
</tr>
<tr>
<td>Castor Bean</td>
<td><em>Ricinus communis</em></td>
<td>Richters Herbs, Goodwood, ON</td>
</tr>
<tr>
<td>Chili Pepper, Black Pearl</td>
<td><em>Capsicum annuum</em></td>
<td>Stokes Seeds Ltd., Thorold, ON</td>
</tr>
<tr>
<td>Chili Pepper, Purple Flash</td>
<td><em>Capsicum annuum</em></td>
<td>Stokes Seeds Ltd., Thorold, ON</td>
</tr>
<tr>
<td>Gerbera Daisy, Festival</td>
<td><em>Gerbera jamesonii</em></td>
<td>Growers in the Niagara region of ON</td>
</tr>
<tr>
<td>Feverfew</td>
<td><em>Tanacetum parthenium</em></td>
<td>Richters Herbs, Goodwood, ON</td>
</tr>
<tr>
<td>Sunflower, Choco Sun</td>
<td><em>Helianthus annuus</em></td>
<td>Stokes Seeds Ltd., Thorold, ON</td>
</tr>
</tbody>
</table>

Oviposition suitability

Adult females (<1w) were removed from the colony using an aspirator and starved for 24h. One flowering cutting of each plant species was placed into a cage (made from a 2L plastic bottle) and exposed to 5 females. The cages were placed in a growth chamber (25±1°C, 70±5% R.H., 16L:8D). On day 3, the number and location of eggs were counted by examining the cutting under a microscope. Cuttings were placed back into the respective cages and returned to the growth chamber for 72h. On day 6, cuttings were placed into tightly sealed jars containing an *E. kuehniella* egg strip as a food source for nymphs. On day 8, 70% ethanol (75ml) was placed into the jars and the jars shaken for 60s. Jars were emptied into a funnel lined with filter paper and attached to vacuum to withdraw ethanol. Nymphs remaining on the filter paper were counted in order to assess egg viability on each plant species. The procedure was repeated 8 times for each plant.

Immature survival and development time

Flowering cuttings from each plant species were placed into individual cages made from 250ml cups. Newly hatched nymphs (<24h old) were transferred from the colony to individual cages using a moistened paint brush. The cages were placed in a growth chamber (25±1°C, 70±5% R.H., 16L:8D). Hatched nymphs were monitored daily for development and survival until the adult stage was reached or the nymph died. The tests were repeated until 15 nymphs per plant species had reached the adult stage.
Data Analysis
An ANOVA using the General Linear Model procedure was performed for both tests using SAS, version 9.2. Means were compared using Tukey’s adjusted Least Significant Means comparison with $\alpha=0.05$.

Results and discussion

Oviposition suitability
The mean number of eggs found on each plant species and the mean number of emerged nymphs were analyzed separately. The 7 potential BP species provided equally acceptable locations for *O. insidiosus* to reproduce (Figure 1). Fewer eggs were recorded on the sunflowers than nymphs indicating that the egg counts were inaccurate. The mean number of nymphs on the sunflowers did not significantly differ from the other 6 potential BP species. Each plant species, therefore, provides numerous locations for oviposition and no significant differences were determined for egg viability on the different plant species.

![Figure 1. Mean number of *Orius insidiosus* eggs and subsequent hatched nymphs on seven potential banker plant species in no-choice tests at 25±1°C, RH 70±5%, 16:8h L:D. Bars indicated by the same letter are not significantly different according to Tukey’s test ($P \leq 0.05$) N=8, $\alpha=0.05$.](image1)

Immature survival and development time
Survival of nymphs varied among plant species (Table 2). No nymphs developed into adults on sunflower due to a severe powdery mildew infection. Sunflowers would not make an acceptable BP, however, as they are extremely susceptible to infestation by WFT. Marigold would also not be an acceptable BP as only 12% of nymphs survived to become adults.
Gerbera may be an acceptable BP as survival was the best on this plant at 58%. Development time was quickest on Castor bean (7.77± 0.35d), and slowest on Black Pearl pepper (9.16± 0.22d) which is the current BP standard.

Table 2. Mean development (days) and survival (%) of newly hatched (<24h) *Orius insidiosus* nymphs reared on different plant species at 25±1°C, RH 70±5%, 16:8h L:D.

<table>
<thead>
<tr>
<th>Banker Plant</th>
<th>N</th>
<th>Development (days)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marigold</td>
<td>50</td>
<td>8.97± 0.38 abc</td>
<td>12.0</td>
</tr>
<tr>
<td>Castor Bean</td>
<td>35</td>
<td>7.77± 0.35 c</td>
<td>35.0</td>
</tr>
<tr>
<td>Black Pearl Pepper</td>
<td>32</td>
<td>9.16± 0.22 a</td>
<td>50.0</td>
</tr>
<tr>
<td>Purple Flash Pepper</td>
<td>37</td>
<td>9.06± 0.23 ab</td>
<td>43.2</td>
</tr>
<tr>
<td>Gerbera</td>
<td>31</td>
<td>8.22± 0.20 bc</td>
<td>58.1</td>
</tr>
<tr>
<td>Feverfew</td>
<td>20</td>
<td>8.80± 0.23 abc</td>
<td>42.9</td>
</tr>
<tr>
<td>Sunflower</td>
<td>30</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

*Means followed by the same letter do not differ significantly from each other by Tukey’s test (P≤0.05).

Based on these experiments, it seems that Gerbera and Castor bean are the plants that would optimize *O. insidiosus* population growth by high survival and short development times of *O. insidiosus* nymphs. Further experiments to determine which plant allows a population of *O. insidiosus* to increase the most will be conducted to determine which plant would be the optimal BP species. This plant could then be used in a more efficient biological control strategy for managing WFT in greenhouse ornamental crops.

**Acknowledgements**

The authors gratefully acknowledge the participation of several greenhouse growers from the Niagara region of Ontario. We also thank Angela Brommit for technical assistance. Funding for this project was provided through a University of Guelph and Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) partnership and an OMAFRA - HQP scholarship awarded to M.O. Waite.

**References**

Pest and disease control in sustainable greenhouse production systems

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Abstract: The European protected crop industry faces growing economic, governmental and market pressures to reduce carbon footprints, energy costs and water wastage. To meet these demands sustainable greenhouse structures and management systems are currently being designed for greater energy-efficiency and water conservation. Before such systems are implemented it is prudent to consider the impacts these will have on pest and disease (P&D) control.

As part of the EU funded project, EUphoros, energy-efficient systems for sustainable greenhouse production have been developed focusing on new greenhouse climate management strategies, which incorporate closed or semi-closed greenhouses, and novel photoselective greenhouse cladding. However, these systems will bring about changes to the growing environment in which pests, diseases and biocontrol agents function and reproduce, primarily in terms of temperature, humidity and light. These changes may have important consequences for P&D pressures and their control.

To assess these impacts work focused on two representative P&D systems; a powdery mildew of tomato (Oidium neolycopersici) and the two-spotted spider mite (Tetranychus urticae) on ornamentals and their biocontrol agents, Bacillus subtilis and Phytoseiulus persimilis respectively. Experimental work assessed the impacts on these P&D systems of a range of temperature and humidity conditions relevant to greenhouse production environments as well the effects of manipulating ultra-violet light levels, through the use of novel greenhouse cladding plastics.

Dynamic models of both P&D systems were then constructed using experimental and published data to forecast the pest and disease pressures in sustainable production systems, allowing their impacts to be assessed and optimal control programmes to be developed.

Key words: Tetranychus urticae, Oidium neolycopersici, integrated control, greenhouse climate management, photoselective films, dynamic models
Development of a new banker plant system to control aphids in protected culture

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Abstract: To establish a new banker plant system of an indigenous strain of \textit{Aphidoletes aphidimyza} as a control agent of pest aphids on solanaceous fruit vegetables under high temperature conditions, we conducted life history studies of \textit{A. aphidimyza} reared on \textit{Melanaphis sacchari} on sorghum banker plants. We examined the effect of temperature on development and lifetime fecundity and calculated the intrinsic rate of natural increase. The survival rate from egg to adult eclosion was 0.8-0.87 at 20-30°C. The lifetime fecundity and intrinsic rate of natural increase of \textit{A. aphidimyza} reared on \textit{M. sacchari} were higher than values for \textit{A. aphidimyza} reared on \textit{Rhopalosiphum padi}. The efficiency of the banker plant system of \textit{A. aphidimyza} and \textit{M. sacchari} was evaluated on sweet pepper plants in greenhouses. The density and frequency of occurrence of pest aphids on sweet pepper plants were more effectively suppressed in greenhouses using the system of \textit{A. aphidimyza} and \textit{M. sacchari} on sorghum plants than in those using the system of \textit{Aphidius colemani} and \textit{R. padi} on barley plants.

Key words: \textit{Aphidoletes aphidimyza}, aphids, biological control, banker plant, \textit{Melanaphis sacchari}, sorghum

Introduction

Release programs for the biological control of aphids in greenhouses include the “pest in first method”, the “banker plant system” (open rearing system), and the direct release of beneficial agents either preventively or curatively (Blümel, 2004). Among these methods, the banker plant system appears particularly promising because continuous release of predators, which is an important characteristic of this method, has a stabilising effect on population fluctuations in the aphid-predator system (Yano, 2006).

With the banker plant system, non-crop plants infested with a host insect (a non-pest of the target crop) are placed in the greenhouse, and the host insect provides food and reproductive resources for the parasitoids or predators (van Lenteren, 1995). This system was first evaluated by Hansen (1983), who reared \textit{Aphidoletes aphidimyza} (Rondani) on a non-pest aphid species, \textit{Megoura viciae} Bucken, using broad bean as the host plant. The system yielded successful control of \textit{Myzus persicae} (Sulzer) in sweet pepper greenhouses. Bennison (1992) and Bennison and Corless (1993) developed banker plant systems of wheat seedlings infested with \textit{Rhopalosiphum padi} (Linnaeus) to establish \textit{Aphidius} parasitoids (\textit{Aphidius matricariae} Haliday or \textit{Aphidius colemani} Viereck) and \textit{A. aphidimyza} for the control of \textit{Aphis gossypii} Glover on cucumbers in greenhouses. These banker plant systems have made it possible to achieve rapid and prolonged control of \textit{A. gossypii}.

The banker plant system used to establish \textit{A. colemani} has also been developed to control \textit{A. gossypii} on solanaceous fruit vegetables in greenhouses in Japan using banker plants of wheat, barley, or oat seedlings infested with \textit{R. padi} (Nagasaka \textit{et al}., 2010a). This system has been adopted as a practical method of release of \textit{A. colemani} in Kochi Prefecture. However, two problems have made the system impractical: the outbreak of hyperparasitoids of
A. colemani in warmer seasons and difficulty of controlling aphid pests other than A. gossypii and M. persicae (Nagasaka et al., 2010b). The development of a banker plant system using A. aphidimyza may serve as an alternative method, as this species can avoid such problems related to use of A. colemani. However, due to the environmental risks involved with exotic strains, the use of an indigenous strain of A. aphidimyza should be considered. Additionally, an indigenous strain would be better adapted to the domestic environment of Japan than an exotic strain.

Many ecological traits (i.e. development, reproduction, predation, diapause) of European strains of A. aphidimyza have been previously examined (e.g., Uygun, 1971; Havelka, 1980; Havelka & Zemek 1988 & 1999). Ecological studies of Japanese indigenous strains are essential for the development of a banker plant system using this strain. To this end, Yano et al. (2009) examined the life history of a Japanese strain of A. aphidimyza reared on R. padi for the control of A. gossypii on eggplant in Japan using barley seedlings infested with R. padi. However, use of this banker plant system became impractical due to poor growth of barley plants under high temperature conditions from late spring to early autumn. Comparisons of the reproduction of four non-pest aphid species on two host plants indicated that the reproduction rate of Melanaphis sacchari (Zehntner) on sorghum was highest at high temperatures (Abe et al., unpublished data). Therefore, we examined a banker plant system consisting of sorghum plants infested with M. sacchari to establish A. aphidimyza as a control agent of pest aphids on solanaceous fruit vegetables during the hot season.

**Life history studies of A. aphidimyza reared on M. sacchari on sorghum**

**Material and methods**

*Aphidoletes aphidimyza* larvae attacking Lipaphis erysii (Kaltenbach) were collected from potherb mustard on 25 August 2005 in Nantan, Kyoto Prefecture, Japan. After field collection, larvae were cultured in the laboratory on eggplant at 25°C with a L16:D8 photoperiod. They were fed *Aphis gossypii* that originated from a colony collected from strawberry on 21 April 2006 in Ayabe, Kyoto Prefecture. *Melanaphis sacchari* were collected from sorghum on 28 September 2006 in Ayabe.

In the development experiment for *A. aphidimyza*, a sorghum leaf chip (6 × 1cm) was placed on wet cotton in the bottom of a plastic Petri dish (9cm in diameter, 1.5cm in depth). Within 24h after oviposition, an egg of *A. aphidimyza* was placed on the leaf chip in the Petri dish. The number of *M. sacchari* nymphs serving as food for *A. aphidimyza* was adjusted to 20 per day, including individuals that remained unconsumered. Egg and larval stages of *A. aphidimyza* were reared at 15, 20, 25, 30, and 35°C with a L16:D8 photoperiod. The developmental stage and survival of *A. aphidimyza* were checked daily. After third instar larvae stopped eating and left the leaf chip to form a cocoon, they were moved to glass vials (1.5cm in diameter, 4cm in depth) filled with moistened cotton. They were kept under the same temperature and photoperiod conditions as for egg and larval stages. Adult emergence in the vial was checked every day. The resulting data were used to calculate the developmental zero and thermal constant.

In the oviposition experiment for *A. aphidimyza*, four fresh sorghum leaves infested with 250–300 individuals of *M. sacchari* and three males and one female adult (<24-h old) of *A. aphidimyza* were introduced into an inverted plastic case (13 and 10cm in upper and lower diameter, respectively, 10cm high). A plastic cup filled with water was placed under the bottom lid of the case, and the stems of leaves were soaked in water through a hole in the centre of the bottom lid. Plastic cases were maintained under conditions of 25°C and a
L16:D8 photoperiod. The number of oviposited eggs on leaves was counted, and the survival of adults was checked daily until the death of the female.

The intrinsic rate of natural increase, mean generation time, and net reproductive rate were calculated according to Birch (1948).

Results and discussion

The mean development times throughout the nymphal period decreased from 49.7 days at 15°C to 11.4 days at 30°C but increased to 23.0 days at 35°C. The survival rates from egg to adult emergence were 0.8–0.87 at 20–30°C but fell to 0.36 at 15°C and 0.14 at 35°C. Survival rates of *A. aphidimyza* were higher at 15–30°C but lower at 35°C than when reared on *R. padi* (Nishikawa et al., unpublished data). The thermal constant and developmental zero throughout the nymphal period were estimated to be 211.4 day-degrees and 11.0°C, respectively. Female mean longevity and mean lifetime fecundity at 25°C were 3.0 days and 23.1, respectively. Survivorship and oviposition curves at 25°C are presented in Figure 1. The intrinsic rate of natural increase, mean generation time, and net reproductive rate were calculated as 0.168, 16.9 days, and 17.2, respectively. The lifetime fecundity and intrinsic rate of natural increase of the Japanese strain of *A. aphidimyza* reared on *M. sacchari* were higher than values for *A. aphidimyza* reared on *R. padi* (Nishikawa et al., unpublished data).

![Figure 1](image_url)  
**Figure 1.** Survivorship (*l_x*, solid line) and oviposition curves (*m_x*, dashed line) of *Aphidoletes aphidimyza* at 25°C.

Evaluation of the banker plant system in greenhouse sweet peppers

The efficiency of this banker plant system was evaluated in greenhouses of sweet pepper plants in Maizuru, Kyoto Prefecture. Experimental banker plant systems were composed of *A. aphidimyza* reared on either *M. sacchari* on sorghum plants or *A. colemani* reared on *R. padi* on barley plants as the alternative prey and banker plant. The density and frequency of occurrence of pest aphids on sweet peppers were more effectively suppressed in greenhouses with the sorghum banker system than in those with the barley banker system. In greenhouses with the sorghum banker system, the population of *A. aphidimyza* was maintained for at least 3 months after settlement (Abe et al., 2011).


Bennison, J. A. & Corless, S. P. 1993: Biological control of aphids on cucumbers: further development of open rearing units or “banker plants” to aid establishment of aphid natural enemies. IOBC/WPRS Bull 16(2): 5-8.


