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Integrated Control in Protected Crops, Mediterranean Climate

Lutte Intégrée en cultures Protégées, Climat Méditerranéen

editors:

Cristina Castañé & Abdelhaq Hanafi

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PREFACE

This bulletin contains most of the oral and poster contributions presented at the meeting of the IOBC/WPRS working group on "Integrated Control In Protected Crops, Mediterranean Climate" in Agadir, Morocco. In comparison with previous volumes of the working group, the relative importance of the different subjects have slightly changed, which reflects the changing dynamics of pest and disease problems in protected vegetable crops. This time, a substantial number of contributions deal with whiteflies in comparison to other pest groups, indicating the current concern they represent to our region. Many studies on native natural enemies, both predators and parasitoids, show the increasing importance that their conservation and / or manipulation has to IPM systems in Mediterranean climatic conditions. Other tools for IPM programs including selectivity of insecticides, residual effects of pesticides on natural enemies, identification of quarantine pests or quality control of released natural enemies are also discussed in this meeting. In addition, presentations on plant diseases are included as they have been in other meetings of this working group. These presentations are very welcome although there are not many. Contributions from authors from other regions of the world sharing similar climatic conditions and pest and disease problems are very much appreciated. I would like to thank all authors for their efforts in preparing their manuscripts. In most cases, the text of the manuscript has been modified with minor changes by an experienced English corrector in order to improve the quality of the bulletin.

This working group has been consolidated in the past decade aiming at fruitful exchanges of information among plant protection scientists that study the problems of the Mediterranean horticulture. This has been possible to a great extent thanks to the personal effort of the last convenor of the group, Prof. Ramon Albajes. I would like to thank him for his hard work in motivating the group and also for his collaboration, ideas and suggestions for this meeting and for the bulletin.

On behalf of all participants, I like to thank cordially Abdelhaq Hanafi –the local organizer and second editor of this volume- for his excellent work in organizing the present meeting in Agadir, Morocco.

Cristina Castañé, Convenor
IOBC/WPRS W. G. on "Integrated Control in Protected Crops, Mediterranean Climate"
August, 2003

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An overview of biological control in greenhouse chrysanthemums in Brazil

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Abstract: Chrysanthemum is a very important crop grown under protected cultivation in several regions of Brazil. Many pests appear in this crop, with aphids and thrips being the most important. Frequent spraying with pesticides results in the rapid development of resistance, in killing natural enemies and also creates problems for exporting the product. Implementation of an IPM program in which biological control is the main control method has now become a high priority for growers concerned about the problems associated with the application of pesticides. This paper discusses the biological control of aphid and thrips populations through the respective introduction of *Lysiphlebus testaceipes* and *Orius insidiosus*. This takes the form of seasonal inoculative releases in cut chrysanthemum crops in commercial greenhouses.

Key words: biological control agent, *Orius insidiosus*, *Lysiphlebus testaceipes*, aphids, thrips.

Introduction

Chrysanthemum is cultivated and marketed in several areas of southern and south-eastern Brazil. Approximately 60 cultivars are currently grown in greenhouses with three growth cycles per year.

The major pest problems for chrysanthemum grown for cut flowers are thrips, aphids, mites and white rust disease. *Frankliniella occidentalis* Pergande is the most important pest all year around, but aphids, and particularly *Aphis gossypii* Glover, also play an important role in the development of Brazil's chrysanthemum crop (Bueno 1999, 2000).

Control of these pests traditionally involves chemical and cultural methods, applied either jointly or separately. Pesticide sprays are frequently applied - three or four times per week – throughout the production season, with an alternation of chemicals between applications. This leads to rapid development of resistance to pesticides and also implies problems for exporting the final product. Brazilian producers currently export only 2 to 5% of their flower production. Increasing this percentage depends, amongst other factors, on the establishment of an IPM program for chrysanthemum in which biological control constitutes the main method of pest management. The biological control of pests has become a high priority in Brazil.

A research program on biological and integrated pest control for greenhouse pests began in 1998. It was undertaken by a commercial ornamental flower producer (Fazenda Terra Viva, Schoenmaker group, Holambra, Sao Paulo) and the Department of Entomology of the Federal University of Lavras, Brazil, in collaboration with the Laboratory of Entomology, Wageningen University, The Netherlands (Bueno, 1999).

The following goals were established: (1) to monitor the development of aphids and thrips and native natural enemies in a pesticide-free greenhouse with cut chrysanthemum; (2)

to release the aphid parasitoid *Lysiphlebus testaceipes* (Cresson) and the thrips predator *Orius insidiosus* (Say) as part of a system to control aphid and thrips populations when their numbers were close to the economic threshold density.

Material and methods

Occurrence of aphids, thrips and native natural enemies in greenhouse

A survey was conducted in a greenhouse with several cultivars from 1999 to 2001; this included a number of chrysanthemum cycles. Aphids were sampled by randomly selecting two leaves per bed. Thrips samples were made by tapping two plants per flowerbed per week in a tray. Infestation rates for aphids and thrips and the presence of parasitoids and predators were monitored on a weekly basis.

Release of Lysiphlebus testaceipes

Two introductions of the parasitoid were made in a commercial greenhouse (600m^2) with "White Reagan" and "Sunny Reagan" chrysanthemum cultivars: plants density was 40 plants/m^2. Parasitoid release rates were 0.15 female/m^2 in the fourth week after planting and 0.24 female/m^2 in the eighth week after planting. Aphids were sampled weekly by randomly observing 10-plants/flowerbed: sampling started one week after planting. Parasitism rates for *L. testaceipes* were evaluated by counting the number of mummies found and adding aphids with parasitoid larvae observed by dissection.

Release of Orius insidiosus

Five introductions of the predator were made in a commercial greenhouse (600m^2) with "White Reagan" (36 plants/m^2) and "Yellow Snowdon" (63 plants/m^2) chrysanthemum cultivars. Predator release rates were 1.5 and 2.0 *Orius*/m^2, with a total of 9.5 *Orius*/m^2 released. The thrips population on greenhouse chrysanthemum was a natural infestation. Sampling was conducted by tapping two plants per flowerbed per week. Numbers of thrips and predators were counted.

Results and discussion

Occurrence of thrips, aphids and parasitoids and predators

Monitoring data demonstrated that aphids and thrips were recurrent pests on all chrysanthemum cultivars throughout the year. Thrips were the most important pest. The following thrips species were found: *Caliothrips phaseoli* (Hood), *Frankliniella coccidentalis*, *Frankliniella gemina* Bagnall, *Frankliniella sp.*, *Thrips palmi* Karny, *Thrips tabaci* Lindeman, *Thrips australis* (Bagnall) and *Haplothrips gowdeyi* (Franklin). The following aphid species were also found: *Aphis gossypii*, *Dysaphis* sp. and *Myzus persicae* (Sulzer). Natural enemies found included parasitoids such as *Aphidius colemani* Viereck and *Lysiphlebus testaceipes*, and predators such as *Chrysoperla* sp., *Cycloneda sanguinea* Mulsant, *Scymnus* sp., *Hipodamia convergens* Guérrin-Meneville, *Orius insidious*, *Pseudodorus clavatus* (Fabricius) and *Franklinothrips vespiformis* (Crawford).

Aphids

A. gossypii was the most important aphid species (98.3%) in chrysanthemum. Although it was found in all seasons, we detected that temperature and chrysanthemum cultivar influenced its infestation. According to Soglia *et.al.* (2002, 2003), the development time, survival of initial instars, fecundity and longevity of *A. gossypii* are all affected by these same factors. An

increase in temperature from 15 to 30°C caused a significant reduction in the development time of the instars. The survival of nymphs of 1st and 2nd instars was affected by different densities of trichomes/mm^2 on the leaves present on the different chrysanthemum cultivars. The intrinsic rate of increase (r_m) and net reproduction rate (Ro) values for this aphid developing on "Yellow Snowdon" and "White Reagan" cultivars were 0.31 and 46.08 and 0.22 and 12.07 respectively, at the optimal temperature (25°C) for *A. gossypi* development.

The parasitoid *L. testaceipes* was the most frequent natural enemy of *A. gossypii* under greenhouse conditions. This parasitoid developmment on *A. gossypii* under laboratory conditions and at different temperatures (15, 20, 25 and 30°C) showed the shortest development time (11.3 days) and the highest percentage of parasitism and emergence (> 60%) at 25°C. This temperature could therefore be considered the most suitable for the reproduction of this parasitoid (Rodrigues 2003). Host preference involving *A. gossypii* and *M. persicae* in non-choice and choice tests indicated that *L. testaceipes* preferred *A. gossypii*. Numbers of hosts encountered, probes with the ovipositor, ovipositions and parasitoid larvae found after host dissection were all greater for *A. gossypii* than for *M. persicae* (Bueno et. al. 2003).

The parasitoid *A. colemani* also played an important role as a natural enemy of *A. gossypii*, and the temperature most suitable for the development of this parasitoid was around 22°C. This parasitoid preferred *A. gossypii* to *M. persicae* (we found a higher number of attacked *A. gossypii* and a higher number of parasitoid larvae in this aphid than in *M. persicae* in non-choice and choice tests (Sampaio et. al. 2001)).

Release of L. testaceipes

A satisfactory control of *A. gossypii* populations was achieved releasing the parasitic wasp *L. testaceipes*. We also observed that it was essential to introduce parasitoids at an early stage of aphid infestation.

Population growth of *A. gossypii* on "White Reagan" and "Sunny Reagan" reached its respective peaks during the fifth (4.5 aphids/plant) and eighth weeks after planting (4.0 aphids/plant). At the end of the crop cycle, 0.2 and 0.3 aphids/plant were respectively counted on "White Reagan" and "Sunny Reagan". Parasitism rates on "White Reagan" after the first and second releases of *L. testaceipes* were 55.2% and 7.8%, respectively. Rates on "Sunny Reagan" were 31.9% (first release) and 10.5% (second release) (Figure 1). Other mortality factors, such as the presence of trichomes on leaves and predatory insects in the greenhouse, affected parasitism rates of *L. testaceipes* and the population density of *A. gossypii*. No aphid crop damage was observed. The parasitoid *L. testaceipes* proved an effective biological agent for controlling *A. gossypii* in both chrysanthemum cultivars and in greenhouse.

Thrips

F. occidentalis was the most important pest (96.8%) on chrysanthemum cultivars for all years. It occurred throughout the growing season, though infestation was greatest during the flowering period.

The predator *O. insidiosus* was found in association with thrips in chrysanthemum greenhouses. Other predators, entered greenhouses spontaneously: their numbers were significantly high in greenhouses without chemical control. *O. insidiosus* is widely distributed in Brazil and it is the most common *Orius* species found on crop and non-crop plants. *O. insidiosus* was collected on crops and weeds around greenhouses: it was mainly found on corn crops and on the weed *Bidens pilosa* L (Silveira, 2003). Studies under laboratory conditions showed that the predator did not enter reproductive diapause under photoperiod conditions of between 9 and 14 hours of light at a temperature of 25°C (Argolo et al., 2001).

Release of O. insidiosus

The thrips population peaked in April in both chrysanthemum cultivars (Figure 2). The thrips population decreased from 4.7 to 2.5 per plant in "Yellow Snowdon" and from 2.8 to 1.1 in "White Reagan" after the first release of the predator *O. insidiosus*. After the fourth release (7.5 *Orius*/m^2 in the total): eight weeks after planting, numbers were reduced to 0.3 and 0.4 thrips/plant in "Yellow Snowdon" and "White Regan", respectively. The thrips population was greatly reduced in comparison with the first week after planting (Figure 2). Little thrips damage was found in the crop. *O. insidiosus* was effective as a biological control agent for thrips in cut chrysanthemum. However, at the end of the crop (flowering period), chemical controls of other pests (coleopterans) interfered with biological control of thrips.

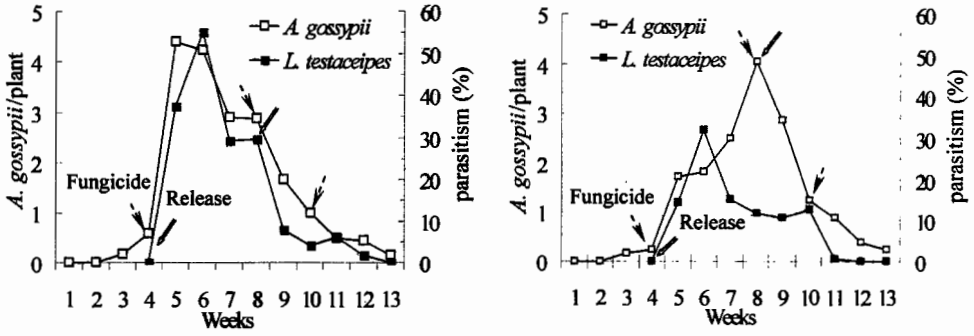

Figure 1. Population dynamics of *Aphis gossypii* and *Lysiphlebus testaceipes* on a chrysanthemum crop in commercial greenhouse (left figure cultivar "White Reagan", right figure cultivar "Sunny Reagan").

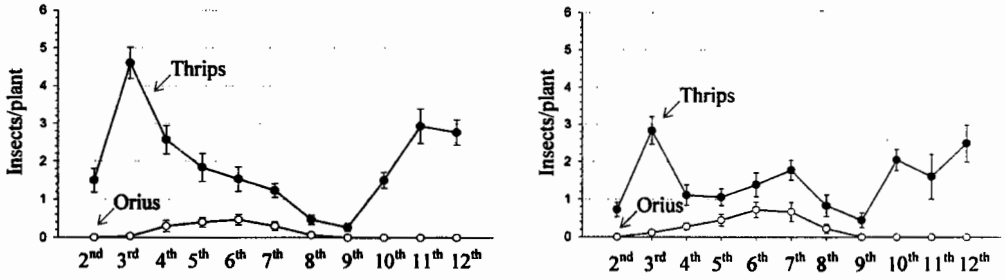

Figure 2. Population dynamics of thrips and *Orius insidiosus* on a chrysanthemum crop (left figure "Yellow Snowdon" and right figure "White Regan"cultivars) in commercial greenhouse.

Conclusion

Biological control of thrips and aphids is possible under greenhouse conditions in Brazil. *L. testaceipes* and *O. insidiosus* are effective biological control agents for these pests. Further research is required into biological and natural controls of other pests in ornamentals in order to develop a full spectrum IPM program, but *Lysiphlebus* and *Orius* can now be evaluated for controlling aphids and thrips on vegetables. We think that aphids may be completely controlled by natural enemies in almost all cases of greenhouse infestation in most of Brazil: many parasitoids and predators spontaneously enter greenhouses. Thrips seem more difficult to control by naturally immigrating beneficials. We therefore propose a combination of natural control and the release of *Orius* predators in the greenhouse when thrips occur very early in the production cycle or when thrips density is very high.

Acknowledgements

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References

Argolo, V.M., Bueno, V.H.P. & Silveira, L.C.P. 2002. Influência do fotoperíodo na reprodução e longevidade de *Orius insidiosus* (Say) (Heteroptera; Anthocoridae). Neotropical Entomology 31: 257-261.

Bueno, V.H.P. 1999. Protected cultivation and research on biological control of pests in greenhouse in Brazil. IOBC/WPRS Bullettin 22(1): 21-24.

Bueno, V.H.P. 2000. Controle biológico de pragas: produção massal e controle de qualidade. Editora UFLA, Lavras, 196p.

Bueno, V.H.P., Carnevale, A.B. & Sampaio, M.V. 2003. Host preference of *Lysiphlebus testaceipes* (Cresson) (Hym.: Aphidiidae) for *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover (Hem.: Aphididae). (in press).

Soglia, M.C.M., Bueno, V.H.P. & Sampaio, M.V. 2002. Desenvolvimento e sobrevivência de *Aphis gossypii* Glover (Hemiptera: Aphididae) em diferentes temperaturas e cultivares comerciais de crisântemo. Neotropical Entomology 31: 211-216.

Soglia, M.C.M., Bueno, V.H.P., Rodrigues, S.M.M. & Samapio, M.V. 2003. Fecundidade e longevidade de *Aphis gossypii* Glover (Hemiptera: Aphididae) em diferentes temperaturas e cultivares comerciais de crisântemo (*Dendranthema grandiflora* Tzvelec). Rev. Bras. Entomol. 47: 49-54.

Rodrigues, S.M.M. 2003. Avaliação de *Lysiphlebus testaceipes* (Cresson) (Hym.:Aphidiidae) como agente de controle biológico de pulgões em cultivos protegidos. Tese de Doutorado, UFLA, Lavras, 106p.

Sampaio, M.V., Bueno, V.H.P. & van Lenteren, J.C. 2001. Preferência de *Aphidius colemani* Viereck (Hym.: Aphidiidae) por Myzus persicae (Sulzer) e Aphis gossypii Glover (Hemiptera: Aphididae). Neotropical Entomology 30: 655-660.

Silveira, L.C.P. 2003. Registro e associação de espécies de *Orius* Wolff com tripes, influência do fotoperíodo na reprodução e avaliação de *Orius insidiosus* (Say) (Hem.: Anthocoridae) no controle biológico de tripes (Thysanoptera) em casa de vegetação. Tese de Doutorado, UFLA, Lavras, 104p.

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Managing pests and diseases on greenhouse tomato in Morocco

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Abstract: By virtue of living in the Souss Valley, we are blessed with a mild climate favorable for continuous cropping all year round. Unfortunately, the same virtue extends to insects, fungi, bacteria, and viruses that populate this region exceptionally well. Moroccan farmers are already faced with numerous pests and diseases that can limit crop production and for which there are few effective management options, especially under EUREPGAP requirements. In addition, new pests and diseases continue to be introduced into Morocco as a consequence of intensive movements of people and agricultural products. The last decade has seen the introduction of more tomato pests and diseases than ever before. We have reported the introduction of three major insect pest species, five fungal pathogens and three viral diseases. There have been two new species of leaf miners: *Liriomyza huidobrensis* (1993) and *Liriomyza trifolii* (1996) and one species of thrips: *Frankliniella occidentalis* (1994). The five new fungal pathogens are: *Fusarium oxysporum* sp. *radicis lycopersici* (1998); *Pyrechaeta lycopersici* (1999); *Oidium lycopersici* (2000); *Verticillium albo-atrum* (2001) and the biotype A2 of *Phytophthora infestans* (1999). The three virus diseases are: Tomato Yellow Leafcurl Virus (1998), Tomato Chlorosis Virus (2001) and Pepino Mosaic Virus (2002).

Key words: Morocco, Tomato, Greenhouse, Pests, Diseases, Management

Introduction

While the pests and diseases that Moroccan growers currently face can be severe, Tomato Yellow Leafcurl Virus (TYLCV) currently constitutes a major challenge for certain production systems such as open field tomatoes and basic plastic shelters. Since the virus cannot be controlled with chemicals, a farmer's only solution is to manage the vector. *Bemisia tabaci* (Gennadius) management has become a major challenge in greenhouse crops and now accounting for 93% of all insecticides used on tomato in each crop cycle. Chemical control of *B. tabaci* often significantly reduces their numbers and effectively delays TYLCV epidemics on tomato. However, once harvest begins, few chemicals are allowed on tomatoes, but the problem of whiteflies and TYLCV transmission still persists. Consequently, the plant protection department of the Ministry of Agriculture has encouraged the registration of new insecticides that can be safely used after harvest begins and which meet EUREPGAP requirements. One of the insecticide products currently under registration in Morocco is AGRI-50 (Cal Agri Product, LLC, Los Angeles, California, USA) which kill whiteflies through a physical mode of action.

In addition to chemical control, farmers rely on a number of IPM tactics including mechanical exclusion using insect nets, rouging infected plants early in the season, eliminating weeds and mass trapping. Over the last five years we have tested the effectiveness of various insect nets at excluding *B. tabaci* and TYLCV. Results demonstrated that the finest mesh size (50 mesh) provided adequate exclusion of *B. tabaci* and TYLCV. Unfortunately, the use of these fine mesh screens coupled with the dusty conditions of the South of Morocco

can create climatic conditions that favor foliar diseases. But, even with high airflow resistance, adequate ventilation can be maintained by covering at least 30% of the greenhouse surface area with screens to maintain adequate natural ventilation for disease control.

Biological control

a) Pests

In Morocco, biological control is used against major greenhouse pests in over 300 ha of crops, of which, 200 ha are greenhouse tomatoes. The decision threshold and the number of natural enemies required for pest control are usually provided by companies that produce biological control agents (such as Biobest Maroc and Koppert Biological Systems). The most commonly used biocontrol agents for greenhouse tomato are *Eretmocerus* sp. and *Diglyphus* sp. *Eretmocerus eremicus* Rose & Zolnerowich and *Eretmocerus mundus* Mercet are released for control of *Trialeurodes vaporariorum* Westwood and *B. tabaci* in greenhouse crops in Morocco. *Macrolophus caliginosus* Wagner is used to a lesser extent for whitefly management because of its longer establishment period.

Diglyphus isaea (Walker) was among the first natural enemies to be used in large-scale biological control programs against leafminer on greenhouse tomato. For greenhouse peppers, the most commonly used biological control agents are *Aphidius colemani* Viereck, *Aphidoletes aphidimyza* (Rondani) and *Orius laevigatus* (Fieber). *Chrysodeixis chalcites* (Esper), and *Spodoptera littoralis* (Boisduval) are controlled using *Trichogramma* sp. and *Bacillus thuringiensis* Berliner treatments.

b) Diseases

Biological control of soil and airborne pathogens is gaining increasing interest with farmers and some biological agents for greenhouse crops have become available during the last 3 years. Examples include Trichodex, *Trichoderma harzianum* Rizai which has been registered for the control of *Fusarium* root and crown rot. Other biological control agents that are available are often commercialized as organic fertilizers to avoid the long administrative procedure of pesticide registration.

Intelligent chemical control

a) Selective mode of application

It is unfortunate that many growers continue to use high volume (HV) spraying (>1000 l/ha of spray solution) which can waste as much as 70-90% of the chemical application which drips to the ground. The overall contamination of the greenhouse area with pesticides can hinder the successful integration of pollinators and biological control agents. An alternative to HV spraying is the use of thermal foggers which a few farms have begun using during 2002. Early results indicate that foggers give growers clear savings in both the amount of pesticide used and the time and labor required to apply them. However, their use seems to be limited to relatively well-enclosed greenhouses.

Alternatives to spray treatments include the application of powdered sulfur for disease control and the use of chemigation using drip irrigation systems to apply systemic pesticides. Nowadays chemigation is becoming popular among tomato growers in Morocco because of its cost effectiveness and reduced toxicity for workers and beneficial organisms. However, some drip-applied systemic pesticides can still be harmful to beneficial organisms.

b) Selective timing and spraying

A popular saying is that "Timing is what IPM is all about" applies not only to pesticide treatments but also to other components of IPM (biological control, cultural practices, sanitation, pollination, etc.). The timing of the pesticide treatment is crucial in order to increase its effectiveness. Pest monitoring programs are currently only employed by a few greenhouse farmers who have hired specialized technicians for pest and disease monitoring. When coupled with selective spraying, pest monitoring has shown to be a cost-effective way of managing pests and diseases within an IPM program.

Pesticide resistance

The first cases of insecticide resistance in *B. tabaci* were observed in greenhouse tomatoes in the Souss Valley in 1999. This was the result of heavy chemical spraying following the introduction of TYLCV to Morocco. Since then we have reported several cases of *B.tabaci* exhibing varying degrees of resistance to pyrethroids and neonecotinoids and to insect growth regulators. Other cases of insecticide and fungicide resistance are suspected but are less well documented in Morocco.

The development of resistance is now more frequent because of the use of fine mesh screens in greenhouse tomato, which causes high selection pressure on a confined pest population. Pest and disease resistance will no doubt continue to present a significant challenge for chemical control programs involving greenhouse tomato in Morocco.

Needs and challenges of IPM in greenhouse tomato in Morocco

The change in pest and disease status as a result of accidental introductions of new pests and diseases will continue to constitute a real challenge to any IPM program. In Morocco, the recent introduction of TYLCV has disrupted existing IPM programs by increasing the number of insecticide applications used against the vector (from 9 to over 60 insecticide applications per tomato crop cycle). There is no doubt that the vast movement of people and agricultural products between Morocco and distant geographical areas will continue to provide tremendous opportunities for pests and diseases to move between widely separated geographical areas. Awareness of these threats is critical to all involved in the greenhouse industry (transporters, farmers, plant material producers, government agencies, etc).

In many tomato greenhouse operations in Morocco, farmers unfortunately still believe in a zero tolerance approach to pest control: this makes biological control and IPM difficult to apply. Another limitation is the lack of trained specialists required for technical supervision during IPM implementation. Even so, several incentives are pushing Moroccan tomato growers to apply IPM methods, reduce reliance on toxic pesticides and adopt more sustainable approaches to crop production. The most important incentive is that of making their products more competitive on world markets. Consumers are increasingly concerned with how fruit and vegetables are grown and how production practices affect the health of consumers and workers and the natural environment. Changing consumer demand was a major reason for the establishment of labels such as EUREPGAP. A key advantage of adopting IPM practices is that these programs can help to meet the demand for crops produced under programs such as EUREPGAP. This assures production practices that minimize exposure of greenhouse staff to toxic pesticides, that have fewer residues in the marketed product and that minimize threats to the environment. Many greenhouse operations are currently subject to certification schemes and this trend is expected to continue until 2005

when the majority of export farms in Morocco are expected to be growing according to these practices.

Section II. Whiteflies

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The potential application of entomopathogenic fungi isolates to control *Lecanoideus floccissimus* Martin *et al.* (Hemiptera: Aleyrodidae)

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Abstract: The pathogenic activity of five local isolates of entomopathogenic fungi were analysed against first stages of the "Spiralling whitefly" *Lecanoideus floccissimus* Martin *et al.*

Key words: Whiteflies, *Lecanoideus floccissimus*, microbial control, Canary Islands

Introduction

The "Spiralling whitefly" *Lecanoideus floccissimus* Martin *et al.* (1997) is a highly polyphagous pest and is responsible for great economic losses in the Canary Islands (Spain). It particularly affects banana crops and ornamental plants in greenhouses (Hernández-Suárez & Carnero, 2000).

Although it was first described with relation to the Canary Islands (Spain), it is generally considered a New World species (Martin *et al.*, 1997). Whitefly adults and nymphs cause damage by direct feeding on plants when present in very large numbers. Secondary problems are associated with the copious waxy flocculent material secreted by nymphs and the excretion of honeydew, which promotes the growth of sooty mould that in turn reduces the photosynthetic capacity of affected plants and interferes with chemical and biological control of this and other pests.

The whitefly is present throughout the year, though populations are highest in summer. Surveys for natural enemies of this pest in the Canary Islands showed the presence of several indigenous predators (coccinellids and neuropterans), but predator populations remained low and had little or no impact on whitefly, despite their severe incidence in crops (Hernández-Suárez *et al.*, 2002).

Chemical and biological control methods have been previously tried. Chemical control has proved ineffective on many occasions, furthermore, it is uneconomic and impractical due to the pest's broad host range (Hernández-Suárez *et al.*, 2002). Classical biological control has been attempted, including the introduction of the exotic parasitoid *Encarsia guadeloupae* Viggiani (Hymenoptera: Aphelinidae) (Nijhof *et al.*, 2000), but no significant degree of control has been achieved yet (Hernández-Suárez *et al.*, 2002). For the first time, a laboratory experiment was carried out in order to study the impact of several local isolates of entomopathogenic fungi on the first stages of whitefly development.

Material and methods

Investigations were carried out to assess the potential of *Beauveria bassiana* (Balsm)

Vuillemin (G1), *Aspergillus flavus* Link (TF71), *Lecanicillium lecanii* (Zimm) Zare & W. Gams (VH), *Metarhizium anisopliae* (Metsch) Sorokin (P58) and *Paecilomyces lilacinus* (Thom) Samson (G9) for microbially controlling the first developmental stages of *L. floccissimus*. These isolates were from different environments on the Canary Is., where they had been isolated from different soil types and insect species.

Bioassays were performed on detached leaf disks of *Strelitzia reginae* Banks carrying homogenous whitefly populations of 30 eggs. Entomopathogenic fungi suspensions from seven-day-old colonies on CMA were applied by spraying 36 µl per cm^2 at a standard spore concentration of 10^5 per millilitre with a commercial hand sprayer. Leaf disks were left to dry in order to prevent saprophyte growth and placed in moist chambers to maintain 100% relative humidity. Incubation was carried out in the dark at 25°C. The study consisted of five treatments and the control was replicated twice. The control involved the application of sterile distilled water.

The number of hatched eggs and live and dead first nymphal instar was assessed each day. Recording finished on the 12^{th} day after inoculation. The data resulting from the experiment was statistically analysed with non-parametric Kruskal-Wallis Analysis ($p \leq 0.05$) for not normally distributed data, using the SYSTAT 10 program (SPSS Inc., 2000).

Results and discussion

In general, mortality at the egg stage was low for all treatments. The highest cumulative mortality occurring at this stage (30%) corresponded to *Paecilomyces lilacinus* (G9) (table 1).

Table 1. Mean % of cumulative mortality (mean ± SE) of eggs and first instar of *Lecanoideus floccissimus* among treatments after 5 and 12 days inoculation (dpi).

Treatments	Egg	First instar	
	12 dpi	5 dpi	12 dpi
Control (C)	33.30 ± 0	0	0
Beauveria bassiana (G1)	11.66 ± 1.66	32.87 ± 7.87	84.03 ± 4.86
Aspergilus flavus (TF71)	15.00 ± 1.67	27.39 ± 3.39	72.40 ± 8.39
Lecanicillium lecanii (VH)	23.33 ± 0	43.48 ± 8.69	100 ± 0
Metarhizium anisopliae (P58)	23.33 ± 0	75.60 ± 1.68	88.94 ± 1.97
Paecilomyces lilacinus (G9)	30.00 ± 6.67	49.43 ±18.98	90.40 ± 0.93

Mean daily % mortality at the egg stage after 12 days of incubation showed no significant differences between treatments. However, *L. lecanii* (VH), *M. anisopliae* (P58) and *P. lilacinus* (G9) showed significant differences with respect to the control when the mean % daily mortality for the last 9 days of incubation was analysed (mortality was only recorded from the 3^{rd} day after inoculation). In both cases, no significant difference was observed between fungal isolates (fig. 1).

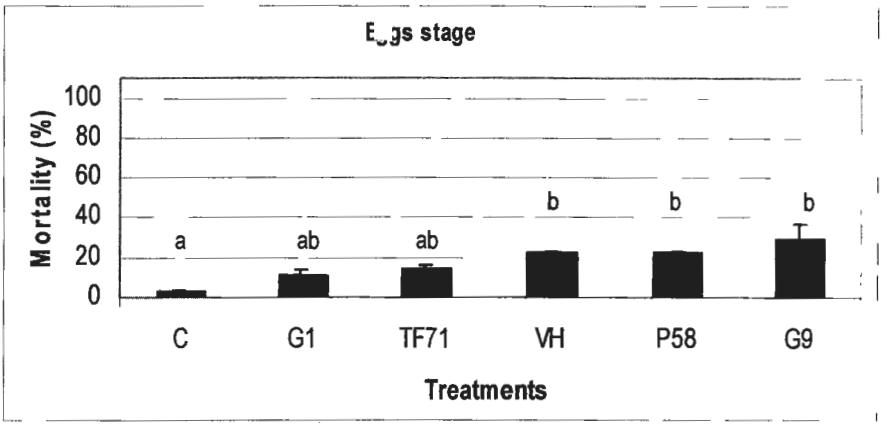

Figure 1. Mean % cumulative mortality (mean ± SE) between treatments at the egg stage 12 days after inoculation. Different letters indicate the existence of significant differences among treatments (Kruskal-Wallis test, $p \leq 0.05$)

Although the entomopathogenic fungi had little effect on egg hatching, they were able to infect 1st instar *L. floccissimus* nymphs after hatching. The mean cumulative mortality of the hatched 1st instar nymphs ranged from 72.4% (*A. flavus*) to 100% (*L. lecanii*) at the end of the study, while 100% of the nymphs in the control survived (table 1). *L. lecanii* was found to cause the highest level of mortality: its application resulted in 100% nymphal mortality (figure 2).

All treatments induced significant mortality with respect to the control but, the mean % of daily mortality between fungal isolates was not significantly different (Kruskal-Wallis test = 4.22; df = 4; P= 0.38).

As speed of kill could be considered as important as total mortality, the mean % of daily mortality after the first 5 days of incubation was also analysed. This showed that *M. anisopliae* produced significantly greater mortality than the other fungal treatments (table 1; figure 2).

L. lecanii and *M. anisopliae* had also been recorded as microbial agents for other whitefly pests such as *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) (Monzón, 2001; Skrobek, 2001), but no previous record had been found of their pathogenicity on *L. floccissimus*. In fact, no records were found about any microbial control agents against this whitefly species; only *Paecilomyces farinosus* (Holm.) Brown & Smith had been quoted as a biological control agent for the other "spiralling whitefly", *Aleurodicus dispersus* Russell (Ramani *et al*, 2002).

The application of *L. lecanii*, *M. anisopliae* and *P. lilacinus* showed great control potential for *L. floccissimus*, especially at the 1st nymphal instar. Investigations into the performance of these fungi with respect to other whitefly stages are currently being carried out.

References

Hernández-Suárez, E. and Carnero A. 2000. Descripción y biología de las especies de mosca blanca (Hemiptera: Aleyrodidae) de mayor interés económico en Canarias (III): ornamentales. La granja nº7. Cabildo Insular de Gran Canaria. . Consejería de Agricultura, Ganadería y Pesca. 59-64.

Hernández-Suárez, E., Carnero, A., Febles-González, J.C., Brito-Herrera, P., Medina-Alonso, G., Suárez Báez, J.M. and Amador Martín, S. 2002: Situación actual de las moscas blancas espirales en platanera. Actividades del ICIA en platanera. Instituto Canario de Investigaciones Agrarias. Gobierno de Canarias. Consejería de Agricultura, Ganadería, Pesca y Alimentación. 83-95.

Martin J.H., Hernández-Suárez, E. and Carnero A. 1997: An introduced new species of Lecanoideus (Homoptera:Aleyrodidae) established and causing economic impact on the Canary Island. Journal of Natural History. 31, 1261-1272.

Monzón, A. 2001: Producción, uso y control de calidad de hongos entomopatógenos en Nicaragua. Manejo Integrado de Plagas (Costa Rica) 63: 95-103.

Nijhof, B.W., Oudman, L., Torres R. and Garrido, C. 2000: The introduction of *Encarsia guadeloupae* (Hymenoptera, Aphelinidae) for control of *Aleurodicus dispersus* and *Lecanoideus floccissimus* (Homoptera: Aleyrodidae) on Tenerife. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society. 11: 41-47.

Ramani, S., Poorani, J. and Bhumannavar, B.S. 2002: Spiralling whitefly, *Aleurodicus dispersus*, in India. Biocontrol News and Information. 23 (2): 55-62.

Skrobek, A. 2001: Investigations on the effect of entomopathogenic fungi on whiteflies. Hohen Landwirtschaftlichen Facultät der Rheinischen Friedrich-Wilhelms-Universität zu Bonn. 114.

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Mise en évidence d'un déplacement orienté des aleurodes entre l'oasis et les serres dans le sud tunisien

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Résumé: L'étude de la dynamique de population des aleurodes entre l'oasis et les serres éloignées d'environ 800 mètres a confirmée l'hypothèse du rôle relais que joue l'oasis pour la contamination des serres dès le démarrage des cultures d'arrière-saison. En effet, les analyses montrent qu'une population importante de mouche blanche quitte les serres à la fin de la saison culturale pour se diriger vers l'oasis qui est très riche en plantes hôtes et offre des conditions climatiques très favorables. Mais le plus important et le plus grave c'est la population qui quitte l'oasis pour se diriger vers les serres une fois les cultures sont mises en place. C'est ainsi que dès le démarrage des cultures d'arrière-saison, l'attaque par le virus TYLC devient la règle dans les serres de tomate. Pour empêcher ou du moins diminuer l'importance des dégâts, il est devenu nécessaire de prendre les mesures qui empêchent le retour des aleurodes dans les serres à partir des oasis.

Mots clés : oasis, géothermie, serre, mouvement orienté, plante hôte refuge

Introduction

L'exploitation des eaux géothermiques en agriculture pour la production des primeurs s'est réellement développée en Tunisie avec la réalisation des forages au niveau de la nappe du complexe intercalaire dans le sud tunisien depuis 1986 (Ministère de l'Agriculture 1992). Ce nouveau écosystème agricole présente des spécificités climatiques et techniques, permettant l'installation d'un certain nombre de parasites et maladies difficiles à combattre moyennant les techniques appliqués en serriculture froide pratiquée au nord et au sahel de la Tunisie.

Entre autres problèmes phytosanitaires portant préjudice aux cultures maraîchères protégées et chauffées par les eaux géothermiques, les aleurodes prennent d'une année à l'autre plus d'importance. Extrêmement polyphages, ces insectes sont susceptibles de s'attaquer à la plupart de nos cultures, en particulier la tomate et le melon qui constituent les principales productions géoserricoles destinées à la l'exploitation.

Depuis son démarrage, le secteur géoserricole a connu un développement très rapide dans le sud tunisien. C'est ainsi que la Tunisie est placée parmi les premiers pays dans l'exploitation de l'énergie géothermique pour le chauffage des serres. Durant la campagne 2001-2002, 96,2 ha sont exploitées avec une production de 9370 tonnes (Ministère de l'Agriculture 2003). Au cours du 10ème plan de développement économique en Tunisie, il est programmé d'aménager 55 hectares pour la géoserriculture (Ministère de l'Agriculture 1992).

A la fin de la saison culturale les agriculteurs cessent de traiter dans les serres. On constate par conséquent la présence d'une population très importante de la mouche blanche sur les cultures. Cette population, qui est constituée en grande partie par des adultes et des pupes, sera éliminée des serres après l'arrachage des plantes qui vont finir par se dessécher. Les adultes présents, ainsi que ceux qui vont émerger des pupes, vont chercher d'autres cultures hôtes pour assurer leur maintien et leur développement.

Avec le démarrage des cultures d'arrière-saison vers la fin du mois d'août début du mois de septembre, une population d'adulte s'installe, provoquant des dégâts très importants par la

transmission du TYLC sur les jeunes cultures. La question qui se pose c'est l'origine de cette population si l'on connaît déjà que les adultes de la mouche blanche sont incapables de se maintenir dans la nature sans la présence des plantes hôtes et que durant la saison estivale les plantes hôtes refuges sont absentes dans les serres.

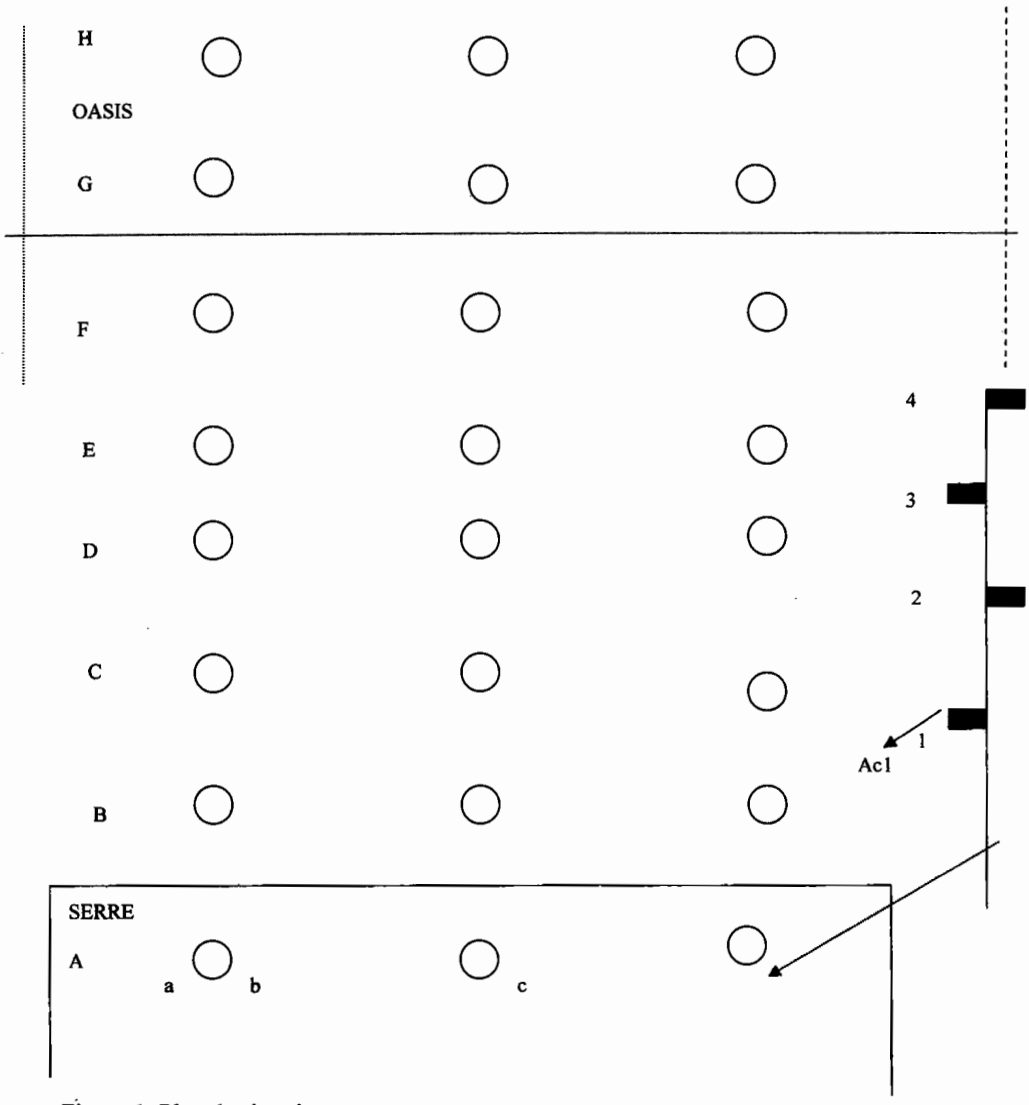


Figure 1. Plan de situation.

Du fait que les forages profonds sont réalisés essentiellement soit pour combler le déficit en eau des oasis existantes, soit pour la création de nouvelles oasis, les sites de géothermie sont généralement implantés au voisinage des ces dernières. C'est pourquoi on a pensé qu'il existe

une relation entre l'écosystème oasien et les serres pour le maintien de la mouche blanche. Pour étudier, le rôle des oasis dans l'état d'infestation des serres, des essais ont été entrepris dans un site représentatif dans la région de Kebili situé à Stiftimi. Cette étude vise à déterminer le niveau de déplacement des adultes de la mouche blanche et ses auxiliaires et la dynamique de déplacement des adultes entre les serres et l'oasis en fonction du temps.

Matériels et méthodes

Durant la saison estivale, juste après l'arrachage des cultures dans les serres au début du mois de juillet jusqu'a la fin du mois d'octobre après le bon démarrage de la plantation de l'arrière-saison, 24 poteaux en fer ont été installés en 8 lignes parallèles espacées de 100 mètres. Chaque ligne comporte 3 poteaux espacés d'environ 50 mètres. Le poteaux est d'une hauteur de 2 mètres et comporte 4 supports rectangulaires fixés horizontalement à différent niveaux sur le poteau. Le premier est à 0.50 mètre, le deuxième à 1 mètre, le troisième à 1.5 mètres et le quatrième à 2 mètres du sol. Les supports sont conçus pour la fixation d'une plaque jaune engluée sur les deux faces de 0,25 mètre de longueur et 0 ,20 mètre de largeur. Les plaques jaunes sont renouvelées chaque semaine pour être analysées sous loupe binoculaire au laboratoire. L'analyse consiste à identifier les différents insectes piégés et surtout le nombre de mouche blanche et ses auxiliaires.

Résultats et discussion

Mise en évidence d'un déplacement orienté des adultes des aleurodes (oasis – serre, serre – oasis)

Les données ont été arrangées et analysées d'une façon à permettre la mise en évidence du mouvement des adultes de la mouche blanche entre les oasis et les serres durant la saison estivale.

Les résultats obtenus suite à une analyse des variances selon la statistique (logiciel SAS) montrent qu'il y a une différence hautement significative entre les populations globales piégées sur les plaques des trois rangées. On constate que les populations sur la rangée (a) située du coté ouest sont plus importantes que les populations sur la rangée (b) située au milieu qui sont plus importantes que celles de la rangée (c) située plus à l'est.

Ce ci pourrait s'expliquer par le fait que les adultes de la mouche blanche ne sont pas de bons voiliers et sont généralement transportés par le vent. C'est ainsi que, les adultes sont transportés de Nord est et se trouvent en majeure partie sur les plaques situées le plus à l'ouest. Ce résultat oriente d'avantage l'emplacement des pièges pour le suivi de la dynamique des populations pour déterminer un programme de contrôle.

L'analyse des données selon la statistique SAS, montre l'existence d'un déplacement orienté entre les oasis et les serres. Au niveau des poteaux sur les lignes A et H les populations sont toujours relativement importantes sur les deux faces, puisque ces poteaux sont placés dans les périphéries respectives des serres et des oasis ou les adultes se trouvent en tourbillon. C'est au niveau des plaques sur les poteaux intermédiaires (B, C, D, E, F, G) que le sens de mouvement des adultes en fonction du temps est bien défini.

Sur la face sud des plaques qui est orientée du coté des serres on enregistre une population très importante au début de l'été juste après l'arrachage des cultures sous serres, puis elle diminue d'une façon brutale pour rester à un niveau très faible durant toute la saison estivale pour augmenter légèrement au cour du mois de septembre et diminuer à nouveau à partir du mois d'octobre.

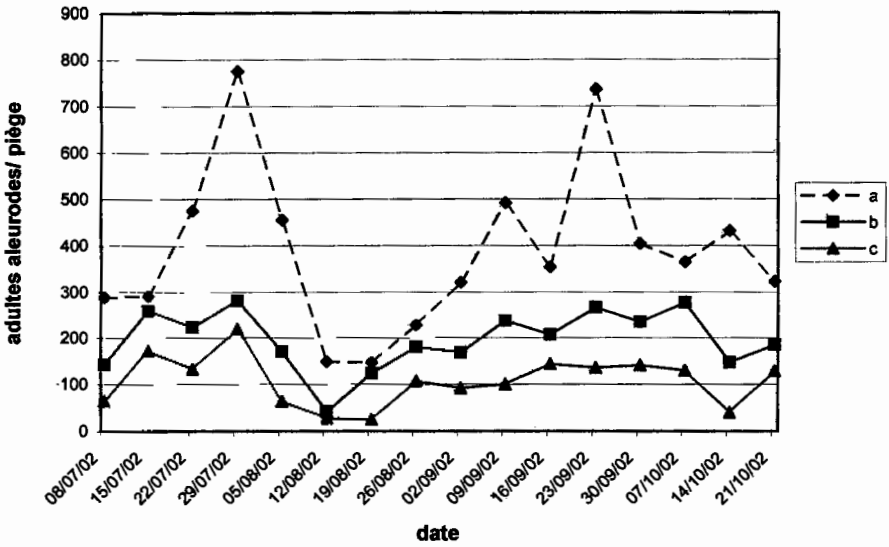
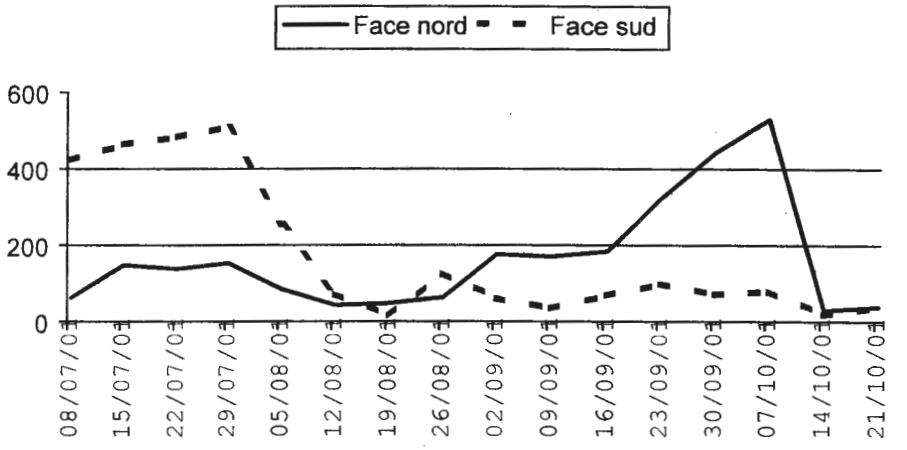


Figure 2. Nombre moyen d'adultes piégés dans des plaques jaunes engluées situées au long de trois rangées, (a) ouest, (b) centre, (c) est.

A la fin des cultures du primeur dans les serres, qui coïncide avec des conditions climatiques très favorables à la multiplication des aleurodes, les populations deviennent très importantes surtout sur les cucurbitacées. Ces populations sont généralement représentées par des adultes et des larves de dernier stade et des pupes. Au début de l'été lorsqu'on arrache les plantations, les pupes vont donner des adultes qui s'ajoutent à la population adulte existante.

Ces adultes qui exigent des végétaux hôtes pour se nourrir et se multiplier vont se trouver dans un biotope pauvre en végétaux et seront obligés de quitter vers un biotope favorable représenté dans notre cas par les oasis qui sont au voisinage et qui sont botaniquement riches en végétaux hôtes des aleurodes. C'est cette nécessité qui a fait qu'on enregistre une population importante qui s'oriente vers les oasis, juste après l'arrachage. Ceci a été justifié par les populations piégées sur la face sud des plaques qui est le sens du coté des serres. La faible population enregistrée sur cette face au cours de la saison estivale pourrait s'expliquer par la présence d'un mouvement tourbillonnaire entre les poteaux.

La légère augmentation de la population sur la face sud au mois de septembre s'explique par l'importance de population qui s'installe au début de l'arrière- saison sur des cultures à un stade végétatif encore insuffisant pour satisfaire leurs besoins. C'est ainsi que les populations enregistrées sur la face sud, lorsque la végétation devient importante diminuent d'une façon très remarquable.



Sur la face nord des plaques qui est le sens du coté des oasis, on enregistre un niveau de population relativement plus important durant toute la période estivale avec un pic très important au début de repiquage des cultures dans les serres au cours du mois de septembre.

L'oasis constitue en effet un milieu très favorable de point de vu richesse en plantes hôtes et de point de vu conditions climatiques au cours de la saison estivale, et permet aussi la production d'une population très importante de la mouche blanche dont une grande partie sera attirée par les nouvelles plantations dans les serres.

Références

Ministère de l'Agriculture 1992. Stratégie Nationale d'Utilisation des eaux géothermales en agriculture. DG/PDIA Tunisie pp 52

Ministère de l'Agriculture 2003. Rapport sur le secteur géoserricole campagne 2002-2003. DGPA Tunisie.

Oriented-movement of whiteflies between oasis and greenhouses in southern Tunisia

Abstract: Whiteflies were monitored along all the season in a greenhouse crop in southern Tunisia by means of yellow sticky traps. The study confirmed that an oasis located at a distance of 800m from the greenhouse play a role as an intermediate habitat of whiteflies allowing these to infest late season crops (sown late August- early September). Results show that a high number of whiteflies may leave greenhouses to move to oasis, that is rich in whitefly hosts and offer good climatic conditions. Later whiteflies go back to greenhouses and late season crops become soon infested being tomatoes infected by Tomato Yellow Leaf Curl Virus. In order to decrease virus incidence, it is necessary to adopt measures to prevent whiteflies coming back to greenhouses from oasis.

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New developments in *Bemisia tabaci* resistance to insecticides in greenhouse tomato in Morocco

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Abstract: In this study, 10 populations of whiteflies *Bemisia tabaci* were collected at conventional farms in the Souss-Massa Valley of Morocco and bioassayed for resistance to three commonly used insecticides (imidacloprid, thaimethoxam and methomyl). Using adult leaf-dip bioassay, we concluded that all the populations tested were resistant to the three compounds. The highest level of resistance was to imidacloprid followed by thiamethoxam and methomyl. Population *Bchk* was very resistant to both imidacloprid (RF = 39) and thiamethoxam (RF = 12). A very high resistance to methomyl was expressed by two populations: *Bcht* and *Nac* (RF = 4).

Keywords: *Bemisia tabaci*, insecticides, resistance, TYLCV, tomato, neonicotinoides, methomyl and cross-resistance.

Introduction

The Souss valley of Morocco has experienced a severe crisis since 1998 with the introduction of TYLCV (Tomato Yellow Leaf Curl Virus) (Hanafi 1999). Since then, farmers have relied heavily on insecticides in order to control the vector. *Bemisia tabaci* (Gennadius) has the capacity to rapidly develop resistance to many insecticides (Dittrich *et al.* 1990a; Dennehy *et al.* 1997). In 2001, the first cases of resistance were confirmed with laboratory bioassays (Hanafi *et al.* 2002; Hanafi *et al.* 2003; Bouharroud *et al.* 2003) in the Souss valley.

In this work, we report on continuing efforts – carried out at 10 separate locations - to trace *B. tabaci* resistance to the following compounds: imidacloprid, thaimethoxam and methomyl.

Material & Methods:

Insects

The populations of *B. tabaci* used in this study were collected at 10 privately owned farms located in the Souss valley. A susceptible population was reared without insecticide applications in the glasshouse at the Agronomic & Veterinary Institute (Complexe Horticole d'Agadir).

Insecticides

The following formulated insecticides were used for bioassays: thiamethoxam "Actara 25 WP", imidacloprid "Confidor 200 SL" and methomyl "Lannate 20".

Bioassays of adult B. tabaci

The leaf-dip bioassay method described by Cahill *et al.* (1995) was used with slight modifications. Leaf discs (50 mm in diameter) were cut from cucumber leaves and dipped in different concentrations of insecticides for 5 to 10 seconds. The leaf discs were then dried in the ambient air in the laboratory. The bases of small Petri dishes (55 mm in diameter) were filled with 7 ml of agar gel (15 g/l). The leaf discs were placed on the agar with their upper sides face down. In order to facilitate insect manipulation, we placed *B. tabaci* adults in the freezer for 10 minutes. In this protocol we used low temperature to immobilize whiteflies instead of anaesthetising them with CO_2. Using a fine brush, 20 to 30 females were moved into the Petri dish containing the treated leaf discs and then initial mortality was evaluated after 1 hour. The dishes were placed upside down to stimulate the normal feeding orientation of the whiteflies. Each bioassay was replicated three times using at least 5 concentrations (2 X to 0.0625 X, with X being the recommended concentration) and four replicates for each concentration. We used distilled water as a control for leaf discs. Mortality was assessed after 48 h.

Data analysis

Probit analyses of concentration-dependent mortality data were made using POLO-PC (Anon., 1987). Resistance factors were calculated at LC_{50} relative to the susceptible population (1)

$$RF = \frac{\text{LC50 of population X}}{\text{LC50 of Susceptible population}} \qquad (1)$$

Results and Discussion

Neonicotinoids

All populations bioassayed were resistant to neonicotinoids tested (Table 1). The highest level of resistance to imidacloprid was recorded with population *Bchk* (RF = 39) and the lowest was expressed by population *IAV* (RF = 2). RFs related to thiamethoxam ranged from 2 (*IAV*, *CMV* and *Cht1*) to 12 (*Bchk*). We should add that the slope-values of both imidacloprid and thiamethoxam were generally low.

Carbamates

Methomyl is known to be more toxic than the other two compounds tested. The level of resistance to methomyl was generally the least variable in all the populations tested, and the RF interval ranged from 2 to 4 (Table 2). Three populations (*CMV*, *IAV* and *Bogr*) were relatively susceptible, though their LC_{50}s were respectively 74, 83 and 107 with respect to the susceptible population (65,3)

Several authors have confirmed *B. tabaci* resistance to neonicotinoids (Williams *et al.* 1996; Elbert *et al.* 1996). Dittrich *et al.* (1990b) tested many *B. tabaci* populations from Sudan and other countries and reported that all these populations have a substantial variability in response, especially to Organophosphorus.

Elbert *et* al. (1996, 2000) revealed significant variation in the response of *B. tabaci* populations from Almeria, in southern Spain, to imidacloprid. Horowitz *et al.* (1998) reported a higher level of *B. tabaci* resistance to methomyl than we found in the Souss Valley.

Table 1. Log-dose probit mortality data for adult *Bemisia tabaci* treated with two neonicotinoids

	Imidacloprid				Thiamethoxam			
Populations	**LC_{50}**	95% cf. limit	Slope±SE	**RF**	**LC_{50}**	95% cf. limit	Slope±SE	**RF**
CMV	79.0	60.5-100.1	1.8±0.2	**3**	30.2	23.9-37.1	1.9±0.2	**2**
Cht1	520.5	128.5-246.1	1.6±0.3	**21**	33.2	25.6-42.2	1.6±0.2	**2**
Cht3	487.0	239.6-2067.5	0.8±0.2	**19**	64.4	43.7-107.3	1.1±0.2	**4**
Bcht	337.6	176.5-1077.7	1.8±0.2	**13**	74.4	51.1-120.6	1.3±0.3	**4**
Bchk	977.3	159.3-1107.5	1.8±0.3	**39**	196.1	48.3-121.6	1.2±0.2	**12**
Nac	176.1	128.6-272.4	1.5±0.3	7	79.2	52.2-140.1	1.2±0.2	**5**
Baa	91.0	72.3-110.1	2.3±0.3	**4**	55.8	42-74.1	1.7±0.3	**3**
Bog	117.0	78.0-198.9	1.1±0.2	**5**	55.5	43.3-70.6	2.1±0.3	**3**
Dur	215.4	142.1-424.2	1.2±0.2	**9**	72.0	53.1-104.1	1.6±0.3	**4**
IAV	62.7	46.8-82.1	1.4±0.2	**2**	26.3	21.1-32.2	1.9±0.2	**2**
Susceptible	25.3	19.6-31.2	1.9±0.2	-	16.7	13.6-20.0	2.2±0.2	-

Table 2: Log-dose probit mortality data for adult *Bemisia tabaci* tested with methomyl

Insecticide	**Methomyl**			
Populations	**LC_{50}**	95% conf. limit	Slope±SE	**RF**
CMV	74.0	47.2-84.5	1.6 ± 0.2	**1**
Cht1	112.6	84.0-146.1	1.6 ± 0.2	**2**
Cht3	162.8	115.6-229.5	1.3 ± 0.2	**2**
Bcht	270.1	190-412.1	1.4 ± 0.3	**4**
Bchk	239.2	168.3-358.4	1.3 ± 0.2	**3**
Nac	265.3	187.8-406.9	1.3 ± 0.2	**4**
Baa	189.3	143-243.2	1.9 ± 0.3	**3**
Bog	107.0	85.1-130.6	2.0 ± 0.2	**1**
Dur	147.8	112.9-187.7	1.7 ± 0.2	**2**
IAV	83.0	63.8-103.7	1.7 ± 0.2	**1**
Susceptible	65.3	54.5-98.1	1.1 ± 0.1	-

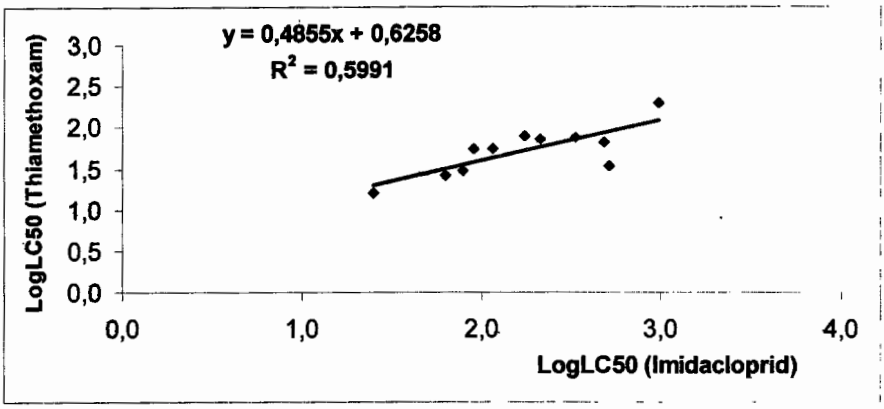

Figure 1: Relationship between LC_{50} of imidaclporid versus thiamethoxam for 11 populations of *Bemisia tabaci.*

Cross-resistance

A comparison of $logLC_{50}$s for all populations showed a very highly significant ($P< 0.001$) positive correlation between $logLC_{50}$s of imidacloprid and thiamethoxam ($r = 0.774$) (Figure 1). Those two compounds belong to the same family (neonicotinoids) which causes over-stimulation and blockage of the acetylcholine receptors (Mullins, 1993).

References

Anon. (1987) POLO-PC – a user's guide to Probit or Logit analysis. 22 pp. LeOra Software, California.

Bouharroud R., Hanafi A., Murphy B., Haouass F. and Serghini M. A. 2003. Evidence of *Bemisia tabaci* resistance to some commonly used insecticides in greenhouse tomatoes in Morocco. Abstract Proceeding of the 3rd International *Bemisia* Workshop Barcelona 17-20 March, 2003

Cahill, M., Byrne, F.J., Gorman K., Denholm, I. And Devonshire, A.L., (1995) Pyrethroïd and organophosphate resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae). Bulletin of Entomological research 85: 181-187.

Dennehy, T. J. and Williams, L. 1997. Management of resistance in *Bemisia* in Arizona cotton. Pesticide Science 51, 398-406

Dittrich, V., Uk, S. and Ernst, G. H. 1990a. Resistance mechanisms in sweetpotato whitefly (Homoptera : Aleyrodidae) populations from Sudan, Turkey, Guatemala and Nicaragua. Journal of Economic Entomology 83, 1665-1670

Dittrich, V., Uk, S. and Ernst, G. H. 1990b. Chemical control and insecticide resistance of whiteflies. In *Whiteflies : their systematics, pest status and management* (ed. D. Gerling), pp. 263-285.

Elbert A. and Nauen R. 1996. Bioassays for Imidacloprid for resistance monitoring against the whitefly Bemisia tabaci. Proceeding of Brighton Crop Protection conference – Pests and Diseases.

Elbert A. and Nauen R. 2000. Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern spain with special reference to neonicotinoids. Pest Management Science 56 : 60 – 64.

Hanafi A., Bouharroud R. and Haouass F. 2002. Evaluating *Bemisia tabaci* resistance to the most common neocotinoids used in Protected Cultivation in Morocco. European Whitefly Studies Network. pp.13-15. EWSN Abstract Compendium 245 p.

Hanafi A., Bouharroud, R. and Murphy, B. 2003. *Bemisia tabaci* insecticide resistance in Morocco. European Whitefly Studies Network Newsletter Issue N0 15, January 2003.

Hanafi, A., (1999) Oui! Vous pouvez gagner la guerre contre la mouche blanche et le TYLCV : 54-56.

Hanafi, A., (2001) Mouche blanche et TYLCV quel management? La lutte chimique. Attention aux problèmes de résistance aux insecticides : 49-51.

Horowitz, A.R., Phyllis, G.W., and Ishaaya, I. (1998) Status of Pesticide Resistance in Arthropod pests in Israel. Phytoparasitica 26(3).

Mullins, J. W. 1993. Imidacloprid : a new nitroguanidine insecticide. American chemical society Symposium Series 524 : 183 – 198.

Williams, L, III, T. J. Dennehy and J. C. Palumbo 1996. Whitefly control in Arizona : development of a resistance management program for imidacloprid. Proceeding Beltwide cotton conferences. pp. 752-755.

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Development of light-emitting diode (led) traps for whiteflies and other insects

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Abstract: We equipped yellow sticky card traps and plastic cup traps with 530 nm lime green light-emitting diodes (Nichia America Corp.) (LED-YC and LED-PC traps, respectively) to increase trap efficacies for capturing whiteflies and other insects. More adult greenhouse whiteflies *Trialeurodes vaporariorum* (Westwood), sweetpotato whiteflies (SPW), *Bemisia tabaci* (Gennadius) biotype B (= *B. argentifolii*), cotton aphids *Aphis gossypii* (Glover), and fungal gnats *Bradysia coprophila* (Lintner) were caught in lime green LED-YC traps than unlit YC traps in greenhouse cage studies with shell beans, *Phaseolus vulgaris* (L.) var. *humilis*, or cotton, *Gossypium hirsutum* (L.) cv. Deltapine 5415. In commercial greenhouses, the lime green LED-YC traps did not catch any more *Franklinella occidentalis* (Pergrande) than the unlit YC traps. In a laboratory test, lime green LED-PC traps caught more adult greenhouse whiteflies and SPW than the PC traps with white LEDs or unlit PC traps. *Ertmocerus eremicus* (Rose and Zolnerowich) parasite captures were not significantly different in lime green LED-YC traps compared with unlit YC traps. In greenhouse tests, the lime green LED-PC traps caught fewer *E. eremicus* and *Encarsia formosa* (Gahan) whitefly parasitoids than unlit YC traps. The results demonstrate the potential of using lime green LED-YC traps in greenhouses for insect detection, monitoring, and control. The lime green LED-PC traps appear to be complimentary and compatible for use in combination with releases of *Eretmocerus* or *Encarsia* for whitefly nymph control in greenhouses.

Key words: *Aphis gossypii, Bemisia tabaci, Bradysia coprophila, Encarsia formosa, Ertmocerus eremicus, Franklinella occidentalis, Trialeurodes vaporariorum,* LED-YC traps, YC traps, LED-PC traps

Introduction

Yellow sticky card (YC) traps have been used widely for detecting, monitoring, and controlling whiteflies in greenhouses. Aphids, leaf miners, *Liriomyza sativae* (Blanchard), western flower thrips, *Frankliniella occidentalis* (Pergrande), and whitefly parasitoids, *Encarsia* spp. and *Eretmocerus* spp., have also been reported to be attracted to yellow. Studies on sweet potato whitefly (SPW) and *Bemisia tabaci* (Gennadius) biotype B behavioral characteristics have led to the development of a plastic cup (PC) trap, also known as a CC trap, which is selective for SPW (Chu and Henneberry 1998). Here we briefly summarize studies on the development and demonstration of light emitting diode equipped lime green YC traps and PC traps (LED-YC and LED-PC, respectively) to improve the effectiveness of these traps in greenhouses.

Material and methods

All experiments were conducted in randomized block designs with 4-10 replicates under greenhouse conditions and in cages. The only exception was experiment 1, which was conducted in a dark room. All test insects except fungal gnats were reared in the insectary and released in cages. These gnats developed in the plant growth media. Experiment 1 compared catches of adult whiteflies (greenhouse and SPW) in white and lime green LED equipped PC traps. The white and lime green LEDs (Nichia NSPPG500S and NSPPW500BS, Nichia America Corp., Mountville, PA) were installed in white trap base PC traps. Unlit PC traps were used as controls. Experiment 2 compared lime green LED-clear plastic sticky card traps or lime green LED-YC traps (Figure 1) with unlit clear plastic sticky card traps or YC traps for catches of whitefly species, cotton aphids, and fungal gnats. Experiment 3 compared catches of SPW and whitefly parasitoids *E. eremicus* and *En. formosa* in lime green LED-PC traps (Figure 2) and unlit PC traps or YC traps. Experiments 4 and 5 were conducted to evaluate the efficacies of lime green LED-YC traps and Tangle-foot® (Tanglefoot Co., Grand Rapids, MI) interior coated-PC traps with unlit YC traps for catching SPW, thrips, fungal gnats, and whitefly parasitoids in commercial greenhouses.

Results and discussion

PC traps equipped with lime green LEDs caught significantly more greenhouse whitefly and SPW compared with traps equipped with white LEDs or unlit PC traps (Table 1). The proximity of the unlit-PC to other LED-PC traps resulted in 2.2 lumens measured for the unlit-PC trap. We reported earlier that in the field SPW were attracted to green, yellow, and orange (490-600 nm) in the visible light spectral range. The peak wavelength of our lime green PC base was close to that of the spectral reflectance curve of the underleaf surfaces of green cotton leaves (Chu et al. 2000). Results of lime green LED-clear plastic sticky card traps and lime green LED-YC trap catches in greenhouse cage studies (Table 2) indicate that lime green LEDs have potential for increasing whitefly catches in YC traps. The lime green LED-YC traps were also attractive to cotton aphids. In addition, the lime green LED-YC trap appears to be highly attractive to fungal gnats that are also a nuisance in greenhouses. Lime green LED-PC traps caught significantly more whiteflies than PC traps coated on the interior with Tanglefoot, but few whitefly parasitoids compared with YC traps (Table 3). Results in Table 4 showed that lime green LED-YC traps caught significant numbers of SPW and fungal gnat adults in greenhouse but not thrips. Thrips are known to be attracted to blue and white colors (Chu et al. 2000) so the results were not unexpected. One Tanglefoot coated-PC trap caught as many SPW as one 100 cm^2 YC trap, but 100% and 43% fewer *E. eremicus* and *En. formosa* parasitoids, respectively (Table 5).

Whiteflies are economic pests worldwide. The application of insecticides has not been effective in controlling whiteflies in greenhouses. An integrated approach using biological agents warrants more attention. Results indicate that the lime green LED-PC trap is compatible with the release of whitefly parasitoids for whitefly nymph control in greenhouses. The lime green LED-YC trap and LED-PC traps we have developed may be an alternative to YC traps for increasing trap catches. The cost effectiveness of the two traps requires further study.

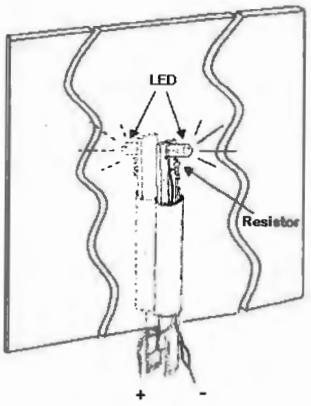

Fig. 1. Light-emitting diodes (LEDs) attached to a yellow sticky card trap using a hair clip assembly with one LED on each side of the sticky card. (Source: Chu et al. 2003a)

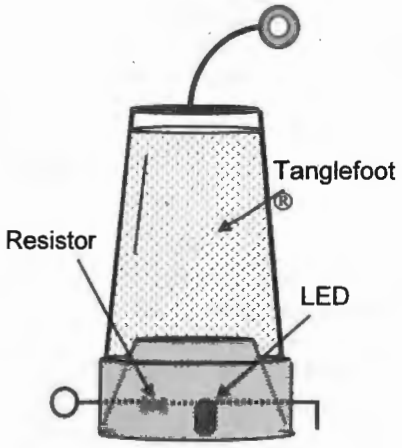

Figure 2. Light-emitting diodes (LED) plastic cup trap. (Source: Chu et al. 2003a)

Table 1. Light intensity and mean number of adult *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B caught in white base plastic cup traps equipped with white or lime green light emitting diode (LED) in a dark room. (Source: Chu et al. 2003b)

Trap type	Lumens	No. adults/trap/day	
		T. vaporariorum	*B. tabaci*
Unlit-plastic cup	2.2	10.0b[a]	91.3b
White LED-plastic cup	2.6	24.3b	52.6b
Lime green LED-plastic cup	2.6	138.1a	270.1a

[a]Tukey's test, $P = 0.05$. Means in a column not followed by the same letters are significantly different.

Table 2. Mean number of adult *Trialeurodes vaporariorum*, *Bemisia tabaci* biotype B, and *Aphis gossypii* caught on lime-green light-emitting diode equipped clear or yellow sticky-card traps in cages in a greenhouse grown with shell bean and upland cotton plants. (Source: Chu et al. 2003b)

Trap type	Insect species	No. adults/100 cm^2/day	
		LED equipped	Unlit
Clear plastic sticky card	*B. tabaci*	74.3a[a]	13.3b
	T. vaporariorum	7.4a	3.9b
	B. coprophila	4.0a	0.8b
Yellow sticky card	*B. tabaci*	388.3a	274.5b
	T. vaporariorum	85.4a	65.3b
	B. coprophila	6.2a	1.3b
	A. gossypii	23.0a	12.7b

[a]*t* - test, $P = 0.05$ Means in a row not followed by the same letters are significantly different.

Table 3. Mean number of adult *Bemisia tabaci* biotype B and *Eretmocerus* and *Encarsia* caught with lime-green emitting diode equipped plastic cup (LED-plastic cup) and unlit yellow sticky card or Tanglefoot coated plastic cup traps in greenhouses grown with bush bean and tomato plants. (Source: Chu et al. 2003a and 2003b)

Trap type	No. adults/100 cm^2 or trap/day[a]		
	B. tabaci	*E. eremicus*	*En. formosa*
Yellow sticky card	232.4a[a]	39.2a	81.6a
LED-plastic cup	189.2a	2.8b	5.3b
Coated plastic cup	497.7b	1.8b	0.0a
LED-plastic cup	3,055.3a	33.4a	0.2a

[a]*t* – test, $P = 0.05$. Means in a column of the paired traps not followed by the same letters are significantly different.

Table 4. Mean number of adult *Bemisia tabaci* biotype B, *Frankliniella occidentalis,* and *Bradysia coprophila* caught with lime-green light-emitting diode (LED) equipped and unlit yellow sticky card traps in commercial greenhouses. (Source: Chen et al. 2003 and Chu et al. 2003b)

Plants	Trap type	No. adults/100 cm^2/ week		
		B. tabaci	*F. occidentalis*	*B. coprophila*
Poinsettia	Yellow sticky card	246.3b[a]	2.2a	8.0b
	LED-yellow sticky card	347.5a	2.5a	44.1a
Gerbera	Yellow sticky card	-	7.3a	1.0b
	LED-yellow sticky card	-	8.4a	2.4a

[a] *t* - test, $P = 0.05$. Means in a column of a plant species not followed by the same letters are significantly different.

Table 5. Mean numbers of *Bemisia tabaci* biotype B and *Eretmocerus* and *Encarsia* caught with Tanglefoot coated plastic cup and yellow sticky card traps in mixed vegetable commercial greenhouse. (Source: Chu et al. 2003a)

Trap types	No. adults/100 cm^2 or trap/day		
	B. tabaci	*E. eremicus*	*En. formosa*
Yellow sticky card	6.7a	4.1a	0.7a
Coated plastic cup	5.5a	0.0b	0.4a

t - test, $P = 0.05$. Means in a column not followed by the same letters are significantly different.

References

Chen, T-Y and C. C. Chu. 2003. Insect survey on poinsettia with yellow sticky card traps equipped with light-emitting diodes. J. Econ. Entomol. (in review).

Chu, C. C., and T. J. Henneberry. 1998. Development of a new whitefly trap. J. Cotton Sci. 2: 104-109.

Chu, C. C., P. J. Pinter, Jr., T. J. Henneberry, K. Umeda, E. T. Natwick, Y.-A. Wei, V. R. Reddy, and M. Shrepatis. 2000. Use of CC traps with different trap base colors for silverleaf whiteflies (Homoptera: Aleyrodidae), thrips (Thysanoptera: Thripidae), and leafhoppers (Homoptera: Cicadellidae). J. Econ. Entomol. 93: 1329-1337.

Chu, C. C., C. G. Jackson, P. J. Alexander, Kamil Karut, and T. J. Henneberry. 2003. Plastic Cup Traps Equipped with Light-Emitting Diodes for Monitoring Adult *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. (in press)

Chu, C. C., A M. Simmons, P. J. Alexander, T. Y. Chen, and T. J. Henneberry. 2003b. Using lime green light-emitting diodes to improve trap catches of *Trialeurodes vaporariorum*, *Bemisia tabaci* (Homoptera: Aleyrodidae), and Other Pests in Greenhouses. J. Econ. Entomol. (in review).

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Encarsia formosa and *Encarsia pergandiella*: addition or substraction

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Abstract: *Encarsia pergandiella* was introduced into Italy in the late 70s and since then has been recorded parasitising whiteflies in several Mediterranean areas. *Ep* is a solitary arrhenotokous autoparasitoid that needs to hyperparasite larvae of its own or other *Encarsia* species in order to produce males. In greenhouse tomato crops in northeast Spain, spontaneous colonization by *Ep* was able to prevent successful establishment and almost replace *E. formosa*. In this work we evaluate *Ep* host killing capacity (host feeding and offspring) when *Trialeurodes vaporariorum* and *E. formosa* pupae were available. When only greenhouse whitefly were available, about 10 % of prey were consumed, whereas consumption was higher when *E. formosa* were added to the arena or when only *E. formosa* were offered as prey. More descendants were produced when primary and secondary hosts were available and fewer primary than secondary hosts were parasitised in arenas with mixed host types. This showed that mated *Ep* females clearly preferred of to oviposit on *E. formosa* pupae.

Key words: greenhouse whitefly, *Encarsia formosa*, *Encarsia pergandiella*, host feeding, sex ratio

Introduction

Encarsia formosa (*Ef*) Gahan (Hymenoptera: Aphelinidae) is successfully used world-wide (van Lenteren *et al.*, 1995; Gabarra & Besri, 1999) to control *Trialeurodes vaporariorum* (*Tv*) Westwood (Homoptera: Aleyrodidae). The exotic species *Encarsia pergandiella* (*Ep*) Howard was introduced into Italy in the late 70s with the objective of complementing whitefly control (Viggiani & Mazzone, 1980). Since then, *Ep* has been recorded parasitising *Tv* and *Bemisia tabaci* Gennadius in greenhouses and open fields in several Mediterranean areas including southern France (Onillon *et al.*, 1994), northeast Spain (Gabarra *et al.*, 1999), and Italy (Mazzone & Viggiani, 1985). *Ep* is a solitary arrhenotokous autoparasitoid that can primarily parasitise several whitefly species and which needs to hyperparasite larvae of its own or other *Encarsia* species to produce males. Consequently, *Ep* may interfere with other parasitoids released for whitefly control. In greenhouse tomato crops in northeast Spain, spontaneous colonization by the naturalized exotic parasitoid *Ep* was able to prevent the successful establishment of *Ef* and almost replace their inoculative releases (Gabarra *et al.*, 1999). In Italy, it has also been shown that *Ep* may successfully compete with *Ef* for whitefly control in greenhouses (Giorgini & Viggiani, 2000).

This study aimed to evaluate the selective capacity of *Ep* females to feed and oviposit on secondary (*Ef* larvae) as opposed to primary hosts (*Tv* nymphs).

Material and Methods

Encarsia pergandiella used in the experiments came from a continuous rearing established in the laboratory. Insects were originally collected from a local tomato greenhouse (Maresme, Barcelona). *Ef* came from a long term rearing maintained at our Institute. Both parasitoids were reared on *Tv* nymphs in greenhouse cages. Whitefly cultures were maintained under

greenhouse conditions on tobacco. Experiments were conducted in a controlled climatic chamber at 25±1 °C, with a 16:8 light:dark photoperiod and 70±10% relative humidity.

A ventilated plastic cage (7.5 cm Ø) with a tobacco leaflet placed upside down on a 4 mm agar layer (1%) was used as a search arena. Forty black pupae (0 *Tv*: 40 *Ef*), 40 white pupae (40 *Tv*: 0 *Ef*) and 20 white and 20 black pupae (20 *Tv*: 20 *Ef*) were detached from tobacco leaves using a needle and deposited in each arena in four lines of ten pupae. Alternate black and white lines of ten pupae were deposited in the 20 *Tv*: 20 *Ef* treatment. To avoid adult emergence during the experiment white pupae were collected in a transitional substage prior to becoming pharate adults (red eyes visible). In order to obtain unmated females, *Ep* parasitised pupae were kept in gelatine capsules until adult emergence. Females, and males reared on *Ef*, were kept in cages for 24 hours to obtain mated females. One 24-48 hour-old female was placed in the arena and then removed after 48 hours. There were between 13 and 28 replicates for mated and unmated females in each *Tv*/*Ef* ratio. Arenas with black and white pupae but with no *Ep* females were also prepared in order to estimate natural mortality. Arenas were examined every 2 to 3 days until whitefly and parasitoid emergence. Whitefly and *Ef* pupae mortality was corrected with the mortality rate observed in control arenas without *Ep* females. Statistical analyses were conducted using analysis of variance (ANOVA), and means were separated using Tukey's studentised range test. Data were transformed using the log10 (x+1).

Manly's alfa index for a variable prey population was calculated for each prey:

$$\alpha_i = \frac{\log p_i}{\sum_{j=1}^{m} \log p_j}$$

where p_i, p_j are proportions of the prey i or j remaining at the end of the experiment: $p_i = n_i / e_i$ in which n_i is the number of prey i present at the start of the experiment and e_i the number of prey i alive at the end of the experiment. The standard t-test was used to test the mean preference values for the two prey species. Cages with fewer than 10 prey from each species remaining at the end of the experiment were not considered when calculating this index, as is usually required (Krebs, 1989). Host killing capacity (offspring plus host feeding) was analyzed using a paired t-test for the mixed choice tests (Horton 1995).

Results

Encarsia pergandiella females preyed on a similar number of prey independently of whether they were mated or not. However, prey consumption did vary according to relative numbers of greenhouse whitefly and *Ef* in the arena (Fig. 1). Diet composition also varied according to different proportions of host types (Table 1). Fig. 1 shows that when only greenhouse whitefly were available, about 10 % of prey were consumed, whereas consumption was significantly higher ($P<0.05$ for unmated and mated females) when *Ef* were added to greenhouse whitefly in the arena, or when only *Ef* were offered as prey; in these latter cases, prey consumption was 30% or greater. When there was mixed prey to feed on in the arena with both prey species in the same proportion, unmated females consumed a greater number of *Ef* than greenhouse whitefly. This differed from the behaviour of mated females, which consumed non-statistically different numbers of the two types of prey.

Applying Manly's index of preference for host feeding to the case of mixed prey in the arena shows that unmated females clearly preferred to feed on *Ef* than on greenhouse whitefly (α_{Tv}= 0.24, α_{Ef}= 0.76) but that mated females showed no preference between the two (α_{Tv}= 0.47, α_{Ef}= 0.53).

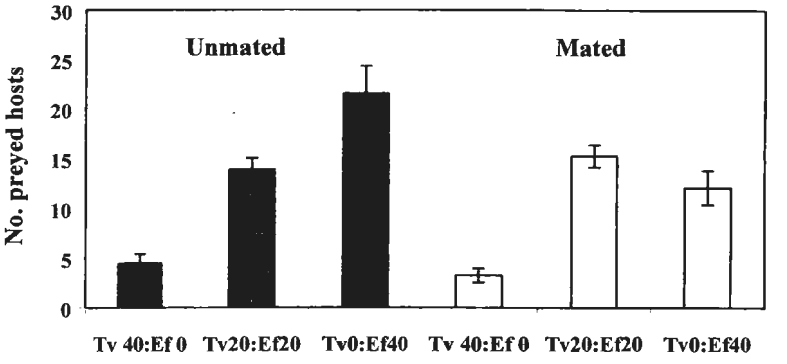

Figure 1. Mean total number (±SEM) of primary and secondary hosts preyed upon in 48h by unmated and mated *Ep* females with different proportions of primary (*Tv*, greenhouse whitefly pupae) and secondary (*Ef*, *E.formosa* pupae) hosts. Means are significantly (P<0.05) different each other within each female status.

Table 1. Mean number (±SD) of primary (*Tv*, greenhouse whitefly pupae) or secondary (*Ef*, *E. formosa* pupae) hosts preyed upon by unmated and mated *Ep* females in 48h. Within each female status and row, means followed by the same capital letter are not significantly (P<0.05) different. Means within a column with the same lower case letter are not significantly (P<0.05) different.

Host (*Tv*:*Ef*) proportion	No. of greenhouse whitefly (*Tv*) or *E. formosa* (*Ef*) preyed upon by *E. pergandiella*			
	unmated females		mated females	
	Tv	*Ef*	*Tv*	*Ef*
40 : 0	4.45±0.93a	-	3.24±0.69b	-
20 : 20	4.41±0.63aB	9.85±0.74bA	7.57±0.72aA	7.63±0.72A
0 : 40	-	21.56±2.75a	-	12.05±1.73

The number and sex of descendants of *Ep* when they were allowed to oviposit on primary and secondary hosts at different proportions are shown in Fig. 2. Unmated females of course only produced male descendants; their number did not significantly increase as a result of greater availability of secondary hosts, whereas mated females produced very few descendants when they only had primary hosts. They had significantly ($P<0.05$) more descendants when they had only secondary hosts and significantly ($P<0.05$) more offspring when both primary and secondary hosts were available. Significantly ($P<0.05$), more secondary than primary hosts were parasitised in arenas with mixed host types, which showed a clear preference for mated *Ep* females to oviposit on the former (Manly's indexes were $\alpha_{\text{primary host}} = 0.06$ vs. $\alpha_{\text{secondary host}} = 0{,}94$). However, it should be taken into account that preyed hosts were not available for egg laying, or that inversely, oviposited hosts were probably avoided as prey, so the preference for feeding on and oviposit in primary vs. secondary hosts is difficult to study separately. The sex ratio (♂/♀) of descendants produced by mated females in the 20:20 (*Tv:Ef*) ratio was 0.09.

The so called host killing capacity (oviposition plus host feeding) was analysed in arenas with mixed primary and secondary hosts by means of a paired Student *t*-test to compare both kinds of hosts and both types of female status (Figure 3): both mated and unmated females had a significant probability of selecting secondary rather then primary hosts (t = 7.43, df= 26, P<0.001; t =11.25, df= 22, P<0.001 respectively) to produce offspring and to prey upon.

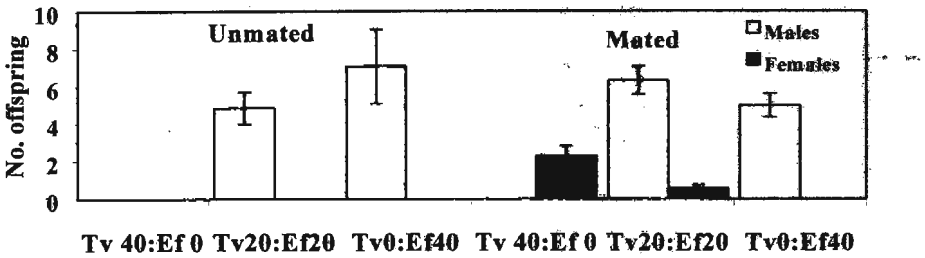

Figure 2. Mean number (±SD) of males and females produced by unmated and mated *Ep* females when these were confined for 48 h in arenas with different ratios of primary (*Tv*, greenhouse whitefly pupae) and secondary (*Ef*, *E. formosa* pupae) hosts.

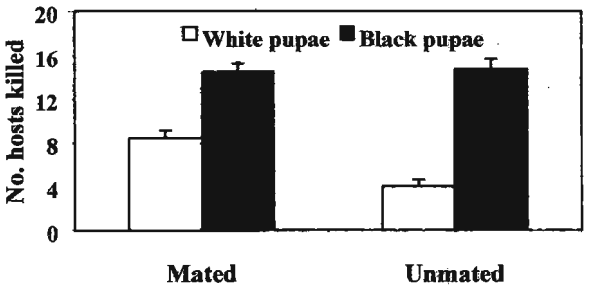

Figure 3. Mean total number (±SEM) of primary (white pupae) and secondary hosts (black pupae) killed to feed upon and to produce offspring in 48 h by unmated and mated *Ep* females at the 20:20 (primary: secondary hosts) ratio

Discussion

The present experiment displayed a clear antagonism between *Ep* and *Ef*. This was evident with regard to both host feeding and oviposition. The host feeding activity of *Ep* on whitefly species, mainly on *B. tabaci*, has been previously reported by several authors (Heinz & Parrella, 1994; Liu & Stansly, 1996; Schuster & Price, 1996; Videllet *et al.*, 1997), but none of them studied the capacity of this autoparasitoid to feed on secondary hosts. Since host feeding in parasitoids is influenced by experimental conditions it is difficult to compare the present results with those reported in the mentioned works. The number of early greenhouse whitefly pupae preyed upon in the 48 h. period of this experiment in which only primary hosts

were available, was similar to those reported by Heinz & Parrella (1994), Schuster & Price (1996) and Videllet *et al.* (1997) and slightly lower than those found by Liu & Stansly (1996). It therefore seems that the presence of *E. formosa* as secondary hosts stimulates host feeding in *Ep* with a preference for feeding on competitors rather than on whiteflies.

Although Buij *et al.* (1981) and Pedata & Hunter (1996) found that *Ep* had no preference for *Ef* or conspecifics when selecting a host for male production, these results show that the presence of *Ef* pupae may lead to biased sex ratios in *Ep* offspring, as mated females mostly parasite *Ef* in detriment to greenhouse whitefly, a similar pattern to that observed by Hunter (1989). However, as noted by Hunter (1989), preferential oviposition of male eggs may not necessarily reflect a lifetime preference, since females were only tested on the first days of oviposition. Although we did not confront *Ep* females with the co-occurrence of their own larvae/pupae and *Ef* larvae/ pupae, a pattern similar to that shown for *E. tricolor*/ *Ef*, in which mated females of the former preferred to hyperparasitise the latter than their own larvae to produce males (Avilla *et al.*, 1991), cannot be rejected.

Some consequences for whitefly biological control may derive from the observed antagonism between *Ef* and *Ep*. Heinz & Nelson (1996) found that multiple releases of *Ef* and *Ep* perform better in biological control than single releases of either of the two parasitoids, which is a surprising result in the light of the present results and those of Gabarra *et al.* (1999) and Georgini & Viggiani (2000), who also observed clear interference between both species. The study of Heinz & Nelson (1996) was carried out over a period of 12 weeks and with high weekly doses of *Ep* and *Ef* (3 mated females/week). This could produce high mortality due to host feeding over too short a period of interaction; broad fluctuations in the sex ratio of successive *Ep* generations have been shown (Onillon *et al.*, 1994) and these can be even more complex when *Ef* co-occur in the greenhouse. Our results would confirm the Mills & Gutierrez (1996) prospective model that predicts the disruption of the control potential of primary parasitoids or obligate autoparasitoids when a facultative autoparasitoid is added.

In conclusion, and as pointed out by other authors (Heinz & Nelson 1996; Gabarra *et al.* 1999), more investigations are needed to further explain the displacement of *Ef* by naturally occurring *Ep* in Mediterranean areas (Gabarra *et al.*, 1999). Even so, it seems that enhanced host feeding activity in *Ep,* due to the presence of *Ef,* may play a role in such a displacement. The extremely male biased sex ratios found in the offspring of *Ep* when they are confronted by mixed primary vs. secondary hosts, may however contradict the results of previous work that showed no preference on the part of this parasitoid for either conspecific or *Ef* larvae to produce males.

Acknowledgements

This work was funded by the CICYT AGL2000-0354 and INTERREG III France-Spain "Control and prevention of *Bemisia* transmited viruses in vegetables" projects. It was made possible thanks to an agreement between the IRTA and the growers associations (ADV) of the Alt Maresme, Baix Maresme and Baix Llobregat.

References

Avilla, J., Anadon, J., Sarasúa, M.J. & Albajes, R. 1991. Egg allocation of the autoparasitoid *Encarsia tricolor* at different relative densities of the primary host *(Trialeurodes vaporariorum)* and two secondary hosts *(Encarsia formosa* and *E. tricolor).* Entomol. Exp. Appl. 59: 219-227.

Buij, M. J., Pirovano, I. & Lenteren, J. C. van 1981. *Encarsia pergandiella*, a posible biological control agent for the greenhouse whitefly, *Trialeurodes vaporariorum*. A study on intra- and interspecific host selection. Med. Fac. Landbouww. Rijksuniv. Gent, 46/2: 465-475.

Gabarra, R. & Besri, M. 1999. Implementation of IPM: Case studies. Tomatoes.. *In* Albajes, R., M. L. Gullino, J. C. van Lenteren, and Y. Elad (eds.). *Integrated Pest and Disease Management in Greenhouse Crops*. Kluwer, Dordrecht. Pp: 420- 434.

Gabarra, R., Arnó, J., Alomar, O. & Albajes, R. 1999 Naturally occurring populations of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in tomato greenhouses. IOBC/WPRS Bulletin 23(1): 253-256.

Giorgini, M & Viggiani, G. 2000. A compared evaluation of *Encarsia formosa* Gahan and *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) as biological control agents of *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) on tomato under greenhouse in southern Italy. IOBC/WPRS Bulletin 23(1): 109-116.

Heinz, M.H. & Parrella M.P. 1994. Poinsettia (*Euphorbia pulcherrima* Willd. ex Koltz.) Cultivar-Mediated Differences in Performance of Five Natural Enemies of *Bemisia argentifolii* Bellows and Perring, n.sp. (Homoptera: Aleyrodidae). Biol. Control 4: 305-318.

Heinz, K. M. & Nelson, J.M. 1996. Interspecific interactions among natural enemies of *Bemisia* in an inundative biological control program. Biol. Control 6: 348-393.

Horton, D. R. 1995. Statistical considerations in the design and analysis of paired-choice assays. Environ. Entomol. 24: 179-192.

Hunter, M. S. 1989. Sex allocation and egg distribution of an autoparasitoid, *Encarsia pergandiella* (Hymenptera: Aphelinidae). Ecol. Entomol. 14: 57-67.

Krebs, C. J. 1989. Ecological methodology. Harper Collins Publishers, New York. 654 pp.

Lenteren, van, J. C., Van Roermund, H. J. W. & Sütterlin, S. 1995. Biological control of greenhouse whitefly (*Trialeurodes vaporariorum*) with the parasitoid *Encarsia formosa*: How does it work?. Biol. Control 6: 1-10.

Liu, T.-X. & Stansly, P.A. 1996. Oviposition, Development, and Survivorship of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in four Instars of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Ann.Entomol.Soc.Am. 89:96-102.

Mills, N. J. & Gutierrez, A. P. 1996. Prospective modelling in biological control: an analysis of the dynamics of heteronomous hyperparasitism in a cotton-whitefly-parasitoid system. J. Appl. Ecol. 33: 1379-1394.

Onillon, J. C., Maignet, P. & Cocquempot, C. 1994. Premiers résultats sur l'efficacité *d'Encarsia pergandiella* (Hymenoptera: Aphelinidae) dans le contrôle de *Bemisia tabaci* (Homoptera: Aleyrodidae) en serres de tomate. IOBC/WPRS Bulletin 17(5): 71-80.

Pedata, P. A. & Hunter, M.S. 1996. Secondary host choice by the autoparasitoid *Encarsia pergandiella*. Entomol. Exp. Appl. 81: 207-214.

Schuster, D.J. & Price, J.F. 1996. Parasitization of *Bemisia argentifolii* (Hom.: Aleyrodidae) by *Encarsia pergandiella* (Hym.: Aphelinidae). Entomophaga 41: 95-103.

Videllet, P., Albajes, R. & Gabarra, R. 1997. Host-feeding activity of *Encarsia pergandiella* Howard on *Bemisia tabaci* (Gennadius). IOBC WPRS Bull. 20(4): 147-153.

Viggiani, G. & Mazzone, P. 1980. Sull'introduzione in Italia de *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae), parassita di *Trialeurodes vaporariorum* (Westw.) (Homoptera: Aleyrodidae). Bolletino del Laboratorio di Entomologia Agraria "Filippo Silvestri" 37: 39-43.

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Performances of two types of insect screens as a physical barrier against *Bemisia tabaci* and their impact on TYLCV incidence in greenhouse tomato in the Souss Valley of Morocco

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Abstract: Tomato yellow leaf curl virus (TYLCV) was introduced to Morocco in 1998. Since then it has become the major challenge for tomato farmers in Morocco. The only way to seriously control the disease is by effectively controlling the vector. Although pesticides remain an important tool for pest management in greenhouse crops, non-chemical methods must be introduced to reduce damage to the environment and to delay the build up of insecticide resistance. Screens have been found to be an efficient method for reducing the entry of greenhouse pests and hence for reducing the number of insecticide applications targeting insect pests and vectors.
In this study, we report on observations from two greenhouses belonging to private farmers and which were equipped with two different types of insect screens 10*14 and 10*20. We report on whitefly captures in sticky traps as well as TYLCV incidence in the tomato crop. The results indicate that the 10*20 insect-net was efficient in excluding whiteflies whereas the 10*14 screen performed very poorly. TYLCV incidence at the end of the crop cycle was estimated at only 23% in the greenhouse equipped with the 10*20 screen compared with 100% in the greenhouse equipped with the 10*14 screen.

Keywords: *Bemisia tabaci*, Tomato, TYLCV, insect screen, IPM

Introduction

Tomato yellow leaf curl virus (TYLCV) a geminivirus exclusively vectored by the sweet potato whitefly *Bemisia tabaci* (Gennadius) was accidentally introduced to Morocco in 1998. Since then it has become the major challenge for tomato farmers in Morocco. The only way to seriously control the disease is by effectively controlling the vector. Since the introduction of TYLCV, farmers have relied heavily on insecticides to manage *B. tabaci* and TYLCV. But they soon realised that this chemical strategy was not only expensive but also failed to achieve the proposed goal. This was due to the continuous spread of TYLCV despite residual populations of the vector and because of resistance to insecticide.

Although pesticides will remain an important tool for pest management in greenhouse crops, non-chemical methods must be introduced to reduce damage to the environment and to delay the build up of insecticide resistance. The need for alternative measures is even more important in the face of growing consumer demand for safer vegetables. Screens have been found to provide an efficient way of reducing the entry of greenhouse pests and hence of reducing the number of insecticide applications targeting insect pests and vectors.
Several commercial screens are commercially available in Morocco, yet very little was known until recently about their effectiveness in excluding *B. tabaci* and reducing TYLCV incidence.

In this study we report on observations from two greenhouses belonging to private farmers and which were equipped with two different types of insect screens: with 10*14 and

10*20 meshes. We report on whitefly captures in sticky traps as well as TYLCV incidence in the tomato crop.

Material and methods

This study was conducted at two private farms located in the Souss Valley in the south of Morocco. On both farms, the type of greenhouse used was the wooden structured canary-type design, and each had an area of 1 ha. On one farm (Benabdeljalil) the greenhouse was equipped with 10*14 insect screen. On the second farm (Baala) the greenhouse was equipped with a 10*20 insect screen. In both greenhouses, the area covered with insect screens amounted to about 22% of the plastic covered area and allowed relatively good ventilation. The insect screens were applied to the laterals and also to the roof ventilation openings. Both greenhouse units were planted with the cultivar Daniella, which is sensitive to TYLCV, in October 2001.

Indoor as well as outdoor populations of whiteflies were monitored at the two sites using 4 yellow sticky cards (dimension 10 x 20 cm) on each direction of the greenhouse and with 4 others in the middle of each quarter ha (greenhouse). Inside the greenhouse, trap height was adjusted according to crop height: 20 cm above the upper part of the Tomato plants. All traps were monitored and changed weekly.

The incidence of TYLCV was monitored weekly, with checks for symptoms and records taken of the number of infected plants.

Results and discussion

In both greenhouses the captures of whiteflies in outdoor traps during the first three weeks were much greater than the captures of whiteflies in indoor traps. Season-long outdoor traps captured significantly greater number of whiteflies than indoor traps, indicating that both types of screens had a certain excluding effect with respect to whitefly *B. tabaci*. However, overall, we registered more whitefly captures indoors in the greenhouse equipped with the 10*14 screen than in the greenhouse equipped with the 10*20 screen.

Site 1: screen 10*20 (figure 1A)

Indoor captures in the greenhouse equipped with the 10*20 insect screen were generally much smaller than outdoor captures, which indicated good exclusion by this type of screen. Towards the end of the crop cycle, indoor captures were slightly greater than outdoor captures because of the absence of insecticide treatment towards the end of the crop cycle. This explains the resurgence of whiteflies, especially in the absence of the action of endemic natural enemies.

Over the whole season, the 10*20 screen provided adequate exclusion of *B. tabaci*. The impact was even more important at the beginning of the crop cycle when plants were young and therefore very sensitive to virus infection.

The relationship between outdoor and indoor captures was later analyzed and the correlation between indoor and outdoor captures was not significant ($r=0.17$).

TYLCV incidence was kept below the level of 10% until about one month before the end of the crop cycle. Final TYLCV incidence was 23.39% which is quite acceptable for farmers.

Site 2: 10*14 screen (figure 1B)

Indoor captures in the greenhouse equipped with the 10*14 insect screen were generally high and were several times greater than those of outdoor traps. In fact, we found a positive

correlation (r=0.5) between captures in outdoor and indoor traps. This indicates that the 10*14 screen was quite permeable to *B. tabaci*.

TYLCV incidence rose beyond 20% 3 months after transplanting and reached 100% by the end of the crop cycle, causing significant crop losses.

The results of this study, which was conducted at farm level and according to standard farming practice, clearly demonstrated that the 10*14screen was neither efficient at excluding *B. tabaci* nor at preventing TYLCV, despite the use of 1.5 times more insecticide applications. The superiority of the 10*20 screen was clearly demonstrated in this study, both in terms of whitefly captures and final TYLCV incidence.

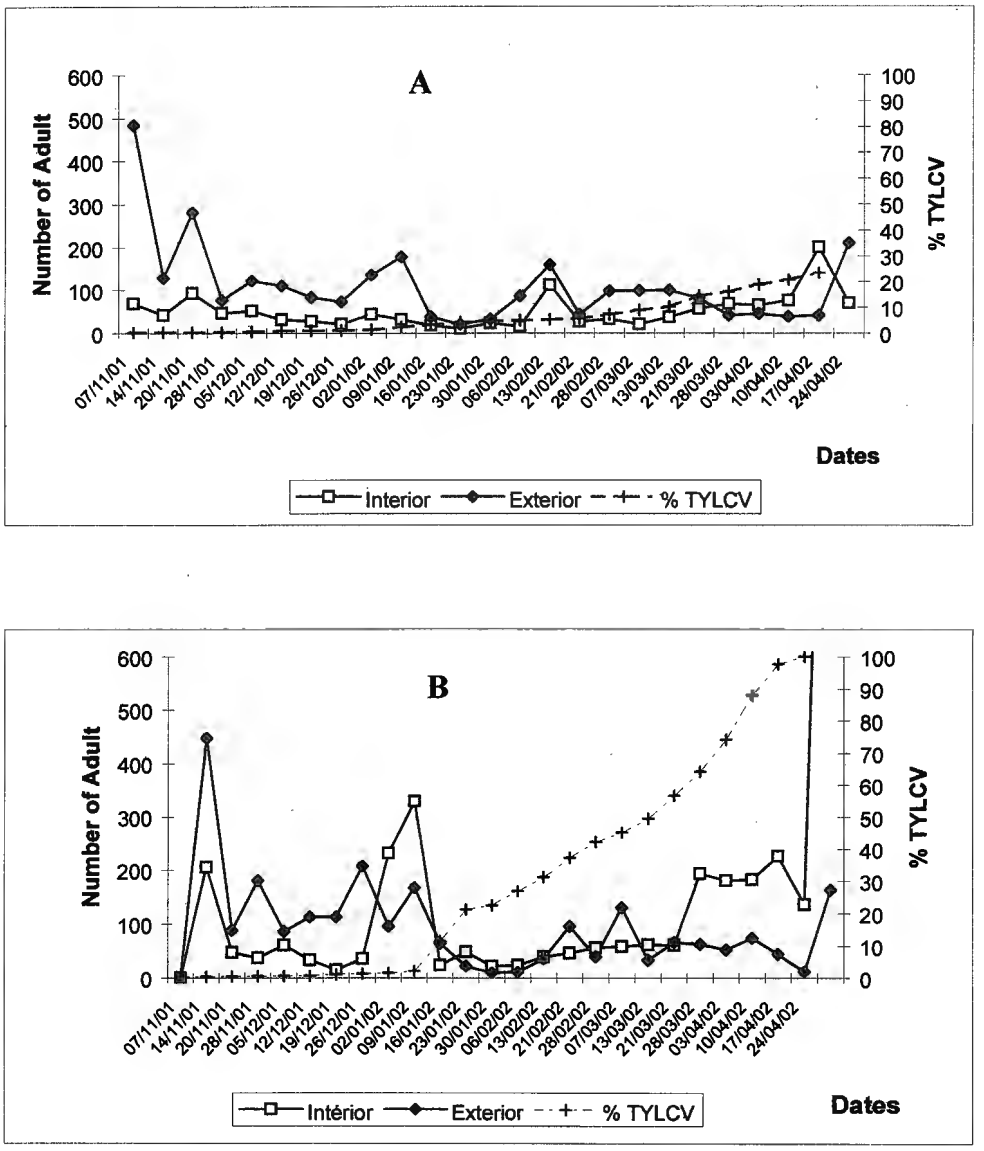

Figure 1. Evolution of mean captures of whitefly on yellow sticky cards and percentage of TYLCV infection in tomato greenhouses equipped with 10*20 (A)10*14 (B) screens.

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Evaluation of different types of insect screens for the exclusion of whiteflies and natural enemies

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Abstract: Under laboratory conditions, we evaluated the effectiveness of different types of insect nets that are commercially available in Morocco to assess their ability to exclude two whitefly species and two natural enemies. The insect nets tested were: 10 X 14, 10 X 20, 10 X 22 and Agryl (P-17). The species of whitefly tested in this study were *Trialeurodes vaporariorum* and *Bemisia tabaci* and the two natural enemies tested were *Diglyphus isea* and *Eretmocerus eremicus*. A simple laboratory design was adopted in this experiment: we used light and yellow sticky traps to encourage insects to move through the insect screens.
The results of these experiments clearly indicate that the 10 X 14 insect nets, which are most commonly used by farmers in greenhouse in Morocco, do not really exclude *B. tabaci*. Only 10 X 20 and 10 X 22 adequately exclude whitefly without impeding the movements of natural enemies.

Key words: Screens, insect nets, mechanical exclusion, tomato greenhouse *Bemisia tabaci, Trialeurodes vaporariorum, Diglyphus isaea, Eretmocerus eremicus,* TYLCV.

Introduction

In greenhouse crops, homopteran insects can cause direct damage by sucking plant sap, but their indirect damage as a virus vector is far more important economically. Growers have long relied on insecticides to manage insect pests in greenhouses but the use of chemicals to manage insect vectors has produced mixed results. Consequently, growers are now seeking preventive measures using insect screens to stop the vectors from entering the greenhouse. This has enabled many farmers to reduce insecticide use, which is not only advantageous from an economic view-point, but also reduces insecticide resistance and environmental pollution. Furthermore, the screens are desirable in greenhouse when using bumble bees for pollination.

Several screens are commercially available but their efficiency for excluding *Bemisia tabaci* (Gennadius) varies considerably. In the past, these screens have been used to exclude certain insect pests that affect greenhouse crops. Optimum screen size evidently depends on the target pest and openings in the screens must be smaller than the insects in question (Table 1).

In Europe and North Africa, screens are identified as 6*9, 10*14 and 10*22 and have rectangular openings: a 10*20 screen measures 10 threads by 20 threads in one centimetre square.

In most instances, the screens are characterised by the term "mesh", which is the number of threads per inch in each direction. For example, a 50-mesh screen has 50 threads per inch of material. If the mesh and thread thickness are known, the size of the opening can be obtained by subtracting the thread size from the reciprocal of the mesh: a 50 mesh screen with a thread thickness of 0.15 mm has openings with a width of 0.35 mm (i.e. 1/50=0.02

inches=0.5 mm; subtracting the thread thickness of 0.15 mm gives 0.35 mm) in each direction. If the openings are rectangular, the screen will have openings of 0.48 mm by 0.27 mm if the thread thickness is 0.15 mm.

Table 1: Width and length (in millimetres) of some important greenhouse crop insect pests.

Insect pest	Width (mm)		Length (mm)
	Thorax	Maximum	
Serpentine leaf miner (*Liriomyza trifolii*)	0.608	0.850	0.177
Sweet potato whitefly (*Bemisia tabaci*)	0.615	0.870	0.181
Melon aphid (*Aphis gossypii*)	0.355	0.239	0.236
Greenhouse whitefly (*Trialeurodes vaporariorum*)	0.288	0.708	0.128
Silverleaf whitefly (*Bemisia argentifolii*)	0.239	0.565	0.107
Western flower thrips (*Frankliniella occidentalis*)	0.215	0.265	0.126

The maximum sizes of the screen openings required to exclude major insect pests are given in table 2. Many insect screens have a regular structure with square or rectangular openings and are made from uniform threads.

Table 2: Maximum dimensions of screen openings required to exclude major greenhouse crop insect pests.

Insect pest	Hole size (mm)	Mesh *
Serpentine leaf miner (*Liriomyza trifolii*)	0.61	34
Sweet potato whitefly (*Bemisia tabaci*)	0.46	42
Melon aphid (*Aphis gossypii*)	0.34	52
Greenhouse whitefly (*Trialeurodes vaporariorum*)	0.29	58
Silverleaf whitefly (*Bemisia argentifolii*)	0.24	66
Western flower thrips (*Frankliniella occidentalis*)	0.19	76

*Based on thread diameter of 0.15 mm

In Morocco, and particularly since the introduction of TYLCV from neighbouring Spain in 1998, the whitefly *B. tabaci* has become the major insect pest on greenhouse tomato. This pest required only one to two applications of insecticide prior to 1998, but the number of insecticide applications targeting *B. tabaci* alone increased 20 to 30 fold during subsequent years when TYLCV reached epidemic proportions. As a consequence, *Bemisia* became resistant to the most commonly used insecticides and this frequently resulted in the failure of the chemical strategy. In 1999, greenhouse growers started using several types of insect screen as a first barrier against the vector of TYLCV. Now, several types of insect screen are fitted in more than 95% of tomato greenhouses in Morocco, though very few farmers have a really clear idea of their purpose.

In this study, we have evaluated the effectiveness of different types of insect screen for excluding two whitefly species and two natural enemies, under laboratory conditions. The 10*14, 10*20 and 10*22 insect screens were evaluated to compare actual insect penetration.

Material and methods

The two whitefly species evaluated in this experiment were the greenhouse whitefly *Trialeurodes vaporariorum* Westwood and the sweet potato whitefly *B tabaci*. Whiteflies were collected from rearing colonies kept in the glasshouse at the IAV Hassan II experiment station in Agadir. The two natural enemies used in this study were *Diglyphus isea* (Walker) (a leafminer parasitoid) and *Eretmocerus eremicus* Rose & Zolnerowich (*E. californicus* Howard)(a whitefly parasitoid). The latter were provided locally by Biobest Maroc.

In this experiment we adopted a simple design using two plastic cups (1 litre) for each replicate. Each screen was tested as a barrier between two compartments formed by inserting one plastic cup within another. The first plastic cup (1 litre) was open at the bottom (7 cm diameter) and we placed the insect screen on its other end (7 cm) as a barrier. A second plastic cup was open at both ends (7.1 cm) and was placed on top of the first cup, just above the insect screen. We placed a Petri dish (9 cm diameter) at its other end and a 7 cm diameter, yellow sticky trap on its inner surface.

Two vertical compartments were formed, with the first, into which insects were released (50 to 100), being below the insect screen (C1). A second compartment (C2) was isolated from C1 by the screen and had a sticky trap positioned at its end. Both compartments were covered with aluminium foil to create darkness and the only light perceived by insects came from the top through the yellow sticky trap placed at the end of C2. The role of the illuminated yellow sticky trap was to attract insects to move through the screen from C1 to C2 and to prevent them from returning to C1 once they had crossed.

We evaluated four treatments: T1 (10*14), T2 (10*20), T3 (10*22); against a positive control (Agryl, (P-17)) and a negative control (6*9). Each treatment was replicated four times.

Results and Discussion

Insect penetration was evaluated by calculating the percentage of insects that crossed the insect screens and which were caught in yellow sticky trap. Percentage penetration was given by the number of insects captured in the yellow sticky traps (C2) divided by the number of insects introduced into C2, X 100.

The results of percentage penetration of whiteflies though the different screens are presented in figure 1. There were no significant differences between percentage penetration of *T. vapaorariorum* and *B. tabaci* for the different types of screens. However, percentage penetration was usually slightly higher for *B. tabaci* than for *T. vaporariorum*, which was related to the smaller size of the sweet potato whitefly.

All three screens had percentage penetration results that significantly differed from those of the positive (6*9) and negative controls (Agryl). Percentage penetration was, however, much higher for the 10*14 screen than for the other two screens (10*20 and 10*22).

Over 77% of *B. tabaci* were able to cross the 10*14 screen, whereas only 6.8% crossed the 10*20 screen and no *B. tabaci* crossed the 10*22 screen. These results indicate clearly that the insect screens most commonly used by farmers (10*14) are easily penetrated by whiteflies of both species (*B. tabaci* and *T. vaporariorum*). Our results also indicate that even the 10*20 screen, which has recently been adopted by many farmers, still allows nearly 7% penetration by the sweet potato whitefly. This percentage penetration, even though small, can still lead to severe TYLCV epidemics if a certain proportion of *B. tabaci* are viruliferous. The only type of screen which completely excluded *T. vaporariorum*, and that was relatively efficient at excluding *B. tabaci*, was the 10*22 one.

The results of this study confirmed that the 10*14 and 10*20 screens do not efficiently exclude *B. tabaci*, the vector for TYLCV. Consequently, only the 10*22 screen could provide adequate exclusion of *B. tabaci*. There is no doubt that this type of screen impedes greenhouse ventilation, but given the presence of TYLCV, the overriding concern should be that of efficacy.

Interestingly, all screen types were permeable to both natural enemies: *D. isaea* and *E. eremicus* (Figure 2). Percentage penetration of *E. eremicus* ranged from 48% for the 10*22 screen and 82% for the 10*14screen. Percentage penetration was lower for the leafminer parasitoid *D. isaea* because of its larger size with respect to *E. eremicus*. Most importantly, these results clearly indicate that, contrary to the common belief, even the finest mesh screens such as the 10*22 - which completely excludes *B. tabaci* - still allow penetration by its parasitoid *Eretmocerus sp.* As *Eretmocerus sp.* naturally occurs in agricultural systems in Morocco, it is still possible for this parasitoid to move into greenhouse systems equipped with even the finest 10*22 screen mesh.

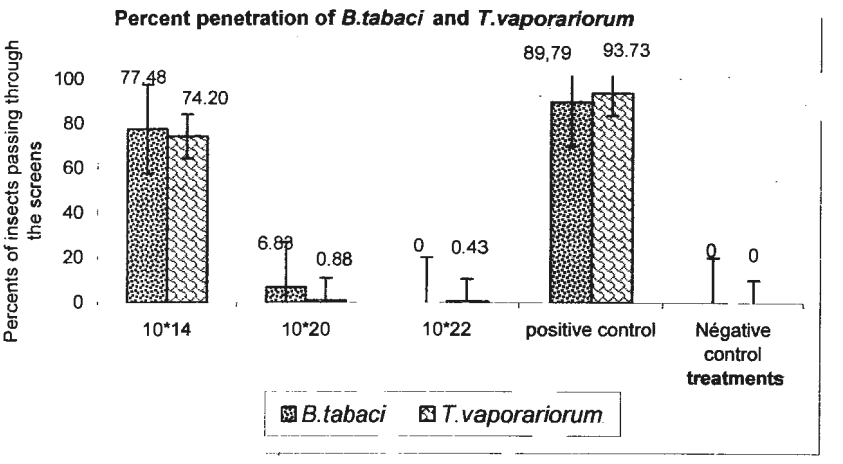

Figure 1: Percentage penetration of *Trialeurodes vaporariorum* and *Bemisia tabaci* through different insect screens (10*14; 10*20 & 10*22) compared with a positive control (6*9) and a negative control (Agryl/P17), under laboratory conditions, IAV Hassan II, Agadir, 2003.

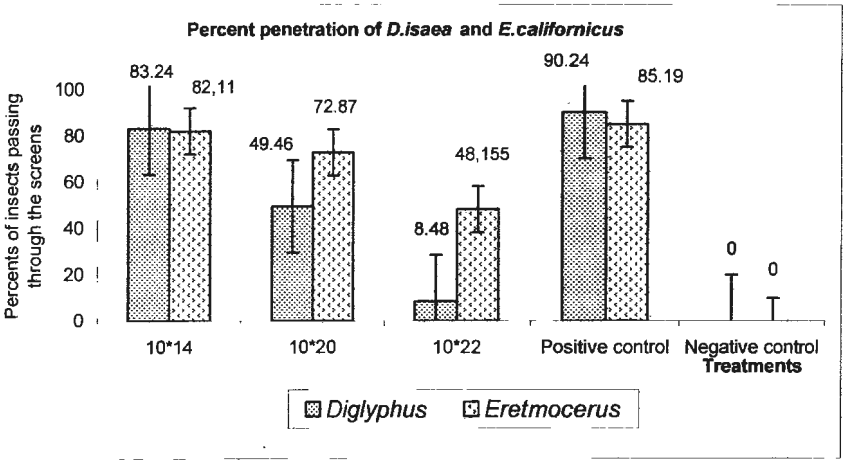

Figure 2: Percentage penetration of *Diglyphus isaea and Eretmocerus eremicus* through various insect screens (10*14; 10*20 & 10*22) compared with a positive control (6*9) and a negative control (Agryl/P17), under laboratory conditions, IAV Hassan II, Agadir, 2003.

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Differential variation in host preference of *Aleyrodes proletella* (L.) on several cauliflower cultivars.

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Abstract: The cabbage whitefly *Aleyrodes proletella* (L) has recently become a very serious pest on certain European brassicae crops. This study investigated the attractiveness of different cauliflower cultivars to this whitefly pest and tried to identify varieties that could contain genes for controlling this whitefly. In the future, this information may prove useful for the development of crop protection strategies.

Key words: *Aleyrodes proletella*, cauliflower, host-preference, pest control, crop protection.

Introduction

In Spain almost 21,000 ha are dedicated to cauliflower production, with a total annual yield of around 374,000 tons (Anonymous, 2000). The cabbage whitefly, *Aleyrodes proletella* (L.) causes serious damage to a wide range of brassicae crops in Europe (Loomans *et al.*, 2002). An increase in the *A. proletella* population has recently been detected on some cauliflower and cabbage crops in different regions of Spain; especially in Galicia, Andalucía, Navarra, Murcia and the Canary Islands (Alcázar & Lacasa, 1999; Hernández-Suárez, 1999; Hernández-Suárez & Carnero, 2000; Lacasa *et al.*, 1998). This study investigated the attractiveness of different cauliflower cultivars to this whitefly pest and aimed to identify varieties that could contain genes for controlling this whitefly. In the future, this information may prove useful for the development of crop protection strategies.

Material and methods

Greenhouse choice assays were carried out to obtain daily infestation rates of *A. proletella* on seven cauliflower cultivars ("Matra", "Freemont", "Nautilus", "Pierrot", "Arbon", "Mayfair" and "Picasso"). Ten replicates of these 48 day-old cultivars were placed in an insect-free greenhouse in a randomised design at 20.6° ± 0.1°C and 68.1 ± 0.1 % r.h. Plants were placed equidistant from adjacent pots and arranged so that their leaves did not touch each other during the period of the experiment. Three days later, all of the plants were infested by releasing *A. proletella* adults from a lab-colony stock. From seven days, the number of adults was counted on all the leaves of all plants. This was carried out *in situ* on a daily basis until the emergence of new adults. The experiments were conducted in a 50 m^2 greenhouse equipped with four benches at the Centro de Ciencias Medioambientales (CSIC) in Madrid, Spain. Average greenhouse conditions were 23.3 ± 0.3°C and 64.1 ± 1.5% r.h.

The relationship between the percentages of infested plants (y) with at least one or more adults, and the number of adults (x) was predicted by a regression analysis where $z = -ax$; $y = 100(1-e^{-ax})$; $z = \ln[1-(y/100)]$; (Nombela *et al.*, 2001). From the regression lines it was

possible to estimate the number of adults needed to infest 50% and 90% of the plants for each cultivar (Muñiz *et al.*, 2002).

Results and discussion

Figure 1 shows the infestation rates of *A. proletella*. A differential variation in the daily percentages of infested plants was observed according to the cauliflower cultivar.

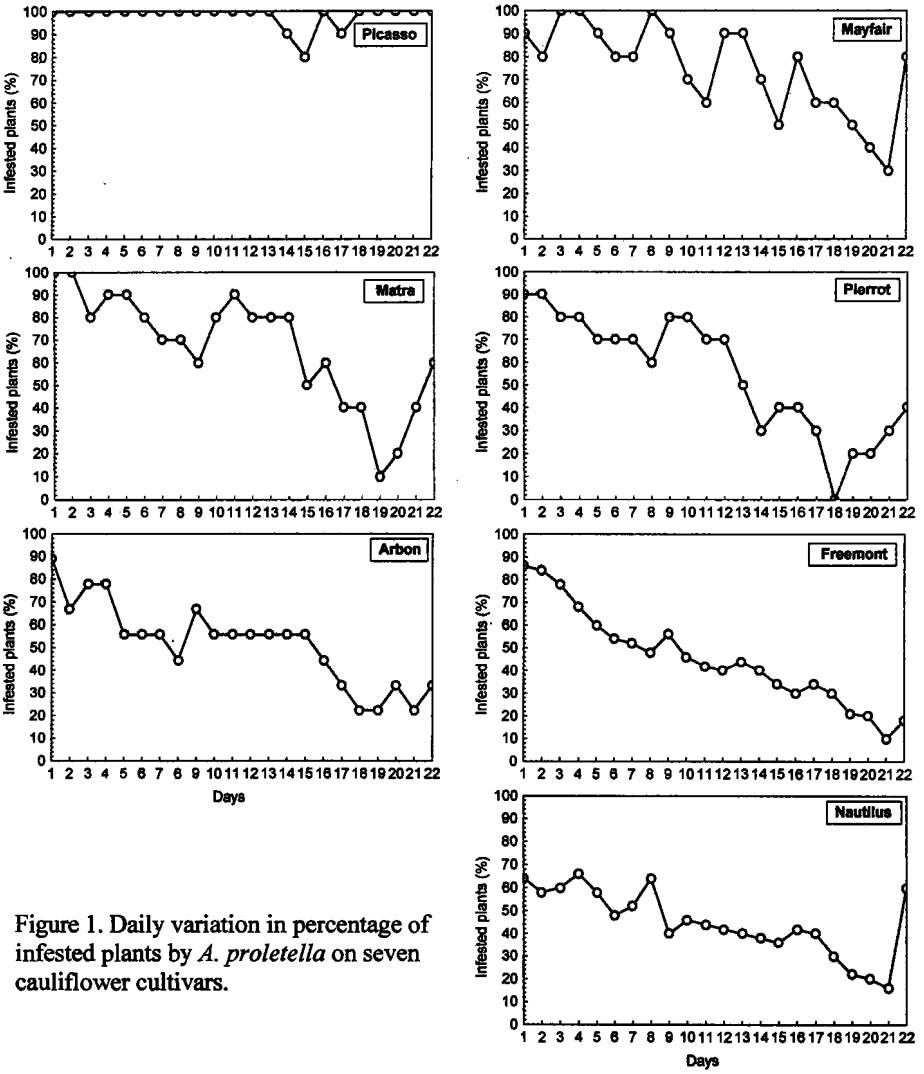

Figure 1. Daily variation in percentage of infested plants by *A. proletella* on seven cauliflower cultivars.

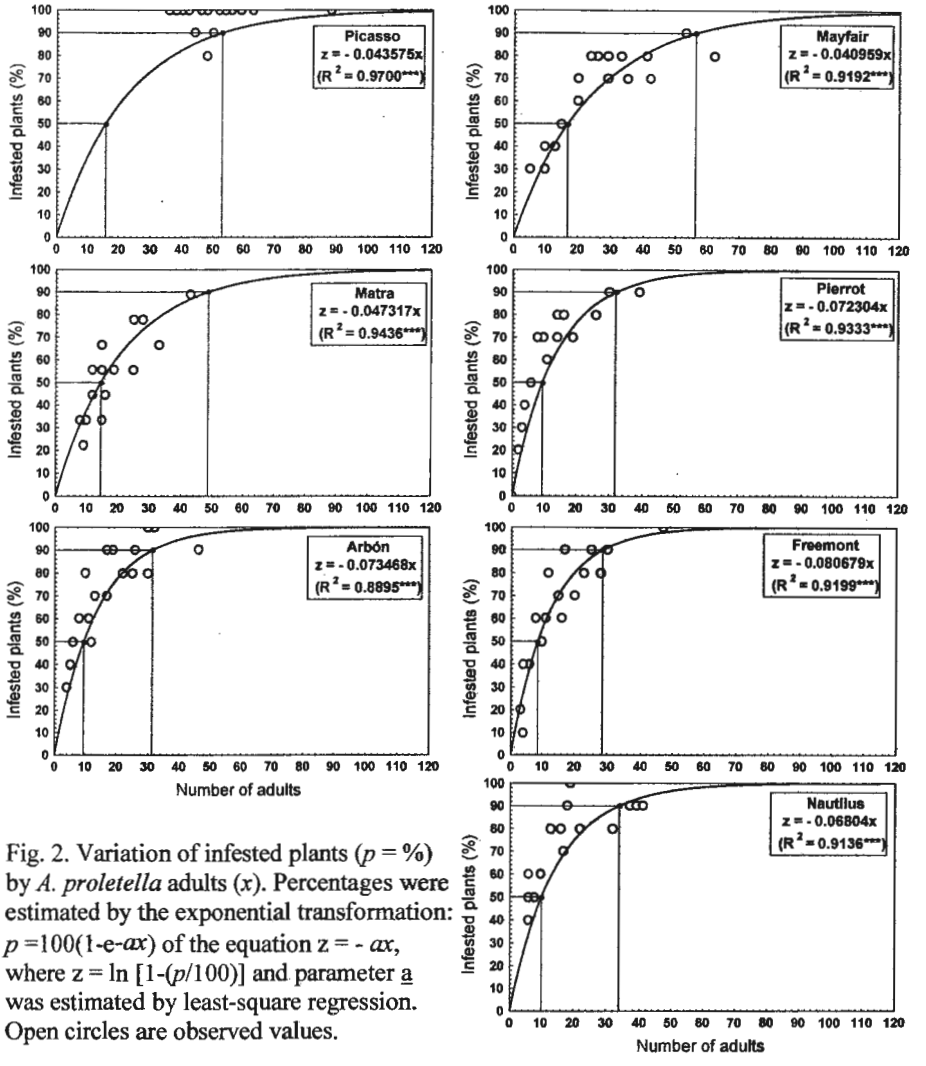

Fig. 2. Variation of infested plants (p = %) by *A. proletella* adults (x). Percentages were estimated by the exponential transformation: $p = 100(1\text{-}e^{-\alpha x})$ of the equation $z = -\alpha x$, where $z = \ln [1-(p/100)]$ and parameter $\underline{a}$ was estimated by least-square regression. Open circles are observed values.

The decreasing daily infestation rates were: Picasso > Mayfair > Matra > Pierrot > Arbon > Freemont > Nautilus" (Table 1).

The numbers of *A. proletella* adults (x) needed to infest 50 and 90 % of plants with at least one or more adults were estimated from the graphs of the exponential lines $y = 100(1\text{-}e^{-\alpha x})$ in Fig. 2. Although the lowest numbers were obtained with "Arbon" and "Freemont", very similar values were obtained for all seven cultivars used in this study (Table 1).

These results suggest the importance of studies focussing on plant-insect interactions on different cultivars of a given crop, because of the variability in insect-pest behaviour according to specific plant characteristics. In our study, "Picasso" and "Nautilus" were respectively the most and least attractive cauliflower cultivars, in terms of the percentage of infested plants. However, further investigations are needed to try to identify useful genes for

controlling this whitefly on brassicae plants. A better understanding of the dynamics of insect–plant interactions would be useful and would help to cope with the threat of losing biodiversity.

Table 1. Infestation rates of *A. proletella* on seven cauliflower cultivars.

Cauliflower cultivar	Infested plants (%) (Mean ± SE; n = 22)	Estimated number of insects to infest	
		50% of plants	90% of plants
Picasso	97.3 ± 1.2 a	16	53
Mayfair	75.4 ± 2.6 b	16	56
Matra	67.5 ± 3.0 c	15	49
Pierrot	55.6 ± 1.6 d	10	32
Arbon	51.4 ± 1.9 d	9	31
Freemont	47.3 ± 2.2 d	9	29
Nautilus	46.8 ± 3.4 d	10	34

Acknowledgements

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References

Alcázar, A. & Lacasa, A. 1999: La mosca blanca de la col *Aleyrodes proletella* (L.) se revela como plaga en los cultivos de bróculi de Murcia. Cuadernos de fitopatología, 1er trimestre: 17-22.

Anonymous, 2000. Anuario de Estadística Agroalimentaria. Ministerio de Agricultura, Pesca y Almentación. Subdirección General de Estadística Agroalimentaria. 693 pp.

Hernández-Suárez, E., 1999. La familia Aleyrodidae y sus enemigos naturales en Canarias. Tesis Doctoral. Departamento de Biología Vegetal. Universidad de La Laguna. 687 pp.

Hernández-Suárez, E. & Carnero, A. 2000. Descripción y biología de las especies de mosca blanca (Hemiptera: Aleyrodidae) de mayor interés económico en Canarias (1). Granja, 7: 50-53.

Lacasa, A., Bielza, P. & Guerrero, M.M. 1998: Evolución de plagas de los cultivos hortícolas en el último decenio. Phhytoma España, 100: 128-140.

Loomans, A.J.M., Staneva, I., Huang, I., Bukovinskiné-Kiss, G. & van Lenteren, J.C. 2002. When native non-target species go indoors: a new challenge to biocontrol of whiteflies in European greenhouses IOBC wprs Bulletin, 25 (1): 139-142.

Muñiz, M., Nombela, G. & Barrios, L. 2002. Within-plant distribution and infestation pattern of the B- and Q-biotypes of the whitefly, *Bemisia tabaci* Homoptera: Aleyrodidae) on tomatoand pepper. Entomol. Exp. Appl. 104: 369-373.

Nombela, G., Beitia, F. & Muñiz, M. 2001.A differential interaction study of *Bemisia tabaci* Q-biotype on commercial tomato varieties with or without the *Mi* resistance gene, and comparative host responses with the B-biotype. Entomol. Exp. Appl. 98: 339-344.

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Comparison of three trap types for catching adult *Bemisia tabaci* whitefly and its parasitoid *Eretmocerus mundus* in tomato greenhouse

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Abstract: The attractiveness of three trap types to *Bemisia tabaci* (Gennadius) B-biotype (= *Bemisia argentifolii* Bellows & Perring) and *Eretmocerus mundus* Mercet adults was compared in two choice experiments in a greenhouse at the Centro de Ciencias Medioambientales, Madrid, Spain. Yellow sticky cards equipped with light-emitting-diodes (LED-YC) caught more adults per trap per day than yellow sticky card (YC) traps. YC traps caught significantly more *B. tabaci* and *E. mundus* adults than plastic cup traps equipped with light-emitting diodes (LED-plastic cup traps). However, the LED-plastic cup traps caught fewer *E. mundus* than the YC traps. The results suggest that LED-plastic cup traps are compatible with *E. mundus* parasites in greenhouses in which parasites are released to reduce the *B. tabaci* nymph population.

Keywords: *Bemisia tabaci*, biological control, *Eretmocerus mundus*, tomato, traps

Introduction

Insecticides have traditionally been the main means of *B. tabaci* control in Spain. However, biological control systems are economically and environmentally more acceptable and often include the use of yellow sticky card (YC) traps to monitor *B. tabaci* population changes. The impact of YC trap catches on *B. tabaci* populations is unknown, and YC traps also catch parasitoids released to control *B. tabaci* nymphs in greenhouses. Chu *et al.* (2003) recently reported that equipping yellow-base plastic cup *B. tabaci* traps with green light-emitting diodes (LEDs) increased trap efficacy by 100% in mixed-crop greenhouses. More importantly, few *Eretmocersus eremicus* Rose and Zolnerowich and *Encarsia formosa* Gahan, (both *B. tabaci* parasitoids) were caught in these LED-plastic cup traps compared with the YC traps, suggesting a potential use for the LED plastic cup trap in greenhouse farming with little or no impact on *B. tabaci* parasitoids.

The objective of this study was to compare LED-plastic cup, YC, and LED-YC *B. tabaci* and *E. mundus* Mercet trap catches in a tomato greenhouse in order to determine the feasibility of developing a compatible *B. tabaci* adult nymph parasite trap control system.

Material and methods

Traps

Three different trap types were compared. The LED-YC trap was a 12.5 x 7.5 cm yellow sticky card (YC) equipped with two 530 nm 10 lumen LEDs (Nichia America Corp. Mountville, PA, USA). One LED was located on each side of each YC trap. The LED-plastic cup (LED-CC) trap (Chu *et al.* 2003) was a yellow-base plastic cup trap with a Tanglefoot® coating on the inside of the cup to catch arthropod insects. Each trap was

equipped with a downward-lo directed 530 nm lime green LED. Standard dimension (12.5 x 7.5 cm) YC traps were considered controls.

The traps were suspended from two parallel steel wires installed horizontally and placed 30 cm apart and approximately one meter above each bench. Electricity for the LEDs was supplied by a 220 volt wall-plug unit with a direct current adapter. Trap locations were re-randomized every five days throughout the experiments to avoid position effects.

Experiment 1 (LED-YC traps* vs. *YC traps)

Tomato seeds cv. Marmande VR were germinated in a climatic chamber under a temperature regime of 25°C: 16 °C (day: night), a photoperiod of 16 L: 8D (light: dark) h, and a relative humidity of 68-75%. Plants were grown in 1:1 soil/perlite mixtures in one-litre plastic pots irrigated with water every other day.

When the potted plants were two-month old, 30 pots were placed on each of the four greenhouse benches. Plants were infested with *B. tabaci* (B-biotype) by releasing large numbers of mature adults in the centre of the greenhouse. Two days later, traps were placed on the wires above the benches in a randomized complete block design with eight replications. One day after placing the traps, , the number of adult *B. tabaci* on each trap were counted *in situ* and these counts were repeated on a daily basis for 20 days. All the traps were removed immediately after the adult count on day 20. Average greenhouse conditions were 20.17 ± 0.18°C and 69.05 ± 1.22% r.h.

Experiment 2 (LED-CC traps* vs. *YC traps)

When the plants were 106-d-old, about 3,000 adult *E. mundus* parasitoids (Eretline, Syngenta Bioline Ltd., Essex, England) were released among the *B. tabaci* infested tomato plants. The traps were suspended above the benches as previously described and daily counts started on the day after trap placement. Numbers of *B. tabaci* and parasitoid adults on each trap were counted for 15 days once numbers of *E. mundus* had decreased to insignificant levels. The YC traps were replaced every five days because of the large number of trapped insects. Average greenhouse conditions were 19.6 ± 0.1°C and 72.3 ± 0.7% r.h.

The number of trapped adult insects was $\log_{10}(x+1)$ transformed and percentages (p) were transformed to arcsine $(p/100)^{0.5}$ before analysis. Data from different trap types were compared using a one-way ANOVA (Statsoft, 1994).

Results and discussion

There were no significant differences in the numbers of adult *B. tabaci* caught in either trap type on a daily basis, except on day 19. However, the mean number of *B. tabaci* captured for all trap days in the LED-YC traps was higher than with the YC traps (Table 1). The average percentage (57%) of the total catches for all trap days in the LED-CC traps was also greater than the 43% caught in the YC traps.

These results suggested that LED-YC traps could perhaps serve an ecological purpose as barriers for adult *B. tabaci* and other insects migrating into greenhouses. The peak wavelength of 530 nm emitted by lime green LEDs is within the range of the peak *Bemisia* spp. phototactic response (El-Helaly *et al.* 1981, Chu *et al.* 2000). The addition of LEDs to the plastic cup traps and YC traps appeared to attract more adults to the traps, particularly at night when adults do not appear to be very active (Liu *et al.* 1994, Chu *et al.* 1998).

YC traps caught significantly more *B. tabaci* adults on a daily basis than LED-CC traps through the whole period of experiment 2 (Table 1). However, the LED plastic cup trap caught fewer *E mundus* per trap per day than the YC trap (Table 1).

Table 1. Average numbers of *Bemisia tabaci* and *Eretmocerus mundus* captured per trap type per day in greenhouse experiments.

Experiment	Trap type	Number of adults per trap per day [a]	
		B. tabaci	*E. mundus*
1	LED-YC	9.6 a	
	YC	8.3 b	
2	YC	77.2 a	8.6 a
	LED-CC	20.9 b	0.3 b

[a] Means in a column for a given experiment followed by a different letter differ significantly ($P<0.05$) by one-way ANOVA

The LED plastic cup trap may be an acceptable alternative to YC traps in greenhouses in which it is aimed to conserve parasitoids. We would recommend the simultaneous use of LED plastic cup traps and biological controls of *B. tabaci* in IPM systems. Our results in a tomato greenhouse confirm an earlier report that the LED plastic cup trap does not attract other *B. tabaci* parasitoids in mixed-crop greenhouses (Chu *et al.* 2003). YC traps used to capture *B. tabaci* adults also trapped *Eretmocerus* sp. and *Diglyphus* sp. in greenhouses in the Almeria area (F. García-Jiménez, pers. comm., 2002) and would probably also do the same in other areas of Spain. Critical questions appear to be: (1) Do the YC traps have an adverse impact on the *B. tabaci* population? and (2) Do the YC parasite trap catches significantly reduce the effectiveness of biological controls?

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References

Chu, C.C., Henneberry, T.J. & Boykin, M. A. 1998: Response of *Bemisia argentifolii* (Homoptera: Aleyrodidae) adults to white fluorescent and incandescent light in laboratory studies. Southwest. Entomol. 23: 169-181.

Chu, C.C., Printer Jr., P.J., Henneberry, T.J., Umeda, K., Natwick, E.T., Wei, Y., Reddy, V. R. & Shrepatis, M. 2000: Use of CC traps with different trap base colors for silverleaf whiteflies (Homoptera: Aleyrodidae), thrips (Thysanoptera: Thripidae), and leafhoppers (Homoptera: Cicadellidae). J. Econ. Entomol. 93: 1329-1337.

Chu, C.C., Jackson, C.G., Alexander, P.J., Karut, K. & Henneberry, T.J. 2003: Plastic cup traps equipped with light –emitting diode for monitoring adult *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. 98: 543-546.

El-Helaly, M.S., Rawash, I.A. & Ibrahim, E.G. 1981: Phototaxis of the adult whitefly, *Bemisia tabaci* Gennadius to visible light. I. Effect of the exposure period on the insects response to different wavelengths of the visible light spectrum using a devised simple technique. Acta Phytopathol. Acad. Sci. Hungar. 16: 181-188.

Liu, T.X., Oetting, R.D. & Buntin, D. 1994: Temperature and diel catches of *Trialeurodes vaporariorum* and *Bemisia tabaci* (Homoptera: Aleyrodidae) adults on sticky traps in the greenhouse. J. Entomol. Sci. 29: 222-230.

StatSoft 1994: Statistica Version 4.5 for the Windows Operating System. Reference for statistical procedures. StatSoft, Tulsa,OK.

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A mutation in the *Rme1* tomato locus reduces *Mi-1.2*-mediated resistance to whitefly *Bemisia tabaci*

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Abstract: The tomato gene *Mi-1.2* is responsible for resistance to three species of root-knot nematodes (*Meloidogyne* spp.), to the potato aphid (*Macrosiphum euphorbiae)* and to the sweetpotato whitefly (*Bemisia tabaci)*. Working with a fast-neutron irradiated population of tomato cv. Motelle (*Mi-1/Mi-1*), we identified a mutant (rme1) that was defective in a locus other than *Mi-1*. We did this on account of its susceptibility to *M. javanica* and to potato aphid, which indicated that *Rme1* is required for *Mi-1*-mediated resistance. Two experimental assays were carried out in order to evaluate the influence of the *Rme1* locus on *Mi-1*-mediated resistance to the B-biotype of *B. tabaci*. In a greenhouse free-choice assay, 10 two-month-old rme1 mutant plants, and the same number of Motelle and Moneymaker (near isogenic susceptible control: *mi-1/mi-*1) plants, were randomised in a complete block design and whitefly adults were freely released in the greenhouse. Five days later, the number of whitefly adults on each plant was counted. This count was then repeated on alternate days over a 15-day period. The mean number of adult whiteflies per plant was significantly greater on rme1 mutants than on Motelle plants and slightly lower, but not significantly different, from on Moneymaker. For the no-choice assay, 11 eight-week-old plants of each genotype were kept in a growing chamber. Five adult female whiteflies were placed in a plastic clip-cage attached to the under surface of one leaf (one cage per plant). After 6 days, the average number of eggs observed on the rme1 mutant plants was almost identical to that on Moneymaker and significantly greater than that observed on Motelle. These results suggest that the *Rme1* locus is also required for *Mi-1*-mediated resistance to the *B. tabaci* B-biotype.

Key words: *Bemisia tabaci*, whiteflies, *Rme1*, resistance, *Mi-1.2* gene, mutant plants, tomato.

Introduction

The tomato (*Lycopersicon esculentum* Mill.) gene *Mi-1* confers resistance to the three most common species of root knot nematodes: *Meloidogyne arenaria* Neal, *M. incognita* (Kofoid & White) and *M. javanica* (Treub). After cloning, *Mi-1* was found to confer resistance to two additional organisms: potato aphid, *Macrosiphum euphorbiae* (Thomas) (Rossi et al., 1998) and whitefly, *Bemisia tabaci* (Gennadius) (Nombela et al., 2000 and 2003).

There is evidence that *Mi-1*-mediated resistance to root-knot nematodes, potato aphids and whiteflies is rather specific, but it is not clear how *Mi-1* mediates this resistance. Furthermore, it is not clear whether the defense responses mediated by *Mi-1* against the three organisms are identical.

Using a genetic screen to identify suppressors of *Mi-1*, we identified a mutant, *rme1* (resistance to *Meloidogyne*), that is compromised in resistance to *M. javanica* and to potato aphids (Martinez de Ilarduya et al., 2001). Here we present data to suggest that *rme1* mutant plants are also compromised in resistance to the sweet potato whitefly *B. tabaci*.

Material and methods

Plants and insects

We worked with the near isogenic tomato (*L. esculentum*) pair Motelle (*Mi-1/Mi-1*) and Moneymaker (*mi-1/mi-1*) and also used a mutant in the background of the wild-type parent Motelle, *rme1*. Tomato seedlings were transplanted into one-litre pots with perlite. Eight week-old tomato plants were used in the experiments

The experiment was conducted with B-biotype *B. tabaci* adults that had been reared on tomato cv. Marmande for more than 30 generations.

Free-choice assay

Ten plants from each genotype were randomised in a complete block design in an insect-free greenhouse with average temperatures of 23°C (day) and 18°C (night) and a relative humidity of 46% to 69%. Plants were infested by releasing mature adult whiteflies in the centre of the greenhouse. After five days, the number of adult whiteflies was counted *in situ* on all leaves. Similar counts were repeated every other day over a period of 15 days.

No choice assay

Eleven plants from each genotype were kept in a growth chamber at 25°C, with a photoperiod of 16-h-light and 8-h-dark and a relative humidity of 68% to 75%. Five adult female whiteflies were placed in a plastic clip-cage, attached to a single leaflet, in such a way that they had access to the abaxial surface of the leaf (Nombela et al., 2001). One plant was used per cage. At the end of the experiment, the number of eggs laid on each plant was recorded.

Data were $\log_{10}(x+1)$ transformed and compared using a one-way ANOVA and Tukey HSD test (Statsoft, 1994).

Results and discussion

Both free choice and no choice experiments indicated that resistance to the *B. tabaci* B-biotype was completely compromised in the *rme1* mutant. In the free choice assay, the daily infestation rates of *B. tabaci* B-biotype on the *rme1* mutant plants were intermediate between those on Moneymaker and Motelle (Table 1). However, the mean values of the number of adults per plant per day on the *rme1* mutant were similar to those on Moneymaker and significantly ($P<0.05$) greater than on Motelle (Table 1).

Table 1. Average daily number of adults of the *B. tabaci* B-biotype observed per plant during the free-choice experiment, and number of eggs per plant laid by 5 females during 6 days under no-choice conditions*.

	Adults per plant (free-choice assay)	Eggs per plant (no-choice assay)
Rme1 mutant	3.74 a	68.82 a
Motelle	2.25 b	36.82 b
Moneymaker	4.72 a	68.18 a

* Means followed by different letters in a column differ significantly ($P<0.05$) by Tukey's HSD.

In the no choice experiment, the average number of eggs observed on the *rme1* mutant plants six days after infestation was similar to that for Moneymaker and significantly ($P < 0.05$) greater than that observed on Motelle (Table 1).

We previously reported that rme1 was compromised in its resistance to *M. javanica* and to potato aphid (Martinez de Ilarduya et al., 2001). Comparing the present results with those for responses to other tomato pests and diseases may help us to determine whether Rme1 is required for *Mi-1*-mediated resistance and for characterising such resistance.

References

Martinez de Ilarduya, O., Moore, A.E. & Kaloshian, I. 2001: The tomato *Rme1* locus is required for *Mi-1*-mediated resistance to root-knot nematodes and the potato aphid. Plant J. 27: 417-425.

Nombela, G., Beitia, F. & Muñiz, M. 2000: Variation in tomato host response to *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to acyl sugar content and presence of the nematode and potato aphid resistance gene *Mi*. Bull. Entom. Res. 90: 161-167.

Nombela, G., Beitia, F. & Muñiz, M. 2001: A differential interaction study of *Bemisia tabaci* Q-biotype on commercial tomato varieties with or without the *Mi* resistance gene, and comparative host response with the B-biotype. Entom. Exper. Appl. 98: 339-344.

Nombela, G., Williamson, V.M. & Muñiz, M. 2003: The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol. Plant Microbe In. 16: 645-649.

Rossi, M., Goggin, F. L. Milligan, S. B., Kaloshian, I., Ullman, D. E. & Williamson, V. M. 1998: The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc. Natl. Acad. Sci. USA 95: 9750-9754.

StatSoft 1994: Statistica Version 4.5 for the Windows Operating System. Reference for statistical procedures. StatSoft, Tulsa,OK.

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Induced resistance in tomato to whitefly *Bemisia tabaci* by Bion®

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Abstract: Chemical elicitors can induce plant resistance to insects and other pests in susceptible plants. They include Benzo [1,2,3] thiadiazole-7-carbothioic acid-S-methyl ester (BTH), which is the active ingredient in the Syngenta plant activator Bion®. Induced plant resistance to the B and Q biotypes of *Bemisa tabaci* after foliar application of Bion® to cv. Marmande tomato plants was evaluated under controlled conditions.

In a free-choice experiment, adult Q-biotype *B. tabaci* significantly preferred control plants to plants sprayed with Bion® at 0.2 g/l. In consequence, the number of eggs laid on treated plants, was lower, though female fecundity was not affected. After 23 days, a decrease in the number of empty pupal cases was also observed on plants treated with Bion® at 0.2 g/l. The effect produced by Bion® applied at 0.1 g/l was not significant.

In a no-choice assay, only one leaflet from each tomato plant was treated with either 1 g/l Bion® or water (control plants). A clip-cage containing 5 *B. tabaci* females (biotipe B) was attached to each treated leaflet and also to another non-treated leaflet from every plant. After 16 days, the total number of immature insects (eggs+L1+L2) on Bion®-treated leaflets was significantly lower than on the water-treated leaflets from the control plants. This difference was mostly due to the number of L1 larvae. The acquired resistance seemed to be very localized (LAR) given the differences between Bion®-treated and non-treated leaflets on the same plants, while no differences were observed between Bion®-treated and control plants in the case of non-treated leaflets.

Key words: *Bemisia tabaci*, Bion®, BTH, induced resistance.

Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important crop pests on a world scale. Damage to tomato plants is especially important in the case of virus transmission.

Induced resistance offers an alternative tool to insecticides in integrated control. Multiple mechanisms are responsible for induced resistance, and different types of interactions between pathways have been described. Pathogens frequently stimulate the pathway mediated by salicylic acid, while herbivores frequently stimulate the pathway mediated by jasmonic acid. However, there are examples in which just the opposite occurs (Karban and Kuć, 1999).

Benzo [1,2,3] thiadiazole-7-carbothioic acid-S-methyl ester (BTH; Bion®) has been described as having an effect on various plant diseases, including TYLCD (Monci, *et al.*, 2003). The effect of BTH treatment on *B .tabaci* has been studied on cotton plants (Inbar *et al.*, 2001).

The aim of this study was to determine whether Bion ® applied to tomato plants affects the performance of *B .tabaci*.

Material and methods

Insect rearing

Two populations of *B. tabaci* belonging to the B and Q biotypes were used in this work Cultures of both biotypes were initially reared on cucumber and after several years these cultures were transferred to tomato cv. Rio Fuego. In 2001, the cultures were transferred to tomato cv. Marmande.

Free-choice assay

Tomato seedlings cv. Marmande with two expanded leaves (15-18 days-old) were sprayed to run-off with Bion ® (50% active product BTH) at 0.1 and 0.2 g/l and also with distilled water (control treatment). After three days, plants were placed, in a randomised block design with ten replicates per treatment, in a rearing cage with an undetermined number of *B .tabaci* whiteflies (biotype Q). After two more days, whiteflies from each plant were carefully counted and eliminated. Eggs were also counted and female fecundity was estimated from the number of eggs per female and day. Plants were then placed in separated cylinder cages and incubated for 23 days. Numbers of L3 and L4 nymph and pupal empty cases were then counted.

Incubation conditions were 25±2°C, 60±10% relative humidity and 16-h-light:8-h-darkness and the experiment was repeated once. Data were analysed by ANOVA. Data relating to the number of eggs per female and day were transformed by sqrt (x+2) prior to analysis. All other variables were transformed by sqrt(x). After analysis, means were compared by a Tukey HSD test ($P<0.05$). All analyses were performed using the Statgraphics package (1997).

No-choice assay

Twenty tomato cv. Marmande plants were used for this assay which was performed in our laboratory at room conditions (average temperature approximately 22°C, with a photoperiod of 16-h-light and 8-h-darkness). When they were two months old, ten plants were treated on a single leaflet with 1 g/l Bion®. Another ten plants were treated with distilled water and considered controls. Five days after the treatments, five adult female whiteflies (B-biotype *B. tabaci*) were placed in a plastic clip-cage and attached to the treated leaflet so that the whiteflies had access to the abaxial surface of the leaf (Nombela *et al.*, 2001). A similar clip-cage containing whiteflies was attached to another non-treated leaflet on each plant. After 24 hours, the whitefly females were removed from the clip-cages. At the end of the experiment - 16 days after whitefly exposure - the number of eggs, L1 and L2 on each leaflet were recorded.

Data were $\log_{10}(x+1)$ transformed and compared by a one-way ANOVA (Statsoft, 1994).

Results and discussion

In the free-choice experiment, treatment with Bion® at 0.2 g/l affected whitefly host preference: the number of whiteflies found on treated plants was significantly ($P<0.05$) lower than on control plants (Table 1). The number of eggs per female and day was not affected, thus the observed reduction in the total number of eggs per plant was produced by the reduction in the number of females attracted to the treated plants and not to a reduction of female fecundity.

A significant ($P<0.05$) reduction in the number of empty pupal cases was also observed at the end of the experiment (Table 2). When Bion® was applied at 0.1 g/l, a reduction in the studied variables was also observed, but differences in this case were not significant.

Table 1. Mean number of adult whiteflies, eggs, and eggs per female and day observed on the Bion ® -treated and control plants 5 days after treatment, in a free-choice experiment.

	Treatment		
	Control	Bion ® 0.1 g/l	Bion ® 0.2 g/l
Adult whiteflies	26.2 a	21.2 ab	15.0 b
Eggs	106.5 a	97.1 ab	57.3 b
Eggs per female and day	3.2 a	3.2 a	3.0 a

Means on a given line followed by a different letter differ significantly ($P<0.05$).

Table 2. Mean number of L3 and L4 nymphs and empty pupal cases observed on the Bion ® treated and control plants 38 days after treatment, in a free-choice experiment.

	Treatment		
	Control	Bion ® 0.1 g/l	Bion ® 0.2 g/l
L3	11.1 a	5.7 a	6.7 a
L4	37.8 a	32.0 a	22.5 a
Empty pupal cases	28.4 a	34.0 ab	11.8 b

Means on a given line followed by a different letter differ significantly ($P<0.05$).

In the no-choice assay, Bion® induced a certain level of resistance to *B. tabaci* because the total number of immature insects (eggs + L1 + L2) significantly ($P< 0.01$) decreased on the leaflets treated with Bion® when compared with the control plants (Table 3). This effect was mostly attributable to the number of first-instar larvae ($P< 0.06$), which seems to suggest that treatment with Bion® mostly impaired or delayed egg hatching to L1.

Table 3. Mean number of eggs, L1 and L2 whiteflies per leaflet observed on the Bion®-treated and control plants at the end of the no-choice assay.

	Individuals per leaflet*			
	Treated leaflet		Non-treated leaflet	
	Bion®	Control	Bion®	Control
Eggs	0.4 a	0.8 a	0.2 a	2.3 a
L1	3.1 b	5.3 a	6.9 a	7.8 a
L2	0.0 a	0.8 a	0.1 a	1.1 a
Total immatures	3.5 b	6.9 a	7.2 a	11.1 a

*Means on a given line from separated treated and non-treated leaflets followed by a different letter differ significantly ($P<0.01$ for Total; $P<0.06$ for L1) by one-way ANOVA

Differences between Bion®-treated and control plants with respect to the number of insects observed on the non-treated leaflets were not statistically significant (Table 3). Moreover, the numbers of L1 and total whiteflies were significantly ($P<0.05$) lower on the Bion®-treated leaflets than on non-treated leaflets from the same plants. This seems to indicate that

resistance induced by BTH is not systemic, but only localized (LAR) to parts of the plant treated with Bion®. Inbar *et al.* (2001) described how treatment of cotton plants with BTH had no systemic effect on whitefly host preference. However, they found a localized reduction in egg density on old leaves. In our free-choice experiment the effects found were also localized, as whole plants were treated with Bion®.

References

Inbar, M., Doostdar, H., Gerling, D. & Mayer, R.T. 2001: Induction of systemic acquired resistance in cotton by BTH has a negligible effect on phytophagous insects. Entomol. Exp. Appl. 99: 65-70.

Karban, R. & Kuć, J. 1999: Induced resistance against pathogens and hervibores: an overview. In: Induced plant defences against pathogens and hervibores, APS Press, St. Paul, eds. Agraval, Tuzun and Bent: 1-16.

Monci, F., García-Andrés, S.., Sánchez, f., Díaz, J., Soria, C., Acebes, A., López, A., Espi, E. & Moriones, E. 2003: Uv-blocking plastic covers and induction of systemic resistance in plants to control *Bemisia tabaci* and geminivirus infections in tomato in commercial plastic houses of southern Spain. 3rd International *Bemisia* workshop, Barcelona, Spain, p. 100.

Nombela, G., Beitia, F. & Muñiz, M. 2001: A differential interaction study of *Bemisia tabaci* Q-biotype on commercial tomato varieties with or without the *Mi* resistance gene, and comparative host response with the B-biotype. Entomol. Exp. Appl. 98: 339-344.

Statgraphics. 1997: Statgraphics Plus for Windows 3.1. Statistical Graphics Corp.

Statsoft. 1994: Statistica Version 4.5 for the Windows Operating System. Reference for statistical procedures. StatSoft, Tulsa, OK.

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Commercial-scale trials of *Eretmocerus* spp. (Hymenoptera: Aphelinidae) for control of *Bemisia tabaci* in tomato and sweet pepper in southeastern Spain.

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Abstract: *Eretmocerus mundus*, is native to the Mediterranean region where it occurs spontaneously parasitizing *B. tabaci* in fruiting vegetables, although only the American species *E. eremicus* was commercially available prior to 2001. Surveys conducted in commercial greenhouses during the 1998/99 and 1999/2000 seasons demonstrated that, despite initial establishment, the exotic parasitoid was steadily replaced by the native one immigrating from outside, providing impetus for commercial production of *E. mundus*. Incidence of whiteflies and other pests were compared in 7 conventionally managed greenhouses and 12 IPM greenhouses using biological control in four growing regions of Spain: Águilas/Mazarrón (Murcia), Almería, Motril (Granada), and the Canary Islands. *E. eremicus* was released in half of each IPM greenhouse and *E. mundus* in the other half using a Latin square design. IPM greenhouses were more likely to use tolerant cultivars, and fewer and more selective insecticides. Little parasitism (3%) was seen in conventional greenhouses compared to IPM greenhouses (50%) where *E. mundus* was dominant. Whitefly control was similar in both. For the pepper trials, each of 12 greenhouses in Campo Cartagena (Murcia, Spain) received *E. mundus* alone, *E. eremicus* alone or a 1:1 mixture of the two in an RCB design with 4 replications. Insecticidal control of whitefly was not required in any of the test greenhouses. Significantly fewer whiteflies were observed in greenhouses were *E. mundus* or the mixture was released compared to those receiving only *E. eremicus*. *E. mundus* rapidly displaced *E. eremicus* where both were released and eventually where only *E. eremicus* was released, attesting to a significant immigrant component of the *E. mundus* population.

Key words: *Eretmocerus mundus, Eretmocerus eremicus, Bemisia tabaci,* biological control, sweet pepper, tomato, IPM.

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Life history of *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) on *Bemisia tabaci* biotype "Q" (Homoptera: Aleyrodidae) using sweet pepper and tomato

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Abstract: *Eretmocerus mundus* is native to the Mediterranean region where it spontaneously parasitizes *B. tabaci* in greenhouse-grown fruiting vegetables. Fecundity on tomato and pepper was evaluated by placing newly emerged couples (n = 15) of *E. mundus* on leaf disks infested with second instar *B. tabaci,* the preferred stage, maintained at 25°C and changed daily until the female died. All whitefly nymphs were observed for host feeding and inverted to count parasitoid eggs. Adult longevity was estimated at 10.1±1.0 days (mean ± SEM) in pepper and 7.3 ± 0.81 days in tomato. Fecundity (number of hosts) was estimated 171.1 ± 22.8 per female in pepper and 147.8 ± 13.5 in tomato. Host feeding incidence was 15.6 ± 0.98 nymphs per female in pepper and 10.7 ± 1.3 in tomato. No significant differences were detected in the duration of life stages between tomato and sweet pepper. Preimaginal survivorship estimated in clip cages starting with 66 eggs in pepper and 59 in tomato was 81.0% and 64.4% respectively. Most of the difference were due to 17% mortality during the pupal stage in tomato possibly due to leaf degradation and not seen in pepper. R_o in pepper was estimated at 67.50 ± 8.71 (mean ± SD) which was significantly greater than 47.00 ± 4.02 in pepper. However, generation time (T) was also significantly greater in pepper (19.40 ± 0.46) than in tomato (18.10 ± 0.36). As a consequence of these two opposing factors, the estimate of intrinsic rate of increase (r_m) was not statistically different in pepper 0.218 ± 0.005 than in tomato (0.214 ± 0.004). These values are well above those reported for *B. tabaci* on any crop indicating the potential of *E. mundus* to control this pest. **Key words**: *Eretmocerus mundus, Bemisia tabaci*, biological control, life history, sweet pepper, tomato.

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Calibration of release rates of *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) for control of *Bemisia tabaci* in sweet pepper and tomato

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Abstract: The efficacy of *E. mundus* was evaluated in an air conditioned plastic greenhouse in Águilas (Murcia; Spain) divided with fine screen into 24 compartments, 8 m^2 each, containing 10 plants. Two experiments were conducted, one in fall and the other in spring, the two main horticultural cropping seasons in southern Spain. Two factors were evaluated: host plant (tomato and sweet pepper) and release rate of *E. mundus* (0, 1.5 ind./m^2 and 6 ind/m^2) using a split plot design with 4 replicates. In fall, female whitefly adults were released only once at 4.8 ind/m^2 two weeks after transplanting, whereas 3 weekly whitefly releases were made in spring to simulate typical immigration. Parasitoid releases were initiated 2 weeks after whitefly entry and continued weekly for 6 weeks in sweet pepper and 11 and 9 weeks in the fall and spring tomato trials respectively. Control infestations were highest in fall, reaching more than 700 nymphs/leaf in tomato and 150 nymphs/leaf in pepper. Nevertheless, upwards of 95% whitefly control was achieved in sweet pepper with the low release rate, although the high rate was required to obtain this result in tomato. The low rate was adequate in both crops during spring. These experiments suggested that an intermediate release rate of 3 ind./m^2 for approximately 6 weeks should be adequate for most situations if initiated early, although higher rates could be necessary in fall tomato when temperatures are high and immigration often intense.

Key words: *Eretmocerus mundus, Bemisia* tabaci, biological control, calibration rates, sweet pepper, tomato.

Section III. Mites, leafminers, aphids and thrips.

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Successful sustainable control of spider mites in greenhouse cut roses by successive releases of two predatory mite species

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Abstract: Successive releases of the predatory mite species *N. californicus* (prey generalist) and *P. persimilis* (prey specialist) were evaluated in two different greenhouses and over two successive growing seasons as a control strategy for the rapid and long-term suppression of *T. urticae* infestations on greenhouse cut roses. Regularly distributed, preventative introductions of the prey generalist at the start of the growing season (March 2001) were followed by spot releases of *P. persimilis* into high-density infestation patches. Despite a total release of 22.2 resp. 17.8 *N. californicus*/m² and 49.4 resp. 41.1 *P. persimilis*/m² spider mite control during this growing season was only achieved in combination with repeated acaricide treatments in mid of July, when the predator/pest ratio had reached >1:10. In contrast, in 2002, *T. urticae* populations were sufficiently suppressed by the release of a total of 11 resp. 13 *N. californicus*/m² and 42 *P. persimilis*/m². The first visible occurrence of the spider mite was observed at the end of May; two months later than in the previous year. During late spring and summer the prey specialist was present in higher densities than the prey generalist, whereas the opposite was observed at the end of the growing season, when *T. urticae* densities declined to their minimum level. Assessments of per leaf densities of spider mites and predatory mites (n = 180 per greenhouse) were carried out at fortnightly intervals, as were assessments of the percentage of infested rose shoots. Reasons for the failure of rapid control of *T. urticae* during the first growing season are discussed.

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Evaluating the effects of five strawberry cultivars on the biology of *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae) using detached leaves in greenhouse conditions

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Abstract: The commercial strawberry cultivars, Camarosa, Chandler, Selva, Sweet Charlie and Pajaro were chosen as hosts for comparing the biology of *Tetranychus cinnabarinus* Boisduval on detached leaves under greenhouse conditions in Southern Turkey. *T. cinnabarinus* females completed their development in 9.00, 9.18, 9.25, 9.38 and 9.45 days on Selva, Pajaro, Sweet Charlie, Camarosa and Chandler, respectively, whereas corresponding male development times were slightly shorter for all tested cultivars. These were 8.47, 8.53, 8.67, 8.87 and 8.89 days on Chandler, Sweet Charlie, Pajaro, Camarosa and Selva, respectively. Male and female development times did not show any statistically significant differences for the cultivars compared. On average, mated females laid 7.39, 7.69, 7.73, 8.42 and 8.57 eggs per female per day and 51.71, 53.85, 54.13, 59.00 and 60.00 eggs during the first seven days of the oviposition period on Camarosa, Selva, Pajaro, Sweet Charlie, and Chandler, respectively. The females ratios of these species were 0.73, 0.73, 0.73, 0.72 and 0.78 in the same order as above. During the experiment greenhouse temperatures varied from 15 to 54 °C and RH ranged from 19 to 58%.

Key words: strawberry cultivars, *Tetranychus cinnabarinus*, greenhouse, Turkey

Introduction

Due to its favourable climatic conditions, the Mediterranean coastal region is one of Turkey's main strawberry-growing (*Fragaria x ananassa* Duch) areas. In this region, strawberries are produced in open field conditions, but also in greenhouses to obtain earlier fruit. Since 1985, cultivars such as Douglas, Chandler, Camarosa, and Sweet Charlie have been imported from the USA. Importing appropriate cultivars for the market has played an important role in increasing the strawberry-growing areas and total yields. Now more than 60 thousand tons of strawberries are produced in the region, 5 thousand of which are grown in greenhouses (DIE, 1999).

In Turkey, strawberries are traditionally produced in uncontrolled greenhouse conditions in which temperature and humidity fluctuate over a fairly wide range. As temperatures increase, *Tetranychus cinnabarinus* Boisduval becomes a serious pest on strawberry and affects almost every greenhouse each year. As in many other agricultural areas of the world, in this region *T. cinnabarinus* is generally controlled by the application of acaricides (Kazak *et al.*, 2002). However, previous open field trials have shown that strawberry production using cv. Camarosa is possible without taking measures to control *T. cinnabarinus* because of its resistance to spider mite damage (Kazak *et al.*, 2000). Thus, development time, fecundity and sex ratios of *T. cinnabarinus* have been studied on five strawberry cultivars in the greenhouse environment, in order to obtain a better understanding of the biology of this mite on this crop and developing future greenhouse IPM programs.

Material and methods

The commercial strawberry cultivars, Camarosa, Chandler, Selva, Sweet Charlie and Pajaro were chosen as hosts for comparing the biology of *T. cinnabarinus* on detached leaves under greenhouse conditions in Southern Turkey.

The leaves of greenhouse-raised strawberry cultivars were cut and 20 mm diameter leaf discs were obtained from each cultivar using a cork borer. Leaf discs were placed abaxial side up on a sheet of blotting paper, which was placed on top of a water-saturated thick sponge. The leaves were then placed in a plastic tray (25x15x5 cm) which was filled with water in order to maintain the mites on the individual discs. Fully developed leaflets were used to standardise leaf age. One gravid female was put on each leaf disc in order to obtain eggs and then placed in the greenhouse on a mosquito net-covered bench. The leaf discs in the culture dishes were checked after 24 hours. When there was more than one spider mite egg (F_2) per leaf disc, excess eggs were removed, leaving one per leaf disc. Once one egg was established on each leaf disc, female mites were removed. The spider mite eggs (F_2) on each leaf disc were observed twice a day (at 8 am and 4 pm) from their egg to adult stages. When females reached adulthood, two spider mite males were introduced to each leaf disc in order to mate with them. The number of eggs laid by the mated females (F_2) was recorded on a daily basis. Females were then transfered onto other clean leaf discs. The old leaf discs, containing the eggs, were placed on freshly prepared leaf disc arenas. Eggs were kept on the same cultivar until adult emergence. Sex ratio was then calculated for F_2 mites for each cultivar. These observations continued until at least the seventh day of female oviposition on each cultivar.

Time intervals of the different biological stages of male spider mite development were also studied. The immature development time for each stage was evaluated according to the method described above. Greenhouse temperatures and humidities were measured at one-hour intervals throughout the experiment using a HOBO data logger (Onset Corp., MA, USA). During the experiment greenhouse temperatures ranged from 15 to 54 °C and RH from 19% to 58% (Fig. 1). Statistical analyses were conducted using one-way ANOVA: means were separated using Duncan's Multiple Range Test at 5%.

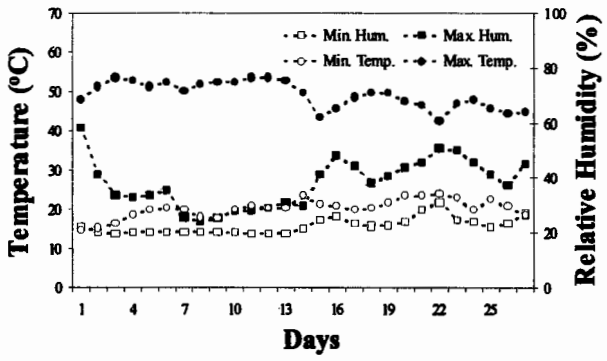

Figure 1. Daily maximum and minimum temperatures and relative humidities in the greenhouse during the experiment.

Results and discussion

Tables 1 and 2 show the mean development times for each immature stage and the total development of male and female *T. cinnabarinus* on five strawberry cultivars. The longest development periods were observed in the egg stage, followed by deutonymphal stages on all cultivars. There were, however, no significant differences in the development times of the egg, larval, protonymph, deutonymph and quiescent stages for female and males on the strawberry cultivars tested. *T. cinnabarinus* females completed their development in 9.00, 9.18, 9.25, 9.38 and 9.45 days on Selva, Pajaro, Sweet Charlie, Camarosa and Chandler, respectively (table 1).

In contrast, *T. cinnabarinus* male development times were relatively shorter than those of females on all tested cultivars. These were found to be 8.47, 8.53, 8.67, 8.87 and 8.89 days on Chandler, Sweet Charlie, Pajaro, Camarosa and Selva, respectively (table 2). Combined male and female development times did not show any statistically significant differences for any of the cultivars. Female preoviposition periods were 0.92, 1.00, 1.03, 1.04, and 1.08 days on Pajaro, Selva, Camarosa, Chandler and Sweet Charlie, respectively. On average, mated females each laid 7.39, 7.69, 7.73, 8.42 and 8.57 eggs per day and 51.71, 53.85, 54.13, 59.00 and 60.00 eggs during the first seven days of the ovipositional period on Camarosa, Selva, Pajaro, Sweet Charlie, and Chandler, respectively (table 3). The sex ratios were 0.73, 0.73, 0.73, 0.72 and 0.78 females (table 3). Similarly, there were no significant differences in the lengths of the preovipositional periods, daily average number of eggs laid per female, or average total number of eggs laid.

Strawberry cultivars vary in susceptibility to twospotted spider mite infestations. Short-day cultivars are generally more tolerant to mite feeding than day-neutral cultivars, particularly later in the fruit-production season (Zalom *et al.*, 1991). An experiment comparing the resistance of strawberry cultivars to *T. urticae* showed that the day-neutral cultivar Selva was more susceptible to the mite than the short-day cultivars Sweet Charlie and Pajaro (MacFarlane & Hepworth, 1994; IFAS, 2003). On the other hand, Gimenez *et al.* (1994) indicated that the short-day cultivar Chandler and the day-neutral cultivar Selva showed intermediate resistance patterns with respect to *T. urticae*. Except Selva, all the strawberry cultivars evaluated in this study were short-day cultivars and there were no evident differences in the biological characteristics of *T. cinnabarinus* between short-day and day-neutral cultivars. It is generally accepted that levels of strawberry leaf chemicals (farnesol, total and catechol-based phenolics and sugar) and both glandular and nonglandular trichomes play an important role in determining host plant resistance to tetranychids (Poe, 1971; Regev, 1978; Luczynski *et al.*, 1990). As stated above, these experiments were conducted in an uncontrolled greenhouse environment. Extremely high temperatures (figure 1) could therefore possibly explain the change in plant physiology that was probably responsible for host resistance to *T. cinnabarinus*. Finally all the strawberry cultivars tested here showed similar effects on the biological characteristics of *T. cinnabarinus*. Before drawing any final conclusions, it is necessary to study population development of the pest on these cultivars under both controlled laboratory conditions and uncontrolled greenhouse conditions.

Table 3. Daily means, total number of eggs, and sex ratios of *Tetranychus cinnabarinus* on five strawberry cultivars under greenhouse conditions (no. of eggs±SEM).

Cultivars	n	Daily*	Total[1]*	Sex ratio
Selva	13	7.69±0.51	53.85±3.57	0.73
Pajaro	8	7.73±0.57	54.13±4.01	0.73
Chandler	7	8.57±0.69	60.00±4.82	0.78
Camarosa	7	7.39±0.49	51.71±3.41	0.73
Sweet Charlie	11	8.42±0.59	59.00±4.16	0.72

*Means in each column are not statistically different (P>0.05; Duncan's multiple range test).
[1]for seven days in the oviposition period.

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References

DIE. 1999: The summary of Agricultural statistics. State Institute of Statistics Prime Ministry Republic of Turkey (1979-1998), 49 pp.

Gimenez Ferrer, R. M., Erb, W. A., Bishop, B. L. & Scheerens, J. C. 1994: Host-pest relationships between the twospotted spider mite (Acari: Tetranychidae) and strawberry cultivars with differing levels of resistance. J. Econ. Entomol. 87(1): 168-175.

IFAS. 2003: http://strawberry.ifas.ufl.edu/breeding/Sweetcharliefull.htm

Kazak, C., Sekeroglu, E., Karut, K. & Kasap, I. 2000: Efficiency of native *Phytoseiulus persimilis* strain on tetranychid mites and determination of establishment possibilities in nature by Climex Model. TUBITAK/TARP Project No: 2373. 54 pp.(Unpublished project report).

Kazak, C., Karut, K., Kasap, I., Kibritci, C. & Sekeroglu, E. 2002: The potential of the Hatay population of *Phytoseiulus persimilis* to control the carmine spider mite *Tetranychus cinnabarinus* in strawberry in Silifke Icel, Turkey. Phytoparasitica. 30(5): 451-458.

Luczynski, A., Isman, M. B., Raworth, D. A., & Chan, C. K. 1990: Chemical and morphological factors of resistance against the twospotted spider mite in beach strawberry. J. Econ. Entomol. 83(2): 564-569.

MacFarlane J. & Hepworth G. 1994: Population trends of twospotted spider mite (Acari: Tetranychidae) on Four Resistant Strawberry cultivars and their relationships to fruiting. J. Econ. Entomol. 87(3): 817-820.

Poe, L. S. 1971: Influence of the host plant physiology on populations of *Tetranychus urticae* (Acarina: Tetranychidae) infesting strawberry plants in peninsular. Fla. Entomol. 54(2): 183-186.

Regev, S. 1978: Difference in farnesol content in strawberry varieties and their susceptibility to the carmine spider mite *Tetranychus cinnabarinus* (Boisd.) (Acari: Tetranychidae). Ent. Exp. Appl., 24: 22-26.

Zalom, F. G., Phillips, P. A., & Toscano, N. C. 1991: UC IPM Pest Management Guidelines: Strawberry. Insects and Mites. http://www.ento.vt.edu/Fruitfiles/Strw2Spotted.html

Table 1. Mean development time (days) of *Tetranychus cinnabarinus* females on five strawberry cultivars under greenhouse conditions (days±SEM).

Cultivars	n	E*	L*	QL*	P*	QP*	D*	QD*	Total*	PO*
Selva	15	4.47±0.07	0.53±0.07	1.00±0.00	0.69±0.02	0.40±0.04	0.96±0.03	0.96±0.04	9.00±0.06	1.00±0.00
Pajaro	11	4.48±0.09	0.52±0.09	1.00±0.00	0.70±0.03	0.39±0.06	1.03±0.07	1.06±0.06	9.18±0.09	0.92±0.35
Chandler	11	4.55±0.12	0.61±0.09	1.00±0.00	0.82±0.13	0.52±0.09	0.97±0.03	1.00±0.00	9.45±0.25	1.04±0.14
Camarosa	13	4.67±0.20	0.62±0.09	0.95±0.05	0.74±0.04	0.46±0.07	1.00±0.00	0.95±0.05	9.38±0.31	1.03±0.11
Sweet Charlie	12	4.56±0.15	0.56±0.12	0.94±0.05	0.69±0.05	0.50±0.08	1.00±0.00	1.00±0.00	9.25±0.16	1.08±0.08

*Means in each column are not statistically different (P>0.05; Duncan's multiple range test). (E: egg, L: Larvae, QL: Quiescent larvae, P: Protonymph, QP: Quiescent protonymph, D: Deutonymph, QD: Quiescent deutonymph, PO: Preoviposition) (n: number observed).

Table 2. Mean development time (days) of *Tetranychus cinnabarinus* males on five strawberry cultivars under greenhouse conditions (days±SEM).

Cultivars	n	E*	L*	QL*	P*	QP*	D*	QD*	Total*
Selva	3	4.22±0.22	0.78±0.22	0.89±0.11	0.67±0.00	0.33±0.00	1.00±0.00	1.00±0.00	8.89±0.11
Pajaro	5	4.07±0.22	0.80±0.13	0.87±0.13	0.67±0.00	0.33±0.00	0.93±0.06	1.00±0.00	8.67±0.14
Chandler	5	4.27±0.26	0.60±0.16	0.93±0.06	0.60±0.06	0.47±0.13	0.80±0.13	0.80±0.13	8.47±0.34
Camarosa	5	4.53±0.13	0.33±0.00	1.00±0.00	0.67±0.00	0.33±0.00	1.00±0.00	1.00±0.00	8.87±0.13
Sweet Charlie	5	4.27±0.26	0.47±0.13	1.00±0.00	0.73±0.06	0.40±0.06	0.80±0.13	0.87±0.13	8.53±0.32

*Means in each column are not statistically different (P>0.05; Duncan's multiple range test). (E: egg, L: Larvae, QL: Quiescent larvae, P: Protonymph, QP: Quiescent protonymph, D: Deutonymph, QD: Quiescent deutonymph, PO: Preoviposition) (n: number observed).

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Role of the parasitoid *Diglyphus isaea* (Walker) and the predator *Macrolophus caliginosus* Wagner in the control of leafminers

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Abstract: *Liriomyza* spp. are pests on several vegetable crops and in some cases, such as tomato, their incidence is increasing. We conducted a survey in order to determine the species of leafminers and their parasitoids present in the vegetable production area of Catalonia, in fall 2001. The impact of two populations of the parasitoid *Diglyphus isaea* (field and mass reared populations) and the predator *Macrolophus caliginosus* on leafminers was evaluated. Laboratory experiments were organized to compare parasitism rates and host-feeding by parasitoid females of both populations, and predation by *M. caliginosus* on *L. trifolii* larvae. According to the survey results, the most abundant leafminer and parasitoid species were *L. bryoniae* and *D. isaea* respectively. *Liriomyza huidobrensis* was not found in any of the samples. Parasitism rates were generally low and pest control was mostly due to mortality (without oviposition) of leafminer larvae. Laboratory experiments revealed no differences in parasitism and host-feeding between field and mass reared *D. isaea*. The mirid bug *M. caliginosus* produced similar mortality to leafminer larvae than *D. isaea* and could therefore be considered an important biological control agent for this pest.

Key words: *Liriomyza*, *Diglyphus isaea*, *Macrolophus caliginosus*, leafminers, biological control, vegetables

Introduction

Leafminers, *Liriomyza bryoniae* (Kaltenbach) and *L. trifolii* (Burgess), were some of the main pests on spring tomatoes in Mediterranean greenhouses between 1989 and 1992 when an Integrated Pest Management (IPM) program based on the inoculation of *Encarsia formosa* Gahan was implemented to control *Trialeurodes vaporariorum* (Westwood). Natural parasitism by *Diglyphus isaea* (Walker) was found in the majority of the greenhouses in which leafminers occurred; increased releases of the parasitoid were therefore only required in 25% of them (Albajes *et al.* 1994). *Encarsia formosa* has not been released in spring tomato greenhouses since 1997 due to competition from naturalized *Encarsia pergandiella* Howard (Gabarra *et al.* 1999). Since then, control of greenhouse whitefly has been attempted using *Macrolophus caliginosus* Wagner. According to IPM advisors, the importance of leafminers has increased and augmentative releases of mass reared *D. isaea* in IRTA-Cabrils no longer seem to be as successful as they once were. The aims of this study were: (1) to determine the different species of leafminers present in the vegetable producing area of Catalonia and their parasitoids and, (2) to evaluate the respective impacts on the target pest of a field and a mass reared population of the parasitoid *D. isaea* and the predator *M. caliginosus*.

Material and methods

Survey

A survey of commercial vegetable crops infested with leafminers was conducted in two vegetable producing areas around Barcelona (Baix Llobregat and Maresme) in September and October 2001. The survey considered 13 tomato greenhouses and 2 tomato fields, 1 cucumber greenhouse and 1 bean greenhouse. *Diglyphus isaea* had not been released in any of these fields or greenhouses. To estimate natural *Liriomyza* spp. larvae mortality, 100 bean and cucumber leaves or tomato leaflets were collected from 100 different plants from each greenhouse or field. Fifty mines from at least 25 leaves or leaflets were dissected under a stereomicroscope. Numbers of live, dead and parasitized leafminer larvae and empty mines were separately recorded. The remaining leaves were kept in order to obtain *Liriomyza* puparia, adults and parasitoids and were used for species identification. Leafminer species were discriminated from possible leafminers, *L. trifolii*, *L. bryoniae* and *L. huidobrensis* (Blanchard), by two characters: the number of pores in the posterior spiracles of their puparium (3 for *L. trifolii* and 8-11 for both *L. bryoniae* and *L. huidobrensis*) and, the color of their third antennal segment (yellow for *L. bryoniae* and *L. trifolii* and brown for *L. huidobrensis*) (Spencer 1973, Martinez 2001). We observed 184 puparia and 134 adults. The identity of *D. isaea* was confirmed by the Natural History Museum in London.

Laboratory experiments

Two *D. isaea* populations were compared. One was mass reared at IRTA for more that 10 years and periodically re-infested with field individuals. The other (field population) was specially reared for this experiment (for 6 months) and was started with adults obtained from tomato leaflets collected in the Maresme area in October 2001.

Six-day-old *D. isaea* females were isolated in Petri dishes (12 cm. Ø) containing an infested bean leaf, and a small strip of paper spread with honey on a base of moistened filter paper. These dishes were sealed with parafilm and kept in a climatic chamber (25°C, 16L:8D). Bean leaves were obtained from plants previously infested with *L. trifolii* adults for 5 days and kept at 25°C to ensure the presence of second and third instar larvae. The mean number of mines per leaf was 55. The leaves in the Petri dishes were replaced each day for 4 consecutive days and checked for parasitized larvae and killed larvae due to host-feeding. Females dead or lost during these 4 days were excluded from the analysis. Between 11 and 12 females were tested from each population.

The same test was conducted with males and females of the predatory mirid *M. caliginosus*. In this case, seven-day-old mirids were used. Individuals were not starved before testing and 13 females and 14 males were tested.

Eight Petri dishes, which did not contain parasitoids or predators, were used as controls to monitor the natural mortality of leafminer larvae.

Results and discussion

Survey

As observed in Table 1, just 5% of puparia had only 3 pores in their posterior spiracles and were therefore associated with *L. trifolii*. The rest had multiple pores in their spiracles and were associated with either *L. bryoniae* or *L. huidobrensis*. All of the observed leafminer adults had yellow third antennal segments, which indicated the absence of *L. huidobrensis*.

In the case of parasitoids, *D. isaea* was present in samples from 16 greenhouses and fields (14 tomato, 1 cucumber and 1 bean) and the pupal parasitoid, *Dacnusa sibirica*, was

found in a sample from a tomato greenhouse. In samples from crops that had not been sprayed with insecticide, average rate of natural parasitism was 7.72 ± 4.95% and dead but not parasitized larvae accounted for 32.84 ± 4.76 % (Figure 1).

Table 1. Leafminer species composition in vegetable crops. September-October 2001.

	Puparia			Adults		
Area	n	*L. trifolii*	*L. bryoniae* *L. huidobrensis*	n	*L. bryoniae* *L. trifolii*	*L. huidobrensis*
North Maresme	42	5	95	32	100	0
South Maresme	104	0	100	69	100	0
Baix Llobregat	38	0	100	33	100	0

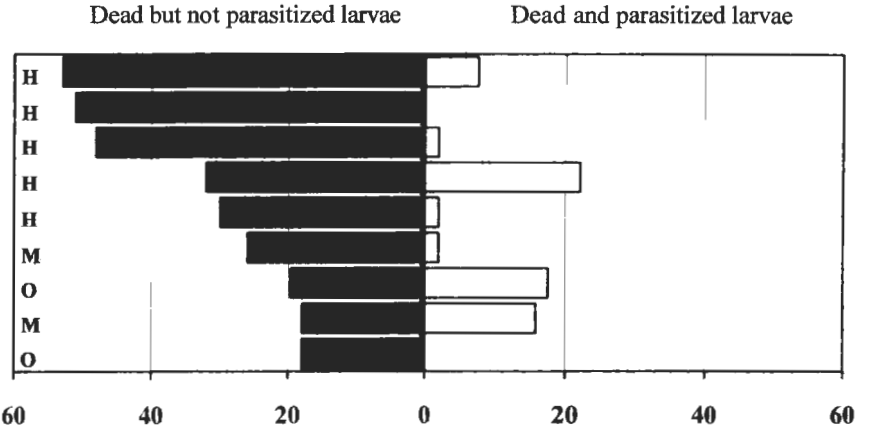

Figure 1. Percentage of dead (non-parasitized and parasitized) larvae in samples that were not treated with insecticide. Letters in the columns refer to the abundance of mirid bugs according to estimations made by IPM advisors: (O) none or few, (M) medium levels and (H) high levels.

The results of this survey indicate that *L. bryoniae* was the most abundant species and that *L. trifolii* was present in a very small numbers. This confirmed the findings of Albajes *et al.* (1994) who recorded a shift in species dominance from *L. trifolii* (neartic origin) to *L. bryoniae* (native species) between 1989 and 1993. However, larval mortalities in our study (including parasitized and non-parasitized larvae) were quite low in comparison with mortality rates higher than 50% recorded by these authors for greenhouse spring tomato crops in July. The high proportion of *L. bryoniae* found in the present study may, as indicated by the previous mentioned authors, have adversely influenced parasitism rates since the percentage of leafminer larvae killed by *D.isaea* is lower for *L. bryoniae* than for *L. trifolii*.
Interestingly, in many of the samples, high mortality rates were associated with low parasitism rates (and therefore also with low parasitoid presence). IPM advisors were asked to

classify the greenhouses and fields from which samples were obtained in terms of the following classification of mirid bug abundance: (O) none or few, (M) medium levels and (H) high levels. It is evident that high mortalities and low levels of parasitism were often associated with high mirid bug populations. These observations suggested the important role that *M. caliginosus* predation may play in controlling *Liriomyza* spp. This is similar to what was observed in Dutch greenhouses (Klapwijk 1999).

Laboratory experiments

Natural mortality of *L. trifolii* larvae in control Petri dishes was low (1.1%, n =353 larvae) and was therefore not considered in the calculations.

Table 2. Average number (±SE) of *L. trifolii* larvae killed by host-feeding or parasitized by a *D. isaea* female in 4 days, number of eggs per female, and percentage of egg-laying females.

D. isaea population	n	Number of *L. trifolii* larvae killed		Fecundity (eggs/female)	% egg-laying females
		host-feeding	parasitization		
Field	11	6.91 ± 1.75	6.73 ± 2.52	6.73 ± 2.52	63.64
Mass reared	12	4.17 ± 1.18	5.83 ± 1.89	5.83 ± 1.89	75.00

No significant differences were found between field and mass reared *D. isaea* populations with respect to the number of leafminer larvae killed by host-feeding (F=1.47;d.f.=22,1; P=0.201), number of leafminer larvae parasitized (F=0.11;d.f.=22,1; P=0.745) and female fecundity (F=0.08;d.f.=22,1; P=0.777). The number of eggs/leafminer larvae was not significantly different for the *D. isaea* populations (F=1.93;d.f.=15,1; P=0.186) and was in average 1.20 ± 0.09. Non-egg-laying females have been included in our analysis. It is important to bear this in mind because non-egg-laying *D. isaea* females kill a much smaller number of larvae for host-feeding than egg-laying females (F=23.15;d.f.=22,1; P<0.001) (Figure 2).

Figure 2. Average number (±se) of *L. trifolii* larvae preyed upon by non-egg-laying (n=7) and egg-laying (n=16) *D. isaea* females.

According to our results, control of *L. trifolii* by *D. isaea* owes as much to parasitism as to host-feeding. Results from other authors about the relative importance of parasitization and host-feeding in the control of leafminer are very controversial. For six to ten-day-old *D. isaea* females Nicoli & Pitrelli (1993) recorded higher host feeeding than fecundity rates (between 2 and 2.5 times). On the contrary, Minkenberg (1989) reported about 3 or 4 times lower host-feeding than fecundity rates for females of the same age. In any case, the parasitism and host-feeding rates found by us were quite low compared with those reported by both Minkenberg (1989) and Nicoli & Pitrelli (1993). Several factors in the experimental settings may be responsible of these differences (e.g. sugar or honey supply to the parasitoid, the *Liriomyza* species studied, leafminer larval density and instar composition).

Table 3. Average number of *L. trifolii* larvae (± se) killed by *M. caliginosus* in 4 days.

	n	*L. trifolii* larvae killed
Females	13	15.23 ± 2.99
Males	14	5.71 ± 0.81

The number of *L. trifolii* larvae killed by *M. caliginosus* females was significantly higher than the number killed by males (F=8.38;d.f.=26,1; P=0.008) (Table 3). The average number of leafminer larvae predated by *M. caliginosus* adults (whether male or female) was similar to the number of larvae that a *D. isaea* female can kill by host-feeding and parasitization. These results support data obtained in our survey, in which host-feeding by *D. isaea* did not seem to explain the large number of dead larvae found without oviposition, and confirmed the important role that *M. caliginosus* play in the control of leafminer populations.

Nedstam & Johansson-Kron (1999) previously observed the activity of this predatory bug on leafminers and their interaction with the parasitoid in commercial tomato greenhouses. The same authors observed that ratios between parasitized and, dead but non-parasitized *L. bryoniae* larvae decreased in greenhouses in which both *D. isaea* and *M. caliginosus* were released. In contrast, when no *M. caliginosus* were present, the ratio remained around or above one. Presumably, the interaction between *M. caliginosus* and *D. isaea* was not only because mirid predation reduces host availability for parasitazion, but also because this bug feeds on parasitized larvae. That is the case of the interaction between *M. caliginosus* and *E. formosa* parasitizing greenhouse whitefly (Castañé *et al.* 2000). However, predation on parasitized whitefly pupae was lower than on non-parasitized pupae and these authors suggest that the differences may be due to the difficulty in penetrating the pupal case of the parasitoid in addition to the host cuticle. For *Liriomyza* larvae this would have no effect as *D. isaea* is an ectoparasioid and does not penetrate the host. Feeding upon dead individuals is common practice among mirids (Wheeler 2001).

According to our results, the reduced abundance of native *D. isaea* and poor establishment of released mass reared *D. isaea* recorded by IPM advisors may be due to several different factors. Shifts in leafminer species composition from *L. trifolii* to *L. bryoniae* may have adversely influenced parasitism rates. In addition, increasing numbers of greenhouses and fields in which *M. caliginosus* are being released or conserved as a biological control agent may have negatively influenced native *D. isaea* populations and hampered the establishment of this parasitoid in vegetable crops.

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References

Albajes, R., Gabarra, R., Castañé, C., Alomar, O., Arnó, J., Ariño, J., Bellavista, J., Martí, M., Moliner, J. & Ramirez, M., 1994: Implementation of an IPM program for spring tomatoes in Mediterranean greenhouses. OIBC/WPRS Bull. 17(5): 14-21.

Castañé, C., Alomar, O., Goula, M. & Gabarra, R. 2000: Natural populations of *Macrolophus caliginosus* and *Dicyphus tamaninii* in the control of the greenhouse whitefly in tomato crops. IOBC/WPRS Bull. 23(1): 221-224.

Gabarra, R., J. Arnó, Ò. Alomar & R. Albajes. 1999 Naturally occurring populations of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in tomato greenhouses. IOBC/WPRS Bull. 22(1): 85-88.

Klapwijk, J.N., 1999. Biological Control of Tomato Pests in the Netherlands. OIBC/WPRS, 22(1): 125-128.

Martinez, M., 2001: Les mouches mineuses agromyzides nuisibles aux cultures ornementale et maraichère sous serre. PHM, 430: 9-13.

Minkenberg, O.P.J.M., 1989: Temperature effects on the life history of the eulophid wasp *Diglyphus isaea*, an ectoparasitoid of leafminers (*Liriomyza* spp.), on tomatoes. Ann. appl. Biol. 115: 381-397.

Nedstam, B. & Johansson-Kron, M., 1999. *Diglyphus isaea* (Walker) and *Macrolophus caliginosus* Wagner for biological control of *Liriomyza bryoniae* (Kaltenbach) in tomato. OIBC/WPRS Bull. 22(1): 185-187.

Nicoli, G. & Petrelli, P., 1993: Parasitization and predation of *Diglyphus isaea*. In: Proceedings of the 7th workshop of the IOBC global working group "Quality control of mass reared arthropods" Rimini (Italy) September 13-16, 1993, eds. Nicoli, Benuzzi and Leppla: 161-170.

Spencer, K.A., 1973. Agromyzidae (Diptera) of economic importance. Series Entomologica 9. Junk W.B.V. (ed.). The Hague.

Wheeler, A.G.Jr., 2001. Biology of the plant bugs. Cornell University, Ithaca, New York.

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Thermal requirements of three populations of *Aphidus colemani* Viereck (Hym.: Aphidiidae)

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Abstract: The parasitoid *Aphidius colemani* plays an important role as a natural enemy of *Aphis gossypii*. The optimal temperature for its development is around 22°C, and high mortality occurs at higher temperatures. However, parasitoids from different climatic regions of Brazil have demonstrated different behaviour related to temperature. The aim of this work was to determine the thermal requirements of *A. colemani* collected from three localities in south-east Brazil (Juramento, Lavras and São Gotardo(MG)).The locality of Juramento has the highest annual mean temperature compared to Lavras and São Gotardo. After 3 generations reared on *A. gossypii* on cucumber plants, the individuals were evaluated at 16, 19, 22 and 25±1°C, RH 70±10% and 12hr photophase. The lowest temperature thresholds for development of *A. colemani* were 4.30, 2.19 and 2.36°C for individuals from Juramento, Lavras and São Gotardo, respectively. The thermal constants of individuals from Juramento, Lavras and São Gotardo were 217.39, 238.1 and 238.09 degrees-days, respectively. The higher temperature threshold and the lower thermal constant showed in individuals from Juramento indicated that the population could be more suitable to control *A. gossypii* in protected cultivation at temperatures higher than 22°C.

Key words: temperature threshold, thermal constant, parasitoid, biological control agent

Introduction

The aphid *Aphis gossypii* Glover is among the main pests in protected crops, such as cucumber and chrysanthemum in Brazil (Bueno 1999). The parasitoid *Aphidius colemani* Viereck is one of the dominant species in South America (Starý & Cermeli, 1989), showing great potential to be used in the biocontrol of *A. gossypii* (Sampaio et al. 2001). The failure of the biological control of *A. gossypii* with *A. colemani* has been related to high temperatures, especially due to the fact that those temperatures cause a high mortality rate in the parasitoid (Toussidou et al. 1999). The optimal temperature for the *A. colemani* development is around 22°C.

Insect populations may show different responses to temperature according to the latitude at which they occur (Taylor, 1981). Thus, the lower temperature threshold for development is proposed as an indication of the insects' adaptability to temperature, such as in the case of the parasitoids, which may aid the detection of more adjustable strains to specific climatic conditions (Royer et al. 2001).

This work had as its purpose the establishment of the thermal requirements of *A. colemani* populations from three different climatic regions in the south-east of Brazil (State of Minas Gerais), aiming to use the parasitoid as a biological control agent of *A. gossypii* in protected cultivation.

Material and Methods

Parasitoid collecting

A. colemani were collected in three counties of the State of Minas Gerais (Brazil, south-east region): the counties of Juramento, Lavras and São Gotardo. The county of Juramento is situated at 16°50'53'' south latitude and 43°35'13" west longitude, with weather characterized as Aw (rainy tropical, mega-thermal with a dry winter season and a temperature in the coldest month of above 18°C). The county of Lavras is situated at 21°14'43" south latitude and 44°59'59" west longitude with weather characterized as Cwb, mild-seasoned (meso-thermal), rainy with a dry winter season and an average temperature in the coldest month of between 3 and 18°C, and an average temperature in the hottest month of below 22°C. The county of São Gotardo is situated at 19°18'40" south latitude and 46°02"56" west longitude with weather characterized as Cwa, mild-seasoned, different from the weather in Lavras only as regards the average temperature in the hottest month, which is above 22°C (Aspiazú *et al.* 1990). Juramento has the highest annual average temperatures (22.4°C), ranging from 16.7°C to 29.3°C and Lavras the lowest, with an average of 19.4°C (Table 1).

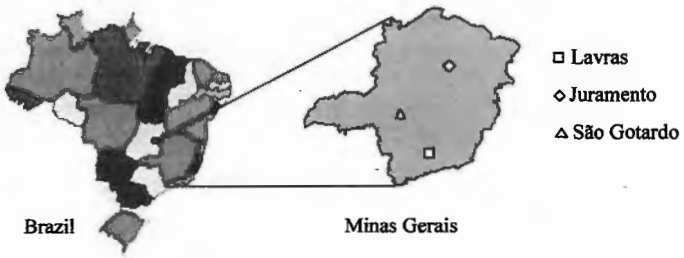

Figure 1. Location of the counties of Lavras, Juramento and São Gotardo in the State of Minas Gerais, Brazil.

Table 1. Annual average temperatures from 1961 to 1990 in the counties of Juramento, Lavras and São Gotardo (source: Agriculture and Agrarian Remodeling Ministry, 1992).

County	Annual average temperature (°C)		
	T_{high}	T_{median}	T_{low}
Juramento	29.3	22.4	16.7
Lavras	26.1	19.4	14.3
São Gotardo	27.8	21.1	16.3

Rearing of A. gossypii and A. colemani

A. gossypii was kept in cucumber plants (*Cucumis sativus* L.), at room temperature. The *A. colemani* parasitoids were reared for three generations over colonies of *A. gossypii* in cucumber plants in a controlled conditions chamber (22±2°C, 70±10 RH and 12 hr

photophase), before being used in the experiment.

Establishment of thermal requirements

A mated, 24-48h old *A. colemani* female was released in a Petri dish (15 cm in diameter) containing 20 nymphs of the 2nd instar of *A. gossypii* in a cucumber foliar disk (4 cm) under a 1% agar-agar layer. After a 1-hour period, the female was taken out and the aphid kept in a controlled conditions chamber until the formation of mummies, which were individualized in glass tubes (10 x 8mm) until the adults' emergence. The aphids were transferred to new Petri dishes containing new foliar disks whenever necessary.

Four temperatures, 16, 19, 22 and 25°C, as well as 70 to 118 individuals from each region were used for each evaluated temperature. For the calculation of the lower temperature threshold for development and the thermal constant, the hyperbole method, according to Campbell et al. (1974) and Haddad & Parra (1984), was used.

Results and discussion

The *A. colemani* lower temperature threshold for development was 4.30, 2.19 and 2.36°C and the thermal constant was 217.39, 238.10 and 238.09 degrees-days for parasitoids from the counties of Juramento, Lavras and São Gotardo, respectively (Table 2). According to Honek & Kocourek (1990), insects from regions where the average temperature is higher should show a higher lower development threshold and a lower thermal constant. This was observed for parasitoids collected in the county of Juramento, which is a climatic region with higher average temperatures (Table 1); however, we should highlight that the lower temperature threshold and the thermal constant of *A. colemani* collected in the counties of Lavras and São Gotardo were quite close to each other, even though they come from different climatic regions.

The detection of different parasitoid populations according to their response to temperature can be performed (Botto et al. 1988). However, more biological studies rather than just thermal requirements may be required (Royer et al. 2001). Thus, more detailed studies of the biology of *A. colemani* from the counties of Lavras and São Gotardo will be able to provide further information on the characteristics of such parasitoids regarding temperature.

Table 2. Lower temperature threshold for development (LDT), thermal constant (K), linear regression of development rate and determination coefficient (r^2) of *Aphidius colemani* from three climatic regions in the state of Minas Gerais, Brazil.

Counties	LDT (°C)	K (degree-days)	Linear regression	r^2
Juramento	4.30	217.39	$y = 0.0046x - 0.0198$	0.8871
Lavras	2.19	238.10	$y = 0.0042x - 0.0092$	0.9253
São Gotardo	2.36	238.09	$y = 0.0042x - 0.0099$	0.8811

The thermal constant and the lower temperature threshold for the development of *A. colemani* changed according to the region where it was collected, and this may show the existence of populations adapted to temperatures higher than 22°C. Studies have shown that the *A. colemani* parasitoid collected from such regions shows a development time of about 12 days and a low mortality rate at 22°C (Sampaio & Bueno, unpublished data). Therefore, *A.*

colemani from the county of Juramento may be regarded as a population which is more tolerant to high temperatures than the populations from the counties of Lavras and São Gotardo. The parasitoid effectiveness may increase in the summer season, or in regions where temperatures are higher than 22°C.

The use of strains adjusted to temperatures higher than the optimum temperatures for development and reproduction is desirable for year-round use, in several regions of Brazil, as a biological control agent in protected cultivation.

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References

Aspiazú, C., Ribeiro, G.A., Vianello, R.L., Ribeiro, J.C., Valente, O.F. & Paula Neto, F. 1990: Análise dos componentes principais aplicada na classificação climática do Estado de Minas Gerais. Teste metodológico. Rev.Árvore 14: 1-15.

Botto, E.N., González, D., Bellows, T. 1988. Effect of temperature on some biological parameters of two populations of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). In: Advances in Parasitic Hymenoptera Research, ed Grupta: 367-377.

Bueno, V.H.P. 1999: Protected cultivation and research on biological control of pests in greenhouses in Brazil. Bull. IOBC/WPRS, 22: 21-24.

Campbell, A, Frazer, B.D., Gilert, N., Gutierrez, A.P. & Mackauer, M. 1974: Temperature requirements of some aphids and their parasites. J. Appl. Ecol. 11: 431-438.

Haddad, M.L. & Parra, J.R.P. 1984: Métodos para estimar as exigências térmicas e os limites de desenvolvimento dos insetos. Piracicaba, FEALQ, 45p.

Honek, A. & Kocourek, F. 1990: Temperature and development time in insects: a general relationship between thermal constants. Zool. Jb. Syst. 117: 401-439.

Ministério da Agricultura e Reforma Agrária, 1992. Normais climatológicas: 1961-1990. Brasília, Mara, 84p.

Royer, T.A., Giles, K.L. Kindler, S.D & Elliott, N.C. 2001: Developmental response of three geographic isolates of *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae) to temperature. Environ. Entomol. 30: 637-641.

Sampaio, M.V. Bueno, V.H.P. & van Lenteren, J.C. 2001: Preferência de *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) por *Myzus persicae* (Sulzer) e *Aphis gossypii* Glover (Hemiptera: Aphididae). Neotrop. Entomol. 30: 655-660.

Starý, P. & Cermeli, M. 1989: Parasitoides (Hymenoptera: Aphidiidae) de áfidos en plantas cultivadas de Venezuela. Bol. Entomol. Venez. 5: 77-80.

Toussidou, M., Williams, M.C. & Leather, S. 1999: Life history parameters of *Aphidius colemani* (Hym.: Aphidiidae) on sweet pepper in different temperature regimes. Bull. IOBC/WPRS, 22: 255-258.

Taylor, F. 1981: Ecology and evolution of physiological time in insects. Am. Nat. 117: 1-23.

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Distribution and diel movement of the predatory mite, *Neoseiulus cucumeris*, on greenhouse sweet pepper – preliminary study

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Abstract: *Neoseiulus cucumeris* is used for biological control of phytophagous mites - such as the broad mite (*Polyphagotarsonemus latus*) - and thrips - such as the western flower thrips (*Frankliniella occidentalis*) and the onion thrips (*Thrips tabaci*) - on greenhouse cucumbers and sweet peppers. The distribution of *N. cucumeris* on cucumbers has been studied, but there is a lack of data relating to sweet peppers. Five hundred *N. cucumeris* were released on sweet peppers and allowed to establish. Samples of flowers and leaves from the top, middle and bottom of plants of each variety and from each tunnel were taken shortly after sunrise (0630), at noon (1200) and shortly before sunset (1630). Significantly more ($P < 0.05$) mites were found on flowers than on leaves sampled from the top, middle and bottom of the plants. Furthermore, significantly fewer ($P < 0.05$) mites were found on leaf and flower samples at mid-day than early in the morning: mite populations on late afternoon samples were not significantly different from morning populations. Broad mites were not found in flowers, but thrips (*F. occidentalis*) were. The highest percentages of *N. cucumeris* were also associated with flowers.

Key words: *Neoseiulus cucumeris*, predatory mite distribution

Introduction

Neoseiulus (= *Amblyseius*) *cucumeris* (Oudemans) is a polyphagous predatory mite, which feeds on a number of mites and insects and on pollen (McMurtry & Croft, 1997). It has been successfully used in greenhouses to control mites, such as *Polyphagotarsonemus latus* (Banks) (Weintraub et al., 2003), and thrips, such as *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindeman (Brodsgaard & Hansen 1992; Gillespie & Quiring 1992; Ramakers 1988). Studies have shown that some *Neoseiulus* species are sensitive to low humidity levels in open fields and orchards (Bakker et al. 1993; Croft et al. 1993), but because of the hairs, trichomes, domatia and other leaf depressions, the microclimate on the surface of greenhouse plants is favorable for their survival.

In a previous study we examined the efficacy of *N. cucumeris* in controlling the broad mite (*P. latus*) on sweet peppers by sampling apical leaves, but found very few *N. cucumeris* (Weintraub et al., 2003). We wanted to determine the distribution of predatory mites on the plant, and in this study we report on the diel movement of *N. cucumeris* in sweet peppers.

Materials and Methods

All trials took place at the Yair Research and Development Farm in the Arava Valley (between the Dead Sea and Red Sea), Israel. Plants were fertilized and watered by a drip irrigation system, and *Orius laevigatus* Fieber were released for thrips control, according to standard agricultural practices for the area. It was not necessary to treat for any other pests. Four walk-in tunnels (7 x 15 m) were planted with two varieties of organically grown sweet

pepper seedlings: 'Nibla' (yellow fruit) and 'Parker' (red fruit) in three double-row beds on 2 September 2002. Ninety 'Nibla' seedlings were planted in the northern half of each tunnel and 90 'Parker' seedlings in the southern half.

N. cucumeris were obtained from Bio-Bee Biological Systems, Kibbutz S'deh Eliyahu, Israel. The predators were reared and packaged for release in wheat bran containing the storage mite, *Tyrophagus putrescentiae* (Schrank), as prey. Approximately 500 *N. cucumeris* were released on the top leaves of each plant on 26 September and allowed to establish. Samples of flowers and leaves were taken from the top, middle and bottom of plants of each variety and from each tunnel, shortly after sunrise (0630), at noon (1200) and shortly before sunset (1630) on 15 October, 29 October and 5 November. Sampled leaves and flowers were placed in jars containing 80% EtOH. In the laboratory, the leaves were removed and the contents of each container were examined for the presence of mites under a dissecting microscope at 25x. Additionally, HOBO data loggers (Onset Computer Corporation, Cape Cod, Massachusetts) were placed in the top, middle and bottom of the canopy of the plants in one tunnel and set to record temperature and relative humidity every 20 minutes from 5 – 10 November.

Data were analyzed using CoStat Statistical Software (Minneapolis, MN, U.S.A.). Analysis of replicates was carried out by completely randomized 1-Way ANOVA. Means were separated by Tukey-Kramer at $\alpha = 0.05$.

Results

Two-way ANOVA (main effects: time of day, height of leaf/flower sample) showed that, for both varieties, significantly fewer mites were sampled at mid-day (at all heights) than in morning ($P < 0.05$). For 'Parker' (for all time periods) significantly more *N. cucumeris* were found in flowers than on leaves ($P < 0.05$) (Table 1). However, the number of mites found in 'Nibla' flowers (Table 2, for all time periods) was significantly greater than on leaves at the top or bottom - although not in the middle - of the plant.

F. occidentalis thrips were only found in flower samples (Tables 1 and 2) except on one occasion where one thrips was found on a middle Parker leaf in a morning sample. One-way ANOVA showed that there was no significant difference between the three sampling times in terms of the number of thrips found in flowers ('Nibla' $F = 0.87$, $P = 0.42$; 'Parker' $F = 1.84$, $P = 0.16$).

Discussion

N. cucumeris were released in a wheat bran mixture that contained the mite *T. putrescentiae* as a food source. As this loose bran is not contained in a sachet that maintains its humidity, it typically dries to the extent that virtually all *T. putrescentiae* die within about a week; this is, however, sufficient time for *N. cucumeris* to disseminate and become established.

The distribution of *N. cucumeris* within plants has mainly been studied on greenhouse cucumber. Steiner (1990) found no significant difference between mite numbers recovered from apical, middle and basal leaves, in spite of the fact that the larval thrips that *N. cucumeris* prey upon – *F. occidentalis* - were more abundant in the middle leaves. Gillespie (1989) found the highest mite populations on the uppermost leaves, which coincided with *T. tabaci* populations. Higgins (1992) sampled the middle leaves and flowers of greenhouse cucumbers and peppers for *F. occidentalis* and *N. cucumeris*. She found higher percentages of thrips in cucumber flowers than in pepper flowers, as opposed to leaves but the majority of *N. cucumeris* were on the leaves of both cucumber and pepper. In field-grown eggplant,

Castineiras et al. (1997) found that *T. palmi* Karny was most abundant on leaves (as opposed to fruit or flowers), but that *N. cucumeris* was most abundant on fruit and was not found in flowers at all. Furthermore, percentages of both predator and prey were lowest on the youngest leaves and highest on the oldest. In none of these reports was there any indication as to the times of day at which samples were taken.

It is well known that the broad mite, *P. latus*, attacks young growing plant parts and that very few individuals cause severe damage to peppers (de Coss-Romero & Pena, 1998). In previous trials (Weintraub et al., 2003), we observed excellent control of the broad mite by *N. cucumeris*, but very few predatory mites were observed in apical leaf samples. The results presented here clearly demonstrate that *N. cucumeris* moves within the plant over the course of a day; at the hottest, driest period (mid-day) they move to the stems or to the ground, but are reduced in numbers on leaves and flowers. It has been shown that two related species, *N. californicus* (McGregor) (Fauvel et al., 1993) and *N. fallacies* (Garman) (Croft & McGroarty, 1977), move across the ground (with or without ground cover) and climb trees to seek prey. Although we did not observe this, this is a plausible explanation for the population decline at midday; mites could have moved to the ground, which would have been cooler and also moist due to the drip irrigation system.

While broad mites were not found in flowers, thrips (*F. occidentalis*) were, as were the greatest percentages of *N. cucumeris*. *O. laevigatus* were released for thrips control, as is standard practice in organic agriculture in the area. They are known predators of *N. cucumeris* (Wittmann & Leather 1997), although these are not the preferred prey when thrips are present, they may have accounted for the low numbers of predatory mites sampled.

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References

Bakker, F., Klein, M.M.E,. Esa, N. C. & Braun, A. R. 1993: Saturation deficit tolerance spectra of phytophagous mites and their phytoseiid predators on cassava. Exp & Appl Acarol. 17: 97-113.

Brodsgaard, H.F. & Hansen, L.S. 1992: Effect of *Amblyseius cucumeris* and *Amblyseius barkeri* as biological control agents of *Thrips tabaci* on glasshouse cucumbers. Biocont. Sci. Tech. 2: 215-223.

Castineiras, A., Baranowski, R.M. & Glenn, H. 1997: Distribution of *Neoseiulus cucumeris* (Acarina: Phytoseiidae) and its prey, *Thrips palmi* (Thysanoptera: Thripidae) within eggplants in south Florida. Florida Entomol. 80: 211-217.

Croft, B.A. & McGroarty, D.L.1977: The role of *Amblyseius fallacies* (Acarina: Phytoseiidea), in Michigan apple orchards. MI St. Univ. Agric. Exp. Sta. Res. Rep. 333.

Croft, B.A., Messing, R.H., Dunley, J.E. & Strong, W.B. 1993: Effects of humidity on eggs and immatures of *Neoseiulus fallacies*, *Amblyseius andersoni*, *Metaseiulus occidentalis* and *Typhodromus pyri* (Phytoseiidae); implications of biological control on apple, caneberry, strawberry and hop. Exp & Appl Acarol. 17: 451-459.

de Coss-Romero, M. & Pena, J.E. 1998: Relationship of broad mite (Acari: Tarsonemidae) to host phenology and injury levels in *Capsicum annuum*. Florida Entomol. 81: 525-526.

Fauvel, G., Bourgoin, B., Perron, G. & Rouzet, J. 1993: Importance de la colonization des vergers de pommier et pêcher du sud de la France par *Neoseiulus californicus* (McGregor) et conséquences pour la lutte biologique contre l'araignée rouge *Panonychus ulmi* Koch (Acari: Phytoseiidae, Tetranychidae). Ann. Assoc. Nat'l. Plant Prot. 2-3: 587-596.

Gillespie, D.R. & Quiring, D.J.M. 1992: Competition between *Orius tristicolor* (White) (Hemiptera: Anthocoridae) and *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) feeding on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Canad. Entomol. 124: 1123-1128.

Gillespie, D.R. 1989: Biological control of thrips (Thysanoptera: Thripidae) on greenhouse cucumber by *Amblyseius cucumeris*. Entomophaga, 34: 185-192.

Higgins, C.J. 1992. Western flower thrips (Thysanoptera: Thripidae) in greenhouses: population dynamics, distribution on plants, and associations with predators. J. Econ. Entomol. 85: 1891-1903.

McMurtry, J.A. & Croft, B.A. 1997: Life-styles of phytoseiid mites and their roles in biological control. Ann. Rev. Entomol. 42: 291-321.

Ramakers, P.M.J. 1988: Population dynamics of the thrips predators *Amblyseius mckenziei* and *Amblyseius cucumeris* (Acarina: Phytoseiidae) on sweet pepper. Netherl. J. Agric. Sci. 36: 247-363.

Steiner, M.Y. 1990: Determining population characteristics and sampling procedures for the western flower thrips (Thysanoptera: Thripidae) and predatory mite *Amblyseius cucumeris* (Acari: Phytoseiidae) on greenhouse cucumber. Environ. Entomol. 19: 1605-1613.

Weintraub, P.G., Kleitman, S., Mori, R., Shapira, N. & Palevsky, E. 2003: Control of broad mites (*Polyphagotarsonemus latus* (Banks)) on organic greenhouse sweet peppers (*Capsicum annuum* L.) with the predatory mite, *Neoseiulus cucumeris* (Oudemans). Biol. Cont. 26: 300-309.

Whittmann, E.J. & Leather, S.R. 1997: Compatibility of *Orius laevigatus* Fieber (Hemiptera: Anthocoridae) with *Neoseiulus* (*Amblyseius*) *cucumeris* Oudemans (Acari: Phytoseiidae) and *Iphiseius* (*Amblyseius*) *degenerans* Berlese (Acari: Phytoseiidae) in the biocontrol of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). Exp & Appl Acarol. 21: 523-538.

Table 1. Average number (± S.E.) of *Neoseiulus cucumeris* and *Frankliniella occidentalis* from the top, middle and bottom leaves and flowers (located in the middle of the plant) of 'Parker' sweet peppers at different times during the day on 3 sampling dates. Temperature (°C ± S.E.) and relative humidity (± S.E.) were recorded for the top, middle and bottom leaves from 5 – 10 November. Different letters within a column indicate statistical significance at $P < 0.05$.

	Neoseiulus cucumeris				*Frankliniella occidentalis*
	Top	Middle	Bottom	Flowers	Flowers
0630					
Number	42	42	42	42	180
Average	0.07±0.05b	0.17±0.06b	0.19±0.61b	0.33±0.04a	0.12±0.02
°C	17.8±1.1	18.2±0.9	19.0±0.8		
%R.H.	77.1±6.7	87.5±4.6	*		
1200					
Number	66	66	66	66	240
Average	0.06±0.03a	0.11±0.04a	0.10±0.04a	0.17±0.02a	0.11±0.02
°C	30.7±1.0	25.6±0.6	26.6±0.7		
%R.H.	48.6±4.3	63.5±3.6	*		
1630					
Number	66	66	66	66	240
Average	0.04±0.02b	0.21±0.05a	0.17±0.05b	0.23±0.03a	0.07±0.02
°C	27.8±0.8	26.2±0.3	26.1±0.4		
%R.H.	42.5±2.8	54.6±3.8	*		

*HOBO malfunction, no data recorded

Table 2. Average number (± S.E.) of *Neoseiulus cucumeris* and *Frankliniella occidentalis* from the top, middle and bottom leaves and flowers (located in the middle of the plant) of 'Nibla' sweet peppers at different times during the day on 3 sampling dates. Temperature (°C ± S.E.) and relative humidity (± S.E.) were recorded for the top, middle and bottom leaves from 5 – 10 November. Different letters within a column indicate statistical significance at P < 0.05.

	Neoseiulus cucumeris				*Frankliniella occidentalis*
	Top	Middle	Bottom	Flowers	Flowers
06:30					
Number	42	42	42	180	180
Average	0.10±0.05b	0.21±0.09a	0.09±0.06b	0.29±0.04a	0.16±0.03
°C	17.8±1.1	18.2±0.9	19.0±0.8		
%R.H.	77.1±6.7	87.5±4.6	*		
12:00					
Number	66	66	66	240	240
Average	0.11±0.06b	0.11±0.04a	0.06±0.04b	0.21±0.03a	0.13±0.02
°C	30.7±1.0	25.6±0.6	26.6±0.7		
%R.H.	48.6±4.3	63.5±3.6	*		
16:30					
Number	66	66	66	240	240
Average	0.05±0.03b	0.13±0.04a	0.06±0.03b	0.19±0.03a	0.12±0.02
°C	27.8±0.8	26.2±0.3	26.1±0.4		
%R.H.	42.5±2.8	54.6±3.8	*		

*HOBO malfunction, no data recorded

Section IV. Plant diseases.

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Impact of ultraviolet-absorbing plastic films on insect vectors of virus diseases infecting crisp lettuce

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Abstract: Crisp lettuce is widely grown in northeastern Spain under tunnel-type greenhouses. Insect pests are a major threat to lettuce, and insecticides are widely used to control aphids, thrips, cutworms and other lepidopteran pests. UV-absorbing plastic films have proved useful in controlling insect vectors and associated virus diseases in a number of horticultural crops. A 2-year study was conducted in the area of Sartaguda (Navarra, Spain) to evaluate the impact of a UV-photoselective film on the population density of insect pests and the spread of virus diseases infecting 'Batavia type' lettuce. Two walk-in tunnel greenhouses (40 m x 9 m) divided into 4-6 sectors were used for the study: one was covered with a UV-absorbing film called Ginegar AD-IR AV clear and the other with a standard plastic film (Astrolux type). Insect population dynamics were monitored weekly by means of yellow and blue sticky traps located in each of the sectors. In addition, lettuce plants were sampled (15 plants/sector) to determine the presence of insect pests at different time intervals. Plants were periodically inspected to assess virus spread in both greenhouses. Plants showing virus symptoms were sampled to identify the viruses present by means of ELISA and using specific antibodies. In both years, the UV-absorbing plastic film was able to significantly ($P<0.05$) reduce the percentage of lettuce plants infested with aphids and thrips and also the spread of aphid and thrips-transmitted virus diseases (Potyvirus and *Tomato spotted wilt virus*, TSWV). The number of winged aphids caught in yellow sticky traps in the two-year study was dramatically reduced in the greenhouse covered with UV-absorbing films. In the autumn-sown 2002 crop, 80% of lettuce plants grown under the Astrolux-covered greenhouse were infested by >5 aphids (*Macrosiphum euphorbiae* and *Acyrthosiphon lactucae*), while only 10% were infested under the UV-absorbing film. Virus spread was also significantly reduced for both cropping seasons under the greenhouse protected by UV-absorbing film. In 2002, the total incidence of Potyviruses and TSWV-infected lettuce plants was reduced by 78% and 69%, respectively, in the greenhouse protected with UV-absorbing plastic film. These results suggest that UV-absorbing films are a very good alternative to insecticide sprays and that they effectively protect lettuce crops from insect pests and insect-borne virus diseases.

Key words: pests, virus, UV-absorbing film, photoselective barriers, aphids, thrips, TSWV, Potyvirus

Introduction

Lettuce is often grown under plastic tunnel greenhouses during the spring and autumn seasons in north-eastern Spain. Although most plastic films contain UV-absorbing elements to extend the life of the material, only a few are able to block the transmission of UV light at wavelengths below 380 nm. The latter interfere with insect vision and orientation leading to significant reductions in population density and the spread of insect pests such as aphids and thrips (Costa et al., 2002). Previous works also show a significant reduction in population density and damage caused by whiteflies (*Bemisia tabaci* Gennadius), leafminers (*Liriomyza trifolii* Burgess) and moths (*Laphigma* sp.) under greenhouses protected by UV-absorbing films (Antignus et al., 1996; Antignus, 2000).

Another positive aspect of the use of UV-absorbing films is the effective control of insect-transmitted virus diseases. A clear reduction was observed in the spread of *Bemisia*-transmitted viruses such as *Tomato yellow leaf curl virus* (TYLCV) and *Cucurbit yellow stunting disorder virus* (CYSDV). This significant decrease has been attributed to the impairment of the ability of whiteflies to fly and disperse in the absence of UV-light (Antignus, 2000; Antignus et al., 2001).

Most of the information relating to the effects of UV-light absorbing plastic films on insect populations and virus spread has focussed on tomato and cucurbit crops. To our knowledge, there have been no reports of the impact of UV-absorbing plastics on insect pests and virus diseases commonly associated with lettuce crops grown in greenhouse environments. Our objective was to study the population dynamics of insect pests and the spread of insect-transmitted viruses on a lettuce crop grown in a greenhouse covered by a UV-light absorbing film in the Navarra region (Spain).

Material and methods

Cropping Practices

A 2-year field experiment was conducted to evaluate the impact of UV-absorbing plastic films on the population dynamics of insect pests associated with lettuce grown under greenhouse conditions at Sartaguda (Navarra, Spain). A 'Batavia' type crisp lettuce crop was grown in two different seasons: September 9th to November 7th, 2002 and March 28th to May 13th, 2003.

Two different commercial greenhouses (tunnel type, 40 m x 9 m) were used for each growing season: one was covered with a standard polyethylene plastic film (Astrolux), and the other was covered with a UV-photoselective plastic called Ginegar AD-IR AV clear (supplied by Ginegar Plastic Products Ltd.). The latter had similar properties to the Astrolux film: the only difference was the UV-absorbing additive. The two greenhouses had the same orientation and were 50 m apart. They were provided with openings on each side of the tunnel and on the roof to facilitate ventilation. These openings allowed unfiltered light to enter part of the greenhouse structure. The doors were occasionally covered with nets to prevent wind damage to lettuce plants. Each greenhouse was divided into 4-6 sectors (replicates) for sampling purposes. A black plastic mulch was used for weed control and to increase soil temperature.

Spraying was necessary at the beginning of the experiment conducted in autumn 2002 in order to control cutworms (*Agrotis* sp.) and other lepidopteran pests (e.g. *Autographa gamma*). Selective baits and two *Bacillus thuringiensis* sprays were used to control these pests and prevent any detrimental effects to insect vectors of plant diseases. No pesticides were needed in the experiment conducted in spring 2003.

Sampling Methods

Insect population dynamics were monitored in three different ways:

a) Yellow and blue sticky traps were placed in each sector of the greenhouse (4 and 6 replicates were respectively used in the 2002 and 2003 experiments). Plates were replaced every week after plant transplant and the number of aphids, thrips, whiteflies and leafhoppers were counted.

b) A horizontal green-tile trap was placed in the middle of each greenhouse to monitor landing rates of virus vectors (aphids, thrips, whiteflies and leafhoppers).

c) Fifteen plants per sector were inspected every two weeks and also at harvest time to determine the presence/absence of insect pests.

Virus incidence was monitored at different time intervals by inspecting all plants within each greenhouse sector and taking leaf samples of plants showing symptoms of virus disease. Identification of viruses was conducted by ELISA using specific antibodies: *Lettuce mosaic virus* (LMV), *Broad bean wilt virus* (BBWV), *Alfalfa mosaic virus* (AMV), *Beet western yellow virus* (BWYV), *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus* (TSWV) and a general anti-Potyvirus monoclonal antibody. These specific antibodies were selected because the mentioned viruses were the ones most frequently found in a 3-year survey of the major lettuce-producing areas of Spain (Fereres et al., unpublished) previously conducted by our research group.

Results and discussion

Autumn, 2002 assay

The most abundant insect pests detected on lettuce plants were aphids (*Macrosiphum euphorbiae* Thomas and *Acyrthosiphon lactucae* Passerini) and then thrips (*Frankliniella occidentalis* Pergande). Fewer winged aphids were trapped in the yellow plates located under the UV-absorbing film than under the Astrolux control film (overall mean ± SE = 0.7 ± 0.2 vs. 2.4 ± 0.5; F= 8.275; P= 0.006; Figure 1).

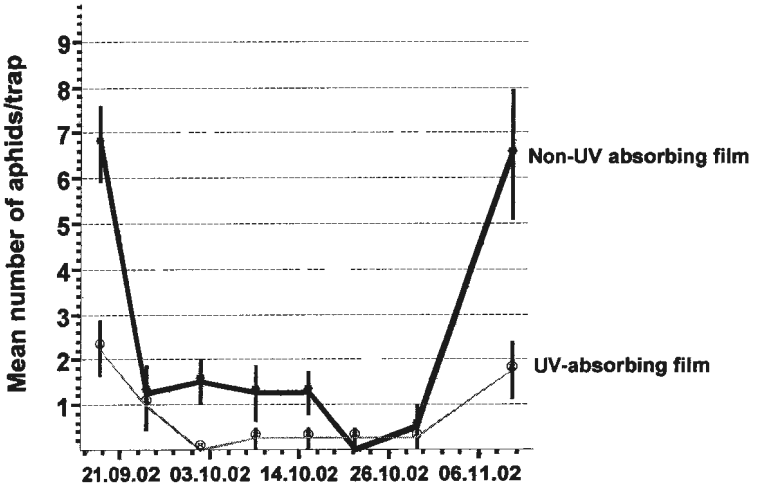

Figure 1. Mean number of aphids per trap and per week caught on yellow sticky plates located in a greenhouse covered with either UV-absorbing film or non-UV absorbing film

No significant differences were observed between the number of whiteflies (*Trialeurodes vaporariourum* Westwood) caught on yellow plates located in the greenhouse covered with UV-absorbing film and the one covered by Astrolux film (overall mean ± SE = 5.7 ± 0.6 vs. 5.4 ± 0.6; F= 0.648; P= 0.424). The number of insects caught in blue sticky traps and by the horizontal green-tile trap was too low to make any statistical comparisons. At harvest, the UV-absorbing plastic film was able to significantly (P<0.05) reduce the number of lettuce

plants infested with aphids and thrips. The percentage of plants infested with at least one aphid was 97.5% in the case of the greenhouse covered with Astrolux film. Only 10% of the lettuce plants grown under UV-absorbing film were infested with 1 or more aphids. Plants with 5 or more aphids were considered unmarketable. A significantly greater percentage of marketable plants were found under the UV-absorbing film than under the Astrolux film (80 ± 11.5 vs. 10.0 ± 7.1; F= 17.121; P= 0.006). The type of plastic film used did not affect the size and weight of the lettuce plants.

Virus incidence was first assessed 10 days after transplant (September 19th). Only a few ELISA-positive samples were detected when using all the commercial antibodies previously mentioned. The only 2 viruses detected in this initial survey were Potyviruses and TSWV: no differences between the two greenhouses were noted in terms of virus incidence at this first sampling date. Further sampling was conducted looking for symptomatic plants after inspection of all the plants present in each greenhouse. One month after the first sampling date (October 22nd), there were clear differences between the number of symptomatic plants under the UV-absorbing and Astrolux film (Table 1). At harvest, differences in virus incidence under each type of film were even more evident. The UV-absorbing film respectively produced 78% and 69% reductions in the total incidence of Potyviruses and TSWV. Most of the Potyvirus-infected plants were infected with LMV. Positive infection of symptomatic plants was confirmed by ELISA.

Table 1. Virus incidence[1] in lettuce grown under UV-absorbing and non-UV absorbing (control) films

Date	TSWV		Potyviruses	
	UV film	Non-UV film	UV film	Non-UV film
9-09-02	4	1	11	6
22-10-02	7	19	7	25
7-11-02	1	19	3	64
Total	12	39	21	95

[1] Total number of plants showing clear symptoms of virus disease. Some of the plants showing symptoms of virus disease died a few days later.

Spring 2003 Assay

For this assay both greenhouses were divided into 6 sectors instead of the 4 used for the autumn assay. Results on aphid numbers trapped on yellow sticky plates were very similar to those obtained for the autumn assay. The number of winged aphids trapped under UV-absorbing film was significantly lower than under the non-UV-absorbing film (overall mean ± SE = 0.6 ± 0.1 vs. 3.8 ± 0.8; F= 13.590; P= 0.001). There were no significant differences between the number of thrips caught under the two types of plastic film.

The most abundant insect pests detected on lettuce plants were aphids (*M. euphorbiae* and *A. lactucae*) and lepidoptera larvae identified as *Autographa gamma* L., which reached damaging levels close to harvest time. A significantly lower percentage of plants were infested by aphids in the greenhouse covered with UV-absorbing films at harvest time (8.9% under UV-absorbing film versus 24.4 % infested under the non-UV-absorbing plastic film). Table 2 shows clear differences in the number of plants infested with *A. gamma* under the two types of plastic film used in the study.

Table 2. Incidence of *Autographa gamma* under UV-absorbing and non-UV absorbing (control) film at harvest time

	Film type			
	UV film	Non-UV film	F	P
Percentage of plants infested with one or more larvae[a]	17.8 ± 4.1	37.8 ± 5.6	8.633	0.015
Mean no. of larvae/plant[b]	0.19 ± 0.04	0.42 ± 0.06	9.496	0.002

[a] Data was transformed by arcsin$\sqrt{x}$ before analysis

[b] Data was transformed by log (x+1) before analysis

Total virus incidence was much lower in the spring than in the autumn experiment. This was probably because insect vectors entered the greenhouse late in the growing season due to low temperatures and/or because of the absence of virus reservoirs after the winter. Nevertheless, the results were similar to those for autumn, and virus symptoms appeared much less frequently in lettuce plants grown under the UV-absorbing film.

In conclusion, the UV-absorbing films tested effectively reduced aphid abundance and the spread of winged forms inside the greenhouse and protected lettuce plants from aphid infestation.These films also proved very useful in reducing the spread of aphid and thrip-transmitted virus diseases infecting lettuce. No effects were observed on whitefly abundance (and/or spread), although population densities were not as high as those observed for aphids and thrips. Our results are consistent with those reported by Costa et al. (2002), who found no reduction in the population of *T. vaporariorum* in commercial greenhouses covered with UV-absorbing films.

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References

Antignus, Y., Mor, N., Joseph, R. B., Lapidot, M. & Cohen, S. 1996: Ultraviolet-absorbing plastic sheets protect crops from insects pests and from virus diseases vectored by insects. Environ. Entomol. 25: 919-924.

Antignus, Y. 2000: Manipulation of wavelength dependent behaviour of insects an IPM tool to impede insects and insect-borne virus. Virus Res. 71: 213-220.

Antignus et al., 2001: Ultraviolet-deficient greenhouse environment affects whitefly attraction and flight-behavior. Environ. Entomol. 30: 394-399.

Costa, H. S., Robb, K. L. & Wilen, C. A. 2002: Field trials measuring the effects of ultraviolet-absorbing greenhouse plastic films on insect populations. J. Econ. Entomol. 95: 113-120.

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Physical and genetic control of *Bemisia tabaci*-transmitted *Cucurbit yellow stunting disorder virus* and *Cucumber vein yellowing virus* in cucumber.

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Abstract: *Bemisia tabaci*-transmitted CYSDV and CVYV are limiting factors in cucumber crop production in greenhouses in the southeast of Spain. From 2002 to 2003, the incidence of disease caused by these viruses was compared in virus-resistant and non-resistant cucumber varieties grown in traditional 'Almeria'-type greenhouses and also in similar greenhouses with an indoor structure of continuous nets and double doors. In the latter greenhouses, natural infestation of *B. tabaci* was greatly reduced with respect to greenhouses lacking such physical-protection measures. Furthermore, the incidence of disease caused by CYSDV and CVYV, and the consequent cucumber yield losses, were insignificant when crops were grown in the adapted greenhouses. Under these conditions, commercial cucumber varieties with proclaimed resistance to CYSDV and/or CVYV did not produce higher yields than a non-resistant variety.

Key words: cucumber, greenhouse, whiteflies, CYSDV, CVYV, control

Introduction

In recent decades, plastic-covered greenhouses have been increasingly used to produce horticultural crops in the Mediterranean area (Castilla & Jarrett, 1995). However, these greenhouses have also proven favourable ecological niches for various species of whiteflies (Homoptera: Aleyrodidae) and in particular the sweetpotato whitefly *Bemisia tabaci* (Gennadius). The Almeria region, in southeast Spain, is important in this respect, as greenhouses cover about 25,000 ha, almost 40 % of which are dedicated to cucurbitaceous crops (Anonym, 2001). Cucumber (*Cucumis sativus* L) is produced predominantly for export the whole year around, in contrast to what happens in regions with a moderate climate.

B. tabaci has been described in Spain since 1943 and became a greenhouse pest in 1988 (Ruiz *et al.*, 1999). This whitefly has proved highly resistant to the majority of phytosanitary products-compounds (Elbert & Nauen, 2000), which has jeopardized its control. The main problem of *B. tabaci* in intensive greenhouse horticulture is associated with its role as a vector for many virus-borne diseases. Such diseases as *Cucurbit yellow stunting disorder virus* (CYSDV) (Célix *et al.*, 1996) have been reported in cucurbita crops since the last decade, while *Cucumber vein yellowing virus* (CVYV) has been introduced more recently (Cuadrado *et al.*, 2001). The two viruses produce diseases that have become the main limiting factors in cucumber production in south-eastern Spain (Janssen & Cuadrado, 2001).

Effectively controlling these diseases would imply various types of action, including the use of virus-tolerant cultivars (Lopez-Sese & Gomez-Guillamon, 2000) and the improvement of physical barriers. At present, farmers have commercial cucumber varieties with acclaimed resistance to CYSDV and CVYV. However, the present paper shows that in the absence of

insecticide applications, physical protection measures adapted to traditional greenhouses can help produce higher cucumber yields than control strategies that only consider the use of virus-tolerant varieties.

Material and methods

Cucumber varieties

Depending on the crop season, one or more of ten commercial cucumber (*C. sativus*) varieties were grown. According to the seed providers, four of these varieties were resistant to CVYV and five to CYSDV/CVYV. Cucumber cv. Marianna RZ, which had no documented tolerance to either CVYV or CYSDV, was used as a virus-sensitive control. The crops were cultivated at a mean density of 1.5 plants per m^{-2}, and according to the usual practices of the region. No insecticides were applied during the cultivation periods.

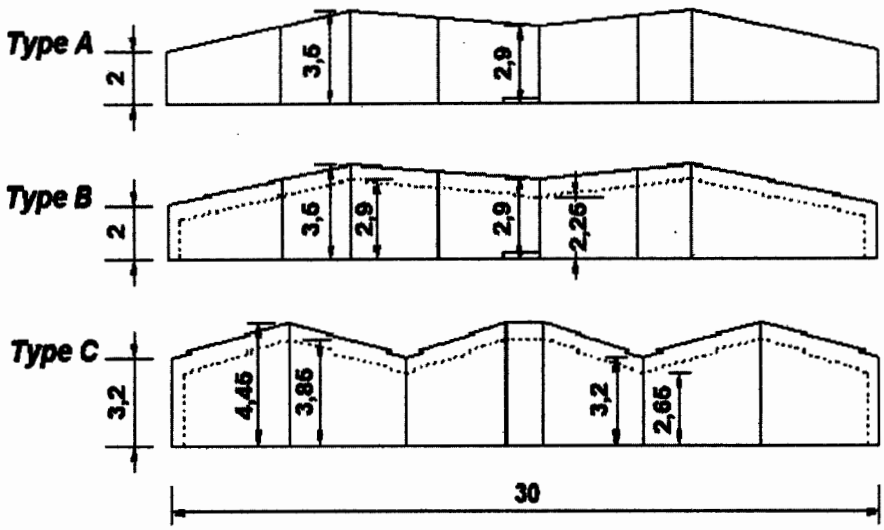

Figure 1. Profile views of the greenhouses. Broken lines represent the internal netting structure. Numbers are measurements in meter.

Greenhouse adaptations

The crops were grown in three greenhouses located at an experimental farm at CIFA, Almeria. The greenhouses all spanned 3,000 m^2 (30 x 100 m) and had the same orientation, but they had different structural features (Figure 1). The first greenhouse (Type A) was a traditional symmetric multi-chapel ('Almería'-type), with a mean height of 2.80 m, wooden sticks, a conventional polyethylene film covering the roof and sides, and nets covering the side windows, and zenital ventilation. The second greenhouse (type B) had identical features to the first, but contained thripsnets (10x20 mesh) continuously mounted on metallic wirings, which formed an internal greenhouse with a mean height of 2.40 m within the main 'Almería'-type structure. The entrance to the second greenhouse incorporated a corridor and a double door. The third greenhouse (Type C) was a modern symmetric multi-chapel, with

metal sticks and an average height of 3.82 m. There was also a corridor inside the greenhouse, a double door and a continuously mounted internal thripsnet structure with a mean height of 3.25 m.

Data acquisition

Cucumber crops were grown in the following periods and greenhouses: spring 2002 (21st March to 25th June) in Types A and B; autumn 2002 (18th October to 10th January 2003) in Types A, B and C; spring 2003 (26th March to 13th June) in Types A and C. Each week, 24 plants of each variety were randomly selected from each greenhouse and the number of adult whitefly on the undersides of the ten uppermost leaves were counted. From all plants (approximately 140 per variety), the symptoms on leaves were annotated. Disease caused by CYSDV was recognized by symptoms that generally initiate on the lower (older) parts of plants: mottled patches that develop to intervenal yellowing where veins remain green (Célix *et al.*, 1996). CVYV infection was detected as vein yellowing symptoms in young shoots (Cohen & Nitzany, 1960). Attribution of symptoms to a particular virus was confirmed by reverse transcriptase polymerase chain reaction assays, as previously described (Ruiz *et al.*, 1999; Cuadrado *et al.*, 2001). Cucumber fruits were collected and weighted twice a week. For data representation and comparison purposes, the number of plants showing disease symptoms caused by virus was presented as a percentage of the total number of diseased plants.

Results and discussion

Chemical control of whiteflies and other greenhouse pests was the method of choice for a long time. However, this type of control has not only proved inefficient, but has also defies the concerns of environmental and health issues of both consumer and producer. Therefore, alternative methods of pest control, such as integrated pest management, are currently applied. In the southeast of Spain, the use of nets fitted to lateral and zenithal greenhouse windows is an essential mechanical control method, creating a physical barrier to prevent pest penetration of greenhouses (Teitel *et al.*, 1999). Although the application of such nets can be a very efficient way to prevent primary pest penetration, some pests still manage to enter, so complimentary control measures, such as the use of crop varieties resistant to pest-borne diseases, remain relevant (Berlinger *et al.* 1991).

In the present paper we investigated whether the physical protection of cucumber crops and the use of varieties resistant to CVYV and/or CYSDV were able to reduce levels of whitefly infestation and the reduction in yields due to *B. tabaci*-borne CYSDV and CVYV, in the absence of insecticide applications.

Cucumber plants grown in the traditional greenhouse (Type A) were readily infested with whitefly. The mean number of *B. tabaci* adults per plant was 10 in autumn 2002, and as high as 30 and 45, respectively, in the crop seasons of spring 2002 and 2003. In contrast, natural *B. tabaci*-infestation was greatly reduced in the greenhouses with indoor structures consisting of nets that covered the entire roof and lateral areas, and that were also fitted with a double door system (Types B and C). Infestation rates varied from 0.2 to 2 adults per plant, according to the greenhouse and the crop season (Table 1) in question. As for whitefly infestation, no significant differences were observed between the different cucumber varieties (results not shown).

Table 1. Mean numbers of *B. tabaci* adults counted per cucumber plant grown in 2002-2003 in a standard greenhouse (A) and in adapted greenhouses (B and C).

		Greenhouse Type		
		A	B	C
Crop season	Spring 2002	45.0	2.0	n.d.[a]
	Autumn 2002	10.0	0.4	0.2
	Spring 2003	30.0	n.d.	0.8

[a] not done

Table 2. Accumulated percentages of cucumber plants with CYSDV and CVYV disease, and total yields obtained in virus-resistant and non-resistant varieties, grown in 2002-2003 in the standard greenhouse (A) and adapted greenhouses (B and C).

Crop season			Spring 2002	Autumn 2002							Spring 2003			
Variety			#1	#1	#2	#3	#4	#5	#6	#7	#1	#8	#9	#10
Resistance[a]			none	none	V	V	V	V, Y	V, Y	V	none	V, Y	V, Y	V,Y
Greenhouse Type	A	CVYV (%)	98	100	98	95	98	98	94	92	100	98	93	100
		CYSDV (%)	99	99	99	96	99	99	76	99	100	100	100	100
		Yield ($kg.m^{-2}$)	6.1	3	4.2	5.3	5.1	5.3	6	4.8	4.4	5.4	5	5.2
	B	CVYV (%)	9	1	2	2	0	1	1	2	n.d.[b]	n.d.	n.d.	n.d.
		CYSDV (%)	10	7	14	16	13	11	3	17	n.d.	n.d.	n.d.	n.d.
		Yield ($kg.m^{-2}$)	12.1	5.4	5.5	6	5.8	6.2	6.1	5.5	n.d.	n.d.	n.d.	n.d.
	C	CVYV (%)	n.d.	1	1	0	1	1	0	1	11	1	0	5
		CYSDV (%)	n.d.	3	8	6	9	5	5	5	2	8	7	3
		Yield ($kg.m^{-2}$)	n.d.	6.9	6.4	6.8	6.9	7.1	7.3	6.8	12.2	11.8	12.4	12.6

[a] as declared by the seed company; V = CVYV; Y = CYSDV

[b] not done

By the end of each crop season, up to 100% of the plants of cucumber varieties grown in the Type A greenhouse showed signs of disease caused by CVYV and CYSDV. Only one variety, with proclaimed resistance to both viruses, showed moderate tolerance to CYSDV, with a maximum infection level of 76% of total plants. The percentage of plants displaying virus disease varied between 0 and 11% for CVYV and between 2 and 17% for CYSDV, when plants were grown in Type B and C greenhouses. The number of plants showing virus symptoms correlated inversely with the yields obtained, which varied between 3.0 and 6.1 $kg.m^{-2}$ when grown in the Type A greenhouse, and between 5.4 and 12.6 $kg.m^{-2}$ when grown in the Type B and C greenhouses. In the productive region, cucumber yields of about 12 $kg.m^{-2}$ were close to those expected for this type of crop in the absence of virus infection. These were generally obtained in the spring crop season in Type B and C greenhouses, and were twice as high as the production level obtained in the Type A greenhouse. Under

conditions of high whitefly incidence, moderately higher yields were obtained from resistant varieties as opposed to non-resistant ones. However, in physically protected greenhouses, no significantly different yields were obtained for these varieties (Table 2).

At present, farmers have commercially available cucumber varieties that are proclaimed resistant, or tolerant, to CVYV and/or CYSDV. In the conditions encountered in the southeast of Spain, using only such resistant varieties was not as efficient a control measure as the physical protection offered by indoor nets. The 'Almeria'-type of greenhouse has structural features that are well consolidated within the productive area of southeast Spain (Sánchez Pérez *et al.,* 2001). We showed that an indoor structure of continuous nets protecting the roof and side apertures could be adapted to this type of greenhouse. The cost of this modification was 1.35 € per square meter, and the results were very positive. There were very significant reductions in both the incidence of disease and in cucumber yield losses attributable to *B-tabaci*-transmitted CYSDV and CVYV, while there was no need to apply insecticides.

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References

Anonym, 2001: Memoria resumen Año 2001. Delegación Provincial de Almería. Consejería de Agricultura y Pesca.

Berlinger, M.J., Mordeshi, S. & Leeper, A. 1991: Application of screens to prevent whitefly penetration into greenhouses in the Mediterranean basin. Proc. Working Group "Integrated Control in Protected Crops Under Mediterranean climate." Bull. IOBC/WPRS, 1991/XIV/5: 105-110.

Castilla, N. & Jarrett, P. 1995: Protected cultivation in the Mediterranean area. Plasticulture 107: 13-20.

Celix, A., Lopez-Sese, A., Almarza, N., Gomez-Guillamon, L. & Rodríguez-Cerezo, E. 1996: Characterization of cucurbit yellow stunting disorder virus, a *Bemisia tabaci*-transmitted closterovirus. Phytopathology 86: 1370-1376.

Cuadrado, I.M., Janssen, D., Velasco, L., Ruiz, L. & Segundo, E. 2001: First report of *Cucumber vein yellowing virus* in Spain. Plant Dis. 85: 336.

Cohen, S. & Nitzany, F.E. 1960: A whitefly-transmitted virus of cucurbits in Israel. Phytopathol. Med. 1: 44-46.

Elbert, A. & Nauen, R. 2000: Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. Pest Manag. Sci. 56: 60-64.

Janssen, D. & Cuadrado, I.M. 2001: Whitefly problems escalate within Spanish cucurbit crops. ESWN Newsletter 10: 1-2.

López-Sese. A.I. & Gomez-Guillamon, M.L. 2000: Resistance to cucurbit yellowing stunting disorder virus (CYSDV) in *Cucumis melo* L. Hort. Sci. 35: 110-113.

Ruiz, L., Janssen, D. & Cuadrado, I.M. 1999: Moscas blancas (*Homoptera: Aleyrodidae*) sobre cultivos protegidos de cucurbitáceas en Almería: Dinámica de las poblaciones de *Trialeurodes vaporariorum* (West.) y *Bemisia tabaci* (Genn.) y caracterización del biotipode *B. tabaci.* Agricola Vergel 18: 660-666.

Sánchez-Pérez, M., Pérez Parra, J.J, Marín Carrillo, G.M. & Céspedes López, A.J. 2001: Estudio de la demanda de inputs auxiliares: Producción y manipulación en el sistema productiva agrícola almeriense. FIAPA, Almería, pp. 201.

Teitel, M.M., Barak, M.J. & Lebiusg-Mordechi, S. 1999: Insect-proof screens in greenhouses: Their effect on roof ventilation and insect penetration. Acta Horticulturae 507: 25-34.

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Effect of kale and lettuce on survival of *Pochonia chlamydosporia* in a field infested with *Meloidogyne javanica.*

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Abstract: The effect of kale and lettuce on the survival of the egg parasitic fungus, *Pochonia chlamydosporia*, and the carry-over effect of application of the fungus on a subsequent tomato crop was evaluated in an unheated plastic house infested with *Meloidogyne javanica.* The fungus was applied once at a rate of 12.5 x 10^6 chlamydospores per plot at the time of planting kale and lettuce. For each crop, fungal colonization of root and soil, initial and final nematode densities and percent egg parasitism were determined. Rizosphere colonization of kale was higher than that of lettuce but there was no difference in soil colonization. Initial and final densities of *M. javanica* did not differ between kale and lettuce. The nematode did not produce eggs on either crop. On tomato, initial and final nematode densities and egg production did not differ among treatments. The preceding crop did not affect fungal parasitism of nematode eggs, but tended to be higher in kale-tomato (9.3%) than lettuce-tomato plots (3.9%). Tomato yield in fungal treated plots was higher ($P < 0.05$) when preceded by kale than by lettuce.

Key words: biological control: plant host; root-knot nematode; tomato.

Introduction

Root-knot nematodes are a major pest of vegetable crops worldwide. Currently, nematode control is based on the use of soil fumigants and nematicides. Nevertheless, the constraints imposed on their use by legislation and consumers require the development of alternative management strategies including biological control. *Pochonia chlamydosporia* Gams is a facultative parasite of nematode eggs that has been extensively studied as a potential biological control agent for cyst and root-knot nematodes (Kerry & Jaffee, 1997). The tri-trophic interaction occurring in the soil between the fungus, the plant and the nematode is complex, and it is affected by several factors. Thus, the plant host affects the growth of the fungus in the rhizosphere, and plant species differ in their ability to support fungal growth (Bourne, *et al.*, 1994; Bourne, *et al.*, 1996). The plant hosts also differ in their susceptibility to infection by *Meloidogyne* spp.

The objective of this study was to determine the effectiveness of kale (*Brassica oleracea* L.) and lettuce (*Lactuca sativa* L.) on the establishment and survival of *P. chlamydosporia* applied at the time of planting in a field infested with *Meloidogyne javanica* (Treub). The carry-over effect of application of the fungus on final nematode densities, plant damage, and crop yield was also determined on a subsequent tomato (*Lycopersicon esculentum* Mill.) crop.

Materials and methods

The experiment was conducted in an unheated plastic house located in Cabrils, Barcelona, Spain, which was infested by *M. javanica*. The soil was sandy loam, pH 8.1, electric conductivity (dS/m) 0.40, and 0.9% organic matter (P/P). The experimental design was randomized stratified blocks with four replicated plots per treatment. Each plot was 4.5 m^2 (1.5 m wide x 3 m long). Treatments included two plants species, kale and lettuce. Plots with or without *M. javanica* were also included to assess the effect of the fungus on the yield of tomato.

Isolate ICAR-Vc10 of *P. chlamydosporia* was provided by B. R. Kerry, ICAR-Rothamsted, UK, and the inoculum was prepared according to Kerry & Bourne (2002) by the Dept. Recursos Naturales, Universidad de Alicante, Spain. The concentration of chlamydospores in the inoculum was estimated in diluted samples using a haemocytometer. The fungus was applied at a rate of 12.5 x 10^6 chlamydospores per plot by removing soil from the first 15 cm of the planting row, mixed thoroughly with the inoculum in a concrete mixer, and returned to the planting row. Kale and lettuce were immediately transplanted to plots inoculated with the fungus. Each plot had two rows of plants spaced 30 cm from each other within the row, and there were 55 cm between rows. To assess initial and final nematode densities, samples from each plot were collected in October 1999 and February 2000, respectively. Individual samples consisted of five soil cores taken from the first 30 cm of soil with a sampling tube (2.5-cm diameter). Soil cores were mixed thoroughly and nematodes were extracted from a 500-cm^3-soil subsample using Baermann trays. Second-stage juveniles (J2) migrating to the water were collected after one week, concentrated on a 25-µm sieve, and counted. The number of J2 was expressed per 250 cm^3 of soil. Root galling from four randomly-selected plants per plot was assessed using a scale from 0 to 10 (Zeck, 1971). Root systems from each plot were then bulked and used for egg extraction by blender maceration in 0.05% NaOCl solution for 10 minutes (Hussey & Barker, 1973) using two 5-gram root subsamples.

In March 2000, tomato cv Durinta was transplanted to plots previously cultivated with kale or lettuce, and they were harvested in July. Plants were spaced 50 cm from each other in the row, and there were 55 cm between rows. Nematode densities were determined before planting and at harvest as described previously. Fungal density in soil and on roots was estimated after cultivation of kale, lettuce, and tomato by dilution plating on a semi-selective medium (Kerry & Bourne, 2002). Parasitism of nematode eggs was assessed on tomato plants according to de Leij & Kerry (1991). Dispersed eggs were spread on a restrictive growth medium (Lopez-Llorca & Duncan, 1986) in three replicated Petri dishes. Percent parasitism was recorded after 48-hour incubation at 25°C. To confirm parasitism by *P. chlamydosporia*, parasitized eggs were transferred individually to a corn-meal agar medium and observed for the presence of chlamydospores one week after incubation at 25°C. To determine tomato yield, fruits produced by four plants were harvested as they matured. Tomatoes were harvested once a week for 6 weeks. Fruits were counted, weighed, and the cumulated yield expressed as kilogram per m^2. Data on fungal survival, egg parasitism, nematode reproduction, and tomato yield were subjected to analysis of variance using the GLM procedure of SAS (SAS Institute, Cary, NC). Data on the fungus and nematodes were transformed to log (x+1), and data on percent egg parasitism to arcsine ($\sqrt{x}$) before analysis.

Results

Initial and final soil population densities of *M. javanica* did not differ between kale and lettuce, and the nematode showed a similar reproductive rate (Pf/Pi) on both crops (Table 1). Some root galling occurred on lettuce but not on kale. Eggs were not recovered from kale or lettuce roots. The number of colony forming units (cfu) per gram of root was higher ($P < 0.05$) on kale than lettuce, but their numbers did not differ in the soil (Table 1).

Table 1. Effect of lettuce and kale on survival of *Pochonia chlamydosporia* and on reproduction of *Meloidogyne javanica* in an unheated plastic house in Barcelona, Spain.

	Juveniles/ 250cm^3 soil [a]		Gall rating	Fungus [b]	
Crop	Pi	Pf		cfu/g soil	cfu/g root
Kale	2960 ±1 520	463 ± 371	0	1560 ± 2794 (3) [c]	750 ± 456* (4)
Lettuce	2060 ± 960	442 ± 95	0.3 ± 0.5	440 ± 239 (4)	63 ± 125 (1)

[a] Values are means ± standard deviation of four replicated plots. Data within a column with * are different according to t-Student test ($P < 0.05$).
[b] Applied at a rate of 12.5 x 10^6 chlamydospores of per plot.
[c] In parentheses, number of plots where the fungus was recovered.

Initial and final soil densities of *M. javanica* and egg production on tomato did not differ among treatments (Table 2). Tomato roots in plots preceded by lettuce showed lower ($P < 0.05$) gall rating than in those with *M. javanica* alone. In fungal-treated plots, the preceding crop did not affect gall rating or egg parasitism on tomato. Percent parasitism ranged from 2.6 to 16% (x = 9.3±5.5) (mean±standard deviation), and from 2.9 to 7.5% (x = 3.9±2.5) on tomato plots preceded by kale or lettuce, respectively. At the end of the study, the fungus was recovered from three of the four plots cultivated with lettuce-tomato but not from those with kale-tomato (Table 2).

Table 2. Initial and final densities of *Meloidogyne javanica* (Mj), gall rating, egg production and percentage of eggs parasitized by *Pochonia chlamydosporia* on tomato cv Durinta preceded by lettuce or kale and in *M. javanica* alone plots in an unheated plastic house in Barcelona, Spain.

	Juveniles/250 cm^3 soil		Gall rating	Eggs/g root[a]	Parasitized eggs (%)	cfu/g soil [b]	cfu/g root
Treatment	Pi	Pf					
Mj + Fungus							
Kale	650	9780	6.7	40580	9.3	0	0
Lettuce	760	9500	6.3	52980	3.9	63±125 (1)	250±204 (3)
Mj alone	520	11100	7.1*	44800	-	-	-

Values are means of four replicated plots. Data within each column with * are significantly different ($P < 0.05$) according to LSD test.
[a] Only healthy eggs included.
[b] In parentheses, number of plots where the fungus was recovered.

Tomato yield and the number of fruits per plant were higher ($P < 0.05$) on nematode-free than on nematode-infested plots (Table 3). Tomato in fungal-treated plots yielded more ($P < 0.05$) when preceded by kale than by lettuce.

Table 3. Yield of tomato cv Durinta preceded by lettuce or kale treated with 12.5 x 10^6 chlamydospores of *Pochonia chlamydosporia* per plot in a plastic house infested with *Meloidogyne javanica* (Mj) in an unheated plastic house in Barcelona, Spain.

Treatment	Yield (kg/m^2) [a]	No. fruit/plant	Average fruit weight (g)	Yield increase (%)
Mj + Fungus				
Lettuce	8.5 ± 2.8 c	31 ± 8 b	95 ± 15 b	97
Kale	11 ± 4.3 b	34 ± 10 b	106 ± 16 a	122
Mj alone	8.8 ± 1.5 bc	30 ± 4 b	101 ± 9 ab	100
Nematode free	14 ± 2.6 a	46 ± 10 a	108 ± 10 a	161

Each value is mean±standard deviation of 16 plants (four plants/plot x four plots/treatment). Data within a column with the same letter are not significantly different ($P > 0.05$) according to LSD test.

Discussion

The virulence of *P. chlamydosporia* against *M. javanica* in the field was confirmed in this study. The fungus was recovered from parasitized eggs nine months after its application to soil, and was compatible with the agronomic practices and environmental conditions typical of vegetable production in plastic houses in north-eastern Spain (Verdejo-Lucas, *et al.*, 2003). However, the expected benefit for nematode control derived from maximizing fungal colonization through the selection of the plant host was not obtained in the study field. Poor colonization of the rhizosphere as suggested by low cfu counts occurred in plots with either plant species, although kale supported more cfu per g root than lettuce four months after fungal application, which confirmed its status as host for *P. chlamydosporia* previously shown in pot tests, (Bourne, *et al.*, 1994; Bourne, *et al.*, 1996). Although fungal colonization of lettuce roots was poor in pot tests (Viaene and Abawi, 2000, Sorribas *et al.*, 2003), low fungal levels were found on tomato roots preceded by lettuce but not by kale nine months after fungal application. The absence of egg production on kale and lettuce probably prevented the fungus from proliferating in the rhizosphere of these crops. It has been shown that the presence of the nematode increased the growth of *P. chlamydosporia* on roots, which was associated with the emergence of egg masses on the root surface (Bourne, *et al.*, 1996). On the other hand, the fungus parasitized 2.3 times more eggs on tomato preceded by kale than by lettuce despite undetectable levels of the fungus in kale-tomato plots at the end of the study. Low recovery of the fungus usually occurs after its application to soil and the inability of *P. chlamydosporia* to establish in soil, and competition with other microorganisms in the rhizosphere has been suggested for reduced fungal establishment. (Godoy, *et al.*, 1983; Viaene & Abawi, 2000), The microflora associated with *Meloidogyne* egg masses may have an antagonistic effect on *P. chlamydosporia* (Kok *et al.*, 2001).

Both kale and lettuce responded as a poor host for the nematode; they decreased population densities of *M. javanica* in soil by 87% and 85%, respectively, and prevented nematode reproduction on roots. Nevertheless, the surviving nematodes reached high

densities, and caused profuse root galling on the subsequent tomato crop. Apparently, kale was a better host for the fungus than lettuce whereas both plant species responded similarly to the nematode. Large-scale experimentation is required to determine how the plant host influences the nematode-fungus interaction in the field, and the extent of the carry-over effect of fungal applications on the following crops. More field data are needed to find out situations where microbial antagonists have real potential for biological control of nematodes.

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References

Bourne, J. M., Kerry, B. R. & de Leij, F. A. A. M. 1994: Organisms and methods for biological control of nematodes. Methods for study of *Verticillium chlamydosporium* in the rhizosphere. Journal of Nematology 26: 587-591.

Bourne, J. M., Kerry, B. R. & De Leij, F. A. A. M. 1996: The importance of the host plant on the interaction between root-knot nematodes (*Meloidogyne* spp.) and the nematophagous fungus, *Verticillium chlamydosporium* Goddard. Biocontrol Science and Technology 6: 539-548.

de Leij, F. A. A. M. & Kerry, B. R. 1991: The nematophagous fungus *Verticillium chlamydosporium* as a potential biological control agent for *Meloidogyne arenaria*. Revue de Nematology 14: 157-164.

Godoy, G., Rodríguez-Kábana, R. & Morgan-Jones, G. 1983: Fungal parasites of *Meloidogyne arenaria* eggs in an Alabama soil. A mycological survey and greenhouse studies. Nematropica 13: 201-213.

Hussey, R. S. & Barker, K. R. 1973: A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57: 1025-1028.

Kerry, B. A. & Bourne, J. M. 2002: A Manual for research on *Verticillium chlamydosporium*, a potential biological control agent for root-knot nematodes. Gent, IOBC/wprs pp

Kerry, B. A. & Jaffee, B. A. 1997: Fungi as biological control agents for plant parasitic nematodes. In: The micota IV Environmental and microbial relationships eds. Wicklow/Söderström. Springer- Verlag. Berlin Heidelberg: 203-218.

Kok, C. J., Papert, A. & Hok_A-Hin, C. H. 2001: Microflora of *Meloidogyne* egg masses: Species composition, population density and effect on the biocontrol agent *Verticillium chlamydosporium* (Goddard). Nematology 3: 729-734.

Lopez-Llorca, L. V. & Duncan, J. M. 1986: New media for the estimation of fungal infection in eggs of the cereal cyst nematode, *Heterodea avenae* Woll. Nematologica 32: 486-490.

Sorribas, F. J., Ornat, C., Galeano, M. & Verdejo-Lucas, S. 2003: Comparisons of native and introduced isolates of *Pochonia chlamydosporia* in growth chamber tests and in a plastic tunnel house infested with *Meloidogyne javanica*. Biocontrol Science and Technology. In Press.

Verdejo-Lucas, S., Sorribas, F. J., Ornat, C., & Galeano, M. 2003: Evaluating *Pochonia chlamydosporia* in a double cropping system of lettuce and tomato in plastic houses infested with *Meloidogyne javanica*. Plant Pathology 52: (in press).

Viaene, N. M. & Abawi, G. S. 2000: *Hirsutella rhosiliensis* and *Verticillium chlamydosporium* as biological control agents of the root-knot nematode *Meloidogyne hapla* on lettuce. Journal of Nematology 32: 85-100.

Zeck, W. M. 1971: A rating scheme for field evaluation of root-knot nematode infestations. Pflanzenschtz-Nachrichten. Bayer AG. 24: 141-144.

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Combined effects of *Trichoderma* applications and soil solarization on *Colletotrichum* crown rot in strawberry plants

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Abstract: The objective of this study was the evaluation of the effect of non-chemical alternatives to control *Colletotrichum* crown rot in strawberry plants. Trials were carried out in Moguer (Huelva, south-west of Spain) during the 2001-2002 season. Treatments consisted of soil solarization and applications of *Trichoderma* strains as a liquid formulation (Tusal®) or as a fungus-enriched compost (Fertusal). *Trichoderma*-treated plots gave less *Colletotrichum* crown rot. Solarized plots showed better results than unsolarized plots. However, *Trichoderma* applications presented better results with unsolarized soil.

Key words: biological control, diseases

Introduction

Methyl Bromide has been extensively used since the 1980s. European Community rules are now more restrictive regarding the use of pesticides. Adequate alternatives for strawberry production must be investigated. The objective of this study was the evaluation of the effect of non-chemical alternatives. Disease suppression by biocontrol agents is the sustained manifestation of interactions between the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant and the physical environment (Handelsman and Stabb, 1996).

Materials and methods

Trials were carried out in Moguer (Huelva, south-west of Spain) during the 2001-2002 season. Thirty-six plots (12.5m x 3.3m), never treated with Methyl Bromide, were planted with strawberry plants cv. Camarosa (four repetitions of nine treatments).

Soil solarization was carried out with transparent polyethylene film during the summer of 2001. Treatments consisting of applications of a cocktail of five *Trichoderma* strains as a liquid formulation (Tusal®), 10^8 conidia/ml, or as a fungus-enriched compost (Fertusal), 5.10^8 conidia/µg, were tested for *Colletotrichum* crown rot.

Fertusal was applied into the whole of plantation. Tusal was incorporated by means of drip irrigation or/and the roots of strawberry plants were immersed in a dilution of this product.

Results and discussion

Trichoderma-treated plots gave less *Colletotrichum* crown rot than *Trichoderma*-untreated plots, showing significant differences between them (figure 1).

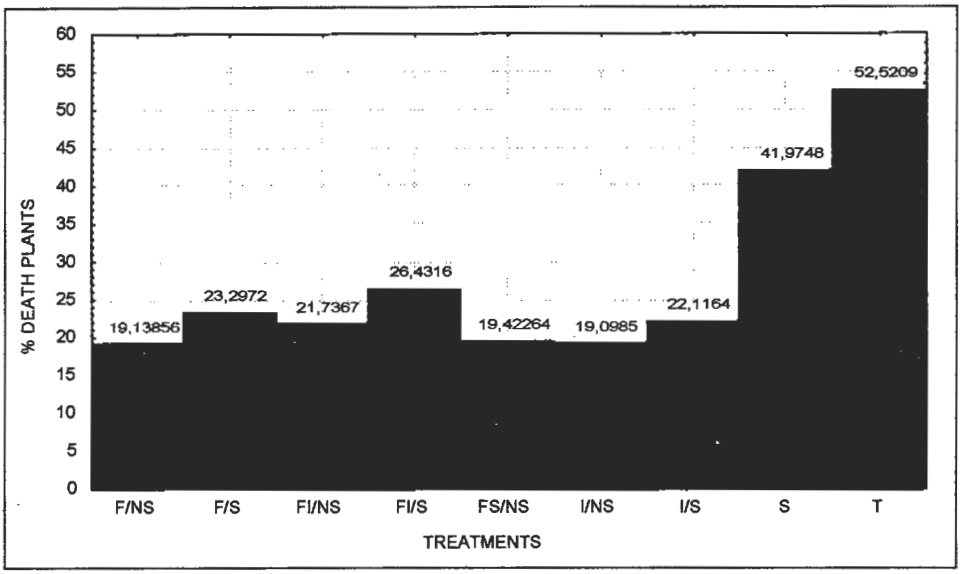

Figure 1. Percentage of dead plants caused by *Colletotrichum* crown rot. (F=Tusal drip irrigation; I=Tusal roots immersion; FI= Tusal drip irrigation+roots immersion; FS=Fertusal; S=Solarization; NS= Non solarization; T=untreated control)

The best results were showed by Fertusal, Tusal drip irrigation and Tusal roots immersion. Solarized plots showed better results than unsolarized plots. However, each *Trichoderma* application presented best results in unsolarized soil.

Each treatment on its own, Tusal drip irrigation or Tusal roots immersion, reduced the dead plants more than both treatments together. The optimum threshold concentration of *Trichoderma* may have been less than that obtained in the combined treatments.

In this way, the competition with other microorganisms in unsolarized plots reduced the proliferation of Trichoderma, showing the best results.

We are currently studying the optimum concentration of *Trichoderma* to obtain the best control of this strawberry pathogen.

References

Handelsman, J. & Stabb, E.V. 1996: Biocontrol of soilborne plant pathogens. The Plant Cell 8: 1855-1869.

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Bacterial soft rot disease of pea in Egypt

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Abstract: Six pectolytic bacteria of *Bacillus* were isolated from pea pods exhibiting symptoms of partial soft rot and discolouration that had been collected from different markets in the El-Minia Governorate, Egypt. Inoculation of the bacteria through wounded pods reproduced soft rot of pods and seeds. The bacterial strains produced blight and discoloration on pods of growing plants under greenhouse conditions. They also proved to be active in degrading the parenchymateous tissues of many plant organs under experimental conditions. Moreover, some bacterial isolates caused decay and damping-off to pea seeds under both laboratory and field conditions. The isolated bacteria were identified as *Bacillus megaterium* and *Bacillus subtilis* on the basis of their physiological and biochemical properties, a pathogenicity test and fatty acid composition analysis. They produced pectic enzymes both *in vitro* and *in vivo*. This is the first time these bacteria have been identified and recorded on pea in Egypt.

Keywords: Pea, *Pisium sativum, Bacillus subtilis, Bacillius megaterium*, blotch, soft rot, maceration.

Introduction

Bacillus spp. have been reported in association with several food crops (Tenne *et al.*, 1975; Ciampi & Huguelet, 1979; Saleh *et al.*, 1996; Saleh *et al.*, 1997). *B. subtilis* is ubiquitous and has been found in association with soybean seed decay, germination and stand inhibition (Schiller *et al.*, 1977; Kulik & Sinclair 1993). It also causes severe storage losses (about 100%) worldwide, and under experimental and field conditions, particularly when soybeans are planted in wet soil under hot moist conditions. Hosford (1982) consistently isolated *B. megaterium* pv. *cerealis* from severely affected wheat seeds. Saleh *et al.* (1997) found that *B. pumilus* degraded potato, garlic, and pome and stone fruit tissues by the production of cell wall degrading enzymes, endopolygalacturonase (EC4.2.2.1) and endopectin lyase (EC4.2.2.3). The objective of this study was to determine the etiology of partial soft rot and spots on pea pods and leaves. The ability of these bacteria to cause seed decay and affect seed germination was also investigated.

Material and Methods

Isolation

Diseased pea pods were collected in 2001 and 2002 seasons from different markets and fields in the El-Minia Governorate and used for isolation. Infected pods were surface sterilized with 1 % Na hypochlorite for 3 min. and washed with sterile water. The infected peel of the pod and the infected seeds inside were separately triturated in a sterilized mortar with small amounts of sterile distilled water. A loop from the resulting suspension was streaked on dry nutrient sucrose agar (NSA), plates and incubated at 30°C. Dominant colonies were restreaked five times to ensure purity. They were subcultured on NSA slants for further study.

Pathogenicity, tests and effects of bacterial isolates on other plants and plant tissues
Unblemished healthy pea pods and leaves (cvs Lincolin and Little Marvel) were collected from the experimental farm at Minia University. Pods were surface disinfected with 95 % ethanol, and inoculated by applying small portions of bacterial growth (24 h old, grown on NSA plates) on a superficial - approximately 1 cm long, 1 mm deep - wound (Ciampi & Huguelet, 1979; Saleh *et al.*, 1996). The inoculated pods were kept in polyethylene bags together with a piece of sterilized moist cotton. Leaves were inoculated by pricking the midrib of the leaflet with sterile needles that had previously been loaded with small portions of bacterial growth. Controls were subject to similar treatment, but without bacterial inoculation. Incubation was performed at 30°C. These experiments were repeated using equal volumes of bacterial suspension (ca 10^8 cfu/ml) as inoculum. The macerating capacity of the bacterial isolates was determined following the Kelman & Dickey (1980) procedure. Isolated bacteria were tested if they were capable of inducing seed decay and damping-off *in vitro* and under green house conditions. Ten surface sterilized seeds were placed in 10 cm Petri dishes (10 seeds per isolate). A volume of 10 ml of bacterial suspension (10^8 cfu/ml) was then added to each Petri dish for the corresponding isolate. The plates were incubated as above for up to 3 weeks at 30°C and inspected on a daily basis for seed decay and disease development. The control treatment was performed in a similar way, but using sterile distilled water. Percentage seed decay and germination were recorded after 3 weeks.

Organs from various plants were inoculated with each of the six isolates to determine their host ranges. The following organs: roots of sugar beet, table beet, fodder beet (*Beta vulgaris*) a radish (*Raphanus sativus*), rape (*Brassicae rapes*), carrot (*Daucus carrota*), and sweet potato (*Ipomea batata*); fruits of cucumber (*Cucumis sativus*), squash (*Cucurbita sp*), pepper (*Capsicum annum*), olive (*Olea europea*) and lemon (*Citrus aurantifolia*); tubers of potato (*Solanum tuberosum*), garlic cloves (*Allium vineale*); and pods of black-eye (*Vigna sinensis*), field bean (*Vicia fabae*), kidney bean (*Phaseolus vulgaris*), lupin (*Lupinus termis*), and soybean (*Glycine max*) were all inoculated either by pricking or by bacterial suspension, as previously mentioned. Pepromia leaves (*Pepromia obvata*) and tobacco leaves (*Nicotiana tabaccum*) were inoculated with a bacterial suspension of the midrib (Saleh *et al.* 1996; Klement *et al.* 1964). Stems of tomato (*Lycopersicon esculentum*) and chrysanthemum (*Chrysanthemum morifolium*) were injected with a bacterial suspension as in Thomson *et al.* (1977) and Saleh *et al.* (1996).

Biochemical and physiological characteristics
The physiological and biochemical characteristics of the isolated bacteria were determined using the methods and techniques recommended in the literature (Smith *et al.* 1952, Hugh & Leifson 1953, Dye 1968,Wolf & Barker 1968, Hunger & Claus 1981; Sneath *et al.*, 1986). Pectic enzyme activities were demonstrated by the Vaerenbergh *et al.*, (1981), Lund *et al* (1981) methods and also viscometrically. Bacterial fatty acids were analyzed in Dr D. Stead's laboratory at the Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK.

Results and Discussion

Isolation, pathogenicity on Lincolin and Little Marvel pea, and effects on other plants
Naturally infected pea typically exhibited brown spots and blotches on mature green pods. The seeds inside were partially rotted and there were large brown spots on leaves. Microscopic examination of crushed, diseased and adjacent tissues revealed the presence of numerous motile and non-motile bacterial cells. Isolation on NSA medium from these tissues resulted in numerous separated single colonies after about 48 h of incubation at 30°C. Water

soaked light-brown spots (ca 5 mm diameter) developed on and around inoculated sites of both attached and detached pods 24 to 48 h after inoculation. These spots enlarged as disease progressed, becoming brown, dry blotches covering large areas of the pods. In both cases, seeds beneath the affected tissue discolored to light brown and partially rotted. Small, water-soaked, light-brown necrotic spots (ca 9 mm) developed on attached and detached leaves about 24 and 48 h after inoculation, respectively. These spots dried and were usually surrounded by irregular yellow haloes. Leaf tendrils were also infected. Inoculation procedures of bacteria that cause water soaking are necessary for some bacteria to be pathogenic (Hosford, 1982). The same author added that chlorosis around spots was due to toxins produced by *B. megaterium*. All bacterial isolates in this investigation were capable of inducing a clear hypersensitive reaction within 12-24 h and this revealed the pathogenic character of the organisms (Vaerenberght *et al.*, 1981). They also caused soft rot, maceration and death to potato tubers and carrot roots. Pathogenicity to potato slices causing soft rot to potato is considered a positive reaction indicating the virulence of bacterial isolate (Alcorn *et al.*, 1991). *Bacillus* species are spore-forming rods that are common in soil; some maybe involved in the post-harvest decay of many plant tissues including vegetables and other crops. *B. pumilus, B. polymyxa, B. megaterium* and *B. subtilis* are known to cause soft rot in potato, carrot, onion, garlic and pome and stone fruits (Obi & Umezurike, 1981; Saleh, *et al.*, 1997). *B. subtilis* and *B. megaterium* have not been previously recorded as pea pathogens in Egypt (Aly *et al.*, 1972). The results reported here added new etiological agents of pea disease and information on the ecology of *B. subtilis* and *B. megaterium*. Isolates 2, 4, 5 and 6 caused a significant reduction in seed germination. The bacterial isolates were able to invade and colonize tissues of tested pea pod, potato tuber, and carrot root slices showing soft rot symptoms. *B. subtilis* and other *Bacillus spp* were found in association with seed decay in many legumes and cereals (Dwivedi & Dwivedi, 1991; Kulik & Sinclair, 1993). All tested isolates produced symptoms of soft rot on the roots of sugar beet, table beet, radish, rape, sweet potato and the fruits of squash, cucumber, lemon, olive, garlic, and pepper 1 to 6 days after inoculation. Inoculated bean, field bean, black eye, lupine, and soybean pods showed dark-green water-soaked areas that subsequently coalesced. The inoculated tissue finally waterlogged and collapsed. Chrysanthemum and tomato stems were only infected by isolates 1, 2, 3, 4 and 5, which produced necrosis of inoculated tissues, and the wilting of inoculated plants 1 to 5 days after inoculation. Inoculation of pepromia leaves with bacterial isolates resulted in water soaked, necrosis, and dead of inoculated tissue. The host range tests conducted in this investigation indicate that the bacterial isolates have a relatively wide host range. (Aly *et al.*, 1972; Bradbury, 1986; Lelliot & Stead, 1987). Sugar beet, squash, cucumber, olive, lemon fruits, carrot, rape, radish, sweet potato, lupine, field bean, black eye and pepromia could be considered as new hosts for *B. megaterium* and *B. subtilis*.

Identification of causal pathogens

The physiological and biochemical properties of the isolates are summarized in Table 1. We conclude that the bacterial isolates could be classified into two groups. Group 1 includes isolates 1 and 2, which were gram-positive rods, single (1-1.03 x 2-3 μ), in pairs or in chains, non-motile and were strictly aerobic. They produced ellipsoidal spores that didn't swell the mother cell. They were catalase positive, VP positive, oxidase negative, and lecithinase negative (egg yolk). They degraded casein and hydrolysed starch and aesculin.They grew in 4, 5 and 7% NaCl without the formation of fragile pellicle. Acid without gas was produced from glucose, galactose, fructose, arabinose, xylose, mannose, mannitol, sucrose and cellobiose but not from maltose, sorbose, α-methyl glucoside, dulicitol or salicin. Good and abundant growth was obtained on nutrient agar, but the addition of glucose did not appreciably improve growth. They also utilized acetate and

Table 1: Physiological and biochemical characteristics of pod and leaf spot bacteria isolated from Little Marvel pea.

Character	Results					
	Isolate					
	1	2	3	4	5	6
Gram stain	+	+	+	+	+	+
Sporulation	+	+	+	+	+	+
Motility	-	-	+	+	+	+
Production of catalase	+	+	+	+	+	+
Production of oxidase	-	-	-	-	-	-
Production of urease (after 2 weeks)	+	+	+	+	+	+
Production of tyrosinase	-	-	-	-	-	+
Production of arginine dihydrolase	-	-	-	-	-	+
2-ketoglutinase test	-	-	-	-	-	-
Degradation of potato, gelatin, casein, aesculin; starch	+	+	+	+	+	+
Reaction on skimmed milk	+	+	+	+	+	+
Lipolytic activity	(+)	(+)	+	+	+	+
Reduction of nitrate	+	+	+	+	+	+
Aerobiosis	-	-	+	+	+	+
Anaerobic production of gas from nitrate and glucose	-	-	-	-	-	-
VP assay	+	+	+	+	+	+
Levan production	-	-	-	-	-	+
Indole formation	+	+	(+)	(+)	(+)	(+)
H_2S production	-	-	-	-	-	-
Reducing substances from sucrose (Bendict)	+	+	+	+	+	-
Tolerance of 4, 7 and 10 % NaCl	+	+	+	+	+	+
Growth factor requirement	-	-	-	-	-	-
Production of acid from: Glucose, galactose, fructose, mannitol, xylose, glycerol, sucrose, cellobiose, melibiose, lactose and arabinose	+	+	+	+	+	+
Myoinositol, sorbose, sorbitol, dulicitol, α-methyl glucoside, maltose and salicin & glycogen	-	-	-	-	-	-
Maximum temperature for growth	40	40	65	65	65	50
Utilization of acetate and citrate	+	+	+	+	+	+
Benzoate, tartrate, succinate and malonate	-	-	-	-	-	-
Utilization of aspargin as C and N sources	+	+	+	+	+	+
Lecthinase (egg yolk)	-	-	-	-	-	+
MR red	+	+	+	+	+	+
pH of VP	8	6.8	8.2	8.2	8.9	7.9
pH of glucose nutrient broth	6.2	6.3	6.2	6.3	6.3	8.9
Sensitivity to erythromycin 50 μg	R	R	R	R	R	R

+: positive reaction, -: negative reaction, (+): delayed or weak positive reaction, R: resistance.

citrate but not benzoate, succinate or tartrate. They grew at 40°C but not at 45°C. The bacterial isolates showed pectolytic activity on pectate gel. From comparison of the bacterial characteristics with those recorded for identification it could be concluded that the isolated bacteria are similar to *Bacillus megaterium* in the majority of their characters (Smith *et al.*, 1952; Wolf & Barker, 1968, Hunger & Claus, 1981; Saleh *et al.*, 1996). Isolates 1 and 2 had 88 and 91 fatty acid homology, respectively, with *B. megaterium.*

Group 2 includes strains 3, 4, 5 and 6, which were gram-positive rods (0.77 - 0.86 x 1.3 - 1.5 μ), and were motile. Bacterial cells are single or in chains, capsulated, catalase positive and facultative aerobic. They produced oval to cylindrical spores. They liquefied gelatin within 2 days, degraded casein and hydrolysed starch. They were VP positive, reduced nitrate, and no gas was produced from nitrate anaerobically. They grew well at 7 and 10 % NaCl with the formation of fragile pellicle. They did not produce oxidase or lecthinase. They grew well on nutrient agar but the addition of glucose did not appreciably improve growth with the formation of dull waxy adhered colonies. They also grew on PDA, PSA and PS broth media at a temperature range from 10 to 55°C, but optimal growth was attained at about 40°C. The bacteria utilized acetate and citrate but not benzoate, tartrate or malonate. They produced acid from glucose, galactose, fructose, mannitol, xylose, glycerol, sucrose, melibiose, lactose, arabinose, cellobiose but not from, sorbose, sorbitol, dulicitol, α-methyl glucoside or maltose. The bacterial isolates showed pectolytic activity on pectate gel and maceration on potato tubers and carrot roots. Accordingly, the isolated bacteria contribute to *B. subtilis* (Smith *et al.*, 1952; Wolf & Barker, 1968, Hunger & Claus, 1981; Saleh *et al.*, 1997). Isolates 3, 4, 5 and 6 had 78.8, 88.8, 72.7 and 71.2 fatty acid homology respectively with *B. subtilis*. Although *B. subtilis* and *B. megaterium* caused large brown blotches and spots on inoculated attached pods and leaves of Little Marvel pea cultivar in the field, they induced soft rot when inoculated in wounded detached pods of Little Marvel pea, leaves and tissues from other plants in the laboratory. This suggests that *B. subtilis* and *B. megaterium* are pathogens capable of inducing the softening of fruits under relatively high humidity conditions as previously reported Saleh et al. 1997. The bacterial isolates in this investigation produced pectic enzymes both *in vitro* and *in vivo*. The high viscometric activity of these enzymes may be an indication that they are of the endo type (Hosford, 1982). It has been established that enzymatic degradation of plant tissue by cell-wall-degrading enzymes is an important process in plant pathogensis caused by soft rot pathogens and could account for their virulence (Saleh *et al.* 1997).

References

Alcorn, S.M.; Orum, T.V.; Steigerwalt, A.G.; Foster, J.L.M.; Fogleman, J.C. and Brenner, D.J. 1991: Taxonomy and pathogeni city of *Erwinia cacticida* sp. Nov. International Journal of Systemic Bacteriology, 41: 197-212.

Aly, M.D.H.; Moursi, A.A.; El-Sherief, M.A.; Diab, M.M. and Abo El-Gar, A.L. 1972: Survey of agriculture research carried out in Egypt (1900-1970). Plant Pathology Science and Technology Research Academy, Egypt.

Bradbury, J.F. 1986: Guide to Plant Pathogenic Bacteria, pp. 1-332, C.A.B. International Mycological Institute, Cambrian News Ltd., Aberystwyth.

Ciampi, L.R. and Huguelet, J.E.1979: Internal bacteria in healthy potato tubers. Fitopatologia 14: 23-28.

Dwivedi, S.K. and Dwivedi, R.S. 1991: Evaluation of aureofungin and agrimycin-c on spermosphere fungi and bacteria of soybean (*Glycine max* (Merr.) L.) seeds. National Academy Science Letters, 14: 433-435.

Dye, D.W.1968: A taxonomic study of the genus *Erwinia*. I-The amylovora group. N.Z.J. Sci. 11:590-607.

Hosford, R.M.Jr. 1982: White blotch in wheat incited by *Bacillus megaterium* pv. *cerealis*. Phytopathology. 72: 1453-1459.

Hugh, R. and Leifson, E. 1953: The taxonomic significance of fermentative vs. oxidative metabolism of carbohydrates by various Gram negative bacteria. J. Bacteriol. 66: 24-26.

Hunger, W. and Claus, D. 1981: Taxonomic studies of *Bacillus megaterium* strains. Pages 218-239 in: The aerobic endospore-forming bacteria (R.C.W., Berkeley and M. Goodfellow, eds) American Press New York.

Kelman, A. and Dickey, R.S. 1980: Soft rot of 'carotovora' group. In: Schaad N.W. (ed.). Laboratory Guide for Identification of Plant Pathogenic Bacteria, pp. 31-35. American Phytopathyology Society St Paul, MN.

Klement, Z.; Farkas, G.L. and Lovreicovich, L. 1964: Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathyology, 54: 474-477.

Kulik, M.M. and Sinclair, J.B. 1993: Bacillus seed decay in compendium of soybean diseases, pp 73-74. Third edition. eds Sinclair J.B. and Bacman P.A. American Phytopathological Society. St. Paul, MN

Lelliot,R.A. and Stead, D.E. 1987: Methods in Plant Pathology. Vol. 2. Methods for the Diagnosis of Bacterial Disease of Plants. Blackwell Scientific Publications, London.

Lund, B.M.; Brocklehurst, T.F. and Wyatt, G.M. 1981: Characterisation of strains of *Clostridium puniceum sp.* nov., a pinkpigmented, pectolytic bacteria. J. Gen. Microbiol. 122, 17-26.

Obi, S.K.C. and Umezurike, G.M. 1981: Pectic enzyme activities of bacteria associated with rotted onions (*Allium cepae*) Applied and Environmental Microbilogy, 42: 585-589.

Saleh, O.I.; Huang, Pi-Yu and Huang, J.S. 1996: Bacterial vascular necrosis and root rot disease of sugar beet in Egypt. Journal of phytopathology, 144: 225-230.

Saleh, O.I.; Huang, PI-YU and Huang, J.S. 1997: *Bacillus pumilus*, the cause of bacterial blotch of immature Balady peach in Egypt. Journal of Phytopathology, 145: 447-453.

Schiller, C.T.; Ellis, M.A.; Tenne, F.D. and Sinclair, J.B. 1977: Effect of *Bacillus subtilis* on soybean seed decay, germination and stand inhibition. Plant Disease Reporter, 61: 213-216.

Smith, N.R.; Gordon, R.E. and Clark, F.E. 1952: Aerobic sporeforming bacteria. Agriculture Monograph no. 16, USDA, Washington, DC.

Sneath, P.H.A.; Mair, N.S.; Sharpe, M.E. and Holt, J.G. 1986: Bergey's Manual of Systematic Bacteriology, Vol. 2, pp. 965-1600, Williams & Wikins, Baltimore.

Tenne, F.D.; Mengistu, A. and Sinclair, J.B. 1975: Occurrence and identification of *Bacillus subtilis* associated with soybean seeds from six geographical countries (Abstract NC-45) Proceeding of American Phytopathological Society. 2: 91.

Vaerenbergh,v.,Welvaert, W. and Vandevelde, R.(1981). On bacterial seed infection of *Capsicum annum* (L). Rijksuniv. Gent, Belgium, 46: 737-747.

Wolf, J. and Barker, A.N. 1968: The genus *Bacillus*: Aids to the identification of its species. In: Gibbs, B.N.D.A. Shapton (eds), Identification Methods for Microbiologists, pp. 93-103, Academic Press, New York.

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Control of powdery mildew on organic pepper

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Abstract: Powdery mildew on pepper, caused by *Leveillula taurica*, is a disease that affects most commercial pepper varieties grown in Israel. Disease symptoms include white powdery mycelial growth on the underside and upper surfaces of leaves, and chlorotic spots that become necrotic. Affected leaves tend to drop off the plant, causing direct sun-scald damage to fruits and reducing the photosynthetic capacity of affected plants. In organic management, sulfur-containing agents efficiently control the disease. However, since sulfur agents may harm beneficial insects introduced into the greenhouse to control pests, our main objective in this study was to search for alternative means of control.

Experiments were carried out at the Yair station under full organic management in protected tunnels. Varieties Fiesta and 107 were used in 1999-2000, and Nibla and Parker in 2000-2001. In the first experiment, the sulfur agents either sprayed onto foliage or fumigated in the greenhouse were, as expected, very efficient in controlling the disease. They performed better than other tested treatments: water extract from cattle manure compost, Kaligrin (potassium bicarbonate) and Rifol (fish oil) (data not shown). In the second year, all treatments -- including Neemgard, water extract from grape marc compost, AQ10 (*Ampelomyces quisqualis*), Kaligrin and Rifol -- significantly reduced the incidence of disease in comparison with the non-treated control; however, the incidence of disease was lowest in plots treated with sulfur. Significantly higher yields were obtained in all treatments (especially in Neemgard) than in the control, but only in the case of var. Nibla.

Key words: *Leveillula taurica*, biological control, neem-oil, potassium bicarbonate, mineral sulfur, compost water extract, *Ampelomyces quisqualis*.

Introduction

Organic greenhouse pepper is an important crop in Israel: last year its total cultivated area increased to 25 ha. It is grown in insect-proof greenhouses or tunnels from September until April-May. Most (76%) organic production is for export to Europe (14,500 tons of organic vegetables, in 2002). Powdery mildew caused by *Leveillula taurica* (Lev.) Arm. is the most destructive pathogen of greenhouse and field-grown peppers in Israel (Reuveni and Rotem, 1973). In association with high temperatures, it causes defoliation, which in turn leads to smaller fruits due to reduced photosynthetic areas and damage by sun-scald. Chemical control is not possible under organic management and no commercially acceptable resistant varieties are available. Although genetic background resistance to the pathogen has been investigated (Daubeze *et al*, 1995), alternative methods for protection are essential. The common method for controlling powdery mildew on organic pepper using sulfur agents may harm beneficial insects introduced into greenhouses to control pests (such as thrips). The main objective of this study was to search for alternative means of controlling powdery mildew.

Materials and methods

Varieties Fiesta and 107 were used in a tunnel experiment in 1999-2000, and var. Nibla and Parker were tested in another greenhouse experiment in 2000-2001. The experiments were carried out at the Yair research station, in southern Israel (Arava region), under organic management conditions. Treatments (in four replications) included Neemgard (97% neem-oil), Sulfo-li (mineral sulfur (650 gr/l)), Kaligrin (potassium bicarbonate), Rifol (70% fish oil), compost water extract (CEX), and AQ 10 (*Ampelomyces quisqualis*). Treatments were applied on a weekly basis, starting 50 days after planting, and disease assessment was carried out every two weeks, by evaluation of the infected leaf area (%), on a sample of 30 leaves per treatment. Fruits were graded and weighed according to their quality.

Results and discussion

Natural infections of powdery mildew appeared on control plants 65 days post planting (dpp). Disease incidence in Nibla and Parker was 41 and 22%, respectively, 86 dpp, and reached 100% after a further 28 days (Fig 1). In all treatments, the maximum infection rate was 25% at the end of the season. Disease incidence was significantly reduced by Neemgard, Kaligrin, AQ10 and CEX treatments, as in the Sulfo-li treatment (Fig 1). The Rifol treatment, which was only applied to the var. Turkal, was also efficient, with 0.25% disease incidence at the end of the season (data not shown).

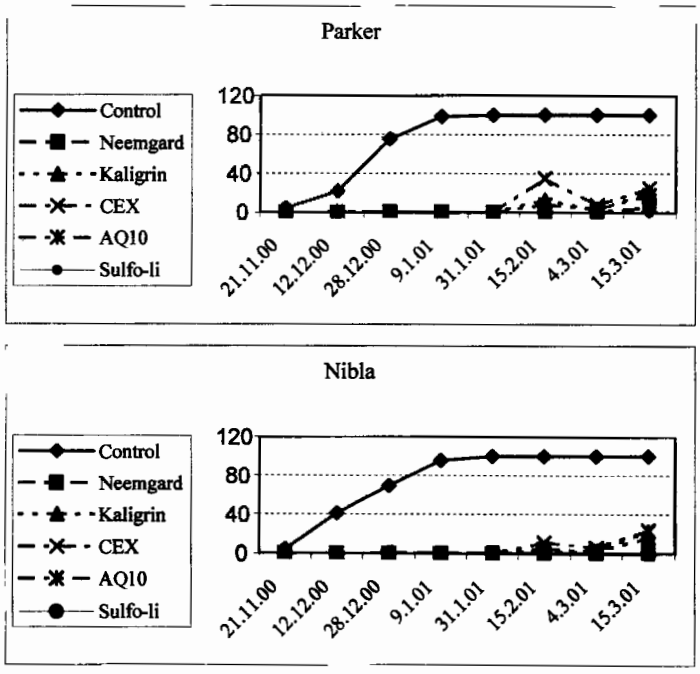

Fig 1: Effect of foliar organic treatments on powdery mildew incidence (% of infected leaf area)

Yields were significantly lower (by 31-41%) on control plants than on all other treatments, only in the case of var. Nibla, because of its relatively high susceptibility to the disease (Table 1). The yield obtained from the var. Parker control plants did not differ from that of the treated plants, indicating its relative resistance to the disease. Yields of grade A fruits (for export) in var. Nibla were 3.2, 3.1, 2.6, 2.5 and 2.5 times higher than in control using Neemgard, CEX, Sulfo-li, Kaligrin and AQ10, respectively. This indicated that all the tested treatments could serve as alternatives to common sulfur treatments for controlling powdery mildew on organic pepper. Previous reports demonstrated the efficiency of CEX in controlling grey mould (*Botrytis cineraea*) on tomato and pepper (Elad and Shtienberg, 1994) and early blight (*Alternaria solani*) on tomato (Tsror and Barak, 1998). Control of various diseases by foliar applications of inorganic salts, such as potassium bicarbonate (Homma and Arimoto, 1990), bicarbonate solutions (Fallik *et al.*, 1997), and mono-potassium phosphate (Reuveni *et al.*, 1998) has also been previously demonstrated. However, in the present study, the experiments were conducted on a large scale under field conditions and full organic management, demonstrating that the control of powdery mildew on peppers is possible using sulfur-alternative agents.

Table 1: The effect of foliar organic treatments on pepper yield levels

Variety	Treatment	Export market		Domestic market	
		Weight (kg/plot)	Number/plot	Weight (kg/plot)	Number/plot
Nibla	Control	5.3 ± 1.3	35.0 ± 8.4	1.5 ± 0.6	13.0 ± 5.3
	Neemgard	16.9 ± 3.9	156.5 ±38.4	1.6 ± 0.4	10.0 ± 2.1
	Kaligrin	13.1 ± 2.3	81.3 ± 15.8	1.4 ± 0.3	9.0 ± 2.6
	CEX	16.2 ± 4.0	93.8 ± 24.2	1.8 ± 0.6	16.0 ± 5.7
	AQ10	13.0 ± 3.0	102.8 ± 23.2	1.1 ± 0.3	5.5 ± 1.3
	Sulfo-li	13.7 ± 2.8	90.0 ± 21.6	0.5 ± 0.1	6.0 ± 1.0
Parker	Control	12.9 ± 2.2	88.5 ± 16.1	3.1 ± 0.7	29.0 ± 6.2
	Neemgard	12.5 ± 2.0	82.5 ± 13.5	1.5 ± 0.3	15.0 ± 2.6
	Kaligrin	14.9 ± 2.3	89.5 ± 14.7	0.6 ± 0.1	4.5 ± 0.8
	CEX	14.9 ± 2.8	91.3 ± 18.8	1.5 ± 0.2	12.0 ± 1.5
	AQ10	13.5 ± 2.6	80.5 ± 16.2	2.3 ± 0.6	14.5 ± 3.9
	Sulfo-li	12.2 ± 1.9	87.0 ± 14.0	1.9 ± 0.6	15.0 ± 4.3

References

Daubeze, A.M., Hennart, J.W. and Palloix, A. 1995. Resistance to *Leveillula taurica* in pepper (*Capsicum annuum*) is oligogenically controlled and stable in Mediterranean regions. Plant Breeding-Zeit. 114:327-332.

Elad, Y. and Shtienberg, D. 1994. Effect of compost water extracts on grey mould (*Botrytis cinerea*). Crop Protection, 13:109-114.

Fallik, E., Ziv, O., Grinberg, S., Alkalai, S. and Klein, J.D. 1997. Bicarbonate solutions control powdery mildew (*Leveillula taurica*) on sweet red pepper and reduce the development of postharvest friut rotting. Phytoparasitica, 25: 41-43.

Homma, Y. and Arimoto, Y. 1990. Mechanisms of plant disease control by potassium bicarbonate. Proceedings, 17th Int. Cong. on Pesticide Chemistry. 2: 94 (abstr).

Reuveni, R. and Rotem, J. 1973. Epidemics of *Leveillula taurica* on tomatoes and peppers as affected by the conditions of humidity. Phytopathol. Z. 76:153-157.

Reuveni, R., Dor, G. and Reuveni, M. 1998. Local and systemic control of powdery mildew (*Leveillula taurica*) on pepper plants by foliar spray of mono-potassium phosphate. Crop Protection, 17:703-709.

Tsror (Lahkim), L. (1999). Biological control of early blight in tomatoes. *Acta Horticulture*, 487:271-273.

Section V: Polyphagous predators

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Macrolophus caliginosus in the biological control of *Bemisia tabaci* in greenhouse melons

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Abstract: This work presents the results of an investigation that aimed to evaluate the effectiveness of releases of the predator *Macrolophus caliginosus* in the control of *Bemisia tabaci* on greenhouse melon. Releases of 6 *M. caliginosus* adults per plant were able to control *B. tabaci*. Results of the lower release rates (2 per plant) were more variable, presumably due to leaf thinning of the crop that may have affected predator establishment. No damaged fruit was recorded. Cage experiments with another zoophytophagous predator, *Dicyphus tamaninii*, indicated that melon fruits were not downgraded. Natural colonization of melons by this predator would therefore not interfere with IPM programs. Laboratory experiments were also carried out to evaluate the toxicity of fungicides commonly used for powdery mildew on survival of *M. caliginosus*. After seven days of exposure, no mortality was recorded for cyproconazole, kresoxim-methyl, nuarimol or triadimenol. Amitraz had low mortality, and sulfur dust had higher mortality after seven days. According to the OIBC toxicity categories, none were harmful to *M. caliginosus*.

Key words: biological control, integrated control, *Macrolophus caliginosus*, *Bemisia tabaci*, melon

Introduction

Bemisia tabaci (Gennadius) is an important melon crop pest in Spanish greenhouses and field crops. In both cases this is due to the development of important populations and the transmission of viral diseases. The development of biological controls for melon crops is therefore much needed. Moreover, in Spain, melon coexists with other neighbouring vegetable crops, and uncontrolled *B. tabaci* populations may enhance the risk of virus transmission and impede successful aplication of area-wide IPM programs (Gerling et al., 2001). Current work in southern Spain has recorded several whitefly parasitoids (Gonzalez Zamora et al., 1996) and predators, including the mirid *Macrolophus caliginosus* Wagner (Namesny, 1997). This predator is widely used on greenhouse tomatoes, but no known trials have assessed its effectiveness on greenhouse melons. These experiments were therefore carried out to determine the potential for using *M. caliginosus* to control *B. tabaci* on melon. Fruits were carefully observed because of the risk of some zoophytophagous mirids causing damage to certain crops (Alomar, 2002).

Another mirid, *Dicyphus tamaninii* Wagner, also spontaneously colonizes vegetable crops and is an effective whitefly predator. However, augmentative releases are not recommended because of the risk of damaging tomato (Lucas & Alomar, 2003). We also determined the risk of damage in order to determine whether natural *D. tamaninii* would interfere with melon IPM programs.

Finally, the success of IPM programs will also depend on using pesticides that are harmless to natural enemies. Powdery mildew is the most frequent melon disease in Spain, and requires frequent preventive treatments (Zafra & Martínez, 2001) that may affect predator

establishment. We therefore also determined the effect of several fungicides commonly used to control powdery mildew on the survival of *M. caliginosus*.

Material and methods

Whitefly control by *Macrolophus caliginosus*

Two successive melon crops were grown in large cages inside a glasshouse. The cages were meshed to prevent predator movement between treatments. Melon plants (cv. Galia, 24 plants per cage) were transplanted early in March (first trial) and July (second trial) in standard growing bags and tied to strings. Fert-irrigation was used to fertilize and irrigate the crop. Side shoots from the main stem were regularly pruned. Flowers were manually pollinated. Once two fruits had been pollinated, the leading shoot was pruned to concentrate on the formation of fruits. Ten adult *B. tabaci* per plant were released in each cage. Two predator release rates (2 and 6 per plant) and a control treatment (no predators released) were tested in both trials. Treatments were randomly assigned to each compartment in a Randomised Block Design with three replications. Whitefly and mirids were monitored weekly by counting their numbers on one middle leaf from six randomly chosen plants per cage. Fruits were observed for any signs of damage.

Potential feeding damage by *Dicyphus tamaninii*

The objective was to test whether *D. tamaninii* would damage young melon fruits. Plants were also grown in bags in small glasshouse compartments. After pollination (see above), flower receptacles (15 to 30 mm diameter) and a neighbouring leaf were individually caged in muslin bags. *Dicyphus tamaninii* adults (5 males and 5 females) were taken from laboratory colonies that were reared on tobacco with *Ephestia kuehniella* (Zeller) and whitefly as prey. After starving for 24 h they were then introduced into each cage for 24 h. In order to force them to feed on the fruit, no prey was present nor was any other food added. After one day, the fruit-cage was opened and all the mirids were removed. Other fruitlets were also caged, but without mirids, and served as controls.

Fungicide test

Table 1 lists the active ingredients tested, their trade names and formulations. Sulfur powder was also studied because of its recommended use against powdery mildew (Zafra & Martínez, 2001). All the ingredients except sulfur were applied together with a wetting agent (Mojante®, ArgEvo, polyethylenglycol nonylphenyl ether) at a rate of 1 cc per litre. Five melon plants per product were sprayed until run-off at the six leaf stage, using a hand held sprayer. Control plants were sprayed with only water and wetting agent. Once dried, one leaf was detached from each plant. Wet cotton wool was wrapped around the petiole, and it was placed in a glass tube filled with water to keep it moist. Each leaf was then isolated in a drum-cell test cage (van de Veire et al., 1996), with ten 5th instar *M. caliginosus* nymphs and *E. kuehniella* eggs as prey. Mirid nymphs were from laboratory colonies. The cells were kept in a controlled climatic chamber at 25°C and for a 16:8 h day:night photoperiod. Each treatment was repeated five times. Mortality was assessed 24 hours, 48 hours and 7 days after exposure and was corrected using Abbot's formula [(%treatment mortality - %control mortality) / (100-%control mortality)] x 100.

Results and discussion

Whitefly control by *Macrolophus caliginosus*

Figure 1 shows control of *B. tabaci* in both experiments as a percentage of final control populations. In the first trial, whitefly control was good for both *M. caliginosus* release rates. Both adult whitefly and pupae were considerably reduced in number with respect to the control cages (average of 24 whitefly adults and 56 pupae per leaf). In the second trial, the high predator release rate also successfully controlled whitely numbers with respect to the control treatment (average of 28 whitefly adults and 113 pupae per leaf). However, whitefly numbers were not controlled at the lower *M. caliginosus* release rate. Although *M. caliginosus* did establish in all predator cages, we also recorded a decrease in predator populations, particularly at the lower release rate. This may have been caused by periodic removal of new shoots, which is common practice in order to favour fruit growth. This de-leafing was especially important in the second trial, because higher temperatures promoted more vigorous plant growth. Excessive de-leafing has been shown to impede the establishment of *M. caliginosus* released on tomato (Ridray & Piron, 2003) and may also have affected our results at the lower release rate in the summer experiment. However, our results are promising and demonstrate the potential for using *M. caliginosus* to control whitefly on melon. No damage to melon fruits was recorded in either of both trials.

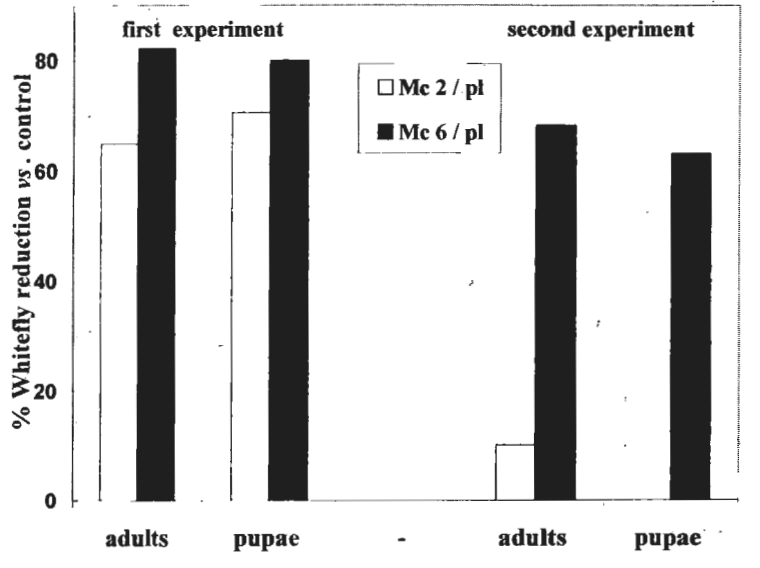

Figure 1. Percentage reduction in number of *Bemisia tabaci* adults and pupae compared with a control without predators in two experiments with two *Macrolophus* release rates (2 and 6 per plant).

Potential of feeding damage by *Dicyphus tamaninii*

Many of the 61 caged fruits did not mature due to the manipulation, but not due to the presence of the predator. After removing the *D. tamaninii* adults, most of the flower receptacles contained a few small red spots with a sticky exudate, but these soon disappeared and left no signs of any feeding. Of the 26 fruits with *D. tamaninii* that reached maturity, only two had small scars, while two others had small holes. However, these injuries were not easily seen, and all fruit was marketable without downgrading. This indicates that spontaneous colonization of melon crops by *D. tamaninii* would not interfere with IPM programs.

Effects of fungicide on *Macrolophus caliginosus*

Table 1 shows the products tested, doses applied, and resulting mortalities. After seven days of exposure, only 2% mortality was recorded in the control, and no mortality was recorded for cyproconazole, kresoxim-methyl, nuarimol or triadimenol. Amitraz had low mortality, while sulfur dust had some mortality rate after seven days. According to IOBC toxicity categories for laboratory testing of residual effects (from 'harmless', <30% mortality, to 'harmful', > 99% mortality (Sterk et al., 1999)), all these products should be classified as harmless and can therefore be used for IPM programs.

Table 1. Tested products and applied doses, and resulting mortality of 5th instar *Macrolophus caliginosus* nymphs after 24 hours , 48 hours, and 7 days.

Active ingredient	Trade name	% a.i. and formulation	Applied rate cc/l	% Mortality (Mean ± SE) [1]		
				24 h	48 h	7 d
Amitraz	Coyote	75 WG	3	2.0±2.00	6.0±4.00	5.31±3.63
Cyproconazole	Atemi	10 WG	0.3	0	0	0
kresoxim-methyl	Stroby	50 WG	3	0	0	0
Nuarimol	Cidorel	12 SC	0.4	0	0	0
Sulfur	Sublimado Flor	99 DP	dusted	2.0±2.00	6.0±4.00	19.23±5.23
Triadimenol	Bayfidan	25 EC	0.5	0	0	0

[1] corrected for control mortality

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References

Alomar, O. 2002: Facultative Predation as a Biological Control. In D. Pimentel (ed.), Encyclopedia of Pest Management. Marcel Dekker, Inc. New York, USA. http://www.dekker.com/servlet/product/DOI/101081EEPM120003824.

Gerling, D., Alomar, O. & Arnó, J. 2001: Biological control of *Bemisia tabaci* using predators and parasitoids. In S. Naranjo & P. Ellsworth (eds.), Special Issue: Challenges and Opportunities for Pest Management of Bemisia tabaci in the New Century. Crop Protection 20(9): 779-799.

Gonzalez Zamora, J.E.; Moreno Vazquez, R., Rodriguez Rodriguez, M.D., Rodriguez Rodriguez, M.P., Mirasol Carmona, E., Lastres Garcia-Teston, J. & Manzanares Ruiz, C. 1996: Evolucion del parasitismo en *Bemisia tabaci* (Genn.) y *Trialeurodes vaporariorum* (West.) (Homoptera; Aleyrodidae) en invernaderos de Almeria. Boletin de Sanidad Vegetal, Plagas 22 (2): 373-389.

Lucas, E. & Alomar, O. 2003: Impact of *Macrolophus caliginosus* presence on damage production by *Dicyphus tamaninii* (Heteroptera: Miridae) on tomato fruits. J. Econ. Entomol. 95(6): 1123-1129.

Namesny A. (ed). 1997: Melones. Compendios de Horticultura num. 10. Ediciones de Horticultura S.L., Reus, Spain.

Ridray, G. & Piron, M. 2003: Le côte obscur des effeuillages sur *Macrolophus*. PHM-Revue Horticole 445: 33-37.

Sterk G., Hassan S.A., Baillod M., Bakker F., Bigler F., Blumer S. Bogenschutz H., Boller E., Bromand J., Brun J., Calis J.N.M., Coremans-Pelseneer J., Duso C., Garrido A., Grove A., Heimbach U., Hokkanen H., Jacas J., Lewis G.B., Moreth L., Polgar L., Rovesti L., Samsoe-Petersen L., Sauphanor B., Schaub L., Staubli A., Tuset J.J., Vainio A., Van de Veire M., Viggiani G., Viñuela E., & Vogt H. 1999: Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". Biocontrol 44: 99-117.

van de Veire, M., Smagghe, G. & Degheele, D. 1996: Laboratory test method to evaluate the effect of 31 pesticides on the predatory bug, *Orius laevigatus* (Het.: Anthocoridae). Entomophaga 41(2): 235-243.

Zafra, J.A. & Martínez, J. 2001: Los cuidados del melón frente a las principales plagas y enfermedades. Vida Rural, 138.

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Nymphal development of three *Orius* species reared on eggs of *Ephestia kuehniella* Zeller.

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Abstract: Little information is currently available about the *Orius* species from the Neo-tropical Region: their occurrence, biological characteristics and their potential as biological control agents. The present work aims to evaluate nymphal development in three species found in agroecosystems in Brazil: *Orius thyestes*, *Orius perpunctatus* and *Orius insidiosus*. The experiment was conducted in a climatic chamber at 25±1°C, with RH 70±10% and with a 12hr photophase. Twenty-five newly-hatched nymphs from each species were individualy kept in Petri dishes (5cm diameter) and fed on *Ephestia kuehniella* eggs. All species presented five nymphal stages and completed their development on *E. kuehniella* eggs. *O. perpunctatus* had the shortest development time (12.1 days). No significant differences were observed between the development times for *O.thyestes* (13.4 days) and *O. insidiosus* (12.7 days). Nymphal survival rates were 92, 88 and 96% for *O. thyestes*, *O. perpunctatus* and *O. insidiosus*, respectively. These *Orius* species will be considered in future studies in order to evaluate their potential as biological control agents for thrips in protected cultivation in Brazil.

Key words: development time, nymphal survival, biological control agent, *Orius* spp.

Introduction

Several *Orius* species have been evaluated and used as effective control agents, especially of thrips, in Europe, USA and in Canada. In Brazil, the reports of *Orius* species are few, though the most common, *Orius insidiosus* (Say), is found on several crop and weed plants (Bueno, 2000).

Information about different aspects of the *Orius* species mostly comes from the Paleartic Region. Only a few species from the Neo-tropical Region are described (Herring, 1966) and there is little information about many aspects of these species (Lattin 2000). There are many questions concerning the potential uses of *Orius* species, such as basic studies of their biology and behavior, nutrition requirements, habitat, mass rearing and quality control.

Silveira *et al.* (2001) reported the first occurrence of *Orius thyestes* (Herring) in Brazil, in association with the *Frankliniella* genus. Herring (1966) reported the occurrence of *Orius perpunctatus* (Reuter) in the states of Santa Catarina and Minas Gerais in Brazil, and described *O. thyestes* as a new species whose holotype was found in Colombia.

According to Silveira (2003), *O. thyestes, O. perpunctatus* and *O. insidiosus* occur simultaneously with several thrips species, including *Frankliniella* sp., *Neohydatothrips* sp. and *Haplothrips gowdeyi* (Franklin). They are found on both crop (corn and soybean) and weed plants. Different *Orius* species may therefore be found in different ecological niches of the same habitat, which would make them potentially useful as biological control agents.

The purpose of this work was to evaluate the nymphal development of *O. thyestes*, *O. perpunctatus* and *O. insidiosus* supplied with eggs of *Ephestia kuehniella* Zeller as prey. This study is part of a research program whose purpose was to select one or more species to release on crops under protected cultivation, especially where thrips were the key pest.

Material and methods

The experiment was carried at the Biological Control Laboratory, Entomology Department, Federal University of Lavras, in a controlled conditions chamber at 25±1°C, with a 12hr photophase and 70 ± 10% RH. The insects used in the experiment were obtained from adults collected from the outdoor weeds *Amaranthus deflexus*, (L) and *Bidens pilosa* (L). Adults from each species were kept in Petri dishes (15 cm diameter) sealed with polyethylene film. *E. kuehniella* sterile eggs were supplied as food every two days and *B. pilosa* inflorescence was used as a substrate for oviposition. The inflorescences were disinfected for 20 minutes in sodium hypochlorite (0.5%) before contact with the predator.

The experimental outline used was a random design with three treatments, represented by the different species, and with twenty-five newly-hatched nymphs (less than 24 hours old) from the F_2 generation of each species as replicates. These were individualized in Petri dishes (5 cm diameter), sealed with polyethylene film, with eggs of *E. kuehniella* as prey and a piece of moist cotton to provide humidity.

The number of instars, individual instar survival, total nymph survival and development time were all recorded on a daily basis. Variance analysis was performed for all the parameters evaluated and the averages were submitted to the Scott & Knott (1974) test at 5% significance.

Results and discussion

All species showed five stages of development, which they completed feeding on the *E. kuehniella* eggs. According to Eubanks & Denno (2000), Lepidoptera eggs have a high nutritional quality, mainly due to their high nitrogen concentration, which furthers the development of most generalist predators.

O. thyestes showed a longer duration at the first instar, compared with the other species (Table 1). No significant differences were noted between the durations of the 2nd, 3rd and 4th instars of the three species studied. However, the fifth instar of *O. perpunctatus* lasted longer than those of *O. thyestes* and *O. insidiosus*. Mendes *et al.* (2002) respectively obtained 2.9, 2.1, 2.0, 2.1 and 3.9 day durations for the 1st, 2nd, 3rd, 4th and 5th instars of *O. insidiosus* supplied with *E. kuehniella* eggs.

The *Orius* species had a high survival rate in all instars. *O. thyestes, O. perpunctatus* and *O. insidiosus* had survival rates of 92%, 92% and 96%, respectively, at the first instar. The mortality rate, which ranged from 4 to 8%, may have been related to the small size of the 1st instar nymphs: on average 0.50mm (*O. insidiosus*) (Isenhour & Yeargan, 1981), which made it difficult for them to handle the prey. The need to adapt to a new nutritional regime could have been another factor. The survival rate for the remaining instars was generally 100%, though *O. perpunctatus* had a survival rate of 96% for the 5th instar.

The shortest period for nymphal development was observed for *O. perpunctatus* (12.1 days). There was no significant difference between the development times for *O. thyestes* and *O. insidiosus*: 13.4 and 12.7 days, respectively (Figure 1). Comparative studies between two species of *Orius* were performed in Europe by Zaki (1989), who showed that the development time for *Orius albidipennis* (Reuter) was 11.1 days and that for *Orius laevigatus* (Fieber) was 14.8 days, when both were fed on *E. kuehniella* eggs. In Brazil, Mendes *et al.* (2002) observed a development time of 13.1 days when *O. insidiosus* was also fed on *E. kuehniella* eggs.

Table 1 – Duration (days) of each instar of *Orius thyestes, Orius perpunctatus* and *Orius insidiosus* fed on *Ephestia kuehniella* eggs (mean ± SE).

Species	1st instar	2nd instar	3rd instar	4th instar	5th instar
O. thyestes	2.8 ± 0.09a	2.1 ± 0.05a	2.0 ± 0.05a	2.2 ± 0.10a	3.5 ± 0.14b
O. perpunctatus	1.9 ± 0.10b	2.4 ± 0.11a	2.1 ± 0.11a	2.2 ± 0.14a	4.2 ± 0.26a
O.insidiosus	2.6 ± 0.09b	2.4 ± 0.10a	2.0 ± 0.08a	2.0 ± 0.10a	3.4 ± 0.10b
	F= 14.682	F= 2.042	F= 0.359	F= 1.316	F= 5.806
	P<0.0001	P<0.1409	P<0.7003	P<0.2776	P<0.005

* Means followed by the same letter in the same column do not differ from one another by the Scott and Knott test at 5% significance.

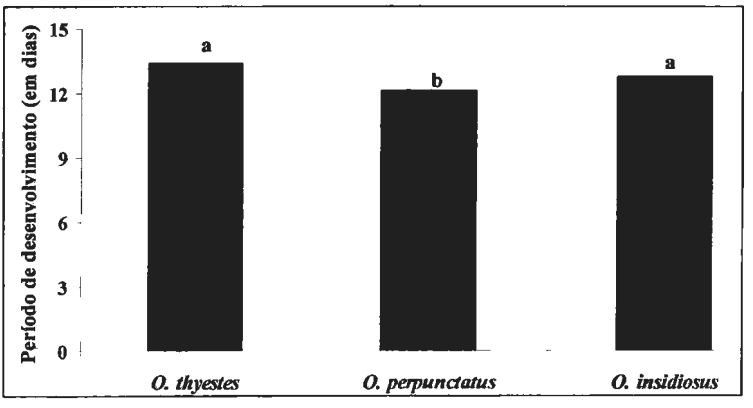

Figure 1. Nymphal development (days) of *Orius thyestes, Orius perpunctatus* and *Orius insidiosus* fed *Ephestia kuehniella* eggs. Means that appear in different columns followed by the same letter do not differ from one another by the Scott and Knott test at 5% significance.

Nymphal survival rates for *O. thyestes. O. perpunctatus* and *O. insidiosus* were 92.88 and 96%, respectively. Chyzik *et al.*, (1995) obtained a survival of approximately 85% for *O. albidipennis* nymphs, when fed on *Ephestia kuehniella* eggs. Mendes *et al.* (2002) showed that a diet based on *E. kuehniella* eggs was recommendable for the development of the *O. insidiosus* due to its high nutritional quality. Kiman & Yeargan (1985) reported that Lepidoptera eggs are appropriate prey onwhich to rear *O. insidiosus,* mainly due to the associated shorter development time and higher nymphal survival rate.

We should highlight the fact that all three *Orius* species were able to complete their respective development cycles when fed on *E. kuehniella* eggs and that this would seem to facilitate mass rearing under laboratory conditions. Even so, further studies need to be performed in order to confirm both the reproductive parameters and the potential use of these predators as biological control agents, especially in the case of thrips on protected crops in Brazil.

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References

Bueno, V. H. P. 2000. Desenvolvimento e multiplicação de percevejos predadores de gênero *Orius* Wolff. In: Controle Biológico de pragas: Produção Massal e Controle de qualidade, ed. Bueno: 69-90.

Chyzik, R.; Klein, M.; Ben-Dov, Y. 1995. Reproduction and survival of the predatory bug *Orius albidipennis* on various arthropod preys. Entomol. Exper. Applic. 75: 27-31.

Eubanks, M.D., Denno, R. F. 2000. Health food versus fast food: the effects of prey quality and mobility on prey selection by a generalist predator and indirect interactions among prey species. Ecol. Entomol. 25: 140-146.

Herring, J. L. 1966. The genus *Orius* of the western hemisphere (Hemiptera: Anthocoridae). Ann. Soc. Amer. 59: 1093-1109.

Isenhour, D.J. Yeargan, K.V. 1981. Effect of temperature on the development of *Orius insidiosus,* with note on laboratory rearing. Ann. Entomol. Soc. Am.74: 114-116.

Kiman, Z.B.; Yeargan, K.Y. 1985. Development and reproduction of the predator *Orius insidiosus* (Hemiptera: Anthocoridae) reared on diets of selected plant material and arthropod prey. Ann. Entomol.Soc.Am. 78: 464-467.

Lattin, J. D. 2000. Economic importance of minute pirate bugs (Anthocoridae). In: Heteroptera of economic importance, eds. Shaefer & Panizzi : 607-637.

Mendes, S.M., Bueno, V.H.P., Argolo, V.M., Silveira, L.C.P. 2002. Type of prey influence biology and consumption rate of *Orius insidiosus* (Say) (Hemiptera: Heteroptera). Rev. Brasil. Entomol. 46: 99-103.

Scott, A. J.; Knott, M. A. 1974. A cluster analyses method for grouping means in the analyses of variance. Biometrics, 30: 507-512.

Silveira, L.C.P.; Bueno, V.H.P.; Mendes, S.M.; Louzada, J.N.C. 2001. *Orius* spp. (Hemiptera: Anthocoridae): habitat e associação com tripes (Thysanoptera). VII Siconbiol. Poços de Caldas-MG. p.327.

Silveira, L.C.P. 2003. Registro e associação de espécies de *Orius* Wolff com tripes, influência do fotoperíodo na reprodução e avaliação de *Orius insidiosus* (Say, 1832) (Hemiptera: Anthocoridae) no controle biológico de tripes (Thysanoptera) em casas-de-vegetação. Lavras-MG: Ufla. 103p. PhD Thesis.

Zaki, F. N. 1989. Rearing of predators, *Orius albidipennis* (Reuter) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) on some insect larvae. J. Appl. Entomol. 107: 107-109.

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Potential risk of damage to zucchinis caused by mirid bugs

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Abstract: Mirid bugs are used in inoculative and conservative biological control strategies on several crops in the Mediterranean area. Due to their zoophytophagous habits, this may imply some risk of crop damage, as has been shown with tomatoes and gerberas. In this work, we studied the potential risk of damage that *Macrolophus caliginosus* and *Dicyphus tamaninii* may cause to zucchini fruits. When high densities of mirid bugs were confined in the apical part of the plant, dimples and pits of various sizes appeared in young fruits. Releasing high densities of either of the predator species tested in exclusion compartment experiments resulted in up to ten percent of unmarketable fruits. Further experimentation is needed, as it is not clear whether the presence of abundant prey prevents damage to fruits. Due to the potential risk of damaging young fruits, the release of these predators is not recommended as a biological control strategy for this crop.

Key words: mirid bugs, *Macrolophus caliginosus, Dicyphus tamaninii,* fruit damage, zucchini.

Introduction

In the Mediterranean region, there is a predatory complex of mirid bugs with zoophytophagous habits that play an important role in pest regulation on vegetable crops. They are used in inoculative and conservative biological control strategies, mainly on tomato crops, and are appreciated by growers because their polyphagy allows them to control more than one pest at the same time. This is important for vegetable crops that simultaneously have several key and secondary pests. Their populations colonize different protected and open-field vegetables since they are abundant on both native flora and commercial crops.

Damage to tomatoes by some species of the predatory complex has been described to take the form of punctures in green fruits due to *Dicyphus tamaninii* Wagner and tissue necrosis around flower petioles due to *Nesidiocoris tenuis* Reuter (Alomar & Albajes, 1996; Wheeler, 2001). In gerbera, damage to flowers by *Macrolophus caliginosus* Wagner has been mentioned by Van Schelt *et al.* (1996). This damage is mainly associated with the scarcity of prey and large predator population.

Therefore, before considering this predatory complex for release or using it as part of conservation strategies, it is important to evaluate the risk of crop damage due its feeding habits. Zucchini is a crop that supports large populations of whiteflies, a preferred prey of mirid bugs: both *M. caliginosus* and *D. tamaninii* have been observed colonizing the crop in our area. In this work we evaluated the susceptibility of this crop to fruit damage caused by these two species.

Material and methods

Description of damage

In a confinement experiment, 25 *D. tamaninii* or *M. caliginosus* adults were introduced into a muslin cloth bag that covered the apical part of zucchini plants (*cv* Diamant and Afrodita).

Few prey were observed inside the covered part of the plant. After 5 days, predators were eliminated with an insecticidal treatment and, in the following two weeks, fruits produced by these plants were checked for quality. Six plants were treated per mirid species and the experiment was repeated during the crop season.

Greenhouse trials

Two experiments were performed using predator exclusion compartments. In the first, we evaluated the respective incidence upon fruit quality of releasing 10 *D. tamaninii,* 10 *M. caliginosus* adults and no predators (control) per plant. In the second experiment, the treatments tested were 10 *M. caliginosus* adults, 3 *M. caliginosus* adults and no predators. The experiments were performed in a 450 m^2 greenhouse, which was divided into 12 compartments with 24 plants in each compartment. After transplanting, twenty greenhouse whitefly adults were introduced onto each plant as prey.

Surveys on commercial crops

Several outdoor crops were periodically monitored for mirid bug abundance and the presence of damaged fruits. A total of 10 fields were visited and 30 plants per crop were examined for the presence of mirid bugs and damaged fruits on a fortnightly basis.

Results

Damage description

In a very artificial situation, such as the one represented by this confinement experiment, we were able to see the different types of lesions that *M. caliginosus* and *D. tamaninii* adults may cause to fruits. The total number of fruits harvested in the experiments were 78 and 77 respectively and these fruits showed important injuries in almost 20% of the yield due to the two mirid species.

Typical damage consisted of dimples and pits of various sizes that sometimes left the endocarp visible. Some fruits were also distorted in an L shape. In many cases a gummy exudate of a light-brown colour was also found, especially in very young fruits. No significant differences were observed between the two cultivars with respect to the amount of damaged fruit and type of fruit damage.

Greenhouse trials

When 10 adult mirid bugs were released in the exclusion compartments at the start of the crop, damaged fruits accounted for 11% of the yield in the *D. tamaninii* treatment and 2.2% in the *M. caliginosus* treatment (figure 1). There was an abundance of prey during the experiment because, although greenhouse whitefly did not establish in the crop, there was a spontaneous population of cotton aphid, *Aphis gossypii.*

In the second experiment in the exclusion compartments, damaged fruit accounted for 10 percent of the yield in the case of 10 *M. caliginosus* adults and 4.6% in the case of 3 *M. caliginosus* adults per plant (figure 2). Again, the greenhouse whitefly did not establish and there was an even greater spontaneous population of cotton aphid at the end of the crop. This also explains the lower number of fruits harvested in this experiment.

Surveys on commercial crops

We found mirid bug populations in five of the ten outdoor crops surveyed, with densities of 0.45 to 3.8 individuals per plant. The main species found were *M. caliginosus* and *D. tamaninii.*

Mirid-damaged fruits (not necessarily unmarketable) ranged form 2.6 to 9.6% of those present in the upper part of the plant.

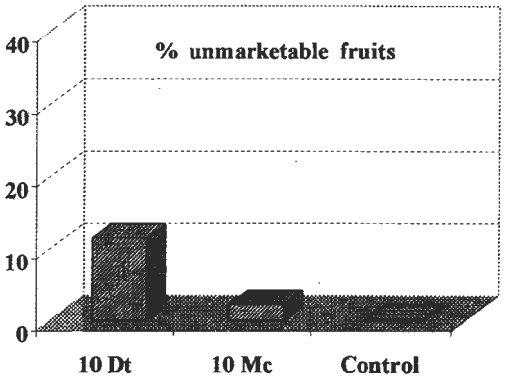

Figure 1. Percentage of unmarketable zucchini fruits after releasing 10 *D. tamaninii* (Dt) adults, 10 *M. caliginosus* (Mc) adults and no mirids (Control) in an experiment with exclusion compartments. The total number of fruits harvested were 363, 417 and 404, respectively, for the different treatments.

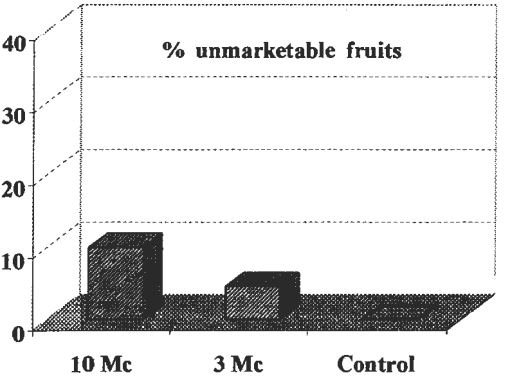

Figure 2. Percentage of unmarketable zucchini fruits after releasing 10, and 3 *M. caliginosus* (Mc) adults and no mirids (Control) in an experiment with exclusion compartments. The total number of harvested fruits were 30, 43 and 27, respectively, for each of the treatments.

Discussion

The amount of damage caused by mirid bugs to the zucchini fruits in these experiments was quite variable, probably because many different factors can influence their appearance. This

damage was produced by high predator populations: these are not common in commercial crops. The percentage of damaged fruits in the confinement experiment was obtained in a setting that forced predators to feed upon the plant since they could not move from the apical part of the plant and few prey were available. In the exclusion compartment experiments, predators were not forced to feed on the plant, as they had been in the previous experiment, and could move from plant to plant in search of prey, but, they were not able to leave the compartment. This was also an artificial setting and quite different from commercial greenhouses, because Mediterranean region greenhouses have ventilation openings that allow predators to freely move in and out. These predators are very active in their movements from crop to crop, as has been shown for spring greenhouse tomatoes (Gabarra *et al.* in press). Although we introduced adult whitefly as prey, the pest established poorly in the two exclusion compartment experiments and the cotton aphid was the main available prey. The abundance of whitefly is a factor that prevents *D. tamaninii* damage to tomatoes (Alomar, O. & R. Albajes, 1996), but it is not known whether this relationship also applies with the cotton aphid or for crops other than tomatoes.

In conclusion, there is a risk of fruit damage to zucchinis when high populations of the two mirid bugs, *M. caliginosus* and *D. tamaninii,* are confined to the crop. These predators are therefore not recommended as natural enemies for controlling zucchini pests in a release strategy. In cases of spontaneous colonization of crops by these predators, some surveillance of their populations is required, especially when they are near a tomato cropping area in which mirid bugs are used for biological control. If mirid predator numbers reach high levels and damage is observed in young fruits, an insecticidal treatment with a short residual life could be used in order to reduce their numbers.

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References

Alomar, O. & Albajes, R. 1996. Greenhouse whitefly (Homoptera: Aleyrodidae) predation and tomato fruit injury by the zoo-phytophagous predator *Dicyphus tamaninii* (Heteroptera: Miridae), pp. 155-177. *En* O. Alomar & R. Wiedenmann, (eds), Zoophytophagous Heteroptera: Implications for Life History and IPM. Thomas Say Special Publications in Entomology, Entomological Society of America.

Gabarra, R., Alomar, O., Castañé C., Goula, M. & Albajes, R. Movement of greenhouse whitefly and its predators between in and outside of Mediterranean greenhouses. Agriculture, Ecosystems & Environment (in press).

Van Schelt, J., Klapwijk, J., Letard, M. & Aucouturier, C. 1996. The use of *Macrolophus caliginosus* as a whitefly predator in protected crops. In: Bemisia: 1995 taxonomy, biology, damage, control and management. Eds. Gerling, D. and Mayer, R.T., pp 702.

Wheeler, A.G. 2001. Biology of plant bugs (Hemiptera: Miridae). Pests, predators, Opportunists. pp 507.

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Biological characteristics of *Macrolophus caliginosus* (Hemiptera: Miridae) when feeding on the non-cultivated plant *Dittrichia viscosa* (Asteraceae)

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Abstract: *Macrolophus caliginosus* Wagner (= *M. melanotoma* Costa) is a polyphagous predator that is commonly used against whiteflies and aphids in vegetable crops. Large numbers of this species have been recorded on several non-cultivated plants found growing on the edges of fields. Of these plants, *Dittrichia viscosa* (L.) W. Greuter is the main host for *M. caliginosus* and contributes considerably to its maintenance in the agro-ecosystem, its winter survival, and its colonization of neighbouring vegetable crops.

The present study investigated the biological characteristics of *M. caliginosus* on *D. viscosa* in the presence of the aphid *Capitophorus inulae* (Passerini), a species commonly found in large numbers on this host plant. The ability of this predator to develop and reproduce on *D. viscosa* leaves without prey was also examined. Experiments were conducted at 25°C, 65±5%RH and with a 16L:8D photoperiod.

Development of *M. caliginosus* nymphs was completed in both the presence and absence of prey, with high rates of survival. The period of nymphal development was significantly shorter in the presence than in the absence of prey. Fecundity was much higher in the presence than in the absence of prey. Female longevity was not significantly affected by the availability of prey, whereas males survived much longer in the presence of prey.

According to the results obtained, *M. caliginosus* can successfully develop and reproduce on *D. viscosa* in the presence of prey species naturally found on this plant. Furthermore, it can complete its development and oviposite a small number of eggs when feeding only on the plant sap of *D. viscosa*. *D. viscosa* is therefore a suitable host plant for development and reproduction of *M. caliginosus*. These results give an insight as to why large numbers of *M. caliginosus* are recorded on *D. viscosa* and indicate the important role of this plant in the conservation or/and increase of *M. caliginosus* populations in the agro-ecosystem.

Taxonomic identity of species in the *Dicyphus hyalinipennis* group (Heteroptera, Miridae)

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Abstract: A correct taxonomic identification of predators is of paramount importance in biological control for both research and the transfer of results to IPM technicians. *Dicyphus* spp. are zoophytophagous mirid bugs that are well known for their role in the control of several horticultural crop pests in Europe. The genus is organized into 4 subgenera and includes more than 20 Mediterranean species. Within subgenera, species can be grouped according to their morphology. In this poster, we focus on the species of the *Dicyphus (Dicyphus) hyalinipennis* group, four of which have been reported as generalist crop predators. The objective of our work was to present a state-of-the-art clarification of the identity of all of the Mediterranean species belonging to the *D. (D.) hyalinipennis* group. General external features (colour, size) are not reliable when identifying *Dicyphus* species, so the main characteristics to be taken into account were biometry (i.e. the relative proportions of certain parts of the body) and the characteristics of the male genitalia. The validity of these characters is discussed for each species.

Key words: *Dicyphus*, Miridae, pest predators, horticultural crops, Mediterranean region, taxonomic characters

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Influence of the presence/absence of males in the oviposition of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae)

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Abstract: Biological studies of *O. insidiosus* have been carried out, but little information is available about its reproductive behavior in different mating conditions. This work aimed to evaluate the following parameters: (1) unmated females, (2) females kept with the male all the time, (3) females kept with the male until the beginning of the oviposition, (4) females kept with the male until the beginning of the oviposition and then kept with the male every seven days. No significant differences in the daily number of eggs (4.01 eggs), eggs hatching (80%) and in sexual ratio (0.52) were found for mated females in all the conditions evaluated. However, the total number of eggs and longevity were lower (23.7 eggs and 13.8 days) for females kept with the male all the time than for females kept with the male until the beginning of the oviposition (39.4 eggs and 17.5 days). The highest total number of eggs (45.2 eggs) and longevity (20 days) were found for females kept with the male until the beginning of the oviposition and kept with the male every seven days. The presence of the male affected the total oviposition capacity and longevity of females of *O. insidiosus*, which interferes with the mass rearing of such a predator.

Key words: mating behavior, predator, longevity, mass rearing.

Introduction

Among the predators of the *Orius* genus, *Orius insidiosus* (Say) is the most common species in Brazil (Bueno 2000). However, not much research concerning this insect has been carried out. The need for biological studies on most of the species of this genus, especially on those from the Southern Hemisphere, is also reported by Lattin (2000). Thus, several questions concerning such predators need to be clarified, one of which is their reproductive behavior in different mating conditions.

It is known that the mass, economic and effective rearing of such predators in laboratory conditions is closely connected to the characteristics of the species, which may influence its reproductive potential. According to Chapman (1998), the possible nutrient effects that accompany sperm transfer in many insects; copulation may also be a trigger for oviposition and sometimes oogenesis. Virgin females do not usually lay eggs. Matting or experimental injection of a component of male accessory glands causes them to oviposit.

The main purpose of this work is to evaluate the effect of different mating conditions on the ability to oviposit of *Orius insidiosus*, which, along with other results from ongoing research in Brazil, will be the support for predator mass rearing, and its consequent evaluation as a biological control agent of thrips in protected cultivations.

Material and methods

The research was carried out in the Biological Control Laboratory of the Entomology Department at the Federal University of Lavras, Brazil. The experiments were carried out in controlled conditions, at 25 ± 1°C, 70±10% RH and 12 hr photophase.

The predator's rearing was performed according to the methodology proposed by Mendes & Bueno (2001). The nymphs were kept together until the 5th instar, and then they were individualized in a Petri dish (5 cm diameter) until they reached the adult stage. Those adults (males and females) were used in the experiments.

The following conditions were tested: (1) unmated females, (2) females kept with the male all the time, (3) females kept with the male until the beginning of the oviposition, (4) females kept with the male until beginning of the oviposition and then kept with the male each seven days.

In all conditions, eggs from *Ephestia kuehniella* Zeller"*ad lilbitum*" were supplied as a source of food and a *Bidens pilosa* L. inflorescence was used as a substrate for oviposition. The food and the oviposition substrate were supplied three times for week.

The evaluations were made three times for week, with the aid of a stereoscopic microscope. The number of eggs per female was recorded. The eggs were kept in a Petri dish (20 cm of diameter) and the nymphs' eclosion was observed; they remained together in the container until the adult stage.

The daily and total number of eggs/female, the egg viability, the sexual ratio as well as the female longevity were evaluated. The data were submitted to variance analysis and further averages tests (Scott & Knott, 1974).

Results and discussion

Unmated females of *O. insidiosus* did not oviposit and showed an average longevity of 21.3 days (Table 1). Ito & Nakata (1998) also observed such behavior among females of *Orius sauteri* (Poppius) and *Orius minutus* (L.).

All the females of *O. insidiosus* (100%) submitted to mating oviposited (Table 1). Ito & Nakata (1998) verified that 91.7% and 93.8% of mated females of *O. sauteri* and *Orius minutus* respectively oviposited. Such behavior may be an indication that mating in this species of predator is essential for the egg fertilization and for the oviposition unchaining. According to van Lenteren (1999), the oogenesis, for the majority of insects, is under the control of external cues, via the neuroendocrine system, such as mating.

For other predator insects, such as the green lacewing, the females are able to oviposit without mating; however, the eggs are sterile (Barbosa et al, 2002). According to Aldrich (1998), *O. insidiosus* exhibits a pronounced sexual dimorphism of the metathoracic scent gland secretion. However, pheromones are unknown for minute pirate bugs, but this situation probably represents a lack of research attention rather than a biological reality.

The presence of the male did not influence the daily number of eggs/female of *O. insidiosus* (Table 1), suggesting that the presence of males close to the females of such predator does not influence the stimulus to oviposition; the opposite situation occurs in green lacewing predators, where the presence of males leads the females to lay more eggs (Barbosa et al, 2002).

Table 1 – Daily and total number of eggs/female and longevity of females of *O. insidiosus* under different mating conditions.

Mating conditions	N	Number of eggs		Longevity
		Daily	Total	
Unmated Females	37	-	-	21.3 ± 0.97 c
Females kept with males	49	3.1 ± 0.27 a	23.7 ± 3.14 a	13.8 ± 0.59 a
Females with males until the beginning of oviposition	41	3.1 ± 0.36 a	39.4 ± 6.11 b	17.5 ± 0.91 b
Females kept with males each 7 days	45	2.8 ± 0.35 a	45.2 ± 7.80 b	20.5 ± 1.23 c

Means followed by the same letter in columns are not different from one another in the Scott & Knott ($P \leq 0.05$) test.

The total number of eggs/female of *O. insidiosus* was lower for females in the constant presence of the male (23.7 eggs) (Table 1) than for females under the different mating conditions. For females kept in the presence of the male until the beginning of oviposition and for females kept with the male every seven days, there was no difference in the total number of eggs (39.4 and 45.2 eggs respectively) (Table 1). These results may show that the fertilization at the beginning of the oviposition period is enough for the female of *O. insidiosus* to lay eggs, or the amount of sperm that the female acquires, once copulated, at the beginning of the adult phase, which could be stored in its spermatheca, is adequate for the oviposition throughout her life.

There was no significant difference in either the egg viability [average of 80% ($F= 3.787$ and $P>0.0413$)], or in the sexual ratio [0.52 ($F = 0.844$ $P> 0.05$)] of the females progeny under the different mating conditions.

The longevity of the females was influenced by different mating conditions (Table 1). For females kept in the constant presence of the male, the longevity was shorter than for other mating conditions (13.8 days). Such behavior may be explained by the individual stress conditions, since the small size of the container in which they were kept (5cm in diameter) may have furthered the number of meetings and contacts between males and females of *O. insidiosus*. According to Schmidt et al. (1998), fighting is regular behavior between adults of *O. insidiosus* and the most extended meetings follow a sort of battle, and the possible outcome is the monopoly of the forage area. Another factor that might lead to stress and to the consequent decrease in longevity is the mating frequency, since, according to Askari & Stern (1972), the females of *Orius insidiosus* always accept the male for mating.

The results regarding the reproductive capacity of *O. insidious* were great for females kept in the presence of the male until the beginning of oviposition, and for those kept with the male every seven days, once there was no influence on the nymphs' eclosion and on the sexual ratio. Such parameters may contribute to future studies on predator mass rearing. The information about reproductive behavior of *O. insidiosus* could be fundamental to the selection and evaluation studies of this predator as control agents in biological control programs.

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References

Aldrich, J. R. 1998. Status of semiochemical research on predatory Heteroptera.. In: Predatory Heteroptera: Their ecology and use in biological control, eds Coll & Ruberson : 21-32.

Askari, A. & Stern, V. M. 1972. Biology and feeding habits of *Orius tristicolor* (Hemiptera: Anthocoridae). Ann. Entomol. Soc. Amer. 65: 96-100.

Barbosa, L. R.;Freitas, S. & Auad, A. M. 2002 Capacidade Reprodutiva e viabilidade de ovos de *Ceraeochrysa everes* (Banks, 1920) (Neuroptera: Chrysopidae) em diferentes condições de acasalamento. Ciênc.agrotec. 26: 466-471.

Bueno, V. H. P. 2000. Desenvolvimento e multiplicação de percevejos predadores do gênero *Orius* Wolff. In Controle biológico de pragas: Produção massal e controle de qualidade, ed. Bueno: 60-90.

Chapman, R. F. 1998. The insects Structure and Function. 4th edition. 768p.

Ito, K & Nakata, T. 1998. Effect of photoperiod on reproductive diapause in the predatory bugs, *Orius sauteri* (Poppius) and *O. minutus* (Linneaus) (Heteroptera: Anthocoridae). Appl. Entomol. Zool., 33: 115-120

Lattin, J. D. 2000. Economic importance of minute pirate bugs (Anthocoridae). In: Heteroptera of economic importance, eds. Schoefer & Panizzi : 607-637.

Mendes, S. M. & Bueno, V. H. P. 2001. Biologia de *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) alimentado com *Caliothrips phaseoli* (Hood) (Thysanoptera: Thripidae). Neotrop. Entomol. 30: 423-428.

Schmidt , J .M.; Taylor, J. R. & Rosenheim J. A. 1998. Cannibalism and intraguild predation in the predatory Heteroptera. In: Predatory Heteroptera: Their ecology and use in biological control, eds. Coll & Ruberson: 133-169

Scott, A. J.& Knott, M. A. 1974. A cluster analyses method for grouping means in the analyses of variance. Biometrics, 30: 507-512.

Van Lenteren, J. C. 1999. Fundamental knowledge about insect reproduction: essential to develop sustainable pest management. Invertebrate Reproduction and development 36:1-3.

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Abundance and wild host plants of predator mirids (Heteroptera: Miridae) in horticultural crops in the Southeast of Spain.

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Abstract: Prospecting for predator mirids with interest in biological pest control on horticultural crops was carried out during 2002 and 2003 in the province of Murcia (southeast Spain) to determine species composition and abundance and host plants. To date little information of this kind was available for the southeast of Spain. Tomato, pepper, squash and eggplant were among the crops more frequently sampled. The most abundant mirids were *Macrolophus* sp., *Nesidiocoris tenuis*, *Dicyphus cerastii*. *Dereaocoris punctulatus* was frequently found but at a low number. *Dicyphus tamanii* was occasionally found. About 100 wild plants belonging to 30 families were sampled in the surrounding of the crops. *Macrolophus* sp. was very abundant on *Dittrichia viscosa*, *Marrubium vulgare*, *Ononis natrix* and *Carduus* sp.; it was found very occasionally on *Tamarix canariensis*. *Dicyphus* was found at a low number on *Withania frutescens Ononis natrix* and *Erodium petraeum*.

Key words: *Macrolophus*, *Nesidiocoris*, *Deraeocoris*, *Dicyphus*, whitefly, biological pest control.

Introduction

Mirids (Hemiptera: Miridae) are one of the most abundant predators in some horticultural crops in the Mediterranean area. A few species are commercially available and commonly released to control whiteflies. However, there are others from the local fauna that colonize the crops spontaneously, which activity as pest control agents depends to a great extent on the health of the surrounding environment and crop management practices (Fauvel, 1999). The advantages of including these natural enemies into integrated pest management (IPM) programmes are, among others, their perfect adaptation to the local environmental conditions and the natural pest control they provide for free.

In the south of Spain control of whitefly (*Trialeurodes vaporariorum* Westwood,) and *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae)) on tomato is mainly done by chemical treatments. The high incidence of virus diseases transmitted by these two whiteflies (TYLCV, TYLCSV, TICV and ToCV), and the high intensification of agriculture are some of the reasons of the lack of diffusion of biological control on tomato. Nowadays, difficulties to control whiteflies by chemical methods, the consumer demand for biological products, yield residue reduction, etc., make it necessary to look for new alternatives. Strategies of conservation and promotion of crop colonization by mirid predators have been reported to provide a good control of whiteflies on tomato in Catalonia (northeast Spain) (Arnó *et* al., 2000). However, the implementation of conservation strategies requires to deepen in some aspects of the biology of the insects such as, population dynamics, host plants and their role as a source of natural enemies depending on crop cycles. In pepper *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) have been reported to satisfactorily control *B. tabaci* (Urbaneja, *et al.*, 2002). However, whitefly predators might enhance whitefly control and increase the stability of the system. In other crops such as eggplant whitefly control using natural enemies

has been little assayed. Squash is normally associated with tomato in traditional crops in the north of the province of Murcia. Although, it is not very important economically, it was considered of interest because of the great amount of mirid predators it harboured.

The objective of this work was to determine the abundance and host plants of predator mirids present in some of the horticultural crops, mainly tomato, pepper, squash and eggplant, and surrounding vegetation in the province of Murcia. To date little information of this kind was available for the southeast of Spain. The aim was to know which are the species of mirid predators that occurs naturally in these crops and which plants may serve as their refuge.

Material and methods

Sampling

Sampling was carried out in 2002 and 2003 in horticultural intensive production areas, concentrated mainly along the coast of the province, and in traditional crops with little use of pesticides, whose production goes almost completely to the local market. More than 30 localities were visited several times a year.

Tomato (*Lycopersicon esculentum* Miller), pepper (*Capsicum annuum* L.), squash (*Cucurbita pepo* L.) and eggplant (*Solanum melanogena* L.) were among the crops more frequently found and sampled. Other crops such as broad bean (*Vicia sativa* L.) , beans (*Phaseolus vulgaris* L.), celery (*Apium graveolens* L.), potatoes (*Solanum tuberosum* L.) etc., were sampled occasionally. About one hundred and ten plans from around 30 families were sampled between crops and natural vegetation. Sampling was done using sweeping nets, tapping or inspecting the plants. Samples were taken to the laboratory where the insects were extracted and preserved in alcohol (70%) or pinned.

For each plant captures were expressed in two ways (1) the number of samples on which a determined mirid species was collected in relation to the number of times this plant was sampled (frequency) and (2) the total number of individuals collected from that plant (abundance).

Identification

Determination of *Dicyphus* Fieber and *Nesidiocoris* Kirkaldy species was based on morphology, measurements of the insects and male genitalia according to the works of Wagner (1964 and 1970). In the case of the species of genus *Macrolophus* Fieber, due to the high variablility found in the characters used to differenciate *Macrolophus melanotoma* (Costa, 1853) (= *Macrolophus caliginosus* Wagner, 1950) from *Macrolophus pygmaeus* (Rambur, 1839), we preferred to consider them as the complex *Macrolophus* spp. (Carapezza, 1995; Josifov, 1992).

Results and discussion

Mirid predators with interest for biological pest control in horticultural crops were found on only 14 of more than one hundred plants sampled (Tables 1 and 2). Among crops, mirid predators were found on tomato, squash, eggplant, potato and pepper. The most abundant species on tomato were *Nesidiocoris tenuis* and *Macrolophus* spp. (Table 1). *Nesidiocoris tenuis* Reuter was more abundant in the centre and the coast while *Macrolophus* spp. was more abundant in the northern parts of the province.

Table 1. List of plants on which mirid predators of interest for biological pest control in horticultural crops were found. *D.punct.= Deraeocoris punctulatus*, *Macroph.= Macrolophus melanotoma/M. pygmaeus*, *D. taman.= Dicyphus tamanii*. Frc= frequency, Abd= abundance, total captures.

		D.punct.		***Macroph.***		***D.cerastii***		***D.taman.***		***N.tenuis***	
Plant	**Family**	Freq	Abd	Freq	Abd	Freq	Abd	Freq	Abd	Freq	Abd
Artemisia spp.	Asteraceae	1/13	1	-	-	-	-	-	-	-	-
Carduus sp.	Asteraceae	-	-	10/16	100	-	-	-	-	-	-
Dittrichia viscosa	Asteraceae	-	-	65/82	845	-	-	-	-	2/82	3
Cucurbita pepo	Cucurbitaceae	-	-	8/23	125	19/23	876	2/23	49	11/23	393
Ecballium elaterium	Cucurbitaceae	-	-	-	-	-	-	-	-	1/5	1
Ononis natrix	Fabaceae	1/17	7	9/17	101	1/17	10	-	-	-	-
Erodium petreum	Geraniaceae	-	-	-	-	1/8	4	-	-	-	-
Lycopersicon estulentum	Solanaceae	-	-	22/31	1305	21/31	149	-	-	27/31	1887
Solanum Melongena	Solanaceae	2/8	10	5/8	17	-	-	-	-	4/8	41
Solanum tuberosum	Solanaceae	-	-	1/3	2	-	-	-	-	-	-
Withania frutescens	Solanaceae	-	-	-	-	1/3	4	-	-	-	-
Tamarix canariensis	Tamaricaceae	1/8	1	-	-	-	-	-	-	-	-
Marrubium vulgare	Lamiaceae	1/25	1	9/25	188	-	-	-	-	-	-
Foeniculum vulgare	Apiaceae	1/2	1	-	-	-	-	-	-	-	-
Capsicum annum	Solanaceae	1/*	10								

* From 3 to 4 greenhouses sampled weekly for more than 6 years.

Dicyphus cerastii Wagner was frequently found on tomato but at a lower number than the other two species. On the contrary, *D. cerastii* was more frequent and abundant than *Macrolophus* and *N. tenuis* on squash. *Dicyphus tamaninii* Wagner was collected only in a couple of occasions. *Nesidiocoris tenuis*, *Macrolophus* spp. and *Deraeocoris punctulatus* Fallen were collected on eggplant and only *D. punctulatus* was very occasionally found on pepper (Table 1).

Table 2. List of sampled plants on which no mirids of interest for biological pest control in horticultural crops were found.

Plant	Family	Plant	Family
Apium graveolens	Apiaceae	*Anthyllis cytisoides*	Fabaceae
Eryngium campestre	Apiaceae	*Bituminaria bituminosa*	Fabaceae
Foeniculum vulgare	Apiaceae	*Dorycnium pentaphyllum*	Fabaceae
Nerium oleander	Apocynaceae	*Genista scorpius*	Fabaceae
Anacyclus clavatus	Asteraceae	*Genista valentina*	Fabaceae
Andryala ragusina	Asteraceae	*Medicago sativa*	Fabaceae
Artemisia barrelieri	Asteraceae	*Ononis natrix*	Fabaceae
Artemisia herba-alba	Asteraceae	*Ononis tridentata*	Fabaceae
Atractylis cancelata	Asteraceae	*Phaseolus vulgaris*	Fabaceae
Calendula arvensis	Asteraceae	*Retama sphaerocarpa*	Fabaceae
Carduus sp.	Asteraceae	*Vicia faba*	Fabaceae
Centaurea sp.	Asteraceae	*Vicia sativa*	Fabaceae
Chrysanthemum coronarium	Asteraceae	*Arundo donax*	Gramineae
Cichorium intybus	Asteraceae	*Hordeum murinum*	Gramineae
Dittrichia viscosa	Asteraceae	*Hyparrhenia hirta*	Gramineae
Helichrysum stoechas	Asteraceae	*Lygeum spartum*	Gramineae
Launaea arborescens	Asteraceae	*Sorgum halepense*	Gramineae
Onopordom corymbosum	Asteraceae	*Stipa tenacissima*	Gramineae
Reichardia tingitana	Asteraceae	*Artemisia barrilieri*	Lamiaceae
Sonchus oleraceus	Asteraceae	*Artemisia herba-alba*	Lamiaceae
Sonchus tenerrimus	Asteraceae	*Lavandula dentata*	Lamiaceae
Echium creticum	Boraginaceae	*Menta sp.*	Lamiaceae
Capparis spinosa	Capparaceae	*Phlomis lychnitis*	Lamiaceae
Arthrocnemum macrostachyum	Chenopodiaceae	*Rosmarinus officinalis*	Lamiaceae
Atriplex halimus	Chenopodiaceae	*Thymus spp.*	Lamiaceae
Beta vulgaris	Chenopodiaceae	*Asphodelus albus*	Liliaceae
Chenopodium album	Chenopodiaceae	*Asphodelus fistulosus*	Liliaceae
Salsola genistoides	Chenopodiaceae	*Gladiolus illyricus*	Liliaceace
Salsola verticillata	Chenopodiaceae	*Malva sp.*	Malvaceae
Suaeda vera	Chenopodiaceae	*Olea europaea*	Oleaceae
Cistus albidus	Cistaceae	*Oxalis pes-caprae*	Oxalidaceae
Cistus clusii	Cistaceae	*Pinus halepensis*	Pinaceae
Fumana ericoides	Cistaceae	*Plantago lagopus*	Plantaginaceae
Helianthemum almeriense	Cistaceae	*Limonium sp.*	Plumbacinaceae
Convolvulus althaeoides	Convolvulaceae	*Coris monspeliensis*	Primulaceae
Convolvulus arvensis	Convolvulaceae	*Rhamnus lycioides*	Rhamnaceae
Diplotaxis sp.	Cruciferae	*Prunus dulcis*	Rosaceae
Eruca vesicaria	Cruciferae	*Rubus ulmifolius*	Rosaceae
Moricandia arvensis	Cruciferae	*Osyris quadripartita*	Santalaceae
Moricandia arvensis	Cruciferae	*Capsicum annuum*	Solanaceae
Rapistrum rugosum	Cruciferae	*Nicotiana glauca*	Solanaceae
Sisymbrium irio	Cruciferae	*Solanum nigrum*	Solanaceae
Cucumis sativus	Cucurbitaceae	*Tamarix boveana*	Tamaricaceae
Cucurbita pepo	Cucurbitaceae	*Daphne gnidium*	Thymelaeaceae
Ecballium elaterium	Cucurbitaceae	*Thymelaea hirsuta*	Thymelaeaceae
Juniperus oxycedrus	Cupressaceae	*Urtica urens*	Urticaceae
Euphorbia serrata	Euphorbiaceae	*Zigophylum fabago*	Zygophyllaceae
Acacia cyanophylla	Fabaceae		

There was a great number of species from the natural vegetation surrounding the crops on which no mirids of interest were collected (Table 2). *Macrolophus* was found in a higher number than the rest of the species (Table 1). The main wild host plants for *Macrolophus* were, in a decreasing order, *Dittrichia viscosa* (L.) W. Greuter, *Marrubium vulgare* L., *Ononis natrix* L and *Cardus* sp.. *Nesidiocoris tenuis* was ocassionally found and at a low number on *Ecbalium elaterium* (L.) A. Rich and *D. viscosa*.

Dicyphus cerastii was found at low number in *O. natrix* and *Withania frutescens* (L.) Pauquy. *Deraeocoris punctulatus* was the species with the greatest range of host plants. It was found on *Artemisia* spp., *Ononis natrix*, *Tamarix canariensis* Willd., *Foeniculum vulgare* Miller and *M. vulgare* (Table 2).

As a conclusion for the prospection carried out over these two years we can say that several species of mirids are potentially good candidates for whitefly control on horticultural crops in the south of Spain, either by release or conservation estrategies. There are also several important wild host plants for mirids. However, for these to be used as insectary plants it is necessary to know more about insect population dynamics and dispersal movements in relation to crop cycles.

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References

Arnó, J., Ariño, J., Español, R., Marti, M. & Alomar, O. 2000. Conservation of *Macrolophus caliginosus* Wagner (Het. Miridae) in commercial greenhouses during tomato crop-free period. IOBC wprs Bulletin. 23: 241-246.

Carapezza, A. 1995. The specific identities of *Macrolophus melanotoma* (A. Costa, 1853) and *Stenodema curticolle* (A. Costa, 1853) (Insecta Heteroptera, Miridae). Naturalista sicil., S.IV, Vol. XIX (3-4): 295-298.

Fauvel, G. 1999. Diversity of Heteroptera in agroecosystems: role of sustainability and bioindication. Agriculture, Ecosystems and Environment. 74: 275-303.

Josifov, M. 1992. Zur taxonomie der paläarktischen *Macrolophus*-Arten (Insecta, Heteroptera: Miridae). Reichenbachia, 29: 1-3.

Urbaneja, A., Calvo, J., Beltran, D., Lara, L., Van der Blom, J. & Stansly. *Eretmocerus mundus*: control biológico de *Bemisia tabaci*. 13° Symposium Internacional: Control biológico de plagas y enfermedades: agentes microbianos y entomófagos. Phytoma ESPAÑA. 144: 139-142.

Wagner, E. & Weber, H. 1964. Héteroptères Miridae. Faune de la France. Fédèration Française des Sociètèss de Sciences Naturelles. Office Centrale de Faunistique. Paris.

Wagner, E. 1970. Die Miridae Hahn, 1831, des Mittelmeerraumes und der Makaronesischen Inseln (Hemiptera: Heteroptera). Entomologische Abhandlungen. 37: 1-484.

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Distribution and population dynamics of *Orius* spp. in sweet pepper greenhouses in north-west Italy

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Abstract: *Orius laevigatus* is one of the most efficient thrips predators, so it is now produced by many biofactories and widely used on IPM vegetable crops. This species is widespread along the Mediterranean and Atlantic coasts and in areas with a marine influence, whereas other *Orius* spp. are more common in Piedmont, an internal region in the north-west of Italy. Research was therefore carried out on sweet pepper in this region in order to assess the natural presence of *Orius* spp. on crops and to compare their colonization and predatory ability with those of the artificially introduced *O. laevigatus*. Surveys were carried out in 8 plastic tunnels in the province of Turin, in 4 of which releases of *O. laevigatus* were made. Thrips and anthocorids were sampled by collecting 10 flowers from 5 sectors per tunnel, every 2 weeks from late June to early October. In the laboratory, the thrips and anthocorids collected from the flowers were counted and adults were identified. Thrips population levels varied greatly in the tunnels investigated. Both *Thrips tabaci* and *Frankliniella occidentalis* were sampled, but severe infestations were only found in the case of the latter species. *Orius* specimens were collected, in highly variable numbers, from all tunnels, regardless of the release. However, the most abundant species was *O. niger*, which was also captured on various wild plants, from which it subsequently moved on to spontaneously colonize crops from June. *O. laevigatus*, on the other hand, was less common and was almost only found in the tunnels in which it had been released.

Key words: *Orius laevigatus*, *Orius niger*, thrips control, colonization ability, host plants

Introduction

Thrips are amongst the most serious pests for sweet pepper, above all since the introduction into Italy of *Frankliniella occidentalis* (Pergande) at the end of the 1980s (Arzone *et al.*, 1989). However, in IPM crops of Liguria (north-west Italy), a native anthocorid - *Orius laevigatus* (Fieber) - proved very efficient in controlling thrips outbreaks when it is not disturbed by chemical treatments (Tavella *et al.*, 1991). This species is now produced by many biofactories and largely used in IPM programmes on account of its predatory effectiveness, which has also been studied in other areas of Europe (Tommasini & Maini, 2002).

O. laevigatus is a west palaearctic species, which is widespread along Mediterranean and Atlantic coasts, in areas with a marine influence; it is recorded in all regions in Italy with the exception of a few alpine ares (Péricart, 1972). In fact, in Piedmont - a more internal region of north-western Italy - where *O. laevigatus* is usually released in IPM pepper greenhouses, other *Orius* species are more common both on crops, and on non crop plants such as *O. niger* Wolff and *O. majusculus* (Reuter). Our research was carried out to assess the natural presence of *Orius* spp. on sweet pepper in this area and to compare their colonization and predatory ability with those of artificially introduced *O. laevigatus*.

Material and methods

Research was performed in 8 sweet pepper plastic tunnels in the province of Turin (north-west Italy) in 2002. The crop location and control strategy are reported in table 1. In this area pepper plants are usually transplanted in mid March. The usual cultural practices were applied during the growing season: for example, 4 tunnels were equipped with nets (2×7 mm) to prevent the entrance of *Ostrinia nubilalis* (Hübner) and, consequently, to avoid the need for chemical treatments. From 1 to 3 releases of *O. laevigatus* (Koppert, The Netherlands) were made in 4 of the tunnels, each at the rate of 0.5 adults/m^2.

Table 1. Locations and adopted control strategies of pepper tunnels investigated in 2002.

Tunnel	locality	*O. laevigatus* release		nets	chemical treatments	
		date	no./m^2		a.i.	date
1	Carmagnola	9/7	0.5	yes	*B. thuringiensis*	weekly from 10/8
2	Trofarello	30/5, 15/6	1.0	yes	azadirachtin indoxacarb pymetrozine cyfluthrin	20/4, 22/5, 10/8 30/6 20/8 27/8
3	Santena	21/6, 8/7, 22/7	1.5	yes	lufenuron	12/6
4	Carignano	27/5, 10/7	1.0	no	indoxacarb pymetrozine	10/6, 29/7 25/6
5	Carignano			no	the same recorded for tunnel 3	
6	Carmagnola			yes	lufenuron hexythiazox chlorpyrifos-methyl imidacloprid	15/5 20/5, 8/7 5/6, 30/6 18/6, 25/7
7	Carignano			no	methomyl *B. thuringiensis*	11/6, 29/6 weekly from 10/8
8	Carmagnola			no	-	-

Surveys were carried out every 2 weeks from mid June to early October in order to monitor thrips and anthocorid populations. During this time, samples of 10 flowers were collected from 5 sectors of each tunnel, placed in 70% ethanol, and transferred to the laboratory. Samplings of *Orius* spp. were also performed on wild flora growing in the surroundings of the crops investigated in order to assess alternative natural host plants to pepper in Piedmont.

In the laboratory, samples collected from both sweet pepper and wild plants were examined to separate and count prey and predator individuals. Thrips and anthocorid adults were then identified according to Palmer *et al.* (1989) and Péricart (1972), respectively.

Results

Sample data, expressed as mean number of thrips and anthocorids collected from 10 flowers per sector, are reported in table 2.

Thrips populations

Population abundance was very variable in the investigated pepper greenhouses: infestation levels oscillated during the growing season, showing maximum values at the beginning and at the end of the cultivation period. In any case, high infestations were only observed in tunnel 3 at the beginning of the crop, and in tunnel 6 throughout the season. Both *Thrips tabaci* Lindeman and *F. occidentalis* were monitored on pepper, but the most severe outbreaks were always due to the latter species, which proved not to be disturbed by the intensive use of pesticides in tunnel 6.

Anthocorid distribution and abundance

Independently of the releases, *Orius* individuals were captured in all the investigated tunnels but in different amounts according to the control methods adopted.

During surveys, 3 other *Orius* species were sampled: *O. niger*, *O. majusculus* and *O. minutus* as well as *O. laevigatus*, (L.) (figure 1). However, *O. laevigatus* was found in very reduced quantities and almost exclusively in the tunnels in which it had been released: in fact, only 1 and 2 females were collected in tunnels 5 and 8, respectively. On the contrary, except for tunnel 7 - where *O. majusculus* prevailed - the predominant species was *O. niger*: 55% of the total number of collected adults belonged to this species. Wild *Orius* species were shown to naturally colonize crops at the end of June and - if not disturbed by chemical treatments - were able to reproduce and develop throughout July and August.

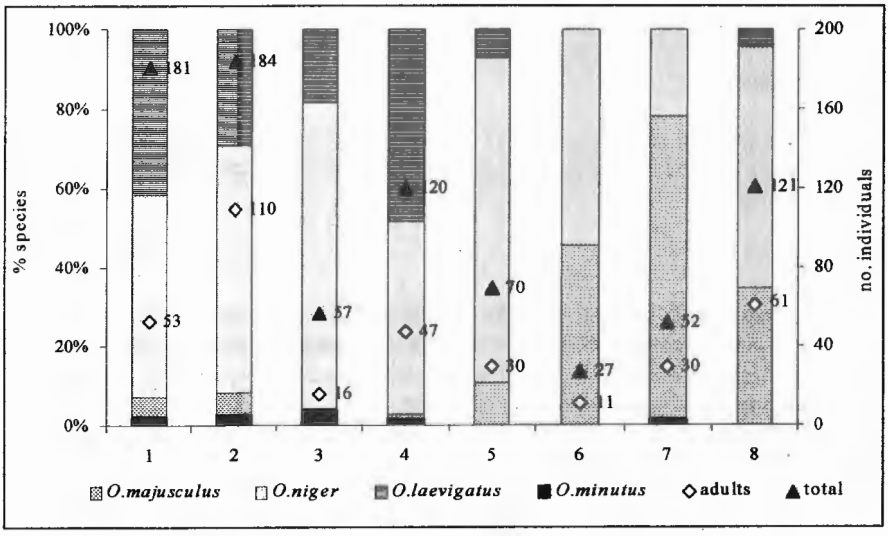

Figure 1. Composition of *Orius* species assessed in the pepper tunnels investigated in 2002.

Anthocorids were collected from several wild plants, belonging to different families: *Amaranthus retroflexus* L. (Amaranthaceae); *Conyza canadiensis* (L.) Cronq., *Erigeron annuus* (L.) Pers., *Galinsoga parviflora* Cav. and *Matricaria chamomilla* L. (Asteraceae); *Brassica napus* L. and *Sinapis arvensis* L. (Brassicaceae); *Trifolium repens* L. (Fabaceae); *Echinochloa crus-galli* (L.) Beauv. (Poaceae); *Polygonum persicaria* L. (Polygonaceae);

Solanum nigrum L. (Solanaceae). They were also captured on neighbouring cultivated plants, such as corn and dahlia and also on wild flora: *O. niger* was the most abundant species, whereas *O. laevigatus* was never found.

Discussion

In Piedmont the presence of thrips and anthocorids on sweet pepper during the growing season varied greatly. In any case, our research showed that a density of 3-5 thrips per flower did not seem to cause damage to the yield, and that chemical treatments were not always able to keep thrips populations under the economic threshold. On the contrary, *Orius* proved to efficiently control the prey regardless of the species: with a density of 0.3 anthocorids per flower, thrips infestations was dramatically reduced.

In the tunnels investigated, the most important role in preying on thrips was carried out by wild *Orius* species, and especially by *O. niger*. From late June onwards, this species naturally colonized crops, and was the most abundant and efficient predator on sweet pepper in Piedmont. Similar cases were observed in central and southern Greece (Barbetaki *et al.*, 1999) and in Belgium (Van de Veire & Degheele, 1992). On the other hand, *O. laevigatus*, was the most common species in Liguria (north-west Italy) (Tavella *et al.*, 1991; 2000) and in north-eastern Spain (Riudavets & Castañe, 1998). It was rarely found on pepper and never on the wild plants surrounding the tunnels in Piedmont. Although this predator was unable to settle on the crops, its introduction a month after transplanting could anticipate the control action before natural colonization by the wild species, but the value of this release has yet to be assessed.

Acknowledgements

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References

Arzone, A., Alma, A. & Rapetti, S. 1989: *Frankliniella occidentalis* (Perg.) (Thysanoptera Thripidae) nuovo fitomizo delle serre in Italia. Informatore fitopatologico 39 (10): 43-48.

Barbetaki, A., Lykouressis, D. & Perdikis, D. 1999: Predatory species of the genus *Orius* recorded in fields of vegetable crops in Greece. IOBC/WPRS Bulletin 22 (5): 97-101.

Palmer, J.M., Mound, L.A. & du Heame, G.J. 1989: CIE Guides to insects of importance to man. 2. Thysanoptera. CAB International Institute of Entomology, London: 1-73.

Péricart, J. 1972: Hémiptères – Anthocoridae, Cimicidae et Microphysidae de l'Ouest Paléarctique. Faune de l'Europe et du Bassin Méditerranéen 7, Masson, Paris: 1-402.

Riudavets, J. & Castañe, C. 1998: Identification and evaluation of native predators of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in the Mediterranean. Environmental Entomology 27 (1): 86-93.

Tavella, L., Arzone, A. & Alma, A. 1991: Researches on *Orius laevigatus* (Fieb.), a predator of *Frankliniella occidentalis* (Perg.) in greenhouses. A preliminary note. IOBC/WPRS Bulletin 14 (5): 65-72.

Tavella, L., Tedeschi, R., Arzone, A. & Alma, A. 2000: Predatory activity of two *Orius* species on the western flower thrips in protected pepper crops (Ligurian Riviera, Italy). IOBC/WPRS Bulletin 23 (1): 231-240.

Tommasini, M.G. & Maini, S. 2002: Thrips control on protected sweet pepper crops: enhancement by means of *Orius laevigatus* releases. In: Thrips and Tospoviruses: Proceedings 7th International Symposium on Thysanoptera. Marullo & Mound Eds., Australian National Insect Collection, Canberra: 249-256.

Van de Veire, M. & Degheele, D. 1992: Biological control of the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), in glasshouse sweet peppers with *Orius* spp. (Hemiptera: Anthocoridae). A comparative study between *O. niger* (Wolff) and *O. insidiosus* (Say). Biocontrol Science and Technology 2: 281-283.

Table 2. Mean number of thrips and anthocorids collected from 10 flowers per sector, and the ratio between prey and predators in the pepper tunnels investigated in 2002.

Tunnel 1

Date	thrips		*Orius* spp.		prey/
	adult	larvae	adult	nymphs	predator
20 Jun					
04 Jul	10.2	30.6	0.8	1.2	20.4
17 Jul	9.2	5.2	1.4	3.6	2.9
01 Aug	1.4	3.6	2.0	5.0	0.7
20 Aug	0.8	2.8	1.6	4.0	0.6
05 Sep	0.0	0.2	2.6	6.2	0.0
20 Sep	0.0	0.4	1.6	4.4	0.1
03 Oct	38.3	61.5	0.8	1.5	41.8

Tunnel 2

Date	thrips		*Orius* spp.		prey/
	adult	larvae	adult	nymphs	predator
20 Jun	1.3	12.7	0.3	0.0	42.0
04 Jul	5.4	10.6	2.8	4.4	2.2
17 Jul	4.0	2.2	4.4	5.2	0.6
01 Aug	0.6	3.0	5.2	1.6	0.5
20 Aug	0.8	0.8	4.2	2.0	0.3
05 Sep	0.4	0.6	4.8	1.4	0.2
20 Sep	0.0	0.0	0.8	0.0	0.0
03 Oct	0.0	0.0	0.0	0.0	0.0

Tunnel 3

Date	thrips		*Orius* spp.		prey/
	adult	larvae	adult	nymphs	predator
20 Jun	13.0	40.7	0.0	0.0	-
04 Jul	74.5	277.0	0.0	0.0	-
17 Jul	92.0	113.6	2.4	4.4	30.2
01 Aug	15.8	20.8	0.4	3.0	10.8
20 Aug	11.8	24.0	0.0	0.6	59.7
05 Sep	15.0	25.5	0.5	0.0	81.0
20 Sep	2.0	14.5	0.5	0.0	33.0
03 Oct	8.0	18.0	0.0	0.5	52.0

Tunnel 4

Date	thrips		*Orius* spp.		prey/
	adult	larvae	adult	nymphs	predator
20 Jun	5.4	3.3	1.3	2.3	2.4
04 Jul	1.0	1.2	2.4	6.4	0.3
17 Jul	3.2	1.2	4.0	5.8	0.4
01 Aug	5.4	2.6	1.4	1.2	3.1
20 Aug	7.4	0.6	0.2	0.0	40.0
05 Sep	5.4	6.8	0.2	0.0	61.0
20 Sep	6.8	8.8	0.2	0.0	78.0
03 Oct	4.6	11.6	0.2	0.0	81.0

Tunnel 5

Date	thrips		*Orius* spp.		prey/
	adult	larvae	adult	nymphs	predator
20 Jun	8.0	4.7	1.3	0.7	6.3
04 Jul	2.0	4.2	1.4	4.0	1.1
17 Jul	6.4	1.4	2.4	2.6	1.6
01 Aug	5.6	2.6	1.0	0.6	5.1
20 Aug	14.4	4.0	0.2	0.2	46.0
05 Sep	7.6	9.2	0.0	0.0	-
20 Sep	5.2	9.2	0.0	0.2	71.0
03 Oct	10.8	14.6	0.0	0.0	-

Tunnel 6

Date	thrips		*Orius* spp.		prey/
	adult	larvae	adult	nymphs	predator
20 Jun	19.0	47.3	0.0	0.0	-
04 Jul	15.4	19.2	0.2	0.0	173.0
17 Jul	5.6	0.4	0.0	0.0	-
01 Aug	67.6	265.0	0.2	0.2	831.5
20 Aug	80.0	39.8	0.4	0.0	299.5
05 Sep	64.4	84.4	0.8	1.4	67.6
20 Sep	129.8	98.8	0.4	1.0	163.4
03 Oct	50.0	79.0	0.2	0.6	161.3

Tunnel 7

Date	thrips		*Orius* spp.		prey/
	adult	larvae	adult	nymphs	predator
20 Jun	0.0	3.0	0.0	0.0	-
04 Jul	1.2	1.8	0.2	1.0	2.5
17 Jul	7.6	7.6	0.0	0.2	76.0
01 Aug	6.6	12.4	2.2	0.6	6.8
20 Aug	4.4	3.4	3.0	1.6	1.7
05 Sep	10.4	4.6	0.2	0.8	15.0
20 Sep	4.2	27.6	0.4	0.2	53.0
03 Oct	4.6	12.6	0.0	0.0	-

Tunnel 8

Date	thrips		*Orius* spp.		prey/
	adult	larvae	adult	nymphs	predator
20 Jun	6.7	9.3	0.3	1.3	9.9
04 Jul	4.0	11.4	2.2	2.2	3.5
17 Jul	2.6	5.6	2.8	2.0	1.7
01 Aug	0.4	5.0	2.8	1.2	1.4
20 Aug	0.6	4.2	1.0	2.4	1.4
05 Sep	23.0	10.6	1.8	3.0	7.0
20 Sep	0.0	0.0	0.4	0.6	0.0
03 Oct	0.2	0.2	1.0	0.0	0.4

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Influence of the prey on the biology of *Nesidiocoris tenuis* (Hem.: Miridae)

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Abstract: *Nesidiocoris tenuis* Reuter is a mirid bug widely distributed in Spain, where it occurs spontaneously in fruiting vegetables. This polyphagous predator could be considered a good natural enemy because it preys on several pests. Nevertheless, due to its zoophytophagous behaviour can produce damages on certain crops under some conditions. In this work, the biology of *N. tenuis*, feeding on four different preys was investigated on tomato: nymphs of the sweet potato whitefly (*Bemisia tabaci* Genn.), eggs of the Mediterranean flour moth (*Ephestia kuehniella* Zeller), nymphs of the Western flower thrip (*Frankliniella occidentalis* Pergande) and nymphs and adults of the twospotted spider mite (*Tetranychus urticae* Koch). A control without prey was also considered. The experiment was conducted under laboratory conditions at 25 ± 1°C , 75 ± 5% and a photoperiod of 16:8 (L:D).

The biological parameters were statistically different depending on the prey consumed. Developmental time was estimated between 31.5 days on *F. occidentalis* and 19.7 days on *E. kuehniella*. Survival was lower on *F. occidentalis* (4.3%) and was higher on *E. kuehniella* (73%). Fecundity was higher when *N. tenuis* fed on *E. kuehniella* (78.6 eggs/female). *N. tenuis* was not able to complete its development cycle feeding only on tomato (without prey). From these results it could be concluded than *N. tenuis* could have an important role in the control of several important pests. For this reason, knowledge about its behaviour and biology will be very important for the success of IPM programs in tomato (conservation strategies).

Key words: *Nesidiocoris tenuis*, *Bemisia tabaci*, *Ephestia kuehniella*, *Frankliniella occidentalis*, *Tetranychus urticae*, conservation biological control, tomato, IPM.

Section VI: Insecticides, molecular techniques and regulation of IPM components

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Biopesticides' Regulation and Use in an IPM Program

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Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. At the end of 2001, there were approximately 250 registered biopesticide active ingredients and close to 1000 products. Biopesticides fall into three major classes:

(1) Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest[s].
(2) Biochemical pesticides Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones, that interfere with mating, as well as various scented plant extracts that attract insect pests to traps.
(3) Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant.

How does EPA encourage the development and use of biopesticides?

In 1994, the Biopesticides and Pollution Prevention Division was established in the Office of Pesticide Programs to facilitate the registration of biopesticides. This Division promotes the use of safer pesticides, including biopesticides, as components of IPM programs. The Division also coordinates the Pesticide Environmental Stewardship Program (PESP).

Since biopesticides tend to pose fewer risks than conventional pesticides, EPA generally requires much fewer data to register a biopesticide than to register a conventional pesticide. In fact, new biopesticides are often registered in less than a year, compared with an average of more than 3 years for conventional pesticides.

While biopesticides require fewer data and are registered in less time than conventional pesticides, EPA always conducts rigorous reviews to ensure that pesticides will not have adverse effects on human health or the environment. For EPA to be sure that a pesticide is safe, the Agency requires that registrants submit a variety of data about the composition, toxicity, degradation, and other characteristics of the pesticide.

Regulation of Biopesticides

Data Requirement for Biopesticides:

* Product Analysis (Chemical identity analysis and certified limits)
* Toxicology (3 tiered system of testing and maximum hazard approach to risk assessment)
* Effects on Non-target Organisms
* Residue Data

* Efficacy Data

What is IPM?

Integrated Pest Management (IPM) is an effective and environmentally sensitive approach to pest management that relies on a combination of common-sense practices. IPM programs use current, comprehensive information on the life cycles of pests and their interaction with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means, and with the least possible hazard to people, property, and the environment.

How do IPM programs work?

IPM is not a single pest control method but, rather, a series of pest management evaluations, decisions and controls. In practicing IPM, growers who are aware of the potential for pest infestation follow a four-tiered approach. The four steps include:

(1) Set Action Thresholds: Before taking any pest control action, IPM first sets an action threshold, a point at which pest populations or environmental conditions indicate that pest control action must be taken. Sighting a single pest does not always mean control is needed. The level at which pests will either become an economic threat is critical to guide future pest control decisions.

(2) Monitor and Identify Pests: Not all insects, weeds, and other living organisms require control. Many organisms are innocuous, and some are even beneficial. IPM programs work to monitor for pests and identify them accurately, so that appropriate control decisions can be made in conjunction with action thresholds. This monitoring and identification removes the possibility that pesticides will be used when they are not really needed or that the wrong kind of pesticide will be used.

(3) Prevention: As a first line of pest control, IPM programs work to manage the crop, lawn, or indoor space to prevent pests from becoming a threat. In an agricultural crop, this may mean using cultural methods, such as rotating between different crops, selecting pest-resistant varieties, and planting pest-free rootstock. These control methods can be very effective and cost-efficient and present little to no risk to people or the environment.

(4) Control: Once monitoring, identification, and action thresholds indicate that pest control is required, and preventive methods are no longer effective or available, IPM programs then evaluate the proper control method both for effectiveness and risk. Effective, less *risky* pest controls are chosen first, including highly targeted chemicals, such as pheromones to disrupt pest mating, or mechanical control, such as trapping or weeding. If further monitoring, identifications and action thresholds indicate that less risky controls are not working, then additional pest control methods would be employed, such as targeted spraying of pesticides. Broadcast spraying of non-specific pesticides is a last resort.

Do most growers use IPM?

With these steps, IPM is best described as a continuum. Many, if not most, agricultural growers identify their pests before spraying. A smaller subset of growers use less risky pesticides such as pheromones. All of these growers are on the IPM continuum. The goal is to move growers further along the continuum to using all appropriate IPM techniques.

How do you know if the food you buy is grown using IPM?

In most cases, food grown using IPM practices is not identified like *organic* food. There is no national certification for growers using IPM, as the United States Department of Agriculture is developing for organic. Since IPM is a complex pest control process, not merely a series of practices, it is impossible to use one IPM definition for all foods and all areas of the country. Many individual commodity growers, for such crop as potatoes and strawberries, are working to define what IPM means for their crop and region, and IPM-labeled foods are available in limited areas. With definitions, growers could begin to market more of their products as *IPM-Grown*, giving consumers another choice in their food purchases.

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Residual effects of some modern pesticides on *Chrysoperla carnea* (Stephens) adults under laboratory conditions.

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Abstract: The evaluation of pesticide effects on important beneficial organisms is an essential step within the framework of an environmental-friendly integrated crop protection strategy in greenhouses. In this work, two acetonic fractions of the Meliaceae *Trichilia havanensis* Jacq, F_{12} (azadirone) and F_{18} [1,7+3,7-di-*O*-acethylhavanensin (4:1)], and four other insecticides (triflumuron, imidacloprid, phloxine B and a natural pyrethrin+PBO) were tested in laboratory residual assays following IOBC recommendations, on adults of the predator *Chrysoperla carnea* (Stephens). The acetonic fractions of *T. havanensis* and phloxine B did not produce any effect on mortality, fecundity or fertility. However, the natural pyrethrin and, to a lesser extent, imidacloprid had a strong effect on survival. Triflumuron totally inhibited egg hatching.

Key words: *Chrysoperla carnea,* adults, *Trichilia havanensis*, triflumuron, imidacloprid, phloxine B, natural pyrethrin, side effects

Introduction

Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) is one of the commonly-used beneficials in greenhouses for the biological control of aphids. Predatory larvae of this species are normally considered the most important life stage for side-effect assessment, but there is an increasing interest worldwide in retaining adults of this predator in agricultural habitats (McEwen et al., 1994), thereby also making essential the knowledge of pesticide effects on this developmental stage.

The success of biological control agents in preventing pest outbreaks in protected crops, has led us to be very aware of the need to apply selective pesticides (Blümel et al., 1999). In the last few years, the search for vegetal molecules with insecticide activity has greatly increased. Two acetonic fractions of the seed kernels of *Trichilia havanensis* Jacq (Meliacea): F_{12} (azadirone) and the mixture F_{18} [1,7+3,7-di-*O*-acethylhavanensin (4:1)], have shown a strong antifeedant activity against two noctuid pests of protected crops in south-east Spain, *Helicoverpa armigera* (Hübner) and *Spodoptera littoralis* (Boisduval) (López-Olguín, 1998). These two limonoids and four other insecticides (triflumuron, a chitin synthesis inhibitor; imidacloprid, a systemic insecticide that interferes with the postsynaptic acetylcholine receptor; phloxine B, one of the most efficient halogenated xanthene pesticides; and a natural pyrethrin+PBO) were tested in laboratory residual assays on *C. carnea* adults following IOBC recommendations to evaluate pesticides in beneficial organisms (Hassan, 1994).

Material and methods

T. havanensis extracts

The acetonic fractions F_{12} and F_{18} were isolated from seed kernels of *T. havanensis* harvested in Puebla (Mexico) in 2001, and diluted in distilled water with 1% of the solvent acetonitrile

and 0.5% of the emulsifier Triton® X-100, at a concentration of 1,000 mg a.i./l (it had shown good potency against some pests) (López-Olguín 1998).

Insecticides

Three commercials: Confidor® (20% imidacloprid, SC, Bayer Hispania, Barcelona, Spain), Pelitre Hort® (4% pyrethrins + 16% PBO, EC, C.Q. Massó, Barcelona, Spain) and Alsystin® (25% triflumuron, WP, Bayer Hispania, Barcelona, Spain), as well as the colorant with insecticidal properties phloxine B (90%, WP, Panreac Química S.A., Barcelona, Spain) were also used. Imidacloprid, pyrethrins and triflumuron were applied at the maximum recommended rates in Spain: 150, 80 and 150 mg of active ingredient (a.i.) per litre, based on a water amount of 1,000 l/ha. Phloxine B, was applied at the same concentration as the extracts: 1,000 mg a.i./l.

Insects

The predator was routinely reared in an environmentally-controlled cabinet [25 ±2 °C; 75±5% r.h.; l6:8 (L:D) photoperiod]. Adults were provided with an artificial diet (Vogt. et al., 2000) and water, and larvae were fed on *Sitotroga cerealella* (Oliver) eggs.

Treatments

To evaluate the residual contact activity, the two square glass plates of the dismountable cages (Jacas & Viñuela, 1994) were treated under a Potter tower with aqueous standard deposits of 1.40±0.10 and 1.54±0.14 mg/cm^{-2} for *T. havanensis* extracts and the rest of compounds (1 ml, 50kPa), and they were mounted as soon as they were dry. The PIEC, predicted initial environmental concentration of µg/cm^{-2} [dose rate (g/ha) x f/100] used in calculations (f= 0.4) (Barret et al., 1994) were 4, 3, 8, 4, and 2.4 µg/cm^{-2} for extracts, imidacloprid, pyrethin, phloxine B and triflumuron, respectively. Per insecticide, 4-5 replicates of 3 couples (<24-h-old) were exposed to the residues and provided with food and water. Cages were kept in a climatic chamber and connected to forced ventilation until the end of the assays.

Adult mortality was monitored on a daily basis and, after three days of exposure, we studied reproduction in survivors (Medina et al., 2001). Fecundity (mean number of eggs per female and day) was checked at least three times in seven days. Egg fertility (percentage of emerged larvae from the different oviposition gauzes after 5-6 days) was tested on the first day of egg laying, and also on the fourth and the seventh days for triflumuron, because this compound had a strong effect on this parameter.

Statistics

The results, presented as means±SD, were analysed by ANOVA and LSD or Bonferroni mean separation using Statgraphics (STSC, 1987). The data were subjected to a Kruskal-Wallis non-parametric test if premises of ANOVA were violated after transformation to arcSen$\sqrt{x}$. Results of *T. havanensis* extracts and other insecticides were analysed separately because experiments were conducted in different periods of time.

Results

The exposure of *C. carnea* adults to *T. havanensis* residues for three consecutive days did not affect mortality. Similarly, no deleterious effect was found on fecundity or fertility (Table 1).

Young adults of the predator exposed to the four insecticides residues were affected differently depending on the compound. The percentage of dead adults after 24 hours of

exposure to the insecticides was 66.6 and 91.6% for imidacloprid and natural pyrethrins respectively. In contrast, phloxine B and triflumuron gave no mortality at all (Fig. 1) and results remained the same 48 and 72 hours after treatment. Fecundity was not affected by any insecticide. However, triflumuron totally inhibited egg hatching when calculated as mean of three days (Fig. 2). There was no egg hatching on the first and fourth days of the recount, but there was a slight sign of recovery on the seventh day (Table 2).

Table 1. Fecundity and fertility of *Chrysoperla carnea* adults exposed to residues of *T. havanensis* acetonic fractions F_{12} and F_{18}, at the selected concentration of 1,000 mg a.i./l

Compound	Eggs/female and day[1]	Egg hatching[2] (%)
Control	27.5 ± 2.87a	78.6 ± 3.12a
F_{12}	28.2 ± 2.96a	75.0 ± 1.76a
F_{18}	26.0 ± 2.33a	79.7 ± 1.50a

Within the same column data followed by the same letter do not differ significantly (P=0.05; Bonferroni mean separation) ^1F =0.18; df=2,12; P=0.83. ^2F=1.16; df=2,12; P=0.34.

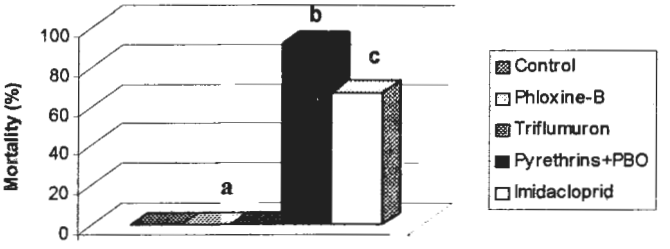

Figure 1. Mortality of *C. carnea* adults after 24 hours of exposure to the maximum field recommended rate in Spain of some insecticides and of 1,000 mg a.i./l of phloxine-B. Columns with different letters differ significantly (P= 0.05; Kruskal-Wallis test; K=18.46).

Discussion

Fractions of *T. havanensis* have been reported to have an effect on some dipteran, lepidopteran and coleopteran pests (Lopez-Olguín 1998; Ortego et al., 1998), but to be harmless to adults of *C. carnea* when applied topically (Huerta et al., 2003a) or via ingestion (Huerta et al., 2003b). As expected, young adults of this predator were not affected by the exposure to residues of these limonoids and their reproduction was normal. In contrast, the ingestion of azadirone (F_{12}) or the commercial azadirachtin by females of *C. carnea* once they have started oviposition gave reductions in their fecundity (Huerta et al., 2003b; Medina et al., 2003). Consequently, related limonoids can show important differences in toxicity towards a chosen species, making obligatory their evaluation, compound by compound and species by species.

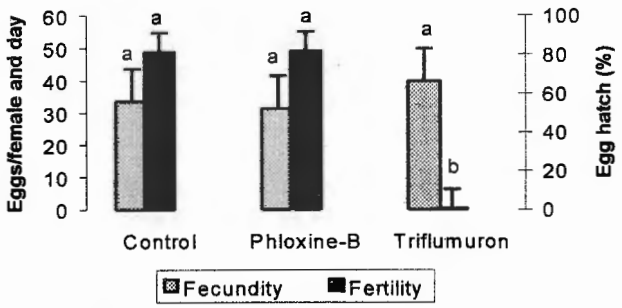

Figure 2. Average fecundity and fertility of *Chrysoperla carnea* after three days of exposure to residues of phloxine B and triflumuron. Within the same parameter, columns with different letters are significantly different. (P=0.05; LSD mean separation; Fecundity: F= 1.36; df=2,8; P=0.30; Fertility: F= 953.87; df=2,8; P=0.00)

Table 2. Fertility of *Chrysoperla carnea* after three days of exposure to residues of phloxine B and triflumuron, measured as egg hatching percentages on three different days.

Compound	**Day 1**	**Day 4**	**Day 7**
Control	87.1 ± 1.8a	81.0 ± 2.7a	75.2 ± 2.9a
Phloxine B	81.0 ± 1.6b	82.9 ± 4.5a	81.2 ± 2.0a
Triflumuron	0.0 ± 0.0b	0.0 ± 0.0b	1.3 ± 0.9b

Within the same column data with the same letter do not differ significantly (P=0.05; LSD mean separation). ^{1}F=1384; df=2,8;; ^{2}F=332; df=2,8; ^{3}F=452.02; df=2,8; $^{1,2,3}P$= 0.00

Phloxine B, the colorant with insecticidal properties, did not show any effect on our predator. However, the neurotoxics imidacloprid and natural pyrethrin were very toxic to *C. carnea* adults. Natural pyrethrins showed an excellent knock-down effect on *C. carnea* adults because they rapidly showed tremors, dying soon afterwards. Triflumuron severely decreased the fertility of this predator, compromising the next generation, as has also been reported for diflubenzuron, another chitin synthesis inhibitor (Medina et al., 2002). In contrast to our results, Senior et al. (1998) found a lesser effect on egg hatching of an acetone solution of triflumuron topically applied to young adults of *C. carnea*. One reason that might be taken into account is that the amount of triflumuron reaching the target inside the insect body is probably higher in a residual than in a topical treatment, because of the length of exposure and, as such, because of a higher insecticide absorption time through the cuticle of the insect. It has been previously reported that this group of ureas is not rapidly absorbed through the integument of *C. carnea* (Medina et al., 2002).

In conclusion, *T. havanensis* fractions and phloxine B are compatible with *C. carnea* adults and imidacloprid, natural pyrethrins and triflumuron are not: IOBC classes 1, 1, 3, 4 and 4, respectively (in the last case because of its effect on egg hatching).

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References

Barret, K.L., Grandy, N., Harrison, E.G., Hassan, S. & Oomen, P. (eds.) 1994: Guidance document on regulatory testing procedures for pesticides with non-target arthropods. SETAC. 51 pp.

Blümel, S., Matthews, G.A., Grinstein, A. & Elad, Y. 1999: Pesticides in IPM: Selectivity, side-effects, application and resistance problems. In: Integrated pest and disease management in greenhouse crops, eds. Albajes, Gullino, Van Lenteren & Elad: 150-167, Kluwer Academic Publishers, Dordrecht.

Hassan, S.A., 1994: Activities of the IOBC/wprs working group *Pesticides and beneficial organisms*. IOBC/wprs Bulletin 17(10): 1-5.

Huerta, A., Medina, P., Castañera, P.& Viñuela, E. 2003a: Topical toxicity of two acetone fractions of *Trichilia havanensis* Jacq. and four insecticides to larvae and adults of *Chrysoperla carnea* (Stephens). Communications in Agricultural and Applied Biological Sciences (in press).

Huerta, A., Medina, P., Castañera, P. & Viñuela, E. 2003b: Lab studies with *Trichilia havanensis* Jacq., a botanical pesticide, and adults of *Chrysoperla carnea* (Stephens). IOBC/ wprs Bulletin (in press).

Jacas, J. & Viñuela, E. 1994: Analysis of a lab method to test the effects of pesticides on adult females of *Opius concolor*, a parasitoid of the olive fruit fly *Bactrocera oleae*. Biocontrol Science & Technology 4: 147-154.

López-Olguín, J.F. 1998: Actividad y modo de acción de productos de *Trichilia havanensis* y *Scutellaria alpina* subsp. *javalambrensis*, sobre *Leptinotarsa decemlineata* y *Spodoptera exigua*. Tesis Doctoral. UPM. Madrid.

McEwen, P., Jervis, M.A., & Kidd, N.A.C. 1994: Used of sprayed L-tryptophan solution to concentrate numbers of the green lacewing *Chrysoperla carnea* in olive tree canopy. Entomologia Experimentalis et Applicata 70: 97-99.

Medina, P., Budia, F., Del Estal, P., Adán, A. & Viñuela, E. 2003: Influence of azadirachtin, a botanical insecticide, on *Chrysoperla carnea* reproduction: toxicity and ultrastructural approach. Journal Economic Entomology (in press)

Medina, P., Budia, F., Smagghe, G. & Viñuela, E. 2001: Activity of spinosad, tebufenozide and azadirachtin on eggs and pupae of the predator *Chrysoperla carnea* under laboratory conditions. Biocontrol Science & Technology 11:597-610.

Medina, P., Smagghe, G., Budia, F., del Estal, P., Tirry, L. & Viñuela, E. 2002: Significance of penetration, excretion, and transovarial uptake of three insect growth regulators in predatory lacewing adults. Archives of Insect Biochemistry and Physiology 51: 91-101.

Ortego, F., López-Olguín, J.F., Ruíz, M. & Castañera, P. 1998: Effects of toxic and deterrent terpenoids on digestive protease and detoxication enzyme activities of colorado potato beetle larvae. Pesticide Biochemistry Physiology 63: 76-84.

Senior, L.J., McEwen, P.K., Kidd, N.A.C. 1998: Effects of the chitin synthesis inhibitor triflumuron on the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae): influence on adult potentialities and offspring. Acta Zoologica Fennica 209: 227-231.

STSC. 1987. User's Guide Statgraphics. Graphic software system STSC Inc. USA.

Vogt H., Bigler F., Brown K., Candolfi M.P., Kemmeter F., Kühner C.H., Moll M., Travis A., Ufer A., Viñuela E., Waldburger M. & Waltersdorfer A. 2000: Laboratory method to test effects of plant protection products on larvae of Chrysoperla carnea (Neuroptera: Chrysopidae). In: Candolfi M.P. et al. (eds.), Guidelines to evaluate side-effects of plant protection products to non-target arthropods, IOBC/wprs, Gent, pp. 27-44.

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Quality control of natural enemies: where are we and where do we go?

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Abstract: The use of biological control agents is increasing worldwide and there are now many companies mass-producing and selling such organisms. However, there is a great need for quality control in the production and use of these natural enemies, because the deterioration of mass reared biological control agents leads to failures in pest management. The area of quality control is rather new for biological control workers. Therefore, a book with contributions from many IOBC partners was prepared on this topic specifically for biological control agents (van Lenteren, editor, 2003).

Introduction

Augmentative biological control, whereby large numbers of natural enemies are periodically introduced, is commercially applied over a large area in various crop systems world-wide (van Lenteren, 2000a; van Lenteren and Bueno, 2003). It is a popular control method applied by professional and progressive farmers, and stimulated by the present international attitudes to policies of reducing pesticide use. Initially, augmentative biological control was used to manage pests that had become resistant to pesticides. Now it is applied because of efficacy and costs, which are comparable to conventional chemical control. Farmers are also motivated to use biological control to reduce environmental effects caused by pesticide usage.

For many years, natural enemies were produced without proper quality control procedures. Poorly performing natural enemies resulted in a failure of biological control and a low profile of these pest control methods (e.g. P. DeBach, Riverside, California, 1976 and P. Koppert, Berkel and Rodenrijs, The Netherlands, 1980, personal communications). Quality control was touched upon by several biological control workers in the 20th century, but the first papers seriously addressing the problem only appeared in the 1980s (van Lenteren, 1986a).

Professional natural enemy producers may have research facilities, procedures for monitoring product quality, an international distribution network, promotional activities and an advisory service. The market for high quality, effective natural enemies will certainly increase with the growing demand for unsprayed food and a cleaner environment. The growing pesticide resistance problems will also move growers to adopt biological control methods.

Initial developments in the area of mass production, quality control, storage, shipment and release of natural enemies have decreased production costs and led to better product quality, but much more can be done. Innovations in long-term storage (e.g., through induction of diapause), shipment and release methods, may lead to a further increase in natural enemy quality with a concurrent reduction in costs, thereby making biological control easier and economically more attractive to apply. Even if the natural enemies leave the insectary in good condition, shipment and handling by the producers, distributors and growers may result in deterioration of the biological control agents before they are released.

The aim of quality control

Quality control programmes are applied to mass-reared organisms to maintain the quality of the population. The overall quality of an organism can be defined as its ability to function as intended after release into the field. The aim of quality control programmes is to check whether the overall quality of a species is maintained, but that is too general a statement to be manageable. Characteristics that affect overall quality have to be identified. These characteristics must be quantifiable and relevant for the field performance of the parasitoid or predator. This is a straightforward statement, but very difficult to actually carry out (Bigler, 1991). The aim of releases of mass-produced natural enemies is to control a pest. In this context, the aim of quality control should be to determine whether a natural enemy is still in a condition to properly control the pest. Formulated in this way, we do not need to consider terms like maximal or optimal quality, but rather acceptable quality. Some researchers believe the aim of quality control should be to keep the quality of the mass-reared population identical to that of the original field population. Not only is this an illusion, it is also an unnecessary and expensive goal to pursue. Another important consideration is that quality control programmes are not applied for the sake of the scientist, but as a mere necessity. Leppla and Fisher (1989) formulated this dilemma as "Information is expensive, so it is important to separate "**need** to know from **nice** to know." Only if characteristics to be measured are very limited in number, but directly linked to field performance, will companies producing natural enemies ever be able to apply quality control programmes on a regular basis.

Mass production of natural enemies

Worldwide, about 150 species of natural enemies are commercially available for augmentative biological control (Anonymous, 2002; Gurr and Wratten, 2000; Chapter 12 van Lenteren, 2003). This form of control is applied in the open field in crops that are attacked by only a few pest species, and it is particularly popular in greenhouse crops, where the whole spectrum of pests can be managed by different natural enemies (van Lenteren, 2000b). Its popularity can be explained by a number of important benefits when compared with chemical control: there are no phytotoxic effects on young plants, premature abortion of fruit and flowers does not occur, release of natural enemies takes less time and is more pleasant than applying pesticides, several key pests can be controlled only with natural enemies, there is no safety or re-entry period after release of natural enemies which allows continuous harvesting without danger to the health of personnel, biological control is permanent, and the general public appreciates biological control.

Natural enemy producers are a rather diverse group. Rearing of natural enemies can be a full-time business or a part-time activity for growers. But natural enemies may also be reared by companies in associated industries like seed companies or producers of fertilisers. In some cases, production of natural enemies has been started by a research group with governmental support and later continued as a private endeavour. The number of biological control agents that are commercially available has increased dramatically over the past 25 years. Today, more than 125 natural enemy species are on the market for biological pest control, and about 30 of these are produced in commercial insectaries in very large quantities (see Chapter 1, van Lenteren, 2003). Worldwide, there are about 85 commercial producers of natural enemies for augmentative forms of biological control: 25 in Europe, 20 in North America, 6 in Australia + New Zealand, 5 in South Africa, about 15 in Asia (Japan, Korea, India etc.), and about 15 in Latin America. The worldwide turnover of natural enemies of all producers was estimated to be 25 million US$ in 1997, and about 50 million US$ in 2000, with an annual growth of 15-20% in

the coming years (K. Bolckmans, Berkel and Rodenrijs, The Netherlands, personal communication 2001).

Currently, more than 75% of all activities in commercial augmentative biocontrol (expressed in monetary value) take place in North Europe and North America. Emerging markets are those of Latin America, South Africa, Mediterranean Europe, and Japan and Korea in Asia. In addition to the commercial producers, there are many natural enemy production units funded by the government, such as in Brazil (40 facilities), China (many, number unknown), Colombia (more than 20 facilities), Cuba (more than 200 facilities), Mexico (30 facilities) and Peru (more than 20 facilities). For prices of natural enemies in Europe and the United States of America see van Lenteren *et al.* (1997) and Cranshaw *et al.* (1996), respectively.

IOBC/EC initiative on quality control

Although augmentative types of biological control of arthropod pests have been applied since 1926, large-scale production of natural enemies began only after the Second World War (DeBach, 1964; van Lenteren and Woets, 1988). Initial mass rearing efforts involved the production of not more than several thousand individuals per week of three natural enemies: the spider mite predator *P. persimilis,* the whitefly parasitoid *E. formosa* and the lepidopteran egg parasitoid *Trichogramma* sp.. None of the early publications on commercial aspects of biological control mention the topic of quality control of natural enemies. Quality control is only mentioned in relation to biological control in the mid- 1980s, and shortly after that the topic gained more interest (van Lenteren, 1986a, b). The 5th workshop of the International Organization for Biological Control (IOBC) global working group "Quality Control of Mass Reared Arthropods" (Bigler, 1991) in Wageningen, the Netherlands, formed the starting point for a heated discussion among producers of natural enemies and scientists on how to approach quality control in the commercial setting at that time.

A series of workshops, some partly, others largely funded by the EC, followed in Horsholm, Denmark, in 1992; Rimini, Italy in 1993, Evora in Portugal, 1994, Antibes, France in 1996, and in Barcelona, Spain in 1997. As a result of these meetings, quality control guidelines were written for 30 species of natural enemies, and these have been tested and adapted by commercial producers of biological control agents in Europe (Chapter 19, van Lenteren, 2003). The guidelines cover features that are relatively easy to determine in the laboratory (e.g., emergence, sex ratio, lifespan, fecundity, adult size, predation/parasitism rate).

Recently, the International Biocontrol Manufacturers Association (IBMA) and the Association of Natural Bio-control Producers (ANBP) have taken the initiative in updating and further developing quality control guidelines. The quality control guidelines for more than 30 species of natural enemies developed so far are presented in Chapter 19.

The worldwide activities in the field of quality control are summarized in Chapter 1, van Lenteren, 2003.

Overview of the first book on quality control for natural enemies

The first section of the book is devoted to the emergence of quality control for natural enemies. The need for quality control for mass-produced biological control agents is discussed, and the aspects of total quality control for the production of natural enemies are described. The second section of the book provides scientific background information for quality control workers. It explains the basis of variability in foraging behaviour of natural enemies and describes technologies illustrating how to manage this variation. This section

makes clear that insight into behavioural variability in the foraging behaviour of natural enemies is a prerequisite for proper mass rearing and efficient application of natural enemies in pest management.

The third section focuses on how to cope with this variation. A population genetic perspective is given on how to manage captive populations. Examples of adaptation to captive rearing and of the trade-off with field performance are presented. Effects of a transfer of natural enemies from the field to a mass production facility are described, such as reduction of fitness and enhancing the possibility of fixation of deleterious mutations in the population by genetic drift. Ways to prevent these negative effects are also presented. Furthermore, the possibilities and advantages of unisexual reproduction for biological control are discussed, and mass production of natural enemies on artificial media is reviewed, particularly with regard to their quality. Finally, pathogens of mass-produced natural enemies and pollinators, and the effects of these pathogens on performance of the infected organisms are discussed.

The fourth section gives an overview of the species of natural enemies that are mass-produced worldwide. The fifth section contains chapters that describe developments towards quality control testing of natural enemies in North America and Europe, and discusses the need for quality testing beyond the Petri dish. The sixth and final section deals with currently used quality control tests (parasitoids, predators, and microbials), and presents basic statistical methods for analysis of the data obtained with the quality control tests.

The future of quality control

Quality control programmes that address not only natural-enemy numbers but also natural-enemy quality (field performance) are a necessity. Therefore, the current quality control guidelines will certainly undergo modifications in the coming years. First, it is expected that simple tests will be included to determine the flight capacity of mass-reared biocontrol agents. Next, semi-field and field performance tests will be developed. Finally, based on extensive ring testing by the mass production industry and comparison of results of the current tests with those of the new flight and performance tests, a new set of criteria is likely to evolve.

It is hoped that IBMA and ANBP, in collaboration with other organizations that are interested in development of quality control of mass-reared insects such as AMRQC/IOBC, will continue to cooperate in a positive way and with strong motivation. Reliable and meaningful quality control programmes will certainly contribute to the urgently needed growth of biological control.

References

Anonymous (2002). 2003 Directory of least-toxic pest control products. *The IPM Practitioner* 24, 1-40.

Bigler, F. (1991). Quality Control of Mass Reared Arthropods. *Proceedings 5th Workshop IOBC Global Working Group "Quality Control of Mass Reared Arthropods"*, 25-29 March 1991, Wageningen, The Netherlands, 205 pp.

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

DeBach, P., ed. (1964). Biological Control of Insect Pests and Weeds. Cambridge University Press, Cambridge: 844 pp.

Gurr, G. and Wratten, S. (eds.) (2000). *Measures of Success in Biological Control*. Kluwer Academic Publishers, Dordrecht, 448 pp.

Lenteren, J.C. van (1986a). Evaluation, mass production, quality control and release of entomophagous insects. In: Franz, J.M (ed.) *Biological Plant and Health Protection*. Fischer, Stuttgart, Germany, pp. 31-56.

Lenteren, J.C. van (1986b). Parasitoids in the greenhouse: successes with seasonal inoculative release systems. In: Waage, J.K. and Greathead, D.J. (eds.) *Insect Parasitoids*. Academic Press, London, pp. 341-374.

Lenteren, J.C. van (2000a). Measures of success in biological control of arthropods by augmentation of natural enemies. In: Gurr, G. and Wratten, S. (eds.) *Measures of Success in Biological Control*. Kluwer Academic Publishers, Dordrecht, pp. 77-103.

Lenteren, J.C. van (2000b). A greenhouse without pesticides: fact of fantasy? *Crop Protection* 19, 375-384.

Lenteren, J.C. van (ed.) (2003). Quality Control and Production of Biological Control Agents: Theory and Testing Procedures. CABI Publishing, Wallingford, UK

Lenteren, J.C. van and Bueno V.H.P. (2003). Augmentative biological control of arthropods in Latin America. BioControl 48: 123-139.

Lenteren, J.C., Roskam, M.M., and Timmer, R. (1997). Commercial mass production and pricing of organisms for biological control of pests in Europe. *Biological Control* 10, 143-149.

Leppla, N.C. and Fisher, W.R. (1989). Total quality control in insect mass production for insect pest management. *Journal of Applied Entomology* 108, 452-461.

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Risks of releasing exotic natural enemies: is there a need to regulate biocontrol agents?

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Abstract: In the past 30 years many exotic natural enemies have been imported, mass reared and released as biocontrol agents for greenhouse pests. Negative effects of these releases for greenhouse biological control have not been reported yet. The current popularity of biological control may, however, result in problems, as an increasing number of projects will be executed by persons not trained in the identification, evaluation and release of biocontrol agents. Therefore, a working group of OECD has developed a guidance document for registration requirements of exotic natural enemies. This guidance document is based on protocols for risk assessment that have been developed within the EU project "Evaluating Environmental Risks of Biological Control Introductions into Europe" [ERBIC]. In this paper, the state of affairs concerning these developments is summarized.

Introduction

In the past 30 years many exotic natural enemies have been imported, mass reared and released as biocontrol agents for greenhouse pests (Albajes et al. 1999). As far as we know, hardly any problems have occurred concerning negative effects of these releases for greenhouse biological control (Lynch et al. 2000; van Lenteren, 2000). The current popularity of biological control may, however, result in problems, as an increasing number of projects will be executed by persons not trained in this field of pest control. An increasing number of countries now apply risk assessment procedures before a new natural enemy can be imported or released. Within an OECD working group and in collaboration with the EU-ERBIC project, guidelines have been developed for harmonized information requirements for the import and release of invertebrate biological control agents used in augmentative biological control.

The first activity of the group was to collect, study and summarise the risk assessment procedures that are currently used by about 25 countries (N.B. less than 10 % of all countries are using some form of regulation concerning the import of exotic biocontrol organisms). Some procedures (e.g. those of Australia, New Zealand and Hawaii; see articles in Lockwood et al., 2001) are so strict that hardly any natural enemy can be introduced. Other countries have no regulations at all, so any species can be imported and released. The aim of the OECD group is, on the one hand, to come up with guidelines that will prevent serious mistakes with importations and, on the other hand, to be able to proceed with safe forms of biocontrol.

Next, a code of conduct and guidelines produced by other organisations (e.g. FAO, EPPO, NAPPO, CABI) were studied and summarised. It was decided that the OECD guidelines would be based on, and for a large part be similar to, the FAO and EPPO guidelines. However, these guidelines are not very specific concerning criteria and methodology, so it was decided to develop clearer guidelines including methodology and criteria based on the work of the EU-ERBIC project (van Lenteren et al, 2003).

Risk assessment procedures for biological control agents are normally characterized by

questions concerning four issues:

1. Characterization and identification of the biocontrol agent (classical methods or molecular techniques, voucher specimens to be deposited, DNA fingerprinting in case of taxonomic problems)
2. Health risks (for invertebrate natural enemies these are usually much easier to determine than for chemical agents)
3. Environmental risks (this is the most difficult part for invertebrate biocontrol agents; see below)
4. Efficacy (will be treated differently to cases with chemical control; as biocontrol agents often form part of an IPM programme, it is often not necessary to reach 90-100% control by the biocontrol agent alone, as long as the total IPM programme results in sufficient reduction of the pest or disease; efficacy of a biocontrol agent is defined as the ability to cause a significant reduction in the number of pest organisms, direct and indirect crop damage, or yield loss.)

The environmental risks assessment developed by the EU-ERBIC group is summarized below.

Environmental risk assessment

The evaluation of risks related to releases of natural enemies demands the integration of many aspects of their biology, as well as information on ecological interactions identified above. For a full risk assessment, three steps are distinguished: (1) the risk identification and evaluation procedure concerning the release of a natural enemy, (2) a risk management plan dealing with risk reduction and risk mitigation, and (3) a risk/benefit analysis of the proposed release of the natural enemy, together with risk/benefit analyses of current and alternative pest management methods.

Risk identification

Normally, for a risk evaluation, one will identify the hazards and determine the probabilities that hazards will materialise. The hazard of a biological control agent can be defined as any imaginable adverse effect which can be named and measured, such as direct and indirect adverse effects on non-target organisms and adverse effects on the environment. The risk of adverse effects of the release of a biological control agent is the product of the impact of the likelihood (probability) and the impact of the magnitude (consequence). Next, a numerical value was added to each criterion (for likelihood: very unlikely = 1, unlikely = 2, possible = 3, likely = 4, very likely = 5; for magnitude: minimal = 1, minor = 2, moderate = 3, major = 4, massive = 5). The likelihood and magnitude of five groups of risks are considered related to the release of exotic biological control agents: establishment, dispersal, host specificity, direct effects and indirect non-target effects. Of these parameters, estimating indirect effects on non-targets will be most difficult, as myriads of indirect effects may occur when generalist natural enemies are introduced. The parameter 'host range' forms a central element in the whole risk evaluation process, because a lack of host specificity might lead to an unacceptable risk if the agent establishes and disperses widely, whereas, in contrast, a monophagous biological control agent is not expected to create a serious risk even when it establishes and disperses well. The environmental risk criteria were then applied to calculate a risk index of a number of natural enemies. The overall risk index for each natural enemy is obtained by first multiplying the figures obtained for likelihood and magnitude, and then by adding the resulting figures obtained for dispersal, establishment, host specificity, direct and indirect effects without weighing. The maximum score is 125 (5 x 5 x 5) (for a detailed description of

the groups of risks and examples of evaluations, see van Lenteren et al., 2003).
Interpretation of risk indices should be done with great care, and can only be done by biological control experts knowing the biology of the natural enemy under consideration. Risk indices should not be seen as absolute values, but as indicators to which a judgement can be connected for granting permission to release or not. The EU-ERBIC project participants propose to use the following risk index categories: risk indices lower than 35 points will generally result in a proposal of no objection to the release of the agent, a risk index higher than 70 points will generally result in advice not to release the agent, and intermediate risk indices between 35 and 70 points will result in advice to come up with additional information before a conclusion concerning release can be drawn. Of the approximately 30 species of natural enemies that were evaluated with this method, it was mainly the larger, generalist predators that fell into the higher risk category. We should, however, not generalize about the risks of certain groups of natural enemies before evaluating many more species.

Risk management
The next step in the risk assessment process is to discuss risk management, including risk mitigation and risk reduction. If an exotic biological control agent is expected to cause significant adverse effects on non-target organisms a permit for releases will not be issued. For an example of risk management issues, I refer to Cross and Noyes (1995). In some cases, risks may be minimised by imposing label restrictions concerning, for example, the types of crops on which the use of the organism is or is not allowed (e.g., treatment of flowering plants with a mycoinsecticide), or by requesting specific application techniques (e.g., soil incorporation only for insect pathogenic nematodes).

Risk/benefit analysis
The final step in making a justified environmental risk analysis for a new biological control agent is to conduct a risk benefit analysis which should include a comparative performance of pest management methods, particularly based on environmental aspects. The environmental benefits of use of the proposed biological control agent should be compared to environmental effects of currently used and other alternative control methods. To be able to make a comparative performance analysis, information as specified below should be available for all control methods: (1) the pest control level that can be obtained, (2) the total cost of applying a pest control method to reach a sufficient level of control (labour, equipment, control agent/pesticide, etc.), (3) the costs to correct for development of resistance, (4) the number of positive effects on the environment (the effect on biodiversity; reduction of environmental pollution) (5) the number of negative effects on the environment (negative effects on biodiversity, such as non-target effects, negative effects on pollinators, fish and wildlife, and negative effects on native natural enemies resulting in a reduction of natural pest control; contamination of soil, water and air; costs to correct for these negative effects), and (6) effects on human health. When data are not available, expert judgement may suffice for some of these items.

Discussion

The topic of implementation of a registration procedure for natural enemies is currently hotly debated by the biocontrol industry, scientists and regulators. The biocontrol industry foresees lengthy, cumbersome procedures leading to high costs, and, thus, in some cases, the impossibility to market an interesting natural enemy because of excessively high costs. Regulators within ministries of environment and agriculture want to prevent unnecessary and

risky releases of exotic organisms. The history of arthropod biocontrol shows that very few mistakes have been made until now. This is a point in favour of the biocontrol industry, and is in strong contrast with the problems that have been created by the accidental importation of pests and diseases by others. The current work by, among others, the EU-ERBIC project will hopefully result in a light and harmonized registration procedure that is not prohibitive for the biocontrol industry and will result in the pre-selection of safe natural enemies.

At the moment, the EU-ERBIC and OECD proposals are being considered for application by several countries. Countries are advised not to apply the full evaluation as proposed in the OECD guidance document to the approximately 150 species of natural enemies that are already in use for a considerable amount of time. In that case, a so-called 'quick scan' method can be used to estimate potential adverse environmental effects based on available information only. We have applied this quickscan method to the 128 species of natural enemies that are currently commercially produced in The Netherlands, and it was concluded that 6 species were considered too risky for release in Holland, and that for several other species more information was needed before being able to conclude that they may be released. The end result of such a quickscan applied in many countries may result in lists of species that can be used in certain, specified regions (ecoareas) of the world, and are exempted from a full environmental risk analysis. The full environmental risk analysis should be applied to new species of natural enemies.

References

Albajes, R., Gullino, M.L., van Lenteren, J.C. and Elad, Y. (eds.), 1999. Integrated Pest and Disease Management in Greenhouse Crops, Kluwer Publishers, Dordrecht.

Cross, A. E. and J. S. Noyes, 1995. Dossier on *Anagyrus kamali* Moursi, biological control agent for the pink mealybug, *Maconellicoccus hirsutus*, in Trinidad and Tobago. IIBC, 16 pp. + 1 Annex.

Lenteren, J.C. van, 2000. Measures of Success in Biological Control Of Arthropods By Augmentation Of Natural Enemies. In: Measures of Success in Biological Control, S. Wratten & G. Gurr (eds.). Kluwer Academic Publishers, Dordrecht: 77-103.

Lenteren, J.C. van, Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S., Loomans, A.J.M., Menzler-Hokkanen, I., Rijn, P.C.J. van, Thomas, M.B., Tomassini, M.C., Zeng, Q.Q., 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. Biocontrol 48: 3-38.

Lockwood, J.A., F.G. Howarth & M.F. Purcell (eds.), 2001. Balancing Nature: Assessing the Impact of Importing Non-Native Biological Control Agents. Thomas Say Publications in Entomology: Proceedings, Entomological Society of America, Lanham, Maryland.

Lynch, L.D., H. M. T. Hokkanen, D. Babendreier, F. Bigler, G. Burgio, Z.-H. Gao, S. Kuske, A. Loomans, I. Menzler-Hokkanen, M. B. Thomas, G. Tommasini, J. Waage, J. C. van Lenteren, Q.-Q. Zeng, 2000. Indirect effects in the biological control of arthropods with arthropods. In: Evaluating Indirect Ecological Effects of Biological Control, E. Wajnberg, J.C. Scott & P.C. Quimby (eds.). CABI publishing, Wallingford: 99-125.

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Molecular identification of insect quarantine pests by RAPD-PCR

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Abstract
The introduction of new pest species or biotypes is an ever-present threat to world agriculture. Increasing levels of world trade and climatic change are just two indications of a growing problem. All attempts at eradication or control require invasion sources and species to be determined from the first moment. There is therefore an evident need for pest diagnostic procedures. Nowadays, DNA based diagnosis can provide a rapid, reliable and cost-effective mean of identification in all possible situations. Thus, we used the polymerase chain reaction (PCR) to generate random amplified polymorphic DNA (RAPD) for three insect pest species: *Bemisia tabaci, Frankliniella occidentalis,* and *Liriomyza huidobrensis*. Genomic DNA was extracted from individual insects and amplified in PCR reactions. For two of the studied species (*B. tabaci, L. huidobrensis*) and for all the primers tested, invariable bands present in 100% of the examined individuals of a given species were found. These markers, if absent in other species, could be considered "diagnostic bands" and would provide useful and reliable diagnosis for quarantine laboratories.

Key words: RAPD-PCR, molecular markers, identification, pest insects

Introduction

The introduction of new pest species or biotypes is an ever-present threat to world agriculture. Increasing levels of world trade and climatic change are just two indicators of a growing problem. Human-mediated pest movements and potential pest organisms constitute long standing agricultural problems. All attempts to eradicate or control these pests first require determination of the invasion sources and species. There is therefore an evident need for pest diagnosis procedures. For many insect pest species visual assessments of specimens from quarantine material are sufficient to permit identification, but for pests which have a complex and difficult taxonomy, or which may be intercepted in a range of developmental forms, or which are incomplete or damaged, a more sensitive diagnosis is necessary. Improper identification and the inability to recognize different populations or biotypes may have drastic and costly consequences for pest control management, especially in protected crops (Menken & Ulenberg, 1987; Hillis *et al.*, 1996; Tan, 2000).

In recent years, integrated control programs have begun to use an ever increasing number of molecular techniques based on DNA technology. Fundamental aspects of these programs can be achieved by means of DNA analysis (McPheron & Steck, 1996; He & Haymer 1999; Ochando & Reyes, 2000; Meixner *et al.*, 2002). Several advantages of technologies based on PCR make these methodologies particularly suitable for the study of pest species (Schiewrater *et al.* 1994; Hoy, 1994; Miesfeld, 1999). One of these - the random amplified polymorphic DNA (RAPD) method – involves the use of the polymerase chain reaction (PCR) to amplify random segments of genomic DNA using a single short primer of arbitrary sequence. The possibility to examine genomic variation without previous sequence information (Williams *et al.*, 1990), the relatively low cost of the technique and the need for only a few nanograms of

template DNA, are all potential advantages of using RAPD in quarantine laboratories (Welsh & McClelland, 1990; Williams *et al.*, 1990; Hadrys *et al.*, 1992). The RAPD-PCR method has therefore been used successfully in a series of different entomological contexts, and particularly as a taxonomic tool (Gawel & Bartlett 1993; Frey & Frey, 1995; Armstrong *et al.*, 1997; Fernández *et al.*, 2001).

Given the possibility of employing RAPD markers to differentiate between species, subspecies, races and even biotypes, the present study sought to use RAPD-PCR to generate informative genetic markers to unambiguously identify three insect pest species that cause considerable economic losses (*Bemisia tabaci* (Gennadius), *Frankliniella occidentalis* (Pergande) and *Liriomyza huidobrensis* (Blanchard)), at any stage of their respective life cycles. Correct identification is the first step towards developing effective specific control strategies.

Material and methods

We analysed adults from *B. tabaci, F. occidentalis* and *L. huidobrensis* that had been preserved in 90% ethanol. In the case of *L. huidobrensis*, also some pupae, were studied. At least three different samples from each species were analyzed, with a total of 80 individuals used from each species.

Total genomic DNA was isolated according to Higuchi (1989) with minor modifications. Four primers from Operon Technologies (Alameda, California) kits A and C were used to generate RAPD profiles. The primer sequences (5'→ 3') were: OPC-11, CAATCGCCGT, OPA-02, TGCCGAGCTG, OPA-07, GAAACGGGTG, OPA-17, GACCGCTTGT. Amplification reactions were performed following the protocol of Williams *et al.* (1990), with slight modifications in 12.5 µl reaction volumes and using the Stoffel Fragment DNA polymerase (PE Applied Biosystems). These amplifications were carried out in a thermal cycler MJ Research PT-100 under the following conditions: preheat at 94°C for 5 min, 45 cycles of amplification (1 min at 94°C, 1 min at 36°C, 6 min at 72°C) and a final step of 6 min at 72°C. The amplification products were resolved electrophoretically on 2% agarose gels, containing ethidium bromide and using a TAE buffer system (40mM Tris, 20 Mm acetic acid and 1 mM EDTA). Gels were visualised with UV light and photographed with a Polaroid camera. A 100 bp DNA Ladder Plus (MBI Fermentas) was used as molecular marker.

Data Analysis

A Molecular Imager System and Image Analysis Software Multi-Analyst Ver 1.1 (BioRad Laboratories, Inc.) were used to analyse the RAPD profiles of the different species. RAPD-PCR products were scored as either present or absent in each fly (variations in intensity were not taken into account). The total number of bands and their frequencies were calculated for each population and species.

Results

According to some authors, the main drawback of the RAPD technique is its sensitivity to changes in reaction conditions, which may affect the reproducibility of amplification products. Reactions were therefore carried out in our laboratory following strict protocols with standardised conditions and repeating each amplification reaction at least twice in order to avoid problems with repeatability. The results were consistently reproducible amplification reactions.

Amplifications of genomic DNA from the 240 specimens studied with the four primers showed clearly different profiles for each species. Representative DNA profiles from all three species obtained using the primers OPA-02 and OPC-11 are shown in Figure 1.

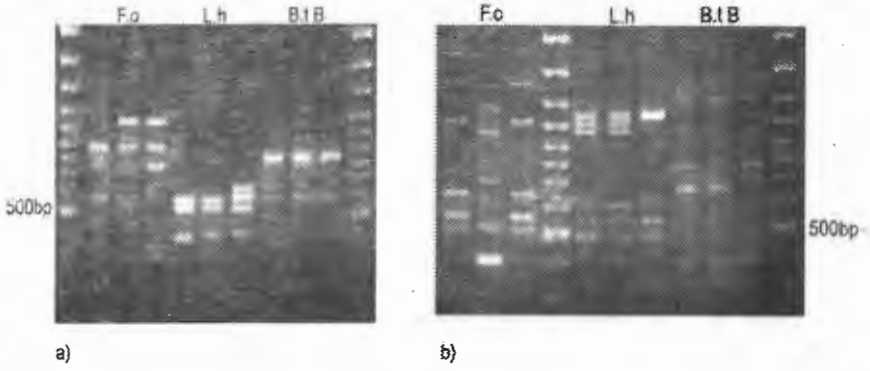

Figure 1.- RAPD profiles with primers OPA-02, (1a) and OPC-11 (1b). Each line consists of DNA from an individual from the specified species. 1a): Lines 1 and 11, molecular size marker 100 bp DNA Ladder Plus, lines 2-4, *Frankliniella occidentalis*, lines 5-7, *Liriomyza huidobrensis*, lines 8-10, *Bemisia tabaci*, biotype B. 1b): Lines 4 and 11, molecular size marker 100 bp DNA Ladder Plus, lines 1-3, *F. occidentalis*, lines 5-7, *L. huidobrensis*, lines 8-10, *B. tabaci*, biotype B.

Monomorphic bands, i.e. invariable bands, that can initially be considered as "diagnostic" markers, present in all of the individuals examined in a given species, were found for all the primers tested and for two of the studied species: *B. tabaci* and *L. huidobrensis*. Thus, seven monomorphic bands were found for *B. tabaci*, seven for *L. huidobrensis*, and none for *F. occidentalis*. Table 1 shows these invariable bands.

Table 1.- Monomorphic bands: bands present in all individuals of the species (by molecular size), with the different primers used. -- No diagnostic bands. * Coincident marker common to two species.

	Primers			
Species	**OPC-11**	**OPA-02**	**OPA-07**	**OPA-17**
Bemisia tabaci	555	600*, 500	890	700, 620, 580
Frankliniella occidentalis	--	--	--	--
Liriomyza huidobrensis	550	600*, 415	590, 490	800, 490

RAPD markers present in a high percentage (≥ 90 %) of the individuals of one species are shown in Table 2. In this case, the total number of bands is greater, 16, considering all three

species and primers. In the case of *F. occidentalis*, which did not display any "diagnostic" bands, a total of 8 high frequency markers were found.

Table 2.- Bands, by molecular size, present in a high percentage (≥90%) of the individuals of the species, with the different primers used. --No high frequency markers.

	Primers			
Species	**OPC-11**	**OPA-02**	**OPA-07**	**OPA-17**
B. tabaci	--	850, 670	750	--
F. occidentalis	1050, 650, 565	1100, 890, 750	425, 400	--
L. huidobrensis	1150, 490	580	1300	500

Discussion

The species studied are responsible for important economic losses in the countries where they are established: they affect fruit and vegetable crops and are expensive to eradicate (McPheron & Steck, 1996; Roderick, 1996; Mumford, 2000; Tan, 2000). There is an evident need for "diagnostic" markers at the lowest possible level. It is possible to morphologically distinguish between the three species used in this work, though this was not the aim of the study. The present work had two main purposes: to test the suitability of the RAPD methodology for generally identifying insect pests; and to provide to insect quarantine laboratories with accurate information valid for all occasions and situations. The combination of morphological identification methods and molecular techniques makes it possible to quickly and accurately discriminate between species and even between biotypes or races.

The findings of this study prove, once more, the usefulness of arbitrary amplified DNA as a valuable source for identification of different insect pests (Gawel & Bartlett 1993; Frey & Frey, 1995; Callejas *et al.*, 1998). From the total number of bands generated, RAPD analysis successfully identified a total of 14 species invariable DNA markers (Table 1, Figure 1). All (except one) of these bands (band OPA02-600 pb, which is common to two of the studied species, *B. tabaci* and *L. huidobrensis*), can be considered, species-specific or diagnostic markers. Notwithstanding, it will be necessary to test for the absence of these bands in other closed species before any definitive conclusions can be drawn. The clear advantage of these diagnostic markers over morphological classification is that identifications can be made with even the smallest pieces of tissue or from damaged specimens at any stage of their development. There are also 16 markers that are present in high frequencies in the different species (Table 2). These bands could be useful for identifying certain populations that in turn prove very helpful in identifying the sources of populations migrating to new areas.

As the number of diagnostic markers increases, so will our ability to make more accurate identifications (Campton, 1987). A single primer, or - depending on the level of identification required - a combination of two or more primers, could be used to reveal clear diagnostic patterns (see Figure 1). RAPD analysis is also a very useful taxonomic tool for identifying insects at any stage of maturity: in our case, we tested pupae and adult insects. This is particularly important where prompt identification is needed, such as in rapid-action pest control management or the analysis of imported products by quarantine laboratories.

The use of RAPD markers to differentiate between species, and even between infraspecific groups, allows a greater degree of flexibility and presents several advantages with respect to other molecular techniques. No prior knowledge of particular sequences or

genes is required, which means that new species can be genetically analysed without referring to a classical genetic database. In contrast to allozyme analysis, this method makes it possible to identify specimens at any stage of maturity, with only a minute quantity of biological material. This facilitates the study of the smallest of insects at the individual level and also means that dead specimens and those conserved in alcohol can also be used (Schierwrater *et al.*, 1994; De Barro & Driver, 1997). Another important advantage of the RAPD technique for population and taxonomic studies is that very high levels of polymorphism are generally detected (Haymer, 1994; Baruffi *et al.*, 1995; Ochando *et al.*, 2003). Reliability can be improved by optimizing experimental conditions and following experimental protocols to the letter.

In summary, RAPD markers are sufficiently reproducible to be a useful tool for the identification and discrimination of insect pest species. The detection of several diagnostic bands for only one taxonomic category (species, population) allows rapid, accurate and unambiguous identification. For recent colonizer pests, these DNA markers could be of great use in genetic, ecological and physiological research. Finally, RAPD markers could also help in assessing the origin and spread of invading populations and in future control programs. Early identification could be vital to specific control strategies designed to prevent the spread of pests and reduce the considerable economic losses they occasion in different crops.

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References

Armstrong, K.F., Cameron, C.M. & Frampton, E.R. 1997: Fruit fly (Diptera: Tephritidae) species identification: a rapid molecular diagnostic technique for quarantine application. Bull. Entomol. Res. 87: 111-118.

Baruffi, L., Damiani, G., Guglielmino, C.R., Bandi, C., Malacrida, A.R. & Gasperi, G. 1995: Polymorphism within and between populations of *Ceratitis capitata*: comparison between RAPD and multilocus enzyme electrophoresis data. Heredity 74: 425-437.

Callejas, C., Roda, P., Reyes, A. & Ochando, M.D. 1998. Identificación genética de *Dacus – Bactrocera- oleae* Gmelin (Diptera: Tephritidae) mediante marcadores RAPD-PCR. Bol. San. Veg. Plagas 24: 873-882.

Campton, D.E. 1987: Natural hybridization and introgression in fishes. In: Population genetics and fishery management, eds. Ryman and Utter: 161-192. University of Washintong Press, Seattle.

De Barro, P.J. & Driver, F. 1997: Use of RAPD-PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Australian J. Entomol. 36:149-152.

Fernández, P., Segura, D. & Ochando, M.D. 2001: Taxonomia y diferenciación genética de *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) y *Bactrocera oleae* (Gmelin, 1790) (Diptera: tephritidae) mediante marcadores moleculares (RAPD-PCR). Bol. R. Soc. Esp. Hist. Nat. 96: 321-329.

Frey, J.E. & Frey, B. 1995: Molecular identification of six species of scale insects (*Quadraspidiotus* sp.) by RAPD-PCR: assessing the field-specificity of pheromone traps. Mol. Ecol. 4: 777-780.

Gawel, N.J. & Bartlett, A.C. 1993: Characterization of differences between whiteflies using RAPD-PCR. Insect Mol. Bio. 2: 33-38.

Hadrys, H., Balick, M. & Schierwater, B. 1992: Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. 1: 55-63.

Haymer, D.S. 1994: Random Amplified Polymorphic DNAs and microsatellites: what are they, and can they tell us anything we don't already know?. Ann. Entomol. Soc. Am. 87, 717-721.

He, M. & Haymer, D. S. 1999: Genetic relationships of populations and the origins of new infestations of the Mediterranean fruit fly. Mol. Ecol. 8: 1247-1257.

Higuchi, R. 1989: Simple and rapid preparation of samples for PCR. In: PCR Technology, ed. Erlich: 31-38. Stockton Press, New York.

Hillis, D.M., Moritz, C. & Mable, B.K., eds. 1996: Molecular Systematics. Sinauer Assoc. Sunderland, Mass.

Hoy, M. 1994: Insect Molecular genetics. Academic Press. San Diego, CA.

McPheron, B.A.& Steck, G.J., eds. 1996: Fruit Fly Pests. A world assessment of their biology and management. St. Lucie Press. Delray Beach, FL.

Meisfeld, R.L. 1999: Applied Molecular Genetics. Wiley-Liss. New York.

Menken, S.B.J. & Ulenberg, S.A. 1987: Biochemical characters in agricultural entomology. Agric. Zool. Rev. 2: 305-360.

Meixner, M.D., McPheron, B.A., Silva, J.G.-, Gasparich, G.E. & Sheppard, W.S. 2002: The Mediterranean fruit fly in California: evidence for multiple introductions and persistent populations based on microsatellite and mitochondrial DNA variability. Mol. Ecol. 11: 891-899.

Mumford, J.D. 2000: Economics of Area-Wide pest control. In: Area-Wide control of fruit flies and other insect pests, ed. Tan: 39-47. Penerbit Universiti Sains Malaysia, Pulau Pinag, Sinaran Bross. Sdn. Bhd.

Ochando, M.D. & Reyes, A. 2000: Genetic population structure in olive fly *Bactrocera oleae* (Gmelin): gene flow and patterns of geographic differentiation. J. Appl. Ent. 124: 177-183.

Ochando, M.D., Reyes, A., Callejas, C., Segura, D., Fernández, P. 2003. Molecular genetic methodologies applied to the study of fly pests. Trends Entomol., in press.

Roderick, G.K. 1996: Population genetic studies of Tephritid flies of economic importance. 1996. In: Fruit Fly Pests. A world assessment of their biology and management, eds. McPheron and Steck: 267-271. St. Lucie Press. Delray Beach, FL.

Schierwater, B., Streit, B., Wagner, G.P. & DeSalle, R., eds. 1994: Molecular ecology and evolution: approaches and applications. Birkhäuser Verlag, Basel.

Tan, K.-H., ed. 2000: Area-wide control of fruit flies and other insect pests. Penerbit Universiti Sains Malaysia, Pulau Pinang. Sinaran Bross. Sdn.Bhd.

Welsh, J. & McClelland, M. 1990: Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213-7218.

Williams, J.K.G., Kubelik, A.R., Livak, K.J., Rafalsky, J.A. & Tyngey, S.V. 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

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Field efficacy of spinosad, an insecticide derived from microbial activity, in protected vegetable crops

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Abstract: The effectiveness of a new insecticide compound derived from metabolites of the naturally occurring bacteria *Saccharopolyspora spinosa,* was tested in Sicily from 1998 to 2002. Experimental plots of protected sweet pepper, eggplant and cucumber were sprayed with different doses of spinosad and it's the effects were compared with those of conventional insecticides. The different flower (thrips) and leaf (noctuids and leafminers) samples were observed to record the number of infested organs and the presence of live specimens of each pest type. Good results, in terms of the reduction of harmful pest populations, were obtained with respect to other standard chemicals used in the same trials. In all cases, EPPO Standard Guidelines were applied and data were statistically analysed.

Key words: spinosad, protected vegetable crops, pests, field test

Introduction

Novel insecticide compounds are currently being presented as promising alternatives to traditional chemical sprays. The new products are progressively offered on the market after testing their efficacy on insect pests and selectivity with regard to natural enemies. This ensures good pest control levels and also compatibility with IPM programs.

Spinosad is an aerobic fermentation product of the naturally occurring actinomycete bacterium *Saccharopolyspora spinosa* Mertz & Yao, which was first found in Caribbean soil in 1982. This microorganism produces over 30 different metabolites (spinosyns); after manipulation, these metabolites can yield even more compounds (spinosoids). The insecticide takes its name from this microbe and contains two chemicals: spinosyns A and D.

Spinosad is more active when ingested than when used as a contact insecticide; it may penetrate the leaf surface by translaminar movement (Bret *et al.*, 1997) and insects such as Lepidoptera, Diptera and Thysanoptera are primary target pests. Furthermore, the unique mode of action of this product, which causes a rapid excitation of the insect nervous system (Salgado, 1997), displays no cross-resistance with any other known synthetic or biological compounds. This makes spinosad particularly valuable as part of insecticide resistance management programs (Tescari *et al.*, 1998, 2000).

In Italy, good results have been obtained against *Spodoptera littoralis* (Boisduval) on lettuce (Sannino, 2001) and against *Frankliniella occidentalis* (Pergande) on grapevine (Tropea Garzia and Buonocore, 2003).

Material and methods

From 1998 to 2002, studies were performed in unheated greenhouses with wooden or steel frames that were covered with polyethylene film, in order to determine the efficacy of spinosad against western flower thrips (*F. occidentalis*), leafminers (*Liriomyza* spp.) and the Egyptian cotton leafworm (*S. littoralis*) on solanaceous and cucurbitaceous crops. All tests were randomised in complete block designs consisting of four replicates, in which European guidelines for the efficacy evaluation of plant protection products were applied, as suggested by the European and Mediterranean Plant Protection Organization (EPPO).

Pest monitoring was carried out on each crop in order to optimise treatment timing; insecticide applications were made using a knapsack sprayer, working at 1 bar and distributing 1000 l/ha of solution.

Samples were taken from the central rows of each thesis and treatments were compared with a control plot sprayed with water alone.

Tables 1-3 show the compounds and rates of commercial products (c.p.) employed each year, timing of applications and also sample sizes and dates (samples were collected and observed according to the pest behaviour: from flowers in the case of thrips, and from leaves in the case of leafminers and noctuids). Information relating to the crop, cultivar, transplanting and plant density is also reported. All data were evaluated using the SNK test and statistical significance was tested at the 5% level.

Results

Thrips

A significant reduction was initially observed on sweet pepper in spinosad plots (at both rates). There was a 100% reduction at the young stages, which statistically differed from results obtained with acrinatrine. The new product showed a good level of efficacy that remained almost invariable for at least 10 days (table 4).

In 2001, two treatments applied on cucumber at 7 days intervals proved better at controlling thrips populations, as shown in figure 1. These results did not significantly differ from the chemical plot, but were significantly different from the control test.

Similar results were obtained in 2002 (figure 2), when the reduced number of flowers on cucumber plants caused a corresponding decline in population towards mid June: this was also observed on the control test plot.

Leafminers

In experiments carried out in 2001 (table 5), when spraying in presence of mines on leaves, spinosad showed appreciable activity, which was statistically different from the untreated plot. As far as the percentage of leaves bearing mines was concerned, an initial increase in numbers was observed but this was maintained at very low levels in all theses. In the cyromazine plot, no positive value was registered at the end of the trial. Significant differences between the chemical plots and the control test plot started from 3 days after the second treatment (10 DAT). Natural parasitism by *Diglyphus isaea* (Walker) was observed in all plots.

A similar population trend was noted in 2002, following the appearance of oviposition stings. In this case, a major reduction was only obtained after the second treatment. The number of damaged leaves constantly increased in the non-treated plot, while it remained at very low levels in the other theses (figure 3).

Table 1. Treatment schedule for thrips control (DAT= days after first treatment).

Active ingredient	Rate c.p. (cc/hl)	Date of treatment	Sample size (flowers)	(DAT)
Year 1998 – Sweet pepper cv Gordo – Transplanting 5/06/1998 – plant density 3/sqm				
Spinosad	25	10th Jul	56/plot	0-2-10-20
Spinosad	50	10th Jul	56/plot	0-2-10-20
Acrinatrin	40	10th Jul	56/plot	0-2-10-20
Year 2001 – Cucumber cv Jazzer – Transplanting 3/04/2001 – plant density 3/sqm				
Spinosad	20	8th May-15th May	60/plot	0-3-7-14
Acrinatrin	80	8th May-15th May	60/plot	0-3-7-14
Year 2002 – Cucumber cv Solverde – Transplanting 6/05/2002 – plant density 2.5/sqm				
Spinosad	20	24thand 30th May -- 7th Jun	60/plot	0-7-14-21
Acrinatrin	80	24th and 30th May –7th Jun	60/plot	0-7-14-21

Table 2. Treatment schedule for leafminer control (DAT= days after first treatment).

Active ingredient	Rate c. p. (cc/hl)	Date of treatment	Sample size (leaves)	(DAT)
Year 2001 – Cucumber cv Jazzer – Transplanting 3/04/2001 - plant density 3/sqm				
Spinosad	50	8th May-15th May	30/plot	0-3-7-10-14-21
Spinosad	75	8th May-15th May	30/plot	0-3-7-10-14-21
Cyromazine	50	8th May-15th May	30/plot	0-3-7-10-14-21
Year 2002 – Cucumber cv Solverde – Transplanting 6/05/2002 – plant density 2.5/sqm				
Spinosad	50	23rd and 30th May	20/plot	0-7-14-28
Spinosad	75	23rd and 30th May	20/plot	0-7-14-28
Cyromazine	50	23rd and 30th May	20/plot	0-7-14-28

Table 3. Treatment schedule for noctuid control (DAT= days after first treatment).

Active ingredient	Rate c. p. (cc/hl)	Date treatment	Sample size (plants)	(DAT)
Year 2001 – Cucumber cv Jazzer – Transplanting 3/04/2001 - plant density 3/sqm				
Spinosad	20	15th May	10/plot	0-3-7-14
Spinosad	25	15th May	10/plot	0-3-7-14
Spinosad	30	15th May	10/plot	0-3-7-14
Methomyl	200	15th May	10/plot	0-3-7-14
Year 2002 – Cucumber cv Solverde – Transplanting 6/05/2002 – plant density 2,5/sqm				
Spinosad	20	30th May –6th Jun	10/plot	0-7-21
Bacillus thuringiensis	200	30th May –6th Jun	10/plot	0-7-21
Methomyl	200	30th May –6th Jun	10/plot	0-7-21

Noctuids

As regards *S. littoralis*, data obtained in 2001 relating to the percentage of damaged leaves evidenced how spinosad (each rate of active ingredient employed) was highly efficacious and differences were observed from 7 days after treatment (figure 4).

In the 2002 experiment, a different control activity evaluated on the percentage of damaged leaves/plot has been registered. Statistically significant differences for

spinosad/*Bacillus thuringiensis* formulation and methomyl/control plot were observed two weeks after the last treatment (21 DAT). Data are shown in table 6.

Table 4. Corrected mortality (Abbott, 1925) of adult and young stages of *F. occidentalis* x days after treatment in 1998 (n.a.= not available (low number of flowers); c. p.= commercial product).

Treatment	Rate c. p. (cc/hl)	2 DAT		10 DAT		20 DAT	
		Adults	Young stages	Adults	Young stages	Adults	Young stages
Spinosad	25	**94.21 a**	**100 a**	**88.21 a**	**97.58 a**	n. a.	n. a.
Spinosad	50	**97.11 a**	**100 a**	**88.61 a**	**98.39 a**	**68.95 a**	**41.88 a**
Acrinatrin	40	**93.06 a**	**60.00 b**	**88.61 a**	**86.86 a**	n. a.	n. a.
Control	-	**0.00 b**	**0.00 c**	**0.00 b**	**0.00 b**	**0.00 b**	**0.00 a**

Means within columns by the same letters are not significantly different (P>0,05).

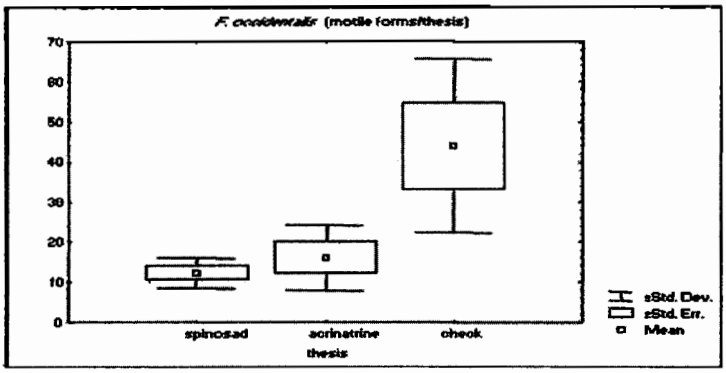

Figure 1. Year 2001. Average number of thrips at the end of the test.

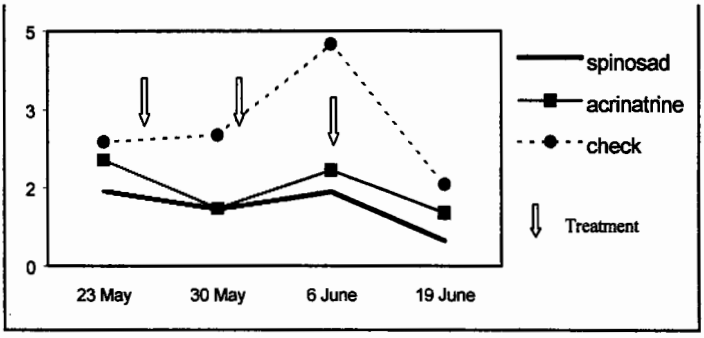

Figure 2. Year 2002. Average number of motile forms of *Frankliniella occidentalis* on cucumber crop (⇩= treatment).

Table 5 – Year 2001. Percentage of leaves infested by *Liriomyza* spp.

Treatment	Sampling date					
	0 DAT	3 DAT	7 DAT	10 DAT	14 DAT	21 DAT
Spinosad 50	14.17 a	7.50 a	12.50 a	7.50 ab	8.33 a	2.50 ab
Spinosad 75	10.83 a	2.50 a	5.83 a	7.50 ab	10.00 a	3.33 ab
Cyromazine	18.33 a	7.50 a	6.67 a	1.67 a	2.50 a	0.00 a
Control	5.83 a	6.67 a	10.00 a	16.67 b	21.67 b	6.67 b

Means within columns followed by the same letters are not significantly different (P>0.05).

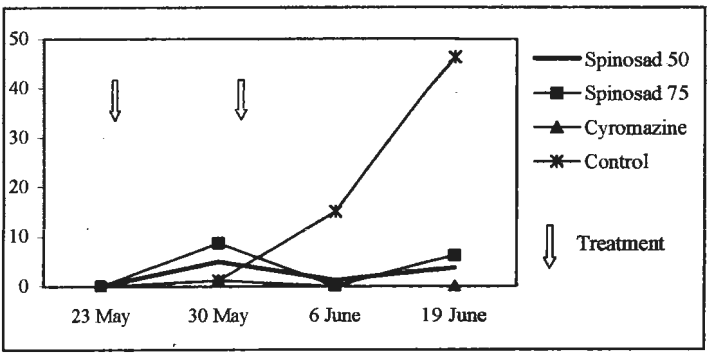

Figure 3 – Year 2002. Percentage of leaves infested by *Liriomyza* spp.

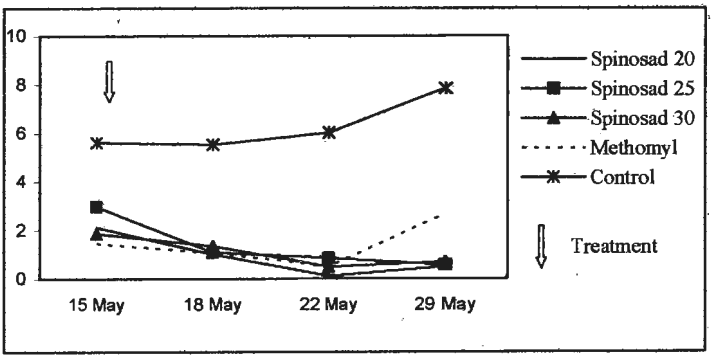

Figure 4 – Year 2001. Percentage of damaged leaves/plot in Noctuids tests.

Table 6 – Year 2002. Percentage of damaged leaves/plot in Noctuids tests.

Treatment	Sampling date		
	0 DAT	7 DAT	21 DAT
Spinosad 20	16.70 a	45.60 a	35.71 a
B. thuringiensis	45.83 a	51.50 a	38.40 a
Methomyl	16.70 a	61.80 a	80.35 b
Control	4.20 a	50.00 a	88.40 b

Means within columns followed by the same letters are not significantly different (P>0.05).

Discussion

Good results were obtained for spinosad: its insecticidal activity was revealed to be similar to those of traditional chemical standards in all trials and it also provided satisfactory controls for pest populations at low rates of application. Thrips were efficaciously controlled by only two treatments, when they were accurately applied at initial pest appearance and after an interval of 7 days. Two similar scheduled treatments, which were applied on the first appearance on the plants of both mines and oviposition stings, successfully also controlled leafminer flies. In the case of noctuids, one or two treatments were necessary to achieve a good control in the initial presence of either low or high percentages of damaged leaves.

Possible side effects of spinosad on beneficial arthropods, and especially the timing of treatments in order to reduce interference with the conservation or distribution of natural enemies, still need to be examined in greater depth on vegetable crops. This needs to be done in order to make a complementary evaluation of this compound and to assure its compliance with ecologically healthy pest control strategies and IPM programs.

References

Abbott, W. S. 1925: A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Bret, B. L., Larson, L. LJ. R., Sparks, T. C. & Thompson, G. D. 1997: Biological properties of spinosad. Down to earth, 52 (1): 6-13.

Salgado, V. L. 1997: The modes of action of spinosad and other insect control product. Down to earth, 52 (1): 35-43.

Sannino, L. 2001: Efficacia contro *Spodoptera littoralis* di un nuovo formulato. L'Informatore Agrario, 5: 74-76.

Tescari, E, Dalla Valle, N. & Guiducci, M. 1998: Spinosad (Tracer*): nuovo agente di derivazione naturale per il contenimento degli insetti dannosi. Atti Giornate Fitopatologiche, 1998: 149-154.

Tescari, E., D'Alessandro, M. & Montagnini, C. 2000: Spinosad (Laser*): ulteriori acquisizioni di effetti collaterali nei riguardi dell'artropodofauna utile. Atti Giornate Fitopatologiche, 2000 (1): 299-306.

Tropea Garzia, G. & Buonocore, E. 2003: Un formulato di origine naturale contro *Frankliniella occidentalis*. L'Informatore Agrario, 7: 57-60.

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