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Integrated Control in Protected Crops

“Mediterranean Climate”

editors:

Ramon Albajes & Erdal Sekeroglu

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at

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PREFACE

The present volume contains most of the oral contributions to the meeting of the IOBC/WPRS working group on 'Integrated Control in Protected Crops, Mediterranean Climate'. If we compare it with the content of the volumes corresponding to the previous meetings, we will find that the authors and the relative importance of the subjects has changed substantially. The considerable number of new authors shows the vitality of the working group. An important development is the incorporation of Turkish researchers, which shows that it was a good decision to hold the meeting in this Mediterranean country. The number of contributions referring to the study and use of Mediterranean native fauna of natural enemies continues to grow. All of this shows the dynamism of integrated pest management in Mediterranean protected crops, though one must rest on our laurels, because there are still some shortcomings that urgently need to be solved in the immediate future. On the one hand we have few colleagues from the southern Mediterranean, whose plastic-covered surface is growing incessantly and whose products are increasingly supplying Europe with early-collected vegetables. This problem is difficult to solve without the clear will of the governments of these countries to accelerate the application of IPM systems; I am sure that the colleagues of the southern Mediterranean will always find in the working group the collaboration that they require. Also, the number of plant pathologists – including plant virologists – is still too low. This time the coincidence of the dates of the meeting with the holding of other meetings specifically devoted to plant pathology explains the absence of colleagues who have been regular participants in the working group in the recent past.

I am sure that these challenges will be taken up by the next convenor of the working group. After ten years convening the group, feel it is time to hand over. I feel that it was worth the effort to consolidate this working group and I want to express my thanks for the efforts of all the colleagues who have organized meetings, participated in meetings and encouraged to the research and application of integrated pest management in the greenhouses of their respective zones. In specific reference to the present meeting in Antalya, Turkey, and on behalf of all its participants, I wish to thank Erdal Sekeroglu –the local organizer and co-editor of this volume – for his excellent work and for having created the possibility for many Turkish colleagues to join the working group. I also think that we can be satisfied with our collaboration with the working group 'Protected Crops, Continental Climate', which until a few months ago was convened by Joop C. van Lenteren. In him I always found unconditional support to carry out joint tasks between north and south. I have no doubt that the cooperation will continue with his successor, Annie Enkegaard.

Our dear colleague Giorgio Nicoli, a habitual and enthusiastic member of the group, left us recently. In addition to the loss of an inestimable scientist, we have lost a friend. It is obvious that we will all greatly miss his contributions, his comments, his good humour, and his contagious optimism.

Ramon Albajes, convenor

IOBC/WPRS W.G. on 'Integrated Control in Protected Crops, Mediterranean Climate'

November 1999

PRÉFACE

Le volume ici-présent contient la majeure partie des contributions orales à la réunion du groupe de travail de la OILB/SROP "Lutte intégrée en cultures protégées, Climat Méditerranéen". Si l'on compare son contenu avec celui des volumes correspondant aux réunions précédentes, on notera un changement substantiel dans les auteurs et la taille relative des différents thèmes abordés. Le nombre remarquable de nouveaux auteurs démontre la vitalité du groupe de travail. Il faut souligner notamment l'incorporation de chercheurs turcs, ce qui confirme le bien-fondé d'avoir organisé la réunion dans ce pays. L'augmentation du nombre de contributions portant sur l'étude et l'emploi de la faune native méditerranéenne d'ennemis naturels se maintient. Tout ceci démontre le dynamisme de la lutte intégrée en cultures méditerranéennes sous abri, mais néanmoins gardons nous d'un quelconque triomphalisme, le travail se poursuit et nous souffrons de plusieurs carences qu'il est urgent de combler et ce dans un futur proche. D'un côté, le manque de collègues du sud de la Méditerranée, avec une superficie agricole sous abri qui ne cesse de croître et dont les produits fournissent de plus en plus l'Europe en légumes printaniers. C'est un problème difficile à résoudre sans la volonté claire des administrations de ces pays d'accélérer l'application de systèmes de contrôle intégré; Je suis persuadé que les collègues de la Méditerranée méridionale trouveront toujours dans le groupe de travail, la collaboration voulue. D'un autre côté, le nombre de pathologistes végétales -incluant les virologistes- demeure excessivement bas. Dans le cas présent, le déroulement simultané de cette réunion avec d'autres spécifiquement dédiées à la pathologie végétale explique l'absence de plusieurs collègues qui participaient régulièrement au groupe de travail.

Je suis persuadé que ces défis seront pris en compte par le prochain coordinateur du groupe de travail. Après près de dix ans d'animation au sein du groupe, je pense qu'il est temps de chercher un successeur. Je pense que l'effort de consolidation de ce groupe de travail aura valu la peine et je voudrais remercier tous les collègues qui ont organisé des réunions, participer à ces mêmes réunions et fait avancer la recherche et l'application du contrôle intégré en serres dans leur pays respectif. En ce qui concerne la présente réunion de Antalya, Turquie, et au nom de tous les participants, je voudrais remercier Erdal Sekeroglu -organisateur local et co-éditeur de ce volume- pour son excellent travail et pour avoir permis à de nombreux collègues turcs d'incorporer le groupe de travail. Je pense également être satisfait de la collaboration que nous avons avec le groupe de travail "Cultures protégées, Climat Continental" que coordonnait jusqu'à voilà quelques mois, Joop C. van Lenteren. J'ai toujours trouvé en Joop un appui inconditionnel pour mener à bien les tâches conjointes entre le nord et le sud. Je n'ai pas le moindre doute que la collaboration se poursuivra avec son successeur Annie Enkegaard.

Dans les derniers mois, nous a quitté notre cher collègue Giorgio Nicoli, membre habituel et enthousiaste du groupe. Outre la perte d'un inestimable scientifique, nous perdons aussi un ami. Il est évident que nous regretterons tous ses contributions, ses commentaires, sa bonne humeur et son optimisme contagieux.

Ramon Albajes, animateur

G.T. OILB/SROP 'Lutte intégrée en cultures protégées, Climat Méditerranéen'

Novembre 1999

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Section I

IPM systems and components in protected crops in the Mediterranean Basin

Section I

Systèmes et composants de lutte intégrée en culture protégée dans le Bassin Méditerranéen

First experiences in Italy of IPM on ornamental cut foliage: *Danae racemosa* and *Fatsia japonica*

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Abstract: On the Northwest coast of Italy, ornamentals and cut foliage are the main cultivated crops. Italian, European and nowadays also North American markets show a big interest in such crops. Among cut foliage crops, *Ruscus* (*Danae racemosa*) and *Fatsia* (*Fatsia japonica*) are two of the most important and to enhance the quality of these productions, an application of IPM using natural enemies has begun. Parasitoids and predators, as for example *Phytoseiulus persimilis* against spider mites, *Aphidius colemani*, *Lysiphlebus testaceipes* and *Harmonia axyridis* against aphids, *Cryptolaemus montrouzieri* against scales and *Heterorabditis* spp. against larvae of weevils (*Otiorrhynchus* spp.), were released. The efficacy of the seasonal inoculative releases of the natural enemies and microbial control agents (e. g. *Bacillus thuringiensis* against Lepidoptera) was generally good and satisfactory for the farmers too. Occasionally, it was necessary to spray some pesticides. Recently, an important new exotic pest, *Protospulvinaria pyriformis*, has been recorded to attack *Fatsia*, increasing problems in defending this crop. Due to the absence of effective natural enemies and to its resistance to selective pesticides, *P. pyriformis* is showing to be now an obstacle to the development of IPM on *Fatsia*. The experience carried out so far seems to show good possibilities for the application of IPM and biocontrol also on cut foliage, although further confirmation is needed and some problems have to be solved yet.

Key words: *Danae racemosa*, *Fatsia japonica*, integrated control, natural enemies.

Introduction

The Italian Riviera is the main floricultural area of Italy. In this region more than 120 species of ornamental cut foliage are grown. Nowadays, the Italian market for cut foliage is showing a positive trend and large quantities of production are absorbed in Europe and recently in the United States too. Among the commercial category of "florists' green", *Ruscus* (*Ruscus racemosus* syn. *Danae racemosa*, 330 ha) and *Fatsia* (*Fatsia japonica* syn. *Aralia japonica*, 15 ha) have an important role on local production and both crops are very popular among consumers as support for cut flower bunches or for flower arrangements. These crops are mainly characterised by their evergreen nature and long harvesting period of several months. The *Ruscus* plants can be exploited for at least 20 years in shade houses, while *Fatsia* is grown for not more than 10 years in greenhouses.

One of the key arthropod pests of *Ruscus* and *Fatsia* is the two-spotted spider mite *Tetranychus urticae*, against which many chemicals are commonly sprayed. This mite feeds on young cladodes, pierces and removes the cell content, leading to characteristic yellow-brown leaf spots. The other harmful pests include aphids (e.g. *Aphis gossypii*), scales (e.g. the mealybug *Planococcus citri*), Lepidoptera moths and weevils. Aphids, thrips and scales can multiply rapidly around the year and they have to be controlled. Occasionally, larvae of

noctuid and tortrix moths may also attack the plants. Adults of weevils (*Otiorrhyncus* spp. and *Neoplinthus tigratus*) can cause severe damage on ruscus; they attack the cladodes during the summer at night, and larvae feed on the roots and burrow into the rhizomes from September to April (Arzone, 1987).

Growers spray pesticides to minimize insect and mite problems, in many cases using chemicals with a high toxicity and without attention towards environmental problems. The situation is made worse because greenhouses are often close to residential areas. Hence, a change of mentality in favour of alternative methods on cut foliage health management, is now desired, also due to the increasing request from European market of "pesticide free" products. One way to solve this problem is to promote the interest in using biological control of arthropod pests. Several parasitoids and predators can be released in our growing situation and some cut foliage crops seem legitimate candidates for arthropod pests biological control. In addition, thanks to the application of IPM, reduction of wide-spectrum insecticides can allow the colonization of ornamental crops by several beneficial species, which are potentially very effective for natural control of some harmful pests, as observed also by Bertaux and Marro (1997). To achieve this, a pilot programme, focused on IPM, formulated by the Experimental Institute for Floriculture of Sanremo and supported by the Regional Government, was established in 1998 on small scale productions of Ruscus and Fatsia. Such programme will continue at least till the end of the year 2000. In the present work the first results are discussed.

Material and methods

Since summer 1998 three Ruscus and as many Fatsia cultivations located in family farms, in the Imperia Province (Liguria Region), were chosen to develop an IPM approach. Shade houses or greenhouses, where Ruscus and Fatsia were grown respectively, ranged in size from 400 to 500 m². The infestation rate of pest species and the presence of natural enemies were monitored weekly in every cultivation. A sampling of 12 ruscus plants/100 m² placed along the diagonals and median lines of each field was examined. On Fatsia this sampling was carried out choosing 15 leaves/100 m², randomly and in different positions on the plant.

In presence of pest infestation a release of a corresponding natural enemy was undertaken, as listed in table 1. The amount of biological control agents released were varied according to the different situations, depending on pest species, surfaces and infestation levels. When no natural enemies were available or because of peculiar conditions, selective pesticides were sprayed. To reduce the presence of ants, which strongly influenced the development and distribution of aphid infestations, baits made with biscuits, butter, bran and boron were used (in number of about 1/10 m²) and replaced every 3-4 weeks in average.

Results and discussion

A strategy on Ruscus and Fatsia was defined according to experiences of biological control carried out in Italy, using natural enemies by seasonal inoculative releases on protected crops (Benuzzi & Tommasini, 1995; Ferrari & Benuzzi, 1996).

Integrated control on Ruscus

The main species observed on this crop during summer and autumn 1998 was *T. urticae*. The predator *Phytoseiulus persimilis* was regularly released as soon as pest mite infestation was detected and the number of predators inoculated was adequate to ensure a satisfactory control in shade house environments (about 7 individuals/m² per release). The same problem occurred

in 1999, in different times and farms. In all situations the predatory mite *P. persimilis* was used successfully; an example is given in figure 1. No pesticides were used against spider mites were IPM was applied.

Different species of weevils (*Otiorrhynchus* spp. and *N. tigratus*) can cause heavy damage on *Ruscus* too. In order to control larvae, treatments by soil drenches with the nematode *Heterorhabditis megidis* are considered the best alternative measures on *Ruscus* (Pasini *et al.*, 1994). Two applications were made, in autumn 1998 and 1999. Our strategy against adults, if the plants are attacked, is to carry out weekly sprayings with Derris (natural insecticide derived from *Lonchocarpus* spp.) or Derris + Pyrethrum. These products have been applied successfully in some recent trials (Sacco *et al.*, in press). Thrips occurred only occasionally, showing to be not a key pest species.

Integrated control on *Fatsia*

Aphids (overall *A. gossypii*) and scales (*Icerya purchasi*, *P. citri*, *Saissetia oleae* and *Protopulvinaria pyriformis*) are recurrent pests on *Fatsia* crops in the Italian Riviera throughout the year.

In autumn 1998, owing to the appearance of aphid colonies a biological control was applied. In accordance with the currently available techniques, the parasitoids *Aphidius colemani* and *Lysiphlebus testaceipes* were released inside the greenhouses. Also the predator's *Chrysoperla carnea* and *Harmonia axyridis* were used in some cases, but they resulted less effective. We observed a negative influence of ants (in the Italian Riviera the species *Iridomyrmex humilis* is present) on biological agents, especially on predators. The visits of the ants on infested leaves were reduced by using baits. A satisfactory and prolonged control of aphid populations was achieved by parasitic wasps *A. colemani* (4-7 parasitoids/m² per release) and *L. testaceipes* (2-3 parasitoids/m² per release), which acclimatised after they were introduced in the crop during September and October (fig. 2). Little or no economic damage was observed in the plants. No further releases of parasitoids were necessary in most *Fatsia* greenhouses and a high parasitization was observed when aphid infestations occurred during spring and further seasons of 1999.

The most difficult situation for biological control on *Fatsia* during 1998 and 1999 was noticed with the scales, and mostly with *P. pyriformis*, which resulted very dangerous and resistant both to biological and to chemical control. This coccid was found firstly in Central Italy in 1993 (Pellizzari Scaltriti, 1993) and a few years later in Sicily (South Italy) (Sinacori, 1995). This pest species is polyphagous, infesting many cultivated and not cultivated plant species. This characteristic can increase the difficulty of control treatments. Recently, some biological control studies have been carried out in Israel, using parasitoids of the genus *Metaphycus*, but no successful results have seemed to be obtained due to encapsulation of the eggs of parasitoid by scales (Blumberg *et al.*, 1993). In South Italy (Sicily) some researches are in progress on indigenous parasitoids of *P. pyriformis* (Sinacori, 1995). Against such pest species a mechanical approach was firstly carried out, i.e. the oldest and unmarketable infested leaves of *Fatsia* were removed, although in some cases localized sprays with imidacloprid plus potassium nitrate were necessary. Releases of predators, mostly of *Cryptolaemus montrouzieri*, were slightly effective in controlling other observed species: *I. purchasi*, *P. citri* and *S. oleae*. These scales were never very dangerous, also because of chemical treatments against *P. pyriformis*.

Population dynamics of phytophagous spider mites on *Fatsia* was found similar to the one on *Ruscus*. The predatory mite *P. persimilis*, used after noticing an increase of *T. urticae* populations, always succeeded in containing infestations (fig. 3).

Lepidoptera infestations appeared occasionally in some greenhouses of Fatsia. Attacks of tortrix moths were observed and the control by the microbial insecticide *Bacillus thuringiensis* sub. *kurstaki* was effective. Noctuid moths were also found, but only an insecticide containing pyrethrum gave a complete control.

Preliminary conclusions

The Italian Riviera is characterized by a mediterranean climatic condition, not very cold in winter and not too warm in summer, giving a very suitable environmental condition for the application of biological control using natural enemies, as it was demonstrated also in southern France by Bertaux & Marro (1997). The experience of biological control carried out up to now on two cut foliage crops, Ruscus and Fatsia, is showing good perspective and benefits. Two of the key pest species, aphids and spider mites, showed to be well controlled by parasitoids and phytoseiids, respectively, all year round. For other species however, as weevils on Ruscus and scale insects on Fatsia, it will be necessary to carry out further studies, examining different aspects connected to new and often unforeseeable dynamics of insect populations. *P. pyriformis* on Fatsia remains perhaps one of the biggest questions to be solved: very few data are available from literature to control *P. pyriformis* and not many experiences on control strategies have been carried out so far in Italy. For other scale species the problem can be less serious, on condition that the suitable natural enemies are introduced when pest populations are still at very low levels.

Table 1- Ruscus (*Danae racemosa*) and Fatsia (*Fatsia japonica*) IPM strategy.

PEST	NATURAL ENEMIES	OTHER METHODS
APHIDS (on Fatsia)	<i>Aphidius colemani</i> <i>Lysiphlebus testaceipes</i> <i>Chrysoperla carnea</i> <i>Harmonia axyridis</i>	sprays with insecticidal soap or natural pyrethrum
SCALES (on Fatsia)	<i>Cryptolaemus montrouzieri</i>	Mineral oil
MOTHS (noctuid and tortricid moths, on Ruscus and Fatsia)	<i>Bacillus thuringiensis</i> sub. <i>kurstaki</i>	Natural pyrethrum
VINE WEEVILS (on Ruscus)	<i>Heterorhabditis megidis</i> (against larvae)	Derris + natural pyrethrum (against adults)
THRIPS (on Ruscus and Fatsia)	<i>Orius laevigatus</i>	Selective insecticides
SPIDER MITES (on Ruscus and Fatsia)	<i>Phytoseiulus persimilis</i>	
TARSONEMID MITES (on Fatsia)		Localized sprays with selective pesticides

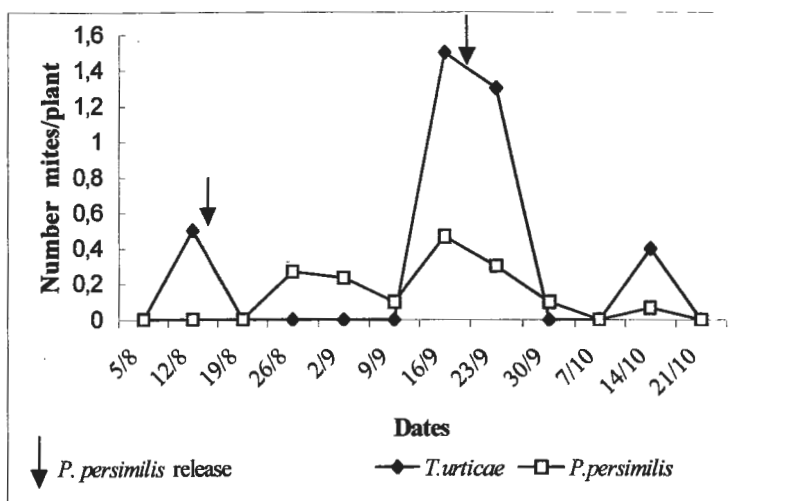


Figure 1. Population dynamics of *T. urticae* and its predator *P. persimilis* on Ruscus crop in a limited period of 1999.

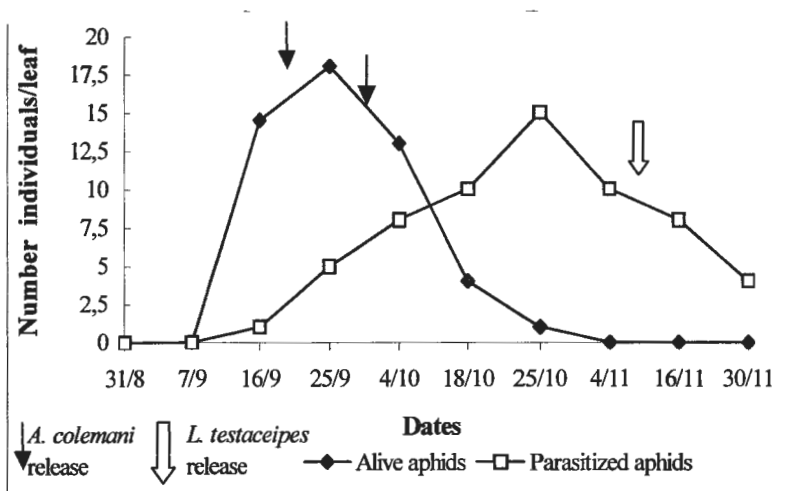


Figure 2. Population dynamics of *A. gossypii* and its parasitoids (*A. colemani* and *L. testaceipes*) on Fatsia crop in a limited period of 1998.

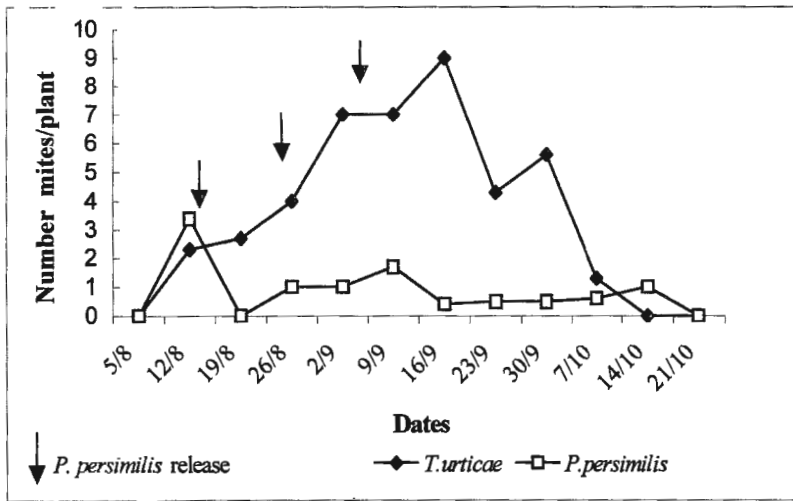


Figure 3. Population dynamics of *T. urticae* and its predator *P. persimilis* on Fatsia crop in a limited period of 1999.

Acknowledgements

This work was funded by Regione Liguria. We appreciate the help of Dr. G. Pellizzari for the determination of *Protospulvinaria pyriformis*.

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Ecological pest management in green and flowering cut foliage in western Liguria

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Abstract: Surfaces of green and flowering cut foliage crops in Italy and particularly in the district of Imperia (Liguria Region) are reported. The main genera grown in Imperia are: *Acacia*, *Genista*, *Pittosporum*, and *Viburnum*. These have replaced more traditional ornamental and flowering crops such as roses, carnation, and gerbera. Major pests of the five most important cut foliage crops are given. The possibility of controlling the pests in different types of farms with low-impact strategies is discussed. Main constraints and needs for a wider application of ecological pest management are reviewed.

Key words: green and flowering cut foliage; pest control; low-impact techniques.

Introduction

The most important obstacle for the implementation of integrated pest management or organic farming techniques in floriculture is the extremely low economic thresholds of pests because the yield is based on aesthetic parameters. This limitation is particularly acute in countries with zero-tolerance that demand products absolutely free of certain pest and diseases. For this reason chemicals are still necessary and integrated pest management strategies have been adopted more successfully than just biological control in ornamental crops and, in particular, in floriculture (Jacobson, 1993; de Goey, 1993; Ravensberg & Altena, 1993; Sopp P., 1993; Vanninen *et al.*, 1993; Wardlow *et al.*, 1990, 1993). Biological control, however, is a principal component of integrated pest management in ornamental crops (Albert, 1993).

As biocontrol aims to manage rather than eradicate pests, its pioneering implementation in floriculture should focus on plant species that can be protected against pests with low-impact chemicals. Flowering and green cut foliage crops seem to satisfy this requirement and they thus may represent a good agroecosystem where to implement integrated pest management strategies.

In this paper we describe the situation of cut foliage crops in western Liguria Region and the main pests that infest them. Then, we show how to proceed with the implementation of low-impact strategies for pest control.

Material and methods

During 1998-99, we recorded data on the main species of cut foliage production and surfaces in the Imperia district. Then, we selected some representative growers and with monitoring techniques (crop inspection and plant sampling) we collected pests for their identification. At the same time we obtained data about chemicals used for pest control.

As result of these observations we defined two main types of growers and we tried to define the most adapted strategies for pest control for each of the two kind of growers.

Results

Principal cut foliage crops and their pests

As in Ireland (Forest, 1999), there are in Italy two factors that have led to the development of a cut foliage production industry: a demand for the product and a suitable climate. Green and flowering cut foliage crops are very important in western Liguria from both environmental and economical points of view. They are grown mainly outdoors in 2,000 ha and represent more than the 80% of the Italian production for several genera (Table 1).

Table 1. Foliage production of the main species in Italy (in ha) and in district of Imperia (in ha and in percentage on total), in the west of Liguria region.

Scientific name	Italian Common Name	ITALY (ha)	IMPERIA (ha)	IMPERIA: INCIDENCE %
<i>Acacia</i> spp	Mimosa	552	500	90,58%
<i>Genista monosperma</i>	Ginestra	1198	1000	83,47%
<i>Ruscus</i> spp	Ruscus	360	300	83,33%
<i>Pittosporum</i> spp	Pittosporo	68	45	66,18%
<i>Viburnum</i> spp	Viburno	70	40	57,14%
<i>Asparagus plumosus</i>	Asparago	74	35	47,30%
<i>Eucalyptus</i> spp	Eucalipto	196	70	35,71%

Data furnished by ISTAT and Agriculture Extension Service of Liguria Region

The main genera grown are *Acacia*, *Genista*, *Pittosporum* and *Viburnum* (Table 2) and in the last few years they have substituted other flower crops (i.e. carnation).

Table 2. Main cut foliage species grown in the district of Imperia with the specification of the type of production

Italian Common Name	Scientific Name	Type of production
Ginestra	<i>Genista monosperma</i>	Cut foliage with flowers
Mimosa	<i>Acacia floribunda</i>	"
	<i>Acacia dealbata</i> cv. "Gaulois"	"
	<i>Acacia dealbata</i> cv. "Tournaire"	"
Viburno	<i>Viburnum opulus</i>	"
	<i>Viburnum tinus</i>	"
Pittosporo	<i>Pittosporum tobira</i>	Cut foliage without flowers
	<i>Pittosporum tenuifolium</i>	"

The number of cut foliage species that are grown by individual farmers varies: a few species in Farms with Cut Foliage Simplified (FCFS) and more species in Farms with Cut Foliage Complex (FCFC) (Fig.1).

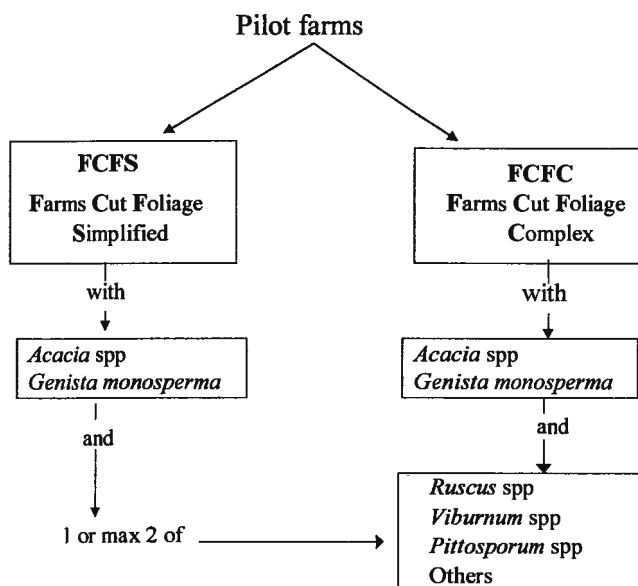


Figure 1. Scheme representing the main cut foliage *genera* grown by two different types of farms (or “pilot farms”) in Imperia.

FCFC are characterised by a variable number of cut foliage species grown, lower grower's specialisation and a higher number of spontaneous species (shrubs and others) around and inside the cut foliage crop.

Cut foliage growers, formerly devoted to grow roses, carnations and gerberas, are generally experienced in applying conventional chemicals. However, in the last years they are increasingly interested in integrated pest management and, in general, in low-impact techniques to satisfy the market demand of products obtained by non-polluting techniques.

Many pests affect cut foliage crops in both FCFS and FCFC in Liguria (Pasini & Rapetti, 1995; Costanzi, 1996), but the most important are aphids and thrips (Table 3). Few field data on population dynamics, eco-ethology and natural enemies of these pests are available. Preliminary field observations demonstrated that natural enemy presence is reduced where the grower's approach to pest control involves chemical spraying and especially in the case of “high-impact” strategies.

Table 3. Pests that frequently infest the main five cut foliage crops (shadow cells remark the most important pests: aphids and thrips).

Ginestra	(1)	(2)	(3)	(4)	(5)	(6)	
Mimosa	(1)	(2)	(7a)	(7b)	(8)		
Ruscus	(1)	(2)	(8)	(9)	(10)	(11)	(12)
Viburno	(1)	(2)	(8)	(9)	(11)		
Pittosporo	(1)	(8)	(9)	(13)			

Legend: (1) Aphids, (2) *Thrips major*, *Thrips flavus*, *Heliethrips haemorrhoidalis*, *Thrips tabaci*, *Frankliniella* spp., (3) *Urosiphya limbalis* = *Mecyna gilvata*, (4) *Agromyza* spp., (5) *Bembecia uroceriformis*, (6) *Agrilus* spp., (7a) *Psylla uncatoides*, (7b) *Metcalfa pruinosa*, (8) Scale insect, (9) *Tetranychus urticae*, (10) *Otiorrhynchus sulcatus* e *Neoplinthus tigratus*, (11) *Eulia* spp and *Epicoristodes acerbella*, (12) Lepidopteran caterpillars, (13) *Cercopis sanguinolenta*.

Pest control strategies

In the consideration of the pest situation described, the purpose of this study was to analyse the possibilities of implementing IPM systems for main cut foliage crops. It is first necessary to focus the attention on key-pests that preliminary observations demonstrated to be aphids and thrips. Against them (Fig.2) we are testing, the efficacy of "low-impact control tactics" such as extracts of plants (i.e. pyrethrins extracted from *Chrysanthemum cinerariaefolium*, preparations from *Derris elliptica*, *Quassia amara*, *Ryania speciosa*, etc.) and the use of natural enemies.

At the same time, we monitor pest population densities in pilot selected farms by means of several monitoring techniques (yellow sticky traps, malaise trap and by beating branches). Monitoring plan and pest control approach distinguish between FCFS and FCFC types of farm. This stage is very important for defining key pests biocology in cut foliage agro-ecosystems and for hypothesizing about "prevention tactics" such as environmental manipulation detrimental to pests (El Titi, 1987) and environmental manipulation advantageous to natural enemies of pests (Van Lenteren, 1987).

Results obtained show that it will be possible, both in FCFS and in FCFC, to individualise the different actions in "control tactics" and in "prevention tactics" (Fig.2).

Discussion

Green and flowering cut foliage agro-ecosystems in Imperia district are very complex from all points of view. Biological control of arthropod pests in cut foliage crops can be successful as it is in vegetable and ornamental crops on the base of the cooperative effort of several people and organizations (consumers, researchers, extension service and growers).

From the point of view of research, it is very important to be able to recommend correct techniques supported by *ad hoc* experimentation. This should pay particular attention to the four main cut foliage genera (*Acacia*, *Genista*, *Pittosporum* and *Viburnum*) and try to study pest problems and design pest control tactics in the framework of the complexity of the whole agro-ecosystem.

It is additionally necessary to form team works that include professional scouting personnel and the grower/nursery owner as it has been done in other countries (Cashion, 1994; Cashion & Osborne, 1993; de Goey, 1993; Shives & Cashion, 1995).

By means of this ecological integrated approach we expect to define optimal low-impact techniques that can satisfy the specific necessities of pest control in each kind of cut foliage grower.

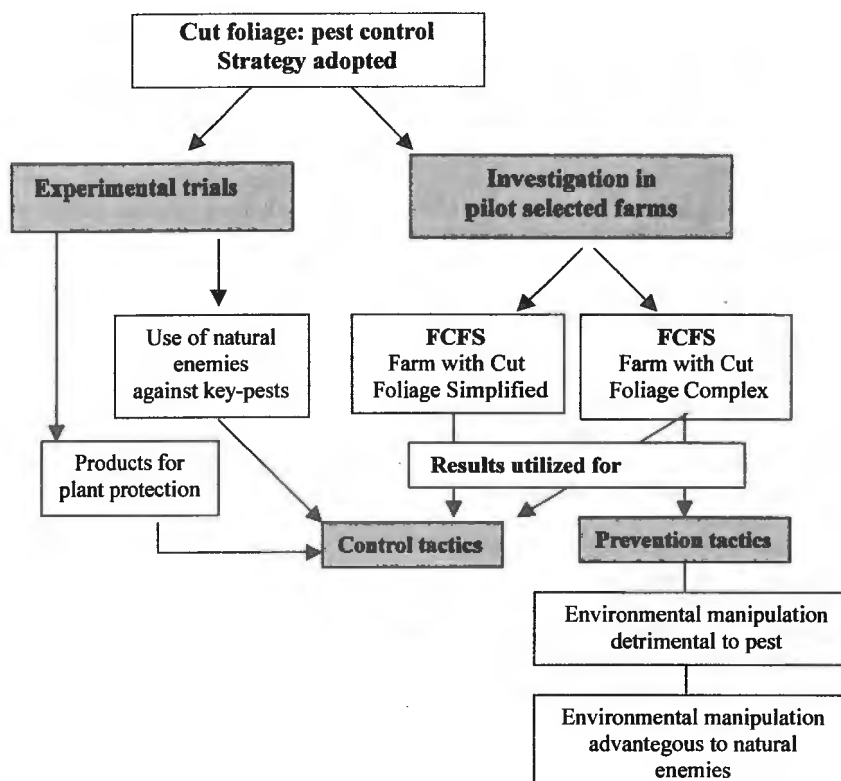


Figure 2. Schematic representation of the strategy adopted for pest control in cut foliage crops

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Integrated Disease Management in Tomato Crops Grown in High Tunnels

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Abstract: The experiments were established in high tunnels with different tomato varieties, Ferguson, Kalimba, Ovacik and Target for 3 years. Tomato diseases, early blight (*Alternaria solani*), leaf mold (*Fulvia fulva*), powdery mildew (*Leveillula taurica* and *Erysiphe cichoracearum*); some pests such as bollworm (*Heliothis armigera*), tomato russet mite (*Aculops lycopersici*) and root-knot nematode (*Meleoidogyne javanica*) and blossom end rot were recorded in the three vegetation period. Solarisation was involved for controlling soil-borne pathogens. Soil temperature in solarised plots for 6-8 weeks was measured as 42.5°C and 39°C in 6 and 15 cm soil depth, respectively. Solarisation and compost application did not have any effect on root-knot nematode but reduced blossom end rot at 56.2%. In addition, compost prepared with cereal straw was applied at 2-2.5 kg/m² for controlling soil-borne pathogens. There was no other soil-borne pest problem in three vegetation periods. In the control of foliar diseases, lower site of plastic cover was opened for ventilation and also some fungicides such as copper compounds, benomyl, chlorothalonil, maneb, triadimefon and sulphur were applied when needed. Fungicide application was made 6 times in the first year, 5 times in the second year and twice in the third year for controlling the diseases. Mites were important problem in the region, and they were tried to controlled with sulphur and other miticides. Root-knot nematode was seen only in the third year. It was assumed that it was because of susceptibility of cultivar Ovacik used in the third year experiment to the nematode. Maximum yield was obtained from the plots solarized and compost applied in an IPM approach. The yield of cultivar Target, which was resistant to root-knot nematode, grown in those tunnels was 360 kg/50 m² which was 103,5-117,7 kg/50 m² higher than in the non-solarised and composted tunnels.

Key words: tomato, disease, integrated disease management, protected crops

Introduction

Tomatoes production is approximately 8.290.000 tonnes a year in Turkey (Anonymous, 1999). Most of these tomatoes are grown as protected crops. Tomato that is most consumed vegetable in our country has important disease problems particularly when grown under cover. Most of them are fungal diseases such as damping off, root rots and foliar damages depending on cultivars and cause important yield losses. Growing the vegetable under cover makes possible to have fresh tomatoes in every season and high income for the growers. Protected growing offers long term production and prevent the crop from unavailable environmental conditions such as temperature and rainfall, and diseases. Since, these places are generally warm, humid and no wind, the plants grow very well but fungal diseases are induced. However, environmental conditions such as temperature, relative humidity and ventilation can be controlled to avoid these diseases. On the other hand, rhizosphere water level can also be controlled with appropriate irrigation regime and soil borne pathogens can be suppressed. Common foliar diseases such as downy mildew, grey mould, early blight, and leaf mould can be controlled with preventing dew occurrence. When organic substances composed are mixed into the soil, they might be stimulatory to antagonistic microorganisms,

so suppressive to pathogens (Jarvis, 1992; Gullino, 1995; Garibaldi, 1995; Malathrakis, 1995). Main fungal diseases of tomato grown under cover in Çukurova Region are foliar diseases such as powdery mildew (*Leveillula taurica* and *Erysiphe cichoracearum*), downy mildew (*Phytophthora infestans*), grey mould (*Botrytis cinerea*), Early blight (*Alternaria solani*), leaf mold (*Fulvia fulva*), and *Sclerotinia sclerotiorum* that attacks root, stem and branches, while soil borne fungal diseases are damping off and root rots diseases (*Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*), fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), *Sclerotinia* rots. Resistance of tomatoes cultivars grown in greenhouses to these diseases is very important in disease management.

Goal of this study was to control some diseases of tomato plants grown in high tunnels with a integrated disease management approach. The tunnels were ventilated in order to reduce relative humidity and prevent the water condensation on the plant surface, therefore to control foliar diseases. Solarisation and compost application were used to control soil borne diseases. Pesticides were applied when needed.

Material and methods

Greenhouse Management

The experiments were established in two high tunnels in 1997 and in three high tunnels each of which was 100 m² in 1998 and 1999. One of the tunnels (Number 2) were solarized in August-September for 6-8 weeks every year. Solarized and non-solarized places were cultivated at the end of November to prepare for planting. Compost prepared with cereal straw were mixed with the soil in solarized plots with the amount of 2.0-2.5 kg/m² in December-January. During composting process, 30 kg bran, 2.5 kg ammonium sulphate and 1 kg urea were mixed into 100 kg cereal straw and then watered to improve composting. Aeration was established in compost mass and left for 10 weeks. Tomato seedling were grown in February and planted in the high tunnels in March. Tomato cultivars, Ferguson in the first year, Kalimba in the second year, Ovacik and Target in the third year were used in the experiments. In the first year, no application was carried out in the first tunnel, while solarisation, and compost and some fungicide applications were done in second one. In the second and third year pesticides for disease and insect control were used in the first tunnel, solarization and compost application in the second one and only insecticide application in the third one. The seedlings were planted as 2.5 seedlings/m².

Tomato growing in the tunnels were carried out according to traditional methods except disease and pest control. The plants were furrow irrigated from March to mid-July every two weeks and fertilised 4 times in the vegetation period. Fertilisers such as 20-20-0, ammonium nitrate, potassium sulphate, urea and super phosphate were applied to obtain 40 kg nitrate, 10 kg phosphor and 6 kg potassium per decar. Cultural practices such as soil pilling and sideshoot removing were also followed,

Disease Management

Lower site of plastic cover of the tunnels were begun to open daily for ventilation to control particularly foliar diseases when seedlings were 15-20 cm length till May. The covers were closed in the evening to avoid cold damage in each year during March and April.

The temperature in the tunnels was recorded, and disease and pest development were also continuously checked during vegetation period, some pesticides were applied when needed. Copper compounds, iprodione (Rovral 50 WP), benomyl (Benlate 50 WP), chlorothalonil (Daconil 2787 W-75), maneb (Rivaneb 80 WP), triadimefon (Bayleton 5 WP) and powder sulphur (Solor 80 WP) were chosen in disease control. Hexythiazox (Nissorun 5 EC) and powder sulphur (Solor 80 WP) for mite control, and deltamethrin (Decis EC 2.5), bromopropylate (Neoron 500 EC) and cyfluthrin (Baythroid EC 50) for bollworm control

were used for 3 years. The tomato fruits were harvested and weighed separately for each tunnel and each cultivar.

Results and discussion

Greenhouse Management

The soil temperature in the plots solarized for 6-8 weeks reached to 42.5°C and 39°C at 6 and 15 cm soil depth, respectively, while 37°C and 32°C in non-solarised plots. The soil temperature at 6-15 cm depth in solarised plots were 6.3°C higher than those in non-solarised plots.

The soil temperature in solarised tunnel increased up to 42.5°C. Thermal death point for many soil-borne pathogens is lower than this temperature, suggesting that soil-borne pathogens of tomato plants might be eliminated. The results of our previous study (Biçici and Erköç, 1986) confirmed this and we found that three isolates of *Rhizoctonia solani* lost viability at 39°C and 50°C for 20 days and 60 min, respectively.

The temperature in the tunnels during tomato vegetation from March to July was varied between 19.2°C and 32.4°C for 3 years. In some years like in 1998, the temperature in March was below 0°C, this caused cold damage on the plots. There was no other negative effect of temperature on the plots in these unheated high tunnels for 3 years. The relative humidity sometimes reached to 98% usually in the afternoons of hot days. When the lower sides of plastic cover of tunnels were opened for ventilation starting from March, the relative humidity were lowered. Therefore, the high humidity was limited with 3-4 hours a day.

Disease and Pest Management

The foliar diseases, early blight (*A. solani*) and leaf mold (*F. fulvum*) were generally seen diseases in 1997 vegetation period. Copper oxychloride, benomyl, iprodione and maneb were applied 6 times in order to control these diseases. The yield of cultivar Ferguson obtained was 443 kg/100 m² and 434 kg/100 m², in solarised and non-solarised tunnels, respectively.

In 1998 vegetation period, early blight, powdery mildew, bollworm and mites were problem on tomato plants grown in all three tunnels. Chlorothalonil, iprodione and copper oxychloride for controlling early blight, triadimefon and benlate for powdery mildew, wettable powder sulphur and hexythiazox for mite control and deltamethrin for bollworm control were used in the first and second tunnels. The pesticides for mite and bollworm control were only used in the third tunnel. The yields of cultivar Kalimba used in 1998 were got 393, 398 and 361 kg/100 m² in the first, second and third tunnels, respectively (Table 3). In 1998, the average yield was less than those in 1997, this might be due to cultivar differences, and also the temperature in March got low as -5 and -3°C twice in 1998. So cold damage might effect the yield.

In 1999 vegetation period, only disease appeared was early blight, mite and bollworm were again problem. In addition, root-knot nematode (*M. javanica*) damage and a physiological disorder, blossom end rot, were seen in this year. In managing this pests, four times pesticide applications were made in the first and second tunnels. Two of them were iprodione and chlorothalonil to control early blight, while the others were sulphur dust against mites and hexythiazox against bollworm. However, in the third tunnel, control one, two application of hexythiazox, and one bromopropylate, one cyfluthrin applications were made to control the mites and bollworm, respectively.

Blossom end rot, a physiological disorder, (Blancard, 1993) to which tomato varieties differ considerably appeared at 31.7% and 42.9% in varieties, Ovacik and Target, respectively. However, these value were found as 36% and 6.7% in solarised and composted tunnels. Blossom end rot was, respectively, determined as 52.5% and 67.2% in the varieties, Ovacik and Target, in the third tunnel where no solarization and compost application made,

but only insecticides were used. Blossom end rot on varieties, Ovacik and Target, grown in the first tunnel, where fungicides and insecticides were used to control fungal diseases and pests but no solarisation and compost applications were made, were found as 6.2% and 54.9%, respectively. When the mean value for both varieties were taken, blossom end rot was 21.4% in solarised and composted plots while 45.2% in non-solarised and non-composted ones (Table 1). The results showed that solarization and compost application reduced blossom end rot at 56.2%.

Table 1. Occurance of blossom end rot of tomato (%) in solarised-composted and non-solarised-composted plots.

Tomato Varieties	first tunnel	third tunnel	second tunnel	mean
	(no sol. and no compost)		(sol+compost)	
Ovacik	6.2	52.5	36.0	31.7
Target	54.9	67.2	6.7	42.9
Mean	45.2		21.4	

In 1999 vegetation period, root-knot nematode, *M. javanica* that had not been found in previous years was markedly seen. Foliar symptoms such as necrosis on the young leaves, saprophytic fungal development on the stems were first seen. When the roots of these plants examined, severe root galling was seen. The nematode symptoms were found on 83.5% of cultivar Ovacik but 3.4% of cultivar Target. Solarisation in the second tunnel did not effect root-knot nematode on cultivar Ovacik because of 93.4% of plants infected with the nematode. However, root-knot nematode symptoms were not seen on cultivar Target grown in solarised and composted plots. We can assume from the results that cultivar Ovacik was much more susceptible to *M. javanica* than cultivar Target, and also solarisation and compost application were not effective on the nematode.

The yield obtained from 3 tunnels in 1999 is shown in Table 2. The total yield of cultivars Ovacik and Target from 3 tunnels were 515 kg and 860.9 kg in the 150 m² covered areas, respectively. The yields of both cultivars grown in solarised and composted plots were 37-44 kg/50 m² for Ovacik and 103.5-117.7 kg/50 m² for Target higher than those grown in non-solarised and non-composted plots. The highest yield was obtained from cultivar Target grown in solarised and composted plots with 360 kg/50 m².

Table 2. Yields of tomato cultivars, Ovacik and Target, grown in insecticides and fungicides applied first tunnel; solarisation and compost applied second tunnel; and only insecticides applied third tunnel in 1999 (kg/50 m²).

Tomato varieties	First tunnel	Second tunnel	third tunnel	Total 150 m ²
Ovacik	161.6	198.7	154.7	515.0
Target	243.0	360.7	257.2	860.9

In conclusion, when cultivar Target resistant to root-knot nematode was grown in solarised and composted plots, yield was 28.6% (105.5 kg/50 m²) and 32.6% (117.7kg/50 m²)

higher than those grown in tunnels where only disease and pest control were made, and where only pest control was made, respectively (Table 3).

Table 3. The yield of tomato cultivars, Ferguson, Kalimba, Ovacik and Target, grown in high tunnels in 1997-1999 (kg/100 m²).

Tomato cultivar	Pest-diseases control, solarisation, composting	only pest-diseases control	only pest control
Ferguson	443	434	-
Kalimba	398	361	393
Ovacik	323	400	310
Target	485	720	514

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Integrated pest management strategies in sweet pepper plastic houses in the Southeast of Spain

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Abstract: In the Southeast of Spain, about 10,000 ha of sweet pepper are grown in plastic houses, in the provinces of Almería, Murcia and Alicante. In the last six years studies to implement Integrated Pest Management have been carried out. The IPM programmes have led to a high reduction in chemical sprays. The use of *Amblyseius cucumeris*, *Orius laevigatus* and *Orius albidipennis* has proved to be successful in the control of thrips and TSWV incidence. Most of the other pests are controlled by natural enemies. Knowledge of thrips population dynamics in relation to temperature can be used to its manage. *A. cucumeris* was successful in reducing the increase of thrips populations in the first few months. Strategies for *Orius* releases have been assayed. Tomato Spotted Wilt Virus (TSWV) vectored by *Frankliniella occidentalis* is the main phytopathological problem; however the removal of plants with TSWV symptoms reduces significantly the disease incidence.

Introduction

The area of pepper crops grown in plastic houses in IPM in the South of Spain has increased remarkably during the last few years. Of 1,930 ha grown in the provinces of Murcia and Alicante, during the 1999 campaign, 2.2% used natural enemies. In the next year a doubling of this area is foreseen. The province of Almería, the most important producer in Spain, 8,030 ha were grown during the 1999 growing season. Although we do not have the data of the area in Almería under IPM, a trend similar to the other two regions is likely.

The change in strategies for the control of pests and diseases has taken place for several reasons: the constantly increasing number of chemical treatments required for control, mainly against *Frankliniella occidentalis* (Pergande), the ever decreasing amount of residuals, the demand for organic products and the farmers' willingness to adopt more convenient control methods. According to the data supplied by a few farmers who grew under chemical control during the 1998 and 1999 growing seasons, an average of 27 treatments were made, with an average of 2.5 products for each spray. Thirtythree of these products were meant to control thrips, 18 were against *Bemisia tabaci*, 4 against caterpillars (*Spodoptera exigua* (Hübner) and *Ostrinia nubilalis* (Hübner)), 5 for fungal diseases and 0.5 against *Tetranychus* spp. In the IPM crops during the same growing seasons, an average of 12 sprays were applied, with an average of 1.3 products each. No treatment against thrips was made in any plastic house; the proportion of products used was: 1.3 against *Bemisia tabaci* (Gennadius), 8.5 *Bacillus thuringiensis* against caterpillars, 5.8 for fungal diseases control and 1 for aphids.

As IPM programmes are relatively new, some practices still lack efficacy data under our conditions. The cost of natural enemies represents the greatest expense in IPM programmes. In the region of Murcia it has been estimated to be 79% of the total cost for pest and diseases

control; thus, it is profitable to optimize the use of natural enemies by releasing only those species that are well adapted to environmental conditions and demonstrate a good response to the specific pest.

Amblyseius (Neoseiulus) cucumeris (Oudemans) is the most common species used for thrips control in horticultural crops; however, its use in the Mediterranean area is limited by the low relative humidity (Rodriguez, 1991; Vacante & Tropea-Garzia, 1993; Rodriguez & Fidalgo, 1993; Van der Blom *et al.*, 1997). In the case of pepper crops, grown between January and October, the use of this species is limited to the first few months. It is also the most costly control used, representing 30-40% (excluding the release expenses) of the total cost of the natural enemies during the entire growing season.

The Tomato Spotted Wilt Virus is the main phytopathological problem of peppers and its control requires the adoption of several practices, each one contributing in some ways to the reduction in the disease incidence (Lacasa & Sanchez, 1999). The good control of *F.occidentalis* is one of the key factors to avoid high disease incidence. An understanding of its population dynamics in relation to the temperature can be used to manage the species and to choose the time of natural enemy releases.

This article is the result of several essays and conclusions drawn from experiments carried out in the last six years. It deals with the influence of the temperature on thrips population dynamics, the response of *A.cucumeris* in thrips control, *Orius* release strategies and cultural practices to be used in order to reduce TSWV incidence.

Materials and methods

Influence of the temperature on F.occidentalis populations dynamics.

The influence of temperature on *F.occidentalis* population dynamics has been determined through the analysis of data collected from plastic house pepper crops over the last six years.

Most of the data come from IPM plastic houses, where *A. cucumeris*, *Orius laevigatus* (Fieber) and *O. albidipennis* (Reuter) were used to control *F.occidentalis*. Other natural enemies, or compatible products, were used for other pests and fungus diseases. Other plastic houses served as control and no specific treatment was made against thrips, while products with low toxicity for thrips were sprayed to control other pests and diseases. For some growing seasons we dispose of thrips population dynamics data in crops where chemical control for thrips was practiced.

The monitoring of *F.occidentalis* population dynamics was made weekly, by sampling flowers and leaves. The samples were carried in refrigerated containers to the laboratory, where the insects were extracted with funnels Berlese incandescent lamps and the individuals were collected in a solution of 10% alcohol and 1‰ Agral ®. Three samples of ten flowers each and three samples of twenty leaves were taken in the experimental plastic houses, and three samples of twenty flowers each and twenty leaves each were taken from the commercial houses.

The temperature and relative humidity were recorded with either a digital datalogger or a hygrothermograph.

Response of A. cucumeris in controlling F. occidentalis

The trial was carried out at the Torreblanca Experimental Station (C.I.D.A.) located in Campo de Cartagena (Murcia) in a 33x11 m plastic house, provided with ventilation in lateral openings 1.2 m wide and 14x10 antitrips mesh. Transplanting took place on January 21, 1999; the variety "Orlando" was used.

The plastic house was divided into two sections by a plastic sheet. On March 18 *A.cucumeris* was released in bags of 500 individuals each, at a rate of 0.5 bag/plant. The assay was considered finished on May 4.

The monitoring of *A.cucumeris* and *F.occidentalis* population dynamics was carried out by taking three samples of 10 flowers each and three samples of 20 leaves each. The extraction was made with Berlese funnels.

Strategies for releasing *Orius* spp

The trial was carried out in the same plastic house as the previous experiment, during the 1999 growing season. It was divided into two sections by a plastic sheet. In section 1 adults of *Orius* spp. were released, while in section 2, nymphs were released. The *O.laevigatus* release took place on April 14 and 27. In both sections *A.cucumeris* were released two weeks before the *O.laevigatus* introduction, in bags of 500 individuals each, at a rate of 0.5 bag/plant. On May 20 a treatment with cipermetrine was made in order to exterminate the *O.laevigatus* and on June 5 the same experiment with *O.albidipennis* was started (Table 1). The *Orius* originated from the insectary of C.I.D.A.'s Crop Protection Department, and had been reared at a temperature of 23°C on *Pelargonium hederifolia* with *Ephestia kueheniella* (Zeller) eggs as a supplementing the diet.

Table 1. Release schedule in Sections 1 and 2.

Dates	Section-1			Section-2		
	n	Species	Stage	n	Species	Stage
14-Apr-98	350	<i>O. laevigatus</i>	Adults	720	<i>O. laevigatus</i>	Nymphs I-II
27-Apr-98	350	<i>O. laevigatus</i>	Adults	380	<i>O. laevigatus</i>	Nymphs III-V
5-Jun-98	300	<i>O. albidipennis</i>	Adults	300	<i>O. albidipennis</i>	Nymphs III-V

Effect of the elimination of the plants showing symptoms of Tomato Spotted Wilt Virus (TSWV) on the disease evolution.

The trial to quantify the effect of the elimination of plants showing symptoms of TSWV on the disease evolution was started on July 14, 1997 and was carried out in the same plastic houses used for the experiments described in the above paragraphs. The plastic house was divided into two sections by a plastic sheet. In one section the plants showing symptoms of TSWV were removed weekly, while in the other, the diseased plants were left in the house. The virus incidence at the beginning of the experiment was 2% in both sections.

The monitoring of *F.occidentalis* population dynamics was done as described in the previous experiment.

Results and discussion

Influence of the temperature on *F.occidentalis* population dynamics.

Plastic house pepper crops in the region of Murcia are grown from December or January to September. During this long crop cycle, *F.occidentalis* appears quite early. However, its density remains low in the winter months, and the exponential increase occurs only in mid spring (Figure 1). The time at which the species' population density sees a continuous and rising increase coincides with the period when the average temperature reaches 20°C (Figure 2) and the portion of the day in which the temperature is below 10°C is very short (Figure 3).

In this period the conditions for species development are optimal. In summer there is a decrease in thrips populations caused by high average daily temperatures of between 25-30°C with almost half of the day reaching above average daily 30°C. This is shown in the experiments in which predators were eliminated in mid July, when the average temperature was 25°C. In this case we first observed an increase in the population density, that reached its maximum (10-14 larvae/flower and 6 adults/flower) four weeks after the predators were eliminated. In the subsequent weeks, however, we observed a reduction in the population density, that became stable at around 4 larvae/flower and 1-2 adults/flower. These values are in clear contrast to the data recorded when the predators were eliminated at the beginning of May, when the conditions for species development are optimal. In this case we observed that *F.occidentalis* density increased drastically, reaching its maximum over 50 larvae/flower and 10 adults/flower two weeks after the extermination of predators.

Response of *A. cucumeris* in the control of *F. occidentalis*.

In the section in which *A.cucumeris* was released, *A.cucumeris* population reached a maximum of 5.4 individuals/flower after 5 weeks (Figure 4) followed by a decrease to 0.5 individuals/flower at the end of the trial. Although, *F.occidentalis* was present from the beginning, its population did not increase noticeably until the first week of April (Figure 4). The highest density was reached four weeks later, at the end of the trail (1.5 larvae/flower and 2.6 adults/flower).

F.occidentalis was also observed from the beginning in the section in which *A.cucumeris* was not released. The thrips population increased from the first week of April, but at a higher rate than in the section with *A. cucumeris* (Figure 4). A maximum of 19 larvae/flower and 11 adults/flower was observed at the end of the experiment.

The results of the trial show that releasing *A.cucumeris* effectively reduces the *F.occidentalis* population. Nevertheless, as it has been observed during these years, the response of *A.cucumeris* is not sufficient to control thrips. One of the main reasons is because the density of *A.cucumeris* is very low when thrips development conditions are optimal. Another predator is needed, such as *Orius* spp., which could work in conjunction with *A.cucumeris* to reduce maximum thrips densities. This is very important for controlling the spread of TSWV.

***Orius* strategies release**

In the section where adults of *O. laevigatus* were released, *O.laevigatus* were first observed two weeks later. Their population increased continuously to reach a maximum of 0.3 individuals/flower (Figure 5), then fell to 0.07 individuals/flower. In the section where nymphs were released, no *O.laevigatus* were observed until 2 weeks later than the house in which adults were released. The maximum density reached was 0.07 *Orius*/flower (Figure 5).

In the second phase of the experiment *O. albidipennis* was released. In the section in which adults were used, the first individuals were observed ten days after the release (Figure 5). From that moment on the population increased continuously reaching a maximum of 0.3 *Orius*/flower. When nymphs were used, they were first observed three days after the release. However, the population did not increase until two weeks later, reaching a maximum of 0.2 *Orius*/flower (Figure 5).

In both phases of the experiment the *Orius* population increase was slower when nymphs were released. The fall of *O.laevigatus* after reaching its maximum density in the section where adults were released, was probably due to the decrease in *A.cucumeris* density and the lack of thrips as a main source of prey. The results agreed with what was expected theoretically. In the case of the adults, the oviposition is immediate, while the nymphs have to complete their development and pass the pre-oviposition period. The idea that the use of

nymphs could give better results, is based on the supposition that the dispersion after release would be and thus establishment would be better. Neither it is certain that there is no dispersion of the nymphs, nor that they remain in the crops once in the adult stage. Besides, their supposed lack of dispersion is thought to imply a direct control of the thrips, although the low densities used in the releases are supposed to contribute very little to thrips control in the establishment phase, being the potential of the *Orius* linked to the increase of its population in response to the increasing number of thrips.

In our opinion none of the two methods is to be excluded and the choice depends on the aim of the release. The state of the individuals to be used must also be taken into account. In quality controls of *O. laevigatus* made from several natural enemies suppliers during the 1999 growing season, it was observed that the females' average longevity at 25°C was 16 days and the average oviposition was 45 eggs/female, which corresponds to a reduction of 50% and 60% respectively, according to data reported for *O. laevigatus* at this temperature by several authors (Alauzet *et al.*, 1994; Sanchez, 1998).

The timing of *Orius* releases can also have repercussions on the global mechanic of the agroecosystem. On some occasions, *Orius* populations increased after their release, in association with a fall in *A. cucumeris* populations. The *Orius* populations then decreased to a low level until it responded to rising thrips density (Figure 1). The interaction between *Orius* and *A. cucumeris* can take place in the absence of thrips (Gillespie & Quiring, 1992). Early *Orius* releases do not guarantee an earlier establishment, nor does having a high *Orius* density prevent the thrips outbreak, which always takes place at a higher or lower level.

It can be therefore said that using *A. cucumeris* in the first phase of the crop can be beneficial to control thrips. Early *Orius* releases can lead to the extermination of the phytoseiid. Since the thrips density is low during the first months due to low temperatures and the initial thrips population increase is slowed by the action of *A. cucumeris*, *Orius* releases can be put off until the average temperature approaches 20°C and the temperature in the crop is >10°C for the majority of the day.

O. laevigatus and *O. albidipennis* are the two most common species of the genus in the Southeast of Spain. *O. albidipennis* is the most common species during the warm season (Lacasa *et al.*, 1996), being better adapted to high temperatures than *O. laevigatus* (Sanchez, 1998). In plastic houses, sweet pepper commercial crops, only *O. laevigatus* is used. It is effective in controlling thrips, and stays at a high density during the period of optimal conditions for thrips development (Sanchez *et al.*, 1997). Generally at the beginning of the summer a decline in its population can be observed. Although the use of *O. laevigatus* is sufficient to control thrips throughout the season, releases *O. albidipennis*, can be beneficial in places where it does not appear naturally, or a decrease in the *O. laevigatus* population level occurs. The presence of *O. albidipennis* can contribute to the system's stability, with a quicker response to perturbation factors, thanks to its better adaptation to environmental conditions of plastic houses in the summer months. In addition, being a general predator, it can contribute to control aphids, *Spodoptera exigua*, *Ostrinia nubilalis*, *Tetranychus* spp. and *Bemisia tabaci*.

Effect of the elimination of the plants showing symptoms of Tomato Spotted Wilt Virus (TSWV) on the disease evolution

The removal of the plants with symptoms of TSWV contribute remarkably to reduce the final incidence of the disease. In the section in which the plants with TSWV symptoms were not removed, the disease incidence eight weeks later was 64%, while in the section where the plants with TSWV symptoms were removed it was 20%. In both sections *F. occidentalis* population dynamics were very similar. The loss in production caused by the virus was higher

in the section where diseased plants were not removed than in the section in which plants with TSWV symptoms were removed (Sanchez *et al.*, 1999).

In Almeria virus incidence is low. However in both Murcia and Alicante regions, TSWV represents the main phytopathological problem (Garcia *et al.*, 1997). Thus, it is necessary to adopt cultural practices contributing to the reduction of disease incidence.

In a few areas with a peculiar epidemiologic situation there is a high immigration rate of viruliferous thrips, which provoke a fast increase in disease incidence, even when the thrips population density is very low. However, in most of the plastic houses the immigration of viruliferous thrips into the crops is low and the disease spread takes place mainly from the internal foci (Sanchez *et al.*, 1998). As a result it is also useful to employ any technique which contributes to reduce the immigration into the greenhouse, such as putting meshes on the ventilation openings (Lacasa *et al.*, 1994). The elimination of the plants with symptoms of TSWV is especially advisable in the first months of the crops, before *F.occidentalis* outbreak. By eliminating the internal source of infection the proportion of viruliferous individuals in the next generations can be considerably lower.

Acknowledgments

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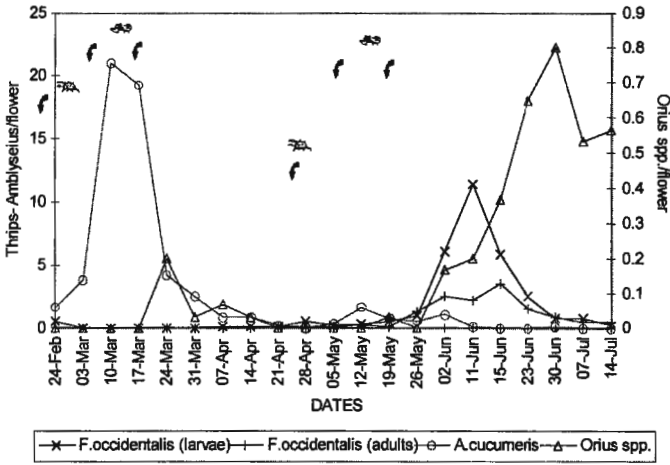


Figure 1. Population dynamics of *F.occidentalis*, *A.cucumeris* and *Orius* spp. Natural enemies release ♣. *Orius* spp. ♠. *Amblyseius cucumeris* ♠.

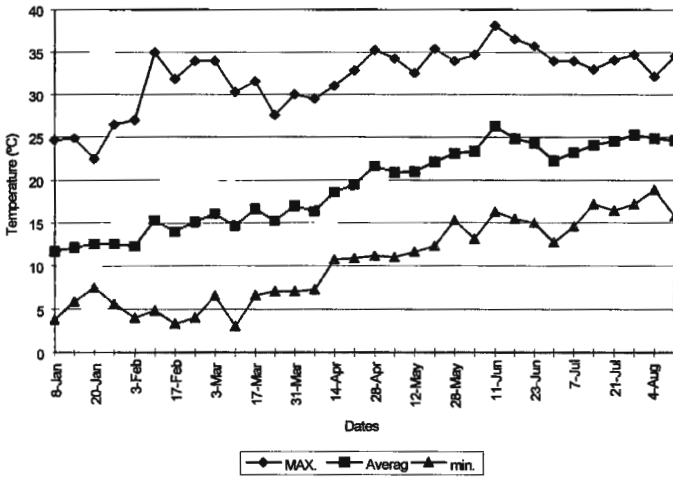


Figure 2. Temperature evolution

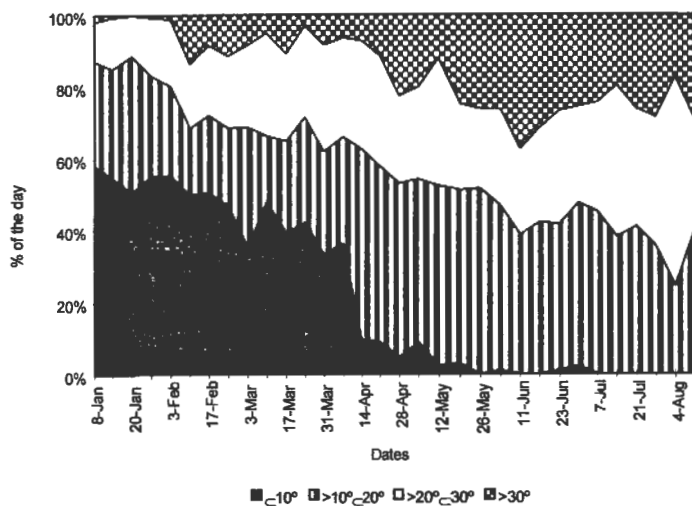


Figure 3. Range of temperature during the day

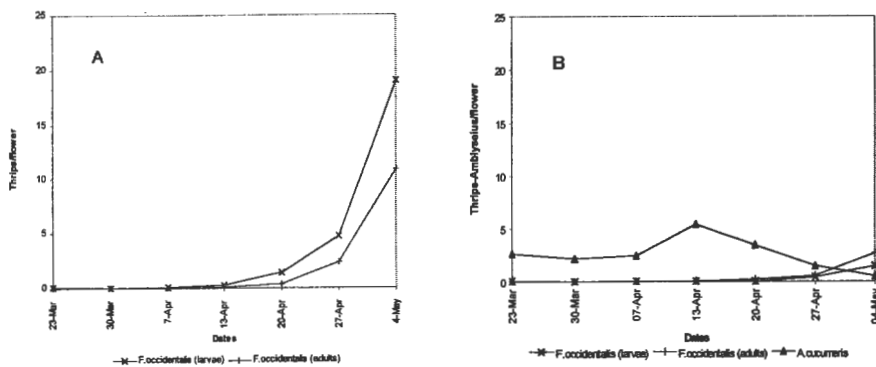


Figure 4. *F.occidentalis* and *Amblyseius cucumeris* Population dynamics. A= Without *A.cucumeris*, B= *A.cucumeris* released

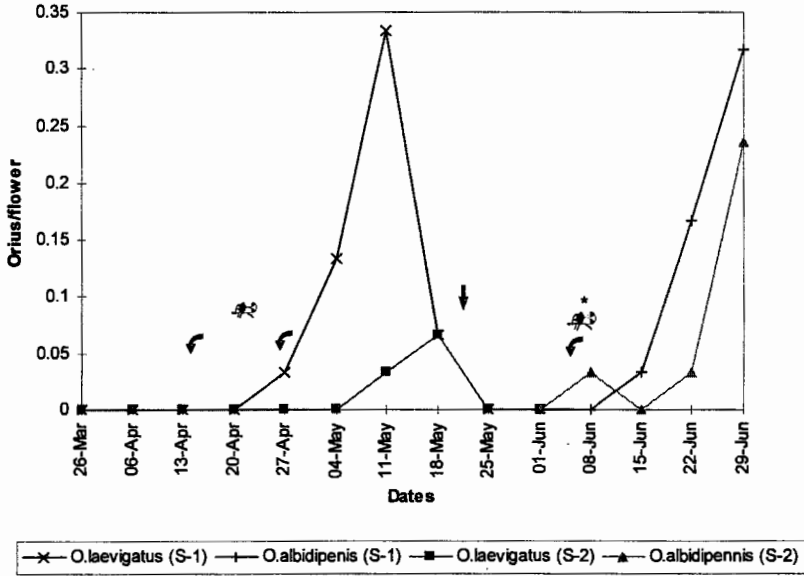


Figure 5. Evolution of *O. laevigatus* y *O. albidipennis*. S-1= nymphs released, S-2= Adults released. \downarrow \times \oplus *Orius laevigatus* and \downarrow \times \oplus *O. albidipennis* releases. \downarrow Spray.

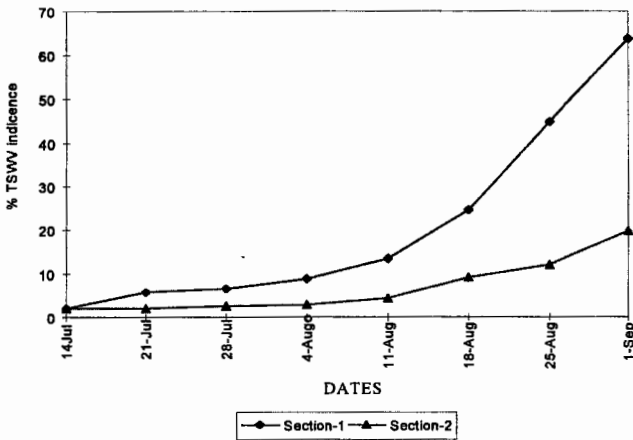


Figure 6. TSWV incidence evolution. Section 1= natural spread, Section-2= plants with symptoms removed.

Technical procedures and constrains for phytosanitary control measures at greenhouses growers' level in the Oeste region of Portugal

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Abstract: During the fieldwork done in the Oeste region with greenhouse growers, studying bioecological aspects of pests and beneficials and their interrelations, substantial differences were noted related with the relative importance of key-pest problems and faunistic biodiversity depending on growers applications of pesticides.

Taking that into account four groups of growers were selected based on their attitudes related with pesticide's use: conventional chemical control; 'conditioned' chemical control; IPM; pest control allowed in organic agriculture

To study potential relations between growers attitudes to pesticides' use and the relative importance of key-pests, the objective and subjective perceptions and the evaluation of the growers and their decision process for selection of control measures, a survey questionnaire was made to sixty growers in order to access and evaluate their technical procedures

The growers' sample was randomly selected through the Oeste region growers and divided in three sub-samples related with three crops: tomato, green beans and lettuce (twenty growers per crop).

The main targets of these questionnaire were define and characterise different cultural practices and phytosanitary measures, the time spent to perform them and when were they done through out the cropping cycle, for the four control strategies defined above.

Data will collected on types and amounts of pesticides used, number of spray applications, time consumed per control measures and costs involved, as well as to clarify technical constrains at the grower's level.

The final goals were designed to understand if such factors and knowledge could be used to speed up the adoption of more environmentally friend and sustainable phytosanitary measures by the growers.

Key words: decision process; technical constrains; pest control strategies

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Pests and their natural enemies on greenhouse vegetables in Antalya

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Abstract: Pests and their natural enemies on greenhouse grown tomato, pepper, eggplant and cucumber at various locations of Antalya were investigated. *Bemisia tabaci*, *Trialeurodes vaporariorum*, *Liriomyza huidobrensis*, *L. trifolii*, *Tetranychus cinnabarinus*, *Polyphagotarsonemus latus*, *Aphis gossypii*, *Myzus persicae*, *Frankliniella occidentalis* and *Thrips tabaci* were found as pests. *Neochrysocharis formosa*, *Diglyphus isaea*, *D. crassinervis*, *Hemiptarsenus varicornis*, *Chrysocharis gemma* were found as parasitoids of leafminers. *Encarsia* sp. and *Eretmocerus* sp. were found as parasitoids of whiteflies. *Asaphes vulgaris*, *Aphelinus* sp., *Syrphophagus aphidivorus*, *Aphidoletes aphidimyza*, and *Chrysoperla carnea* were found as natural enemies of aphids. *Orius minutus*, *O. niger* and *Macrolophus caliginosus* were found as predators of thrips. *Feltiella acarisuga* and *C. carnea* were found as predators of spider mite.

Key words: Greenhouse pests, natural enemies, Antalya

Introduction

Antalya is an important region for production of protected vegetables. An area of approx. 15.000 ha is allocated for protected vegetables and 1.350.000 tons of vegetables are produced annually (Anonymous, 1999). Several insects and mites pests are causing significant losses in these crops. The number of pests in greenhouses tend to increase as the movement of plant propagation material into country from other countries has intensified in recent years. *Polyphagotarsonemus latus* (Banks) and *Frankliniella occidentalis* (Pergande) are the most recent examples of new pests of greenhouses which presumably were imported through movement of such material (Tunc & Gocmen, 1994). Little information is available on native natural enemies of pest of greenhouse crops. The present study was undertaken to fill this gap.

Material and methods

Greenhouses in four districts (Topcular, Uncali, Kumluca and Alanya) of Antalya were surveyed between September (1998) and June (1999). Pests and their natural enemies were sampled using yellow sticky traps, a vacuum suction sampler, and by collecting leaf samples. Parasitoids were obtained using emergence boxes.

Results and discussion

Pests occurring on greenhouse vegetables and their natural enemies are given in Tables 1-4. Greenhouse-grown tomato, eggplant, paper and cucumber were infested by almost the same composition of pest species.

Table 1. Pests and their natural enemies on protected tomato in Antalya.

Pest	Natural enemy	
<i>Bemisia tabaci</i> <i>Trialeurodes vaporariorum</i>	<i>Eretmocerus sp.</i>	Hym.:Aphelinidae
<i>Liriomyza trifolii</i> <i>L. huidobrensis</i>	<i>Neochrysocharis formosa</i>	Hym.:Eulophidae
<i>Tetranychus cinnabarinus</i>	<i>Orius niger</i>	Het.:Anthocoridae
<i>Aphis gossypii</i> <i>Myzus persicae</i>	No	
<i>Thrips tabaci</i> <i>Frankliniella occidentalis</i>	<i>Orius niger</i> <i>Orius minutus</i>	Het.:Anthocoridae
<i>Polyphagotarsonemus latus</i>	No	

Table 2. Pests and their natural enemies on protected eggplant in Antalya.

Pest	Natural enemy	
<i>Bemisia tabaci</i> <i>Trialeurodes vaporariorum</i>	<i>Eretmocerus sp.</i> <i>Encarsia sp</i>	Hym.:Aphelinidae
<i>Liriomyza trifolii</i> <i>L. huidobrensis</i>	<i>Diglyphus isaea</i> <i>D.crassinervis</i> <i>Hemiptarsenus varicornis</i> <i>Neochrysocharis formosa</i> <i>Chrysocharis gemma</i>	Hym.:Eulophidae
<i>Tetranychus cinnabarinus</i>	<i>Chrysoperla carnea</i>	Neu.: Chrysopidae
	<i>Feltiella acarisuga</i>	Dip.: Cecidomyiidae
<i>Aphis gossypii</i> <i>Myzus persicae</i>	<i>Aphelinus sp.</i>	(Hym. Aphelinidae)
	<i>Asaphes vulgaris</i>	Hym.: Pteromalidae
	<i>C. carnea</i>	Neu.: Chrysopidae
	<i>Syrphophagus aphidivorus</i>	Hym. Encyrtidae
	<i>Aphidoletes aphidimyza</i>	Dip.: Cecidomyiida
<i>Thrips tabaci</i> <i>Frankliniella occidentalis</i>	<i>Orius niger</i> <i>Orius minutus</i>	Het.:Anthocoridae
	<i>Macrolophus caliginosus</i>	Het.: Miridae
<i>Polyphagotarsonemus latus</i>	No	

Cotton whitefly *Bemisia tabaci* (Genn.), greenhouse whitefly *Trialeurodes vaporariorum* (West.) leafminer *Liriomyza trifolii* (Burgess), spidermite *Tetranychus cinnabarinus* (Boisd)., broadmite *P. latus*, cotton aphid *Aphis gossypii* Glov., green peach aphid *Myzus persicae* (Sulz.), western flower thrips *F. occidentalis* and onion thrips *Thrips tabaci* Lindeman were reported as important pests of greenhouse grown vegetables (Tunc, 1992; Gocmen, 1995; Tunc & Gocmen, 1994; Yabas & Ulubilir, 1996). *L. huidobrensis* (Blanchard) was formerly recorded by Yabas *et al.*, 1995 in Icel & Izmir but it was not reported in Antalya before this study.

Table 3. Pests and their natural enemies on protected pepper in Antalya.

Pest	Natural enemy	
<i>Bemisia tabaci</i> <i>Trialeurodes vaporariorum</i>	No	
<i>Liriomyza trifolii</i> <i>L. huidobrensis</i>	No	
<i>Tetranychus cinnabarinus</i>	<i>Orius minutus</i>	Het.:Anthocoridae
<i>Aphis gossypii</i> <i>Myzus persicae</i>	No	
<i>Polyphagotarsonemus latus</i>	No	

Table 4. Pests and their natural enemies on protected cucumber in Antalya.

Pest	Natural enemy
<i>Bemisia tabaci</i> <i>Trialeurodes vaporariorum</i>	No
<i>Tetranychus cinnabarinus</i>	No
<i>Aphis gossypii</i> <i>Myzus persicae</i>	No
<i>Thrips tabaci</i> <i>Frankliniella occidentalis</i>	No

A good number of natural enemies species were able to survive the heavy pesticide pressure in greenhouses. *Neochrysocharis formosa* (Westwood), *Diglyphus isaea* (Walker), *D. crassinervis* Erdos, *Hemiptarsemus varicornis* (Girault), *Chrysocharis gemma* (Walker) were found as parasitoids of leafminers. *Encarsia* sp. and *Eretmocerus* sp. were found as parasitoids of whiteflies. *Asaphes vulgaris* (Walker), *Aphelinus* sp., *Stryphophagus aphidivorus* (Mayr), *Aphidoletes aphidimyza* (Rondani), and *Chrysoperla carnea* Stephens were found as natural enemies of aphids. *Orius minutus* (L.), *O. niger* (W.) and *Macrolophus caliginosus* Wagner were found as predators of thrips. *Feltiella acarissuga* (Vallot), and *C. carnea* were found as predators of spider mite.

It seems that there is a rich natural enemy source that may create a potential for natural biological control if pesticidal pressure is ceased in greenhouses in Antalya. Eggplant is relatively less pesticide receiving crops and this was reflected by the highest number of natural enemies species detected on this crops. Absence of natural enemies on cucumber may be due to shorter growing period and heavier insecticide exposure of this crop compared to the others.

Among the parasitoids *D. isaea* and *Dacnusa sibirica* Telenga are used in biological control of *Liriomyza* spp. in several countries (de Goffou, 1991; Leuprecht, 1992). *Encarsia* sp. and *Eretmocerus* sp. were reported as efficient parasitoids of whiteflies (Polaszek *et al.*, 1992; Lopez *et al.*, 1997).

It may be concluded that control programs that protect native natural enemy source may contribute to the reduction of frequency of pesticide applications in greenhouses in Antalya.

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Crop protection techniques in horticultural greenhouses farming systems: A sociological approach of farmers' adoption

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Abstract: This paper describes one of the methodological tools used in a research project still in course in Portugal. The research's goal is to identify and assess the restrictions faced by farmers when adopting a specific crop protection technique. The crop protection techniques under analysis are the conventional chemical control; 'conditioned' chemical control; IPM; pest control allowed in organic agriculture, used in horticultural greenhouses farming systems.

Key words: IPM adoption, growers constrains, technical itinerary, crop protection techniques, survey questionnaire

Introduction

The research is being conducted in the Oeste region, an area located in the littoral centre of Portugal Mainland. According to the last statistical data (1993), 67% of the total Utilised Agricultural Area (UAA) occupied by intensive horticultural farming systems in the country is located in that region. In addition, 70% and 28% of the total UAA cultivated with, respectively, open field and greenhouse horticultural farming systems are located in Ribatejo and Oeste. Tomato, lettuce and green beans are the mainly cultivated vegetables in the region's greenhouses.

The conventional chemical control is the most generalised crop protection technique used by growers in the region's greenhouses. In Portugal, the adoption IPM techniques is still not expanded in greenhouses; However, is expanded fast in perennial crops particularly, vineyards, apple and pear orchards. Organic agriculture is even more restricted in terms either of area or of number of farmers. According to the Portuguese Agri Environmental Programme (Reg. (CEE) 2078/92), elaborated within the context of the 1992 CAP Reform, the agri environmental measures oriented toward a reduction in the use of inorganic fertilisers and pesticides and an increase on the organic production have had a reduced impact among Portuguese farmers.

Material and methods (Theoretical framework)

Taking into account the still reduced adhesion of Portuguese farmers on environmental friendly crop protection farming practices, as well as the non existence of empirical studies oriented to understand the reasons that inhibit farmers to adopt them, the methodological tools used in the research were individual enquiries. Three types of survey questionnaires were

constructed in order to identify the different kind of constraints that might be responsible for farmers' decisions on crop protection practices. These constraints were identified with economic, technical and sociological factors.

The theoretical assumptions underlying the research and, consequently, the applied methodological tools are twofold.

Firstly, the agricultural practices that growers adopt are here defined as the outcome of the reciprocal relationships between the social action and the context in which such adoption takes place. Concerning growers' agricultural practices dealing with environmental problems/concerns, like the crop protection ones, it was considered that farmers are situated within a complex web of relationships like, for instances, governmental agencies and public policies, pesticides suppliers and public and private agricultural technical advisors. All this kind of institutions and social actors influence how growers act and, in the context of the research, were identified with institutional, technical and social exogenous constraints of growers' adoption for crop protection techniques. It is, thus, assumed that the characteristics of the "networks that surround farmers have particular historical trajectories and dynamics and farmers' pesticide practices have to be understood in this context" (Ward, 1995).

It should be noted that the theoretical assumption described above is opposite to those that depict social action as the outcome of individual decisions and assume exogenous causes independent from and without consequences on the endogenous social and technical systems functioning. This last theoretical assumption is underlying predictive models. These models are largely used by neo-classical economics to analyse and to make calculations about, for instances, the costs and benefits of changes on current pesticides practices.

The second theoretical assumption underlying the research is concerned with the endogenous constraints and how they influence growers' agricultural practices. The endogenous constraints considered could be differentiated at two levels: at the farm functioning level and at the individual/ grower level.

At the farm functioning level, the farming system's characteristics, the available farming time and the type of available farming labour force, i.e., waged or familial labour force, were endogenous constraints included in the questions of the individual surveys. Each one of these three issues was assumed as eventual restrictions to the growers' adoption of crop protection technique. For instance, in a farm where greenhouse's horticulture exists as a farming system along with any other agricultural production, particularly those with high demands on labour time and labour force, growers will tend to adopt crop protection techniques which make use of routinized practices and, consequently, not too much exigent in farming time and labour. The conventional chemical control is an example of routinized agricultural practice. Similar individual option might be expected in situations of part-time family farmers, very common in the region where the research is being conducted. Thus, time and labour are important constraints at the farm functioning level that might influence growers' adopting crop protection technique, because "making use of natural processes requires active ecosystem management, which is more than producing a crop. Instead of applying rules (e.g., calendar-based preventive spraying), farmers must be able to apply general principles to their situation and make their own rules" (Röling, 1993).

At the individual/grower level, growers' attitudes toward environment and environmental damages produced by pesticides' use, growers' scientific and technical knowledge and skills and their adjustment to each crop protection technique requirements, the economical costs and labour time required by each crop protection technique were the issues under analysis and included in the individual surveys content.

In order to assess the importance in the individual behaviour of the last three issues identified above, individual questionnaires were elaborated in order to enquire growers. The main theoretical basis of the "technical itinerary" approach used in the research and underlying the enquirer's questions will be described below.

A technical itinerary is a set of logical and ordered technical agricultural operations applied to a cultivated eco-system (Gras *et al.* 1989). For instances, a fertilisation, a pesticide treatment and irrigation are examples of technical operations. In order to fulfil a technical operation several different agricultural practices or tasks must be performed. The kind of agricultural practices done by growers and how they perform them, i.e. which kind of tools they use, are important clues to understand growers' goals and technical knowledge and skills. While technical agricultural operations belong to the knowledge/ scientific domain, agricultural tasks or practices are actions rooted in a particular social context (Cerf, 1996). Thus, agricultural practices translate the individual action and the context in which it occurs.

Within the context of the research, a technical itinerary was constructed for each one of the three most cultivated vegetables in the greenhouses of the region. For each one of the vegetables, the technical operations and agricultural practices concerning each one of the crop protection techniques under analysis were identified. Since all the technical operations and practices were theoretically identified and defined, it should be noted that the schedule is a theoretically technical model. In such context, when asking growers to describe the technical farming operations they perform and how they do them, enquirers follow the previous theoretical technical model as a working framework. The empirical collected data allow us to identify in which steps the differences between the technical model and farmers' model occur. The analysis of such differences may identify growers' lack of technical skills and/or scientific/practical knowledge and suggest, for instances, technical courses or information systems oriented to specific subjects in order to fulfil the identified gaps. By through this process way the links between research, management and policy can be improved.

When describing the technical agricultural operations and practices, growers were asked about the economical costs and labour time involved in each one of the tasks they performed, and who, i.e., waged labour force or in case of family labour force, which household member, performed it. On the basis of the empirical data the economical costs of each crop protection technique can be evaluated and compared in real situations. A similar process will be applied to the labour time requirements. Finally, the identification of who performs the different tasks is important in order to define, in a more comprehensive way, information systems and/or technical courses, because target groups were already identified. As the empirical data already collected shows, the responsibility of doing the several agricultural practices and the decision-making process to perform them are distributed among the farm operator, several household members and waged labour.

Besides improving links between research, management and policy, the technical itinerary approach contributes to develop the knowledge and action needed to bring about constructive change in real situations. In such sense, and also because it does not offer a recipe for what change is desirable but rather a description of an action-oriented process, it can be defined as a "participatory" research.

Results and discussion

The described survey questionnaires have been performed throughout the growers community in the Oeste region and the results achieved will be submitted to this meeting in other paper presentation.

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**Section II
Pollinators**

***Section II
Pollinisateurs***

Bumblebees (*Bombus terrestris* L.) (Hymenoptera: Apoidea) as a potential pollinator for greenhouse muskmelon crops; a behavioural study

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Abstract: The use of bumblebees for muskmelon pollination is beginning to be widely adopted; however, there are still some controversy between growers and technicians about its efficacy. In order to contribute for such controversy this study was made, based on the behaviour analysis of the bumblebee, either at the pollination activity level of the hives or at the individual level of the pollinator during its visit to the flower.

An integrated approach of our results suggests the existence of four major distinct aspects for the understanding of the bumblebee foraging activity on this crop: a) colony state; b) flowers availability; c) short term variations in flowers' reward and optimal foraging; d) learning process. Bumblebees are potential pollinators of the muskmelon either through pollen spreading during their visits to the flowers linked with their grooming behaviour, or the systematic pattern for their visits in time, as a response to variations in nectar amounts and availability, even if small. However, some management practices may be needed to optimise such potential.

Key words: pollination, *Bombus terrestris*, *Cucumis melo* L., foraging behaviour, greenhouse, Portugal.

Introduction

Muskmelon is a non-parthenocarpic cucurbit which needs an external pollinator to produce fruits (Rosa, 1924). This is particularly true in greenhouses where the use of honeybee hives is the widely adopted solution. Honeybee efficiency was studied and demonstrated by several works (Vaissière & Izard, 1995; Sugianna, 1987; Orr & Eizikowitch, 1988) on the muskmelon crop. The recent increase in knowledge about other pollinators, like bumblebees, the establishment of cage rearing systems and good results achieved with other crops, linked with the bioethological characteristics of bumblebees (Fisher & Pomeroy, 1989) made them a potential alternative to honeybees muskmelon pollination. Recent research did show their efficiency on this crop (Fisher & Pomeroy, 1989; Dasgan *et al.*, 1997); however, in Portugal, there are still some controversy amongst growers and technicians related with there efficiency and use.

Under such circumstances this research work was set up to clarify the problem, through the analysis of the bumblebees behaviour under muskmelon greenhouses' conditions, either at the pollination activity level of the hive or at the individual level of the pollinator during its visit to the flower. Through such knowledge it is our intention to assess the potential of bumblebees for muskmelon pollination.

Floral biology of Cucumis melo

Muskmelon, being a monoic plant, have two types of flowers, staminate (S) and hermaphroditic (H) (Rosa, 1924) the latter had larger corolla size (Mann, 1953). Number of S

flowers is higher but the ratio H/S varied from day to day (McGregor & Todd, 1952). Muskmelon flowers open at 8:00h and close in afternoon of the same day (McGregor & Todd, 1952). In the following morning the corolla is withered (Rosa, 1924).

Soon after the flower is open stamens dehisce and nectar secretion begins. In S flowers the secretion ceases at 11:00h but in H flowers it continues all day (McGregor & Todd, 1952). H flowers have three or four times more nectar than S flowers, but the sugar concentration of the nectar is greater in S than in H flowers (McGregor & Todd, 1952; Fisher & Pomeroy, 1989).

The period for stigma's receptivity occurs during the morning, mainly (Pouvreau, 1984a). McGregor & Todd (1952) and Pouvreau (1984a) mentioned that more than one visit is necessary for a successful pollination.

Material and methods

This trial was conducted in Oeste region of Portugal, from 16/5/99 to 4/6/99 in a non-heated plastic greenhouse with the muskmelon "galia". The covered area was 2000 m² and the plant were laid on the soil surface. A commercial hive with bumblebees was positioned on the 16/5 afternoon. In order to increase pollination of the crop, the sugar solution container was removed from the hive.

1 – Pollination activity

A set of 10 squares was defined randomly in the greenhouse, each one with six plants. Plants on each square were observed during two minutes, every hour from the 8:00h to 17:00h (included). The n^o of bumblebees' foragers visiting flowers, n^o of flowers visited by each forager and type of flower (S or H) visited were registered. A total of 16 days of daily activity were sampled. Multiple regression was done to test the effect of temperature and relative humidity on the n^o of bumblebees' foragers per square (Sokal & Rohlf, 1981).

2 – Pollinator behaviour

a) Time per flower

Time spend during the visit to flowers was measured, at different day periods. Apart from that, whenever possible, other variables were registered like presence/absence of pollen loads and their behaviour on the flowers (grooming behaviour and nectar collection). A total of 10 days of time measuring were sampled. The average time spend on the visits to S and H flowers was tested for differences using a two-tailed t test, after log transformation of data (for variances homogeneity) ($p=0.05$) (Sokal & Rohlf, 1981).

b) Visitation rate

Forages were followed up at different day periods, for at least 1 min., during their foraging activities, using a tape recorder. Registered data was related with number and duration of visit to the flowers and period spend between two consecutive visits. Such information provides *a posteriori* information on n^o of visits/min. and interflower time. The average number of morning and afternoon visits was compared with non-parametric Mann-Whitney U-test (two-tailed) (Sokal & Rohlf, 1981). The same statistical procedure was used for the interflower time.

3 – Availability of flowers

The number of open flowers per each type was recorded daily, in each of the 10 squares defined.

4 – Environmental monitoring

Temperature and relative humidity was registered by a termohigrographe during all the time of the trial.

Results

1 – Pollination activity

According to the regression analysis temperature and relative humidity had no significant effect on the bumblebee foraging activity.

Fluctuations in number and foraging period, from day to day, made clear their effects in the S.E. of data presented in fig. 1.

Visits to the S flowers begin at 9:00h with a steady increase up to the peak at 13:00 – 14:00h. After that period the number of flower visits is gradually reduced (fig. 4). The pattern of the visits to the S flowers is basically the same presented by number of forager bumblebees (fig. 1).

Concerning H flowers the visits began later, after noon (12:00h) being intensively visited by 13:00h. During this period the rate of visits to S and H flowers is similar (fig. 4). At late afternoon H flowers are visited again with some intensity, being the day period with higher temperature and lower relative humidity (fig. 2).

The S flowers were visited since the beginning of the study (from 18/5). H flowers, in spite of the fact that first visits were recorded on the 19/5, systematic visits only begin after 25/5 (fig. 5).

In general, days with less open flowers (31/5; 1/6; 3/6; 4/6) coincide with days that were observed more visits (fig. 3 and fig. 5). These same days were, also, those with higher number of visits to H flowers. The number of H flowers visits was a tendency to increase throughout the study.

The number of available flowers was not constant along the days of observation. The number of S flowers was always higher than the H type, in spite of fluctuations on the sex ratio (H/S proportion).

2 – Pollinator behaviour

The general behavioural pattern of forager bumblebees on a muskmelon flower can be described as such: after landing on the flower the insect move to the nectary and inserts the mouthparts on it. Movements of the specimen towards other locations on the nectary can disrupt this phase. A foreleg scraping movement of the mouthparts was observed many times at the end of the visit to the flower. Such behaviour can occur either on the flower or during the fly movements of the forager after the visit. The typical grooming behaviour, extended to the entire body was less frequently observed.

The mouthparts and the ventral side of the body produce the contact with stamens and stigma. During the foraging observations some situations were recorded with bumblebees making a close approach to the flowers without landing or if they landed they immediately take off.

a) time per flower

The average time consumed in a visit to the melon flower was 3.90 ± 0.10 ($\bar{x} \pm \text{s.e.}$). ($n=2264$). The average time per S flowers is 3.90 ± 0.10 ($\bar{x} \pm \text{s.e.}$) ($n = 2141$) and for H flowers is 3.44 ± 0.32 ($\bar{x} \pm \text{s.e.}$) ($n=123$). There was a significant difference on average time of visit between S and H flowers. ($\bar{x} \pm \text{s.e.}$).

During the first phase of trial the average time per visit became gradually smaller (with the exception on the 19/5) and finally stabilised in the days after (fig. 6).

From all the observed foragers visiting flowers 99.1% did collect nectar, 11.32% presented grooming behaviour during the visit and 71.4% had no pollen loads. Furthermore, it should be mentioned that observed pollen loads were not, in their majority, from melon pollen.

b) *visitation rate*

As shown in table 1 the average n° of visits/minute and interflower time is bigger during the afternoon. The maximum n° of visitation rate record was 19.05 visits/minute. The average number of visits/min and the average of mean interflower time before 14:00h is significantly different from the observed after 14:00h ($p=0.0107$ and $p=0.066$ respectively, $p < 0.05$, Mann-Whitney test).

Table 1. Visitation rate and interflower time at different period of day. (Visitation rate (vis/min) - n° of flowers visit per minute per bee; Interflower time (seg) - time between flowers)

Period of day h	Visitation rate (vis/min)		Interflower time (seg)	
	$\bar{x} \pm \text{s.e.}$	n	$\bar{x} \pm \text{s.e.}$	n
9.00 - 14.00	10.30 (± 0.48)	48	2.27 (± 0.08)	619
14.00 - 18.00	12.07 (± 0.43)	47	2.08 (± 0.66)	708

Discussion

Nectar is the major reward provided by *Cucumis melo* flowers. This was the case in this study justified by the high number of visits directed towards the nectar and the number of individuals with pollen loads. According to Mackenzie (1994) the absence of pollen baskets or their small size, if present, indicates a nectar foraging behaviour.

Some aspects of behaviour described in the previous point may contribute to a better pollination. This is the case with foreleg scraping movement of the mouthparts, which according to Jander (1976) in Apoidea is related with pollen scraping and, perhaps, direct eating could be involved. Such behaviour, as well as grooming, in general, in spite of potential interference with viability of the carried pollen (Vassière *et al.*, 1992), can present some advantages to the plant, since the general movement of the forager and the consequent dynamics of the pollen grains can increase the amounts of carried pollen as well as the pollen droppings on the stigma.

Fisher & Pomeroy (1989) in a trial on the same crop mentioned the exit from the greenhouse of many foragers (about 18 to 32% between 12:00 and 20:00h), many of them return with pollen. This situation was scarcely detected in the present work, probably motivated by the high nectar standing crop/ bumblebee available inside the greenhouse structure. The presence of the weed *Solanum nigrum* L. made also available the pollen needed by the insects. The mentioned weed could have been a magnetic plants (Laverty, 1992) responsible for the increase of their residence period inside the greenhouse.

Often, the pollen forages were observed making sporadic visits to *Cucumis melo* for nectar while they forage on the weed flowers. The visits made by such bumblebees linked with the visits made by the nectar foragers contributed to an increase of pollination activity on melon flowers, which is in accordance with the magnetic effects of these weeds. These pollen

foragers were also the responsible for the pollen loads (not being collected on the melon crop as reported before) detected during the flowers' visits.

Temperature and relative humidity are not enough to explain the pollination activity under studied conditions, what is in agreement with others authors' statement like Pouvreau (1984b) and Corbet *et al.* (1979). These authors states that the bumblebees are less dependent on environmental conditions than other insects, owing to their capacity to regulate body temperature (endothermy).

An integrated approach of our results suggests the existence of other fundamental factors to explain the foraging activity of bumblebees, such as:

- a) colony state (energy demand of the colony and worker development);
- b) flowers availability;
- c) short term variations in reward of flowers and optimal foraging;
- d) learning process.

During the hive development period bumblebee energetic needs vary on a daily basis. As a consequence the pollination activity varies, also, according to the fulfilment of the energetic demands. As a result, the number of nectar forager bumblebees working was not constant throughout the study period (fig 1), as stated already by Carter (1992). This author also mentioned that the foraging activity of bumblebees is especially sensitive to small variations on nectar amounts stored in the hive, since this resource is usually stored in small quantities and so easily depleted.

The number of available flower exerts a strong influence on the pollination intensity, with high pollination rates being associated with low number of available flowers (fig 3 and fig 5). Flower sex ratio is also important to explain the increase on visit number to flowers, when they are more abundant, in relation to S flowers.

Hartling & Plowright (1979), Heinrich (1976), Pyke (1978a) and Pyke (1978b) developed the optimal foraging theory based on the principal that bumblebees pollination forage in order to maximise the energetic benefits obtained compared with energetic costs.

Bumblebees do not share information amongst them on food sources (Michener, 1974) and each individual must optimise cost/benefits relation, based on the sampling process (Plowright & Laverty, 1984). Sampling is the process whereby the bees investigate flowers (Heinrich, 1976), in order to obtained reassess information on the reward provided by each one. An explanation for the visits to H flowers after noon (12:00h) could be related with bumblebees capacities to detected small variation in sugar concentration (Pouvreau, 1974) and nectar volume (Hodges, 1985). In this period temperature and humidity conditions will allow bigger sugar concentration in H flowers, and they will present a more advantageous energetic balance. Under this circumstances climatic factors like temperature and relative humidity do not seem to directly influence pollination activity but, instead, their action have an indirect influence on the floral microenvironment, by inducing small variations on the nectar quantity and quality (Corbet *et al.*, 1979). Visits to the flowers begin earlier because they usually present a bigger nectar concentration than H flowers.

Between 13:00 and 14:00h a high number of foragers are active inside the greenhouses and they, probably, cause the first drop in nectar content of the flowers. At 13:00h should be very reward to bumblebees the investment on the small number of H flowers, since they will present a bigger nectar volume, as a result of nectar production being a continuous process. At 14:00h the visits' number to the H flowers drops drastically as a consequence of the fast depletion of such flowers in small number. Because as mentioned above, nectar production is continuous on these flowers, by the end of the day the number of visits increases again.

Through the day period the number of individuals foraging and the visits' intensity increases, during a first period, and as a consequence the amount of nectar is greatly depleted,

which induces a reduction on the number of forages during a second period. During the depleting phase of the flowers each forager increases its visitation rate to compensate energetic costs of foraging (Corbet *et al.*, 1995). This results in a bigger number of visits /min. and in bigger inter flower time in the afternoon. Mann (1953) got similar results with honeybees on the same crop. The observed behaviour of flowers' approach and rejection by the foragers, as stated above, reflects the high intensity of visits and the consequent nectar depletion on those flowers, which is in agreement with observations of other authors (Giurfa & Nunez, 1992).

The average time of visit to H flowers is smaller than to S flowers in spite of the fact that the former have bigger volume of nectar. However, Mann (1953) pointed out for honeybees' longer visits to the H flowers. The number of available flowers could cause the apparent contradiction between both results to each forager. Schmid-Hempel & Schmid-Hempel (1987) discussed this subject, mentioning a reduction on the time per flower linked with an increase on flower density, with simultaneous increase in efficiency (Hodges & Wolf, 1981) also indicate that *Bombus* foragers live the flowers before all the nectar are extracts.

Several authors mentioned the existence of a learning process in bumblebees. Keasar *et al.* (1996) state that naïve forages learn to forage more efficiently as far as experience increases. They became more efficient in flowers visit and in the interflower time. The reduction of time per flower is a cause of the flowers' morphology learning and the related expected amount of nectar (Keasar *et al.*, 1996), which is in agreement of the results obtained in the present study (fig. 6). The time per flower values, which stabilised between 29/5 and 3/6, probably is the optimal time to achieve a maximum foraging efficiency on muskmelon flowers, in these conditions. To be able to achieve such performance a learning period will be necessary (through a sampling process) which implies time and energy cost (Heinrich, 1976).

The H flowers begin to be visited in a systematic way only after 25/5, which also implies a learning process focusing on the best strategy to explore the available resources (fig. 5).

From all the stated above it was concluded that bumblebees are potential pollinator of the muskmelon either through pollen spreading during their visits to the flowers linked with their grooming behaviour, or the systematic pattern for their visits in time, as a response to variations in nectar amounts and availability, even if small. However, some management practices may be needed to optimise such potential in term of crop pollination. These strategies should increase the relative amount of H flowers (higher sex-ratio H/S), should reduce humidity in the morning period and should increase H flowers attractivity when they have the higher stigma receptivity. Godinho & Jansen (1995) for honeybees propose the opening of greenhouse windows as earlier as possible exactly for the purpose of humidity reduction.

Bumblebees need two vital resources: nectar and pollen. Since muskmelon flowers provide small amount of pollen, the closed greenhouse environmental can be a limitante factor on this resource. However, the existence of weeds inside the structure can act as pollen resource, which as the case in the present study. In the absence of pollen sources inside the structure may be advantageous to open the windows for external collection when some phases of the development of the hive makes pollen necessary. Pollen inputs can contribute to increase hives' longevity and profitability in the muskmelon crop.

The use of bumblebee hives in melon crop under field conditions needs further scientific work related with: assessment of necessary number of foragers in the particular area; comparison of bumblebee and honeybees efficiency and the relative increase on yield production in a quantified way.

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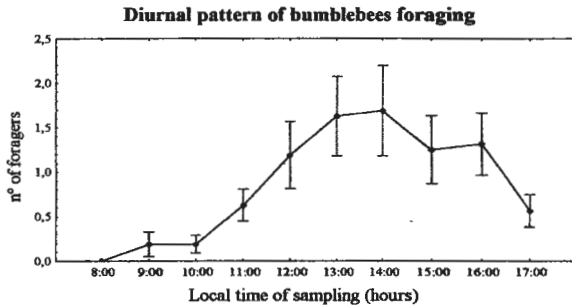


Fig.1- Number of foragers bumblebees on *Cucumis melo* flowers per 2 min. in 10 defined squares, per hour. Data includes averages and S.E. related to 16 days.

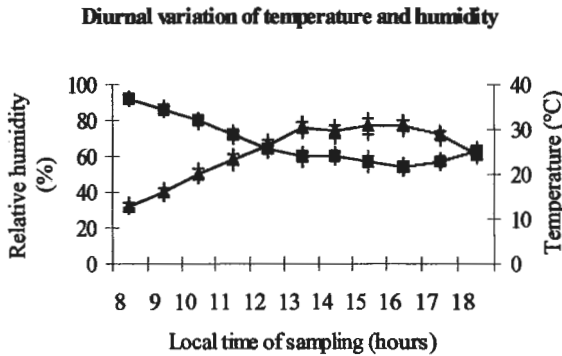


Fig.2- Averages (and S.E., +) of temperature () and relative humidity () during the day.

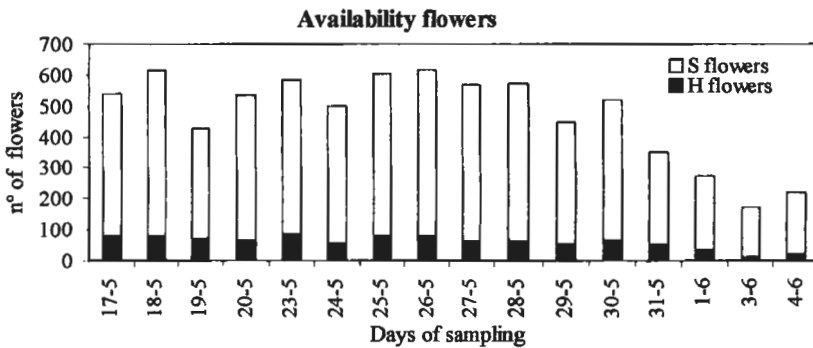


Fig 3- Total availability staminate and hermaphroditic flowers at each day.

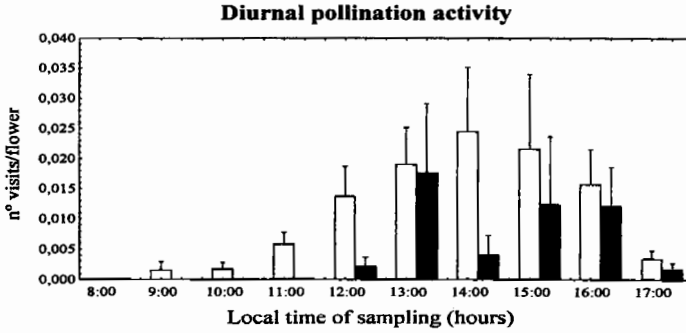


Fig. 4– Number of visits per flower, per 2 min. in 10 defined squares, per hour (■ H= n° visits to hermaphroditic flowers/ total hermaphroditic flowers; S= n° visits to staminate flowers/ total staminate flowers). Data includes averages and S.E. related to 16 days.

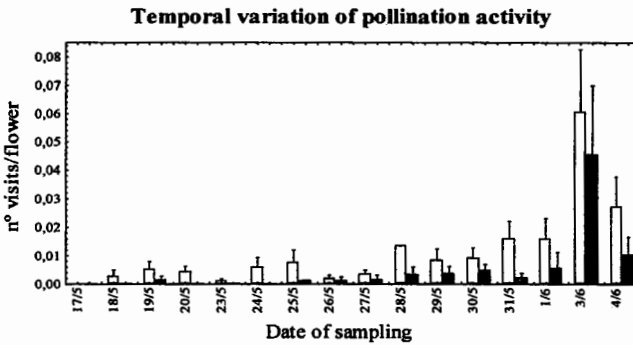


Fig. 5– Number of visits per flower, per 2 min. in 10 defined squares, per hour (■ H= n° visits to hermaphroditic flowers/ total hermaphroditic flowers; S= n° visits to staminate flowers/ total staminate flowers). Data includes averages and S.E. related to 10 hours.

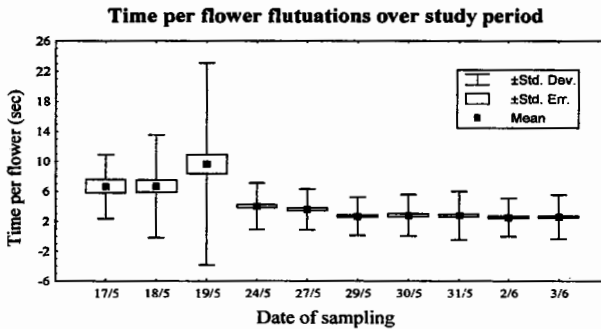


Fig. 6- Mean of time per flower at each day.

Seasonal and density effects in pollination efficiency of bumblebees (*Bombus terrestris* L.) in greenhouse tomato

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Abstract: The use of *Bombus terrestris* L hives became popular through the growers' community in the recent past in the Oeste regions of Portugal, mainly in the tomato crop. The present study focus two factors (density and cropping season) which could explain the observed differences in pollination efficiency reported by technicians and greenhouses growers.

A behavioural analysis of bumblebees was made, particularly to the pollination activity of the hives and to the foraging behaviour of pollinator, in greenhouses with different foragers' density per greenhouses and during different cropping period (Spring and Autumn crops).

Results that related with foragers' densities indicated behavioural differences, which were not related with production differences.

Cropping period effects revealed high significant differences on the foraging behaviour between greenhouses which could be reflected on the obtained yield.

The results supported the importance of both studied factors, which should be taken in consideration when foragers' densities are estimated to optimise pollination in a particular crop and cropping season.

Key words: *Bombus terrestris* ; pollinator efficiency; foraging behaviour; tomato; greenhouse; Portugal.

Section III
Plant diseases

Section III
Maladies végétales

Effect of plant extracts on tomato stem necrosis

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Abstract: Tomato pith necrosis pathogens were isolated from tomato greenhouses in Eastern Mediterranean Region of Turkey. *Pseudomonas viridiflava*, *Pseudomonas corrugata*, *Pseudomonas cichorri*, *Erwinia caratovora* var. *caratovora*, *Erwinia chrysanthemi* were identified by morphological, physiological and pathogenically tests. This is also first report of *Pseudomonas viridiflava* as a tomato pith necrosis pathogen in Turkey. And effects of some plant extracts (garlic, eucalyptus and thyme) on these pathogens were tested with in vitro studies. Filtered and autoclaved eucalyptus extract (50%) was effective on all pathogens. Effect of eucalyptus extract was decreased at 40% ratio and effect was lost at 30% ratio. Filtered garlic extract was (50%) effective to all pathogens except *Pseudomonas corrugata*. When the concentration of garlic extract was decreased, the effect was decreased at the same time. Autoclaved garlic extract was not effective to all pathogens.

Key words: Plant extract, tomato, *Pseudomonas*, *Erwinia*

Introduction

There are many pathogens which are known as causing tomato pith necrosis. For the last five years these pathogens have caused important damages in tomato greenhouses in the East Mediterranean region (Cinar & Aysan 1995; Tokgonul 1995). It is determined that a group of the pathogens belong to *Pseudomonas* and *Erwinia* species cause pith necrosis (*Pseudomonas corrugata*, *Pseudomonas viridiflava*, *Pseudomonas cichorri*, *Erwinia caratovora* var. *caratovora*, *Erwinia chrysanthemi*). Symptoms of the diseases depend on pathogen species but their general symptoms are wilting, flocking on the stem, browning and softening of the pith. These pathogens enter to the plant from pruning wounds and they spread rapidly in greenhouses. Many researchers have used some compounds derived from medicinal plants to control plant pathogen microorganisms. They have found hopeful results. (Ark & Thompson, 1959; Cakir & Yegen, 1991; Cakir, 1992). It has been discovered that some compounds from medicinal plants include therapeutic features. These compounds are volatile oils, organic acids, alcohol, ketoses, phenols e.t.c.. For example in mint (*Mentha piperita*) extract mentol (more than %50), in thyme (*Thymus* spp.) timol and carvacrol (approx. 20%) (Ravid & Putievsky, 1983, 1985), in eucalyptus (*Eucalyptus* sp.) extract cineole (70%) (Baytop, 1974) are antimicrobial compounds from these plants.

Our aim in this study is to determine tomato pith necrosis pathogens in tomato greenhouses in the East Mediterranean region in Turkey and to determine antimicrobial effect of thyme, garlic and eucalyptus extracts against these pathogens. In this study only in vitro preliminary results are given.

Material and methods

Isolation from diseased plants and identification

From diseased plants showing typical disease symptoms, chips of the infected pith were taken and squashed and suspended. Loopfulls were streaked on plates of King's B medium, plates were incubated at 25°C for 48 h. Identification were made according to morphological, physiological and pathogenically tests (Lelliott & Stead, 1987).

Antimicrobial effect of plant extracts against pith necrosis pathogens

50 grams of plant (thyme, garlic and eucalyptus) were blended in 100 ml distilled water each. Rest of the each extract were sterilized by filtering from 0.45 Millipore filter (Yonucu, 1997). Half of the extracts were sterilized by autoclaving at 121°C for 20 minutes and tested separately. Bacteria suspensions prepared from 48 hours cultures (10^6 cfu/ml) were spreaded on King's B medium. On these inoculated plates 1 cm diameter paper discs were placed each. 25 µl extract were given on thee discs. Plates were incubated at 25°C for 48 h. Inhibition zones were measured. In this study original and regional isolates of pathogens (*Pseudomonas corrugata*, *Pseudomonas viridiflava*, *Pseudomonas cichorii*, *Erwinia caratovora* var. *caratovora*, *Erwinia chrysanthemi*) were used.

Results and discussion

Isolation from diseased plants and identification

Two differential bacteria colony were developed as a result of isolations. One of them was cream colored the other was florescent colored. They have tested separately and identified. From cream colored bacteria 21 of them were *Erwinia chrysanthemi*, 3 of them were , *Erwinia caratovora*, 1 of them was *Pseudomonas corrugata*. From florescent colored bacteria 3 of them were *Pseudomonas viridiflava* and 7 of them were *Pseudomonas cichorii*.

Pseudomonas viridiflava was firstly found as a tomato pith necrosis pathogen in the East Mediterranean Region in Turkey. So this is the first report of *Pseudomonas viridiflava* in the East Mediterranean Region in Turkey. This pathogen was also reported to cause tomato pith necrosis in New Zealand (Wielke et al ,1974), USA (Lukezic et al, 1983), Italy (Fiori et al , 1982; Sasa, 1997), Greece (Alivizatos, 1986; Skoudridakis, 1986), France (Coln, 1986) and Bulgaria (Bogatsevskaja et al, 1999).

Antimicrobial effect of plant extracts against pith necrosis pathogens

As we see at table 1 and 2 effect of the extracts depended on the way of sterilization (filtration and autoclave), plant extract and concentration. While the concentration decreasing the effect decreased at the same time for all extracts. Thyme extract was effectiveness for both methods.

Filtered and autoclaved eucalyptus extract (50%) was effective on all pathogens. Filtered eucalyptus extract was the most effective on *Pseudomonas corrugata* and *Pseudomonas cichorii*. Filtered garlic extract was most effective to *Erwinia chrysanthemi* and effect was continued with *Pseudomonas cichorii* and *Pseudomonas viridiflava*. But it was not effected *Pseudomonas corrugata* (Table 1). Effect of garlic extract was disappeared when it was autoclaved. It was certain that heat effected active compounds of garlic (Mangamma & Speeramulu, 1991; Yonucu,1997). On the other hand effect of eucalyptus extract was increased with autoclaving. At 30% concentration effect was disappeared or decreased with all extracts (Table1 and 2). When the incubation time was longed effects of all extracts were disappeared (Cakir & Yegen, 1991, Yonucu, 1997).

Table 1. Effects of filtered extracts on pathogens of tomato stem necrosis

Isolates	Garlic			Eucalyptus			Thyme		
	50%	40%	30%	50%	40%	30%	50%	40%	30%
Regional ECH	0,533	0,500	0,140	0,239	0,100	0,000	0,000	0,000	0,000
Regional ECC	0,300	0,267	0,000	0,350	0,100	0,000	0,000	0,000	0,000
Israel (ECC)	0,200	0,170	0,000	0,150	0,000	0,000	0,000	0,000	0,000
Regional-PCIC	0,356	0,140	0,000	0,356	0,200	0,000	0,000	0,000	0,000
GSPB1224 (PCOR)	0,000	0,00	0,000	0,556	0,444	0,000	0,000	0,000	0,000
Regional PCOR	0,000	0,00	0,000	0,411	0,167	0,000	0,000	0,000	0,000
GSPB 1685 (PVIR)	0,267	0,178	0,120	0,411	0,167	0,000	0,000	0,000	0,000
Regional-PVIR	0,189	0,100	0,000	0,167	0,156	0,000	0,000	0,000	0,000

(ECH : *Erwinia chrysanthemi*, ECC : *Erwinia caratovora* var. *caratovora*, PCIC : *Pseudomonas cichorii*, PCOR : *Pseudomonas corrugata*, PVIR : *Pseudomonas viridiflava*, GSPB : Göttinger Sammlung Phytopathogener Bakterien, Israel ECC isolate from Dr Kritzman, GSPB isolates from Dr Rudolph were obtained)

Table 2. Effects of autoclaved extracts on pathogens of tomato stem necrosis

Isolates	Garlic			Eucalyptus			Thyme		
	50%	40%	30%	50%	40%	30%	50%	40%	30%
Regional ECH	0,000	0,000	0,000	0,367	0,156	0,000	0,000	0,000	0,000
Regional ECC	0,000	0,000	0,000	0,511	0,156	0,000	0,000	0,000	0,000
Israel (ECC)	0,000	0,000	0,000	0,267	0,000	0,000	0,000	0,000	0,000
Regional-PCIC	0,000	0,000	0,000	0,411	0,222	0,000	0,000	0,000	0,000
GSPB1224 (PCOR)	0,000	0,000	0,000	0,567	0,433	0,000	0,000	0,000	0,000
Regional PCOR	0,000	0,000	0,000	0,533	0,378	0,130	0,000	0,000	0,000
GSPB 1685 (PVIR)	0,000	0,000	0,000	0,356	0,267	0,140	0,000	0,000	0,000
Regional-PVIR	0,000	0,000	0,000	0,417	0,278	0,100	0,000	0,000	0,000

(ECH : *Erwinia chrysanthemi*, ECC : *Erwinia caratovora* var. *caratovora*, PCIC : *Pseudomonas cichorii*, PCOR : *Pseudomonas corrugata*, PVIR : *Pseudomonas viridiflava*, GSPB : Göttinger Sammlung Phytopathogener Bakterien, Israel ECC isolate from Dr Kritzman, GSPB isolates from Dr Rudolph were obtained)

Because of these bacteria located in pith and vessels, chemical control with copper compounds has not been effective in greenhouses. Therefore it is necessary to apply new developed control methods including the use of plant extracts. The results of our study on plant extracts are hopeful for the future studies. In the future, we will research on plant extracts on tomato plants with in vivo studies and it is certain that more detailed studies should be carried out.

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Induction of resistance on eggplants against *Verticillium* wilt disease and root-knot nematodes using biotic and abiotic factors

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Abstract: The present study aimed to find out the effect of trifluralin, salicyclic acid (SA) and dead *Verticillium* culture on *Verticillium dahliae* and *Meloidogyne javanica* Race 1, and host resistance to wilt disease and root-knot nematode damage on eggplant. Three doses (0.5 mM, 1 mM and 2 mM) of trifluralin and two doses (0.5 mM and 1 mM) of SA were used to test the direct effect. For the host resistance experiment the 15-cm height eggplants were applied with 1/2x, 1x and 2x of recommended dose of trifluralin and 100 ml of 1 mM, and 2 mM SA to the soil daily for 4 days. The eggplant seedlings were also inoculated with 10^6 spores/ml of dead *Verticillium* culture. Following this inoculations eggplants were re-inoculated with *V. dahliae* and *M. javanica*. Trifluralin and SA at all concentrations significantly inhibited the mycelial growth of *V. dahliae* whereas only SA had resulted 64.4-99.5% of mortality against *M. javanica* at two doses used. Resistance of eggplant to wilt disease was significantly increased by all treatments tested. Trifluralin and SA treatment caused a significant reduction in the appearance of disease at all doses used. The highest effectiveness of trifluralin was obtained with the lowest dose. SA treatment at 1 mM concentration had the greatest inhibitory effect on the wilt disease among the treatments tested. Trifluralin at 2x recommended dose had the greatest inhibitory effect on the appearance of root gall and the reproduction rate of *M. javanica*. SA at both doses significantly resulted decrease of root gall. *M. javanica* did not reproduce in SA treated pots.

Key words: eggplant, *Verticillium* wilt, root-knot nematode, induced resistance

Introduction

Verticillium wilts and root-knot nematodes are soil-borne diseases that cause serious problems in vegetable, field, tree and ornamental crops in tropical and sub-tropical areas (Schnathorst, 1981). They cause important yield losses such as infection of root-knot nematodes may reduce yield of eggplants at 30-60% (Netscher & Sikora, 1990).

Soil fumigants particularly methyl bromide and other pesticides are used in controlling these diseases in the Mediterranean Region of Turkey. As known, the fumigants are not environmentally friendly and not result adequate control of soil borne diseases. The difficulties in controlling wilt pathogens and root-knot nematodes by conventional methods make induced resistance an attractive and useful strategy for the plant disease control. Several kinds of herbicides have been reported to increase or decrease plant diseases especially those caused by soil borne plant pathogens (El-Khadem *et al.*, 1984). Grinstein *et al.* (1984) reported that pre-treating tomato and eggplant seedlings with dinitroaniline herbicides increased their resistance to vascular wilts caused by *Fusarium* and *Verticillium* species. The compounds accumulated in herbicide-treated inoculated tomatoes, were toxic to fungi and extracted by ethanol. Canihos (1997) reported that pre-treating cotton seeds with herbicides, prometryn, linuran and haloxyfop, markedly increased cotton resistance to vascular wilt caused by *V. dahliae*. Salicyclic acid (SA) was reported as both exo- and endo-inducers in

plant resistance to diseases (Raskin, 1992; Malamy & Klessig, 1992). The herbicide trifluralin application to soil resulted the reduction in nematode population (Wafdy *et al.*, 1993).

The purpose of this study was to test, both *in vitro* and *in vivo*, the effect of trifluralin, SA and dead *Verticillium* culture on *Verticillium dahliae* and *Meloidogyne javanica*.

Material and methods

The pathogen *V. dahliae* was isolated from diseased plants and subcultured on Czapek-Dox agar. These subcultures were maintained in the dark at 4 °C. Root-knot nematode *M. javanica* race 1 that is widespread in the East Mediterranean Region was used. Herbicide trifluralin (Treflan), SA and dead *Verticillium* culture were involved as inducers.

Effect of trifluralin and SA on the fungus

Experiments on solid medium were carried out using the method of Grinstein *et al.* (1984). Aliquots of media were prepared containing the different concentration of trifluralin and SA, and a control containing neither trifluralin nor SA. Three doses (0.5 mM, 1 mM and 2 mM) of the herbicide and two doses (0.5 mM and 1 mM) of SA were consistently used and the tests were conducted in 9-cm petri dishes. A 5-mm agar disc cut from a young active culture of *V. dahliae* was transferred to the test medium (five replicate plates of each concentrations and control). The plates were than incubated in the dark at 25 °C for 14 days, after which colony diameters were measured.

The procedure outlined by Awadalla & El-Refai (1992) was adopted to test effect of trifluralin and SA in liquid culture. Each compound was incorporated at the same concentration as above into 250-ml flasks containing 100 ml of Czapek-Dox liquid media after autoclaving. Each flask was inoculated with 5 ml of spore suspension containing 10⁶ spores/ml. The flasks were placed on an orbital shaker at 25 °C in the dark for 14 days. The content of each flask was filtered using Büchner funnel and Whatman No.1 filter paper that have been previously weighed. The filter papers with mycelia were wrapped individually in soil previously weighed, dried in an incubator at 80 °C for 48 h and then weighed to mg. Three replicates were included for each concentration of trifluralin and SA, and three flasks containing neither of compounds were used as controls.

Effect of trifluralin and SA on the root-knot nematode

Direct effect of different concentrations of SA (0.5 mM, 1 mM and 2 mM) and trifluralin (0.5 mM and 1 mM) on second stage juveniles of *M. javanica* Race 1 were studied. For this aim *M. javanica* Race 1 population was grown on eggplants. Gelatinous egg masses were collected from eggplant and second stage juveniles were obtained. One hundred individual was used for each dosage and the experiment was conducted with 4 replicates. After then, the larvae were incubated in solutions given above for 12 h at 26±1 °C. Microscopic examination was done to determine the percentage of mortality.

Effect of the biotic and abiotic factors on host resistance to wilt disease and root-knot nematode

Eggplant seeds were sown in the soil sterilised with MeBr in 25-35 cm pots and incubated at 26±1 °C growth-room. When the seedlings were 10-15 cm height, trifluralin that is a post-emerge herbicide was applied at 1/2x, 1x and 2x of application dose recommended by the firm for eggplant plantations for each pot. Untreated pot was used as a control. After 4 weeks, the seedlings were washed thoroughly to remove traces of herbicide and transplanted into herbicide-free soil in 12-cm pots after inoculating by dipping their roots for 5 min in 10⁶ spores/ml concentration of washed conidia of *V. dahliae*. Four plants from each treatment and

control were inoculated. Disease ratios were recorded according to the degree of chlorosis or wilting symptoms using rating scale from 0-4 (Wilhelm *et al.*, 1974).

SA application was carried out using the method of Özgönen (1998). Eggplant seedlings (10-15 cm height) were grown and transplanted into 12 cm pots as above and 100 ml of 1 mM and 2 mM SA was applied to the soil daily for 4 days. Then the seedlings were inoculated with *V. dahliae* as above. Dead *V. dahliae* culture was applied like pathogen inoculation, and after 7 days incubation, the pathogen was inoculated.

To determine the effect of Trifluralin, SA and dead *Verticillium* conidia on the induced resistance against *M. javanica* Race 1, each plant was inoculated with 1000 second stage juveniles, after the application of different doses of two chemicals and death *Verticillium* conidia to eggplants. Six weeks after inoculation the plants were uprooted, the roots were carefully washed under tap water to remove soil particles. The galling ratio was determined by using of 0-10 scale (Barker, 1985). In addition 100 g (50 cm³) of soil was taken from each pot, and analysed using the modified Baermann funnel technique to determine the population density of second stage juveniles in the soil.

Results and discussion

Effect of trifluralin and SA on V. dahliae and M. javanica

Various concentrations of trifluralin and SA incorporated in Czapek-Dox agar, liquid media, and distilled water to determine their effect on the mycelial growth of *V. dahliae* and on the mortality of *M. javanica*, respectively. Table 1 shows that trifluralin and SA at all concentrations tested significantly inhibited mycelial growth of the wilt pathogen in solid medium. The degree of inhibition was directly related to the herbicide concentration but not related to SA concentration. The maximum inhibitory effect was obtained in trifluralin treatment at 2 mM concentration compared to control. There was no statistical differences between 0.5 mM and 1 mM concentrations of SA. While the lowest concentrations (0.5 mM) of trifluralin did not have any inhibitory effect on the mycelial growth of pathogen in solid medium.

Table 1. Effect of trifluralin and SA at different concentrations on the mycelial growth of *Verticillium dahliae* on solid and liquid media and on mortality of *Meloidogyne javanica* in liquid medium.

Chemicals and dosages (mM)	Diameter of coloni (mm) on solid medium	Mycelial dry weight (g/100ml) in liquid medium	Mortality of <i>M.javanica</i> (%) in liquid medium
Trifluralin			
0.5	75.0 d*	0.888 c	12.0 c
1.0	61.8 b	0.489 b	11.9 c
2.0	47.6 a	0.058 a	16.0 c
SA			
0.5	69.1 c	1.205 d	64.4 b
1.0	69.5 c	1.280 d	99.5 a
Control	77.3 d	1.048 cd	11.2 c

* Means in the same column followed by the same letter are not significantly different as determined by LSD at p=0.05 level

Trifluralin in liquid medium reduced the mycelial dry weight of *V. dahliae* depending on the concentration. Similar results were reported by Grinstein *et al.* (1976). They found that trifluralin inhibited the growth of *Rhizoctonia solani* and *F. oxysporum* f. sp. *lycopersici* (FOL) depending on the concentration. However, they reported that conidial germination of FOL was not affected by trifluralin. El-Khadem *et al.* (1984) also found that trifluralin reduced the growth of *R. solani* and *F. oxysporum* f.sp. *vasinfectum*, but did not effect the spore production in liquid culture. SA in both concentrations did not have any inhibitory effect on the mycelial dry weight of the wilt pathogen (Table 1). It can be said from Table 1 that SA stimulated the mycelial growth of *V. dahliae* in liquid medium. The stimulatory effect of SA on the mycelial dry weight found in the present study agrees with that found by Özgönen (1998) and Kücükkömürçü (1999).

The herbicide, trifluralin did not have any effect on the mortality of second stage juveniles of root-knot nematode, *M. javanica* compared to those of control. However, both concentrations of SA significantly increased the mortality of nematode with 64.4% and 99.5% in 0.5 and 1.0 mM concentrations, respectively. Maheshwari & Anwar (1990) also reported that different SA concentrations (100, 500 and 1000 µg/ml) resulted mortality of *M. incognita*. The mortality in three different concentrations (0.5, 1.0 and 2.0 mM) of the herbicide and controls is assumed to result of the natural death of the nematode.

Effect of trifluralin, SA and dead Verticillium culture on Verticillium wilt disease and root-knot nematode incidence

Resistance of eggplant to wilt disease caused by *V. dahliae* was significantly increased by all treatments tested (Table 2). Trifluralin treatment caused a significant reduction in the appearance of disease on the plants at three doses used, but efficiency was diminished as the dose increased. The highest effectiveness was obtained from the lowest dose (1/2x of recommended dose) with 70.6% disease reduction. El-Khadem *et al.* (1984) found that trifluralin significantly reduced Fusarium disease incidence of cotton at the higher concentration (double of recommended dose). The effect of herbicide on reducing Fusarium wilted plants was attributed to the effect of the herbicide on chlamyospore germination in soil. A similar effect of trifluralin on the resistance of tomato and eggplant to *R. solani*, *F. oxysporum* and *V. dahliae* has also been reported (Grinstein *et al.*, 1984).

Table 2. Effect of trifluralin and SA on Verticillium wilt disease incidence of eggplant

Treatments	Score of disease*	Efficiency of treatment
Trifluralin		
1/2x recommended dose	0.625	70.6
1x recommended dose	1.125	47.1
2x recommended dose	1.5	29.4
SA		
1 mM	0	100
2mM	0.375	82.3
Dead <i>V. dahliae</i> culture	0.875	58.8
Control	2.125	0

* Disease index value at the rating scale from 0-4

SA in both concentration tested had also an effectiveness in controlling *Verticillium* wilt disease of eggplant. SA treatment at 1 mM concentration had the greatest inhibitory effect in the appearance of wilt disease with 100% effectiveness. SA itself was not fungi-toxic to the pathogen, we conclude therefore that effect is most likely due to changes in the resistance mechanisms in plants. SA is well known to induce accumulation of PR as well as to effect flowering, stomata closure and seed germination of plants (Malamy & Klessig, 1992; Raskin, 1992).

Effectiveness of *Verticillium* dead culture was obtained as 58.8% on the wilt disease appearance. The effect might be due to cross protection mechanisms of the plant.

The treatments had different effect on the incidence of gall caused by *M. javanica* and its second stage juvenile population in soil (Table 3). Dead *Verticillium* culture had effect on neither gall incidence nor second stage juvenile population in soil. As expected there was no cross protection effect. Trifluralin at 2x recommended dose had the greatest inhibitory effect on the appearance of gall. The herbicide also reduced second stage juvenile population in correlation with the concentration. Trifluralin itself at three concentrations tested did not have toxic effect on the nematode population (Table 1) suggesting that the herbicide enhanced a biochemical defence within the host. This might be responsible for the observed increase in resistance. Similar effect was obtained by Youmans (1986) who reported that 2x and 1x rate of trifluralin reduced *Heterodera glycines* second stage juvenile population in soil but no effect on the egg hatch at laboratory conditions.

Table 3. Effect of trifluralin, SA and dead *Verticillium* culture on galling of *Meloidogyne javanica* and second stage juvenile in soil.

Treatments	Galling index	Reduction (%)***	2.stage juveniles/ 100g soil	Soil results Reduction (%)***	Reproduction rate (R=pf/pi)****
<i>Trifluralin</i>					
1/2x rec. dose	3.5 a*	0	2605 abc	39.9	2.5
1x rec. Dose	2.7 a	21.4	1670 bc	61.4	1.6
2x rec. dose	2.0 b	42.8	610 c	85.9	0.6
<i>SA</i>					
1mM	2.0 b	42.8	915 c	78.8	0.9
2mM	1.5 b	57.1	820 c	81.1	0.8
Dead	3.5 a	0	3580 ab	17.4	3.5
<i>Verticillium</i>					
Control (M.j.**)	3.5 a		4335 a		4.3

* Means in the same column followed by the same letter are not significantly different as determined by Duncan at $p < 0.05$ level

** inoculated with *Meloidogyne javanica*

*** according to control

**** Pi=initial population (1000 juveniles per plant), Pf=final population

SA had also inhibitory effect on the gall incidence and the juvenile population similar to those observed in double recommended dose of trifluralin. Both concentration of SA tested 1 and 2 mM, respectively, reduced gall incidence at 42.8 and 57.1% and second stage juvenile

population at 78.8 and 81.1%. Similar results were observed by Mashwari & Anwar (1990) who found that SA inoculated *Capsicum frutescens* c. California Wonder plants were less attracted by *M. incognita*.

In conclusion, treatment of eggplant with trifluralin, SA and dead *Verticillium* culture markedly reduced the appearance of wilt disease depending on the concentrations used. SA and trifluralin of 2x recommended dose also had inhibitory effect on gall incidence caused by root-knot nematode, *M. javanica*, and its second stage juvenile population in soil. In light of these results, these chemicals may be used in controlling these diseases in an integrated crop management approach. A more thorough physiological and histological investigations needs to be carried out to explain the nature of resistance to *Verticillium* wilt disease and root-knot nematode in eggplant.

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Control of wilt and root diseases of *Asclepias tuberosa* L.

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Abstract: Chlorosis and wilt, followed by weak flowering or death, have been occurring in *Asclepias tuberosa* plants grown in Israel. Some roots have been rotted and tuberous roots had dark lesions. Binucleate *Rhizoctonia* and *Pythium intermedium* were detected as the primary pathogens. The objective of the present study was to optimize the chemical control of these pathogens: seed or post-emergence treatments and chemical control of *Rhizoctonia* were investigated in two separate field experiments. Inoculation of plants with both pathogens significantly reduced the number and weight of flowers as compared with the non-inoculated plants. However, seed treatment or post-emergence application of fungicides had no significant effect on the yield. The percentage of surviving plants, one year after planting, was higher in the non-inoculated plants, with no significant difference between the seed and post-emergence treatments. The recovery of *Rhizoctonia* from dead plants was higher than the recovery of *Pythium*, in all treatments, except in the case of seed treatment without inoculation. The various fungicides applied to the foliage of non-inoculated plants in experiment II, had no significant effect on the number and weight of flowers, as compared with non-treated control. However, treatment with fludioxonil (Celest) resulted in more flowers than treatment with tolclofos-methyl (Rizolex), in the non-inoculated plots. There was no difference between these treatments in the inoculated plots; this may be explained by the relatively low efficiency of the inoculation. The percentage of surviving plants, one year after planting, was similar in all fungicide treatments and in the control, without significant differences between inoculated and non-inoculated plots. The recovery of *Rhizoctonia* from dead plants was lower in all treatments than the recovery of *Pythium*, in spite of the artificial inoculation with *Rhizoctonia* in part of the plots. It is possible that the climatic conditions during the growing season were more favorable to *Pythium*, or that the effects of the various treatments will be expressed only during the second year of growth.

Key words: diseases, control, binucleate *Rhizoctonia*, *Pythium intermedium*

Introduction

Asclepias tuberosa L., butterfly weed, is a perennial plant native to North America that has been grown in the USA mainly as an outdoor garden ornamental (Lyons, 1985). Because of its attractive orange-red inflorescence, it has been developed recently as a cut-flower crop in Europe and Israel (Ecker & Barzilay, 1993). The plant is propagated sexually by seeds (Borland, 1987) and vegetatively by tuberous root cuttings (Albrecht & Lehmann, 1991). It is self-incompatible for pollination and plants derived from seeds are genetically heterogeneous, particularly in growth habit and in flower color pattern (Wyatt, 1980). In Israel, the flowers are grown in heated greenhouses 25/18C (day/night) with a long-day photoperiod (12-14 h of natural daylight plus incandescent light during the night) (Ecker & Barzilay, 1993). They are grown in sandy soil or in a soilless medium of volcanic stones, and are drip irrigated.

Fumigation of the potting medium with methyl bromide (750 kg/ha) prior to planting is a common practice.

Chlorosis and wilt, followed by weak flowering or death, have been occurring in *Asclepias tuberosa* plants grown in Israel. Roots have been rotted and tuberous roots have shown dark lesions. Binucleate *Rhizoctonia* and *Pythium intermedium* were detected as the primary pathogens. Other organisms were less prevalent (*Myrothecium* and *Fusarium* spp.) (Tsrer et al, 1997). In artificial inoculations of seedlings with *P. intermedium* or binucleate *Rhizoctonia* or both, wilting began 7 days after inoculation, with disease incidence ranging from 25 to 65% and disease severity index ranging from 0.30 to 0.85 (on a scale of 0 to 3).

The objective of the present study was to optimize the chemical control of these pathogens: seed or post-emergence treatments and chemical control of *Rhizoctonia* were investigated in two separate field experiments.

Materials and methods

Greenhouse experiments

Two experiments were conducted in greenhouses at the B'sor experiment station, located in the south-western part of Israel. In each experiment, treatments were arranged in randomized complete blocks, with four replicates. Plot size for each replicate was three beds wide by 2 m long to a final stand of 60 seeds per square meter. Experiment I was sown on 4.6.98; treatments included: control, seed dipping for 10 minutes in a mixture of 2% tolclofos-methyl (Rizolex), 1% benzimidazole (Benlate) and 0.2% captan (Merpan), and post-emergence spray with tolclofos-methyl (Rizolex 1.25 gr/m²) and prothiocarb (Dynone 0.3%). First harvest was on 23.8.98 and regular spray with tolclofos-methyl and prothiocarb was applied after each additional harvest. Experiment II was sown on 2.7.98; treatments included: fludioxonil (Celest 4 l/ha), flutolanil (Moncut 10 l/ha), pencycuron (Monceren 10 l/ha), tolclofos-methyl (Rizolex 15 kg/ha), iprodione (Rovral 10 kg/ha), and non-treated control. Fungicides were applied 31.8.98 and after each harvest. Prothiocarb (Dynone 0.3%) was also applied, to minimize infection with *P. intermedium*.

Inoculation

In both experiments inoculation in several of the plots was carried out prior to sowing by incorporating wheat grains inoculated with *Rhizoctonia* or *P. intermedium*. In experiment I both pathogens were spread, and in experiment II only *Rhizoctonia* was spread.

Yield assessment

In each experiment plants were harvested according the commercial standards. Numbers of flowers, their weight and stem length were determined for each plot.

Disease assessment

Diseased and dead plants were examined in the lab to define the causal agent by placing root or stem segments on potato dextrose agar. After incubation in the dark for 3-7 days, microscopic observation was done. The percentage of surviving plants was calculated by counting the emerging plants after each harvest.

Results and discussion

Experiment I: Effect of seed and post-emergence treatments on yield

Seed and post-emergence treatments were tested because we observed in a previous study that *Rhizoctonia* might be seed-borne (Tsrer et al, 1997). In this experiment, the yield from seed-

treated plots was higher than of the post-emergence fungicides' application plots, but without significant statistical difference (Table 1).

Two-way ANOVA indicated that inoculation of the plants with both pathogens significantly reduced the number and weight of flowers (especially in the third and fourth harvest) as compared with the non-inoculated plants.

Table 1. Effect of inoculation with *Rhizoctonia* and *Pythium* and fungicides application on the accumulated yield

Treatment	Number of flowers/m ²	Weight of flowers (kg/m ²)
Control	415.5	9.4
Post-emergence spray	358.8	8.6
Seed treatment	422.6	9.3
Inoculated with <i>Rhizoctonia</i> and <i>Pythium</i>	272.1	5.5
Inoculated+ post-emergence Spray	275.5	5.6
Inoculated+ seed treatment	287.4	5.8
Two-way ANOVA	F	P
A factor (inoculation)	37.8	0.00
B factor (treatments)	1.08	0.37
AXB	0.48	0.63

Experiment I: Effect of seed and post-emergence treatments on plant survival

The percentage of surviving plants one year after planting was higher in the non-inoculated plants, with no significant difference between the treatments. The recovery of *Rhizoctonia* from dead plants was higher than the recovery of *Pythium*, in all treatments, except in the case of seed treatment without inoculation (Table 2). In both seed and post-emergence treatments in the non-inoculated plots, the percentage of surviving plants was lower than in the controls, and in spite of that, yields were not reduced, indicating the potential efficiency of these treatments.

Table 2: Effect of inoculation with *Rhizoctonia* and *Pythium* and fungicides application on surviving plants and recovery of pathogens.

Treatment	Surviving plants (%)	<i>Rhizoctonia</i> (%)	<i>Pythium</i> (%)
Control	51.2	56.6	6.6
Post-emergence spray	47.2	37.9	16.7
Seed treatment	49.9	15.4	42.3
Inoculated with <i>Rhizoctonia</i> and <i>Pythium</i>	24.6	66.7	14.0
Inoculated+ post-emergence spray	29.1	30.0	13.3
Inoculated+ seed treatment	26.4	57.7	8.5

Experiment II: Effect of fungicide treatments on yield

In commercial greenhouses in Israel the growers treat with Rizolex to reduce infections of *Rhizoctonia*. In the present experiment it was observed that application of the tested fungicides to the foliage of non-inoculated plants had no significant effect on the number and weight of flowers, as compared with the controls, during the first year of the experiment (Table 3). However, in the Rizolex treatment yields were lower than in the controls. Two-way ANOVA between the inoculated and non-inoculated plots indicated that inoculation of the plants with *Rhizoctonia* significantly reduced ($F=5.9$, $P=0.03$) the weight of flowers, but not the number, and that treatment with Celest resulted with significantly more flowers than treatment with Rizolex, only in the non-inoculated plots. There was no difference between these treatments in the inoculated plots, which may be explained by the relative low efficiency of inoculation. It is also possible that the effects will be expressed only during the second year of growth.

Table 3: Effect of inoculation with *Rhizoctonia* and fungicides application on the accumulated yield

Treatment	Number of flowers/m ²	Weight of flowers (kg/m ²)
Control	1003.4	20.4
Rizolex	904.5	19.7
Monceren	1020.9	21.0
Celest	1067.8 *	20.9
Moncut	1048.6	21.3
Rovral	1057.0	21.1
Non-treated and inoculated	981.9	19.3
Inoculated + Rizolex	906.4	18.0
Inoculated + Celest	946.3	17.9

Experiment II: Effect of fungicide treatments on plant survival

The percentage of surviving plants, one year after planting, was similar in all fungicide treatments and in the control, without significant difference between inoculated and non-inoculated plots (Table 4). The recovery of *Rhizoctonia* from dead plants was lower than the recovery of *Pythium* in all treatments, in spite of the artificial inoculation with *Rhizoctonia* in part of the plots. It might be that the climatic conditions during the growing season were more favorable to *Pythium* (in spite of treatment against this pathogen with prothiocarb).

These preliminary results indicate that fungicidal sprays have the potential to control the pathogens and prevent yield loss. Among the fungicides tested, Celest and Rovral are the most promising. These results may become more clear in subsequent years.

Table 4: Effect of inoculation with *Rhizoctonia* and fungicides application on surviving plants and recovery of pathogens

Treatment	Surviving plants (%)	Rhizoctonia (%)	Pythium (%)
Control	74.3	5.5	50.7
Rizolex	73.6	8.3	52.8
Monceren	75.3	4.7	51.8
Celest	73.5	20.0	43.1
Moncut	75.0	21.1	42.1
Rovral	72.9	1.9	57.7
Non-treated and inoculated	68.0	42.6	64.7
Inoculated + Rizolex	62.1	34.4	69.5
Inoculated + Celest	66.9	44.3	75.9

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Combination of *Trichoderma* spp. and soil solarization to control root rot diseases of cucumber in greenhouses conditions

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Abstract: Effects of soil solarization alone and in combination with *Trichoderma* spp. (*T. harzianum* and *T. koningii*) on cucumber root rot pathogens (*Rhizoctonia solani* and *Fusarium solani*) were investigated. The experiments were conducted in greenhouses in the East Mediterranean Region, Turkey. Maximum soil temperature was found to be 43.2 °C and 37.4 °C at 10 cm and 20 cm depths, respectively.

Cucumber root rot disease ratio decreased in all three applications which were solarization, solarization plus commercial preparation of *Trichoderma* spp. and solarization plus in vitro culture of *Trichoderma* spp., compared to those of control. However no difference was found among applications. Effect of treatments on yield was also determined.

Key words: *Trichoderma* spp., solarization, soilborne pathogens, integrated control.

Introduction

Total of 401.325 da of protected cultivation areas has been reported in Turkey, and 273.825 da of this total areas that is approximately 70%, takes place in the East Mediterranean Region (Anonymous, 1998). Diseases caused by soilborne pathogens are one of the most important problem restricting crops grown under protected conditions. Methyl Bromide (MeBr) is extensively used as soil fumigant, however because of its ozone depletion effect, it will be banned in coming 5-8 years in Turkey. Therefore there is a need to develop alternative methods to MeBr usage. Soil solarization employs solar energy to increase soil temperature and it is non-chemical and hydrothermal method. Soil solarization when applied alone may not be consistently effective for controlling soilborne pathogens. In this case, to increase the effect, it is usually combined with cultural, biological and/or chemical (preferably at reduced dosages) methods (Katan, 1996; Coelho, 1999).

It was reported that application of solarization and *Trichoderma harzianum* in a field contaminated with *Rhizoctonia solani* delayed inoculum development and increased disease and pathogen control (Chet *et al.* , 1982; Elad *et al.* , 1980 b). *T. harzianum* applications in potato disease caused by *R. solani* resulted decrease in disease severity (Elad *et al.* , 1986). Same report suggests that combination of *T. harzianum* with solarization or MeBr application increased the efficiency and also resulted better control of *Sclerotium rolfsii* in beans.

The aim of this study was to determine the effect of solarization plus *Trichoderma* spp. combinations on cucumber root rot disease in greenhouses.

Materials and methods

Solarization applications

Before solarization, soil was first crumbled to a depth of 30-40 cm, and then was levelled.

Following, the soil was irrigated to depth of 50-60 cm to increase heat transmission and sensitivity of microorganisms. When the soil reached to the right conditions, 0.05 mm thick polythene sheet was used to cover the soil, and the edges were carefully embedded to avoid air bubble formation (Grinstein & Hetzroni, 1989). Soil type in two locations was organic and sandy, and the pH was 7.0-7.2.

When soil humidity decreased, drip irrigation system established under the sheets was switched on. Solarization was done between 11th July-11th September, in 1997 and 13th July-7th September in 1998, for about 8 weeks. Soil temperature was recorded in parcels that solarization applied and in controls by placing soil thermograph at 10 cm and 20 cm depths.

***In vitro* culture of *Trichoderma* spp.**

Trichoderma spp that was isolated from solarized soil and was found to be effective in dual cultures was grown either on corn flour-perlite mixture (15 g perlite:8 g of corn flour:40 ml of ddH₂O) or wheat bran-peat mixture (1:1 v/v plus 40% w/w ddH₂O) (Wilson *et al.*, 1988; Kleifeld & Chet, 1992).

The isolate was cultured monthly on PDA medium to determine its vitality, and conidial density (conidia/ml) was measured.

Application of commercial preparation of the antagonist

In an experiment carried out in Adanalıoglu-Icel in 1997, *T. harzianum* containing commercial prepare, Trichoderma-2000 (Mycontrol Ltd-Israel) was applied to solarized plots (8x38m) at 30th September 1997. Five litres of prepare was used for total of 600 cucumber plants in 6 repeats. Glasshouse experiments were conducted with 3 characters that were solarization plus commercial *Trichoderma* prepare, solarization alone and control. Each character was repeated six times using randomised block design.

In another experiment done in Yenitaskent-içel in 1998, the commercial prepare named as Promot containing *T. koningii* and *T. harzianum* (JH Biotech Inc. USA) was applied to solarized plots (3x5.5 m).

Antagonist suspension of 1.5 kg/ha was applied to solarized soil by spraying and was then mixed to 10x15 cm depth before planting. Following, the cucumber seedlings were dipped in an antagonist suspension of 10g/L and planted on 18th December 1998.

Evaluation of effects of applications on disease emergence and crop yield

Cucumber plants were observed within 15 days intervals following planting. In an experiment conducted in Adanalıoglu, cucumber plants that were planted as first crop was uprooted at the end of 3 months and evaluation was done by examining vascular tissue. Percentage of disease emergence and effect of applications were determined. Variance analysis were conducted to determine the differences among applications. Yield data's were taken during 3 months (8th March and 10th June, 1999) in experiment conducted in Yenita□kent.

Result and discussion

Solarization applications

Soil temperatures reached during solarization are given in Table 1.

Table 1. Soil temperatures recorded in solarized and non-solarized plots in 1998 (°C).

Soil depth (°C)	Max. mean temperature of soil during solarization (°C)	Max. mean temperature of non-solarized soil (°C)
10	43.2	34.0
20	37.4	32.2

Soil temperature values reached during solarization was similar to those studies reported by Porter & Merriman (1985) and Yücel (1985). Soil temperatures at 10 cm and 20 cm were found to be 34 °C and 32.2 °C in control plots, respectively. The reason for this could be that the top level of the soil become dry and because of having not enough humidity, solar energy may not be transmitted. One of the factor effecting success of solarization is that the right humidity level of the soil. This increases heat transmission and makes resistant forms of microorganism susceptible to heat (Katan, 1996).

***In vitro* culturing of *Trichoderma* spp**

The *Trichoderma* isolate cultured in corn flour-perlite and wheat bran-peat media's continued its vitality by end of 12 months after culturing. The conidial densities were 2.5×10^8 and 5.4×10^8 conidia/ml in corn flour-perlite and wheat bran-peat media's, respectively. These values were found to be similar to those commercial preparate obtained from Israel, in which the conidial density was 10^8 conidia/ml.

Evaluation of the effect of applications on disease emergence and crop yield

Percentages of disease ratio, effect and yield ratios among different applications in 1997 (Adanalioglu-içel) and 1998 (Yenitaskent-içel) are given in Tables 2 and 3.

Table 2. Effect of different applications on cucumber root rot (*R. solani* and *F. solani*) in Adanalioglu-içel.

Characters	Mean disease (%)	Mean effect (%)
Solarization	27.3 a	42.0
Solarization + commercial preparate	24.3 a	48.2
Control	47.8 b	-

As it is shown in Table 2, differences among applications ($p=0.05$) was found to be not significant. Percentage effect of applications were 42.0% and 48.2% in plots treated with solarization and solarization plus *T. harzianum*, respectively.

The differences among treatments was found to be not-significant ($p=0.05$). Percentage effect of applications were 55.3%, 62.8% and 58.8% in solarization alone, solarization plus commercial preparate and solarization plus in vitro culture of *Trichoderma* spp, respectively.

Yield values evaluated from 10 plants in kg's were 43.9, 49.4, 46.7 and 41.2 in treatments of solarization alone, solarization plus commercial preparate and control plots, respectively.

Similar studies carried out by using *T. harzianum* to control *R. solani*, disease emergencies on 11 weeks after planting were 0.87 and 1.12 in plots containing *T. harzianum* and in controls, respectively. Same soil was used to plant cotton and the disease emergencies on 29 days after planting was 23% and 44% in plot containing *T. harzianum* and in controls, respectively (Elad *et al.* . 1980 a).

In the present study, in cucumber, planted as first crop, disease emergence after 3 months of planting were 27.3%, 24.3% and 47.8% in plots had solarization alone, solarization plus commercial preparation of antagonist and controls, respectively.

Table 3. Effect of different applications on cucumber root rot (*R. solani* and *F. solani*) in Adanaliloglu-ıçel

Characters	Mean disease (%)	Mean effect (%)	Fruit yield (kg/10plant)
Solarization	33.3 a	55.3	43.9
Solarization+ commercial preparate	27.7 a	62.8	49.4
Sol.+artificial culture	30.7 a	58.8	46.7
Control	74.5 b	-	41.2

In the case of cucumber cultivation planted as single crop, disease emergencies on 6 months after planting were 33.3%, 27.7%, 30.7% and 74.5% in plots had been treated with solarization alone, solarization plus commercial preparation of antagonist, *Trichoderma* alone and control, respectively. Hadar *et al.* (1979) found that disease emergence caused by *R. solani* in eggplants reduced to 13% from 40% when low dosage of PCNB (1-2 g/kg) in combination with *Trichoderma* preparate (2 g/kg). was used. When *T. harzianum* was applied alone the ratio was 26%.

Effect of soil solarization depends on many factors such as climate, soil structure, resistance or susceptibility of pathogen in soil and population density. It is known that *Fusarium* spp and *R. solani* are resistant to heat. In solarized soil population of microorganisms is reduced and reinfesting such soil with antagonist organisms may result in resistance. However in the case of using such microorganisms it is important to consider the organisms compatibility with soil biomass, high adaptation capability with environmental conditions and its vitality in rhizosphere.

In this study, both commercial preparate and in vitro culture applications of antagonists increased solarization effectiveness. In the case of integrated pest control, combination of biocontrol agent with soil solarization and disinfection using low doses of fumigants have two main advantages. Firstly environmental damage caused by standard doses is reduced and secondly disease control and yield are increased (Sivan & Chet, 1993).

As a result of this study while the main effect was obtained with solarization applications, it was also found that antagonist application following solarization resulted increase in disease control.

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Soil solarization: an alternative control method for *Pseudomonas syringae* pv. *tomato*

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Abstract : Effect of soil solarization on bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* were investigated as alternative control method to the using of methyl bromide in nurseries. Soil solarization was found effective until 30 cm depth of soil, disease incidence and disease severity were reduced as 77 and 24 %, respectively by soil solarization.

Key words : bacterial speck, soil solarization, tomato, nursery,

Introduction

Bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (PST), is an important disease in many tomato growing areas of the world (Good & Sasser, 1980), Turkey included (Cinar, 1977). Bacterial speck of tomato has been a threat to transplant industry, fresh market and processing tomatoes. The ability of PST for surviving epiphytically on symptomless tomato transplants, weeds and non-host plants, on seeds or in seeds and on infested tomato debris. Because it is ubiquitous organism, control of the bacterial speck is difficult by cultural practices. And also, chemical control with copper compounds alone or in combination with dithiocarbamates is not completely satisfactory (Conlin & McCarter, 1983).

Increased social pressure to restrict the use of chemical fumigants has created the development of alternative control methods for soil-borne inoculum. Methyl bromide has been an important soil fumigant for containerized nursery production and greenhouses. With the probability of losing methyl bromide as a soil disinfectant within the next few years, researchers need to carry out development and testing of usable alternatives (DeVay & Steplaton, 1997). Soil solarization is an alternative control method to the using of methyl bromide in disinfestation of the soils. In this technique, thin transparent plastic film is stretched over moist soil during the hottest period of the year and left for several weeks to absorb solar energy. Soil solarization has been successful in controlling soil borne fungal and bacterial pathogens in the Eastern Mediterranean Region of Turkey since 1985 (Erkilic *et al.*, 1995; Pala, 1985).

The objectives of this research were to determine :

- (i) effect of soil solarization on soil temperatures
- (ii) effect of soil solarization on disease incidence
- (iii) effect of soil solarization on survival of PST on tomato plant debris in soil
- (iv) effect of soil solarization on total microorganism populations in soil

A preliminary study was conducted in small containers and preliminary report of this work has already been published in "Second International Conference on Soil Solarization and Integrated Management of Soil-borne Pest" in Aleppo, Syria.

Materials and methods

Effect of soil solarization on soil temperatures

The experiments were conducted in 72 m² (18x4 m) field at faculty farm of Cukurova University in Adana, Turkey in 1995 and 1996. The field was inoculated by infested plant debris and bacterial suspension (8.0×10^7 cfu/ml) by rifampicin-resistant strain of PST. The research area was irrigated prior to solarization and half part of field were covered with transparent polyethylene sheets (30 µm thick) and keep moist with water between first of July and first of September as solarization periods. Other part of the field was not covered as control. Soil temperatures were recorded in solarized and control plots with soil thermometers during 8 weeks (Aysan *et al.*, 1997)

Effect of soil solarization on disease incidence

After soil solarization, tomato seeds (surface sterilized by 1 % NaOCl in 3 min) were sown in solarized and control plots in the following February and covered by polyethylene sheets, forming a low tunnel in order to provide a high relative humidity. After seed germination, infected seedlings and lesions on cotyledons leaves were counted and recorded before the lesions coalesced. Disease incidence was carried out by calculating the ratio of infected plants to the total plants and disease severity was evaluated by 0-3 scale (Aysan, 1999). To confirm PST, the bacteria on lesions were isolated and identified by biochemical and pathogenicity tests.

Effect of soil solarization on survival of PST on tomato plant debris in soil

Before soil solarization, approximately 50 g infected tomato plants by a pathogenic, rifampicin resistant strain of PST (4.5×10^7 cfu/plant debris) were placed in nylon mesh bags and positioned at the soil surface (0 depth) and at depths of 5, 15, 30 and 40 cm for 3 replicates in solarization and control plots, separately (Aysan & Cinar, 1998). After soil solarization, all bags were removed from both depths and isolated on King's medium B supplemented with rifampicin (100 mg/ml).

Effect of soil solarization on total microorganism populations in soil

For soil isolation, 10 g soil samples were taken from 0-40 cm depths before, just after and 6 months after solarization and diluted by serial 10 fold dilutions and spread on King's medium B. The colonies on petri dishes were counted 2-3 days later.

Results and discussion

Effect of soil solarization on soil temperatures

Maximum soil temperatures recorded at depths 5, 10, 15, 30, and 40 cm were 51.6, 51.3, 40.5, 36.7, 36.0 °C in solarized soil respectively; these temperatures were 3.2 - 14.1 °C higher than the corresponding non-solarized plot (Table 1).

Thermal death of various microorganisms in vitro have shown that at or above 50 °C. At temperature of 37-50 °C eradication or marked reduction in population occur within 2-5 weeks (Stapleton & DeVay, 1986). The onset of death of PST in vitro is at 35 °C and total kill has started at 48 °C (Devash *et al.*, 1980). According to data of our study, PST population was started to reduce at 15-30 cm and started to kill at 5-10 cm of solarized soil. Thermal sensitivity of bacteria depends upon the nature of the individual taxa. PST which is a fluorescent *Pseudomonas* sensitive to heat (Stapleton & DeVay, 1986).

Table 1. Maximum and minimum soil temperatures ($^{\circ}\text{C}$) at 5, 10, 15, 30 and 40 cm depths in solarized and non-solarized soils during the solarization period

Depths	Solarized soil ($^{\circ}\text{C}$)			Non-solarized soil ($^{\circ}\text{C}$)			Difference		
	Max	Min	Average	Max	Min	Average	Max	Min	Average
5 cm	51.6	40.1	44.5	41.7	36.7	36.7	9.9	9.4	7.8
10 cm	51.3	36.2	41.2	37.2	32.1	34.6	14.10	4.1	6.6
15 cm	40.5	33.1	38.1	35.6	30.4	32.4	4.9	2.7	5.7
30 cm	36.7	30.0	34.6	33.5	29.2	30.9	3.2	0.8	3.7
40 cm	36.0	29.4	33.8	31.0	27.0	29.9	5.0	2.4	3.9

Effect of soil solarization on disease incidence

When tomato seeds were sown in the research area at February, symptoms on cotyledons were observed after one month. The rate of symptom development on seedlings in solarized plot was significantly less than that on seedlings in non-solarized plot as seen in Table 2. In 1995, totally 21 seedlings out of 929 in solarized soil, and 116 seedlings out of 1084 in solarized soil were recorded as infected plants. In 1996, 21 seedlings out of 825 in non solarized soil were infected. Disease incidence and severity were reduced 75, 79 % and 22, 25 % by soil solarization in 1995 and 1996, respectively. These results indicate a disease reduction of 77 % by solarization according to the results of two years.

Table 2. Effect of soil solarization on disease incidence and disease severity

Years	Treatments	Infected plant/ Total plant	Disease Incidence (%)	Disease Severity (%)
1995	Soil Solarization	116/1084	10.70	66.66
	Control	21/929	2.26	53.33
Effect (%)			78.87	19.99
1996	Soil Solarization	102/825	12.00	75.66
	Control	20/758	3.00	56.66
Effect (%)			75.00	25.11

Effect of soil solarization on survival of PST on tomato plant debris in soil

The population of the pathogen on plant debris in nylon mesh bags that placed in solarized soil at 0, 5, 15 and 30 cm depth was eliminated by solarization. And, at 40 cm depth of solarized soil, population of bacteria was decreased as 10^2 cfu/g plant debris. However the bacteria was survived in non solarized soil during 8 weeks as seen Table 3. After isolation from tomato debris, PST was covered on King's medium B with rifampicin because of high population. The populations of PST could not counted, but bacterium was identified as PST by biochemical and pathogenicity tests.

Soil solarization in Eastern Mediterranean Region of Turkey is sufficient to restrict or eliminate PST populations on the infested plant debris. Infested tomato debris is adequate for initial of epidemics of bacterial speck as an primary inoculum in greenhouses and nurseries in our region (Aysan & Cinar, 1998).

Effect of soil solarization on total microorganism populations in soil

The populations of total microorganisms including *Bacillus* spp., *Pseudomonas* spp., and *Actinomycetes* spp., at 0-40 cm depth of solarized soil were not changed just after and 6 months later. It was found that there was no negative effect of solarization on the bacteria in soil.

Table 3. PST populations on tomato debris in solarized and control plots

Depths (cm)	Soil Solarization 1995 (cfu/g plant debris)	Control-1995 (cfu/g plant debris)	Soil Solarization 1996 (cfu/g plant debris)	Control-1996 (cfu/g plant debris)
0	-	-	-	-
5	-	-	-	-
15	-	+ *	-	+ *
30	-	+ *	-	+ *
40	100	+ *	+ *	+ *

(* : bacteria not counted but bacterium identified)

It was evaluated that soil solarization was very effective in controlling bacterial speck disease in nurseries. This effect may be due to the increase of the soil temperatures. The advantages of this method are simple, relatively cheap, alternative to methyl bromide and non toxic to antagonistic microorganisms.

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The threat of insect-transmitted viruses to vegetable production in Morocco

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Abstract: The vast movement of people and agricultural products between Morocco and distant geographical regions has created unprecedented opportunities for introducing plant viruses and the insects that carry them (vectors) to new areas. To illustrate these points we report two recent introductions of viruses that are threatening vegetable production in Morocco. A devastating plant virus, tomato yellow leaf curl geminivirus was introduced to Morocco. The strain of the insect (*Bemisia tabaci*) that transmits was confirmed to be also present in Morocco. Many farmers had to destroy completely their tomato crops in open fields or in greenhouses when virus incidence had reached high levels. Exact estimates are not available, but no doubt that direct and indirect damage of TYLCV will cost the Moroccan tomato industry millions of dollars. Small farmers who depended on tomato production for much of their living are devastated and some tomato production areas mainly open field tomatoes became economically depressed. The unprecedented movement of agricultural products and propagates (such as seeds, bulbs, tubers and transplants) in late of the twenty century, provides tremendous opportunities for plant viruses and the insects that spread them (vectors) to move between widely separated geographic regions. Clearly, an awareness of these threats and the actions needed to avoid introducing these exotic pests is critical to all involved in horticulture. Morocco must adapt its regulations, human and material resources, to the increasing exchange of horticultural products in order to protect its horticulture.

Introduction

Despite technological advances leading to tremendous yield increases for many crops, modern horticultural production continues to face pest threats, among them insects, plant pathogens, and weeds. Often growers are faced with multiple pests, which exacerbates crop damage and complicates management strategies. A dramatic example of how two types of pests "team up" to cause major problems for Moroccan vegetable production is the case of plant viruses and their insect vectors.

The vast movement of people and agricultural products between Morocco and distant geographical regions has created unprecedented opportunities for introducing plant viruses and the insects that carry them (vectors) to new areas. Outbreaks of new viruses may be favoured in these new areas by crop susceptibility, the presence of particular weeds and certain agricultural practices. In some cases, conditions may be ideal for the emergence of altered plant viruses and new virus/vector relationships. This may result in the appearance of insect-transmitted plant viruses in crops and regions where they have not been seen before. Because plant viruses and their insect vectors are intimately linked, the status of both must be considered in formulating strategies to prevent or slow their introduction, as well as to manage any invasions. To illustrate these points we report two recent introductions of viruses that are threatening vegetable production in Morocco.

First, a devastating plant virus, tomato yellow leaf curl geminivirus was introduced to

Morocco probably from neighbouring Spain in 1998. The strain of the insect (*Bemisia tabaci*) that transmits was confirmed to be also present in Morocco. Second, Cucurbit yellow stunting disorder virus, CYSDV, was identified in Morocco in 1999. The latter is also exclusively transmitted by *B. tabaci* and has caused significant economic damage to cucurbits in Spain. CYSDV is spreading rapidly since the fall 1999 in the Souss Valley, probably *because* of whiteflies resistance to insecticide. This is the first official report of the occurrence of those two viruses in Morocco.

Plant viruses are foreign genetic elements that take over a plant's cellular machinery and use it to produce their own genetic material and proteins. This has dire consequences for the plant, altering many normal plant functions such as cell division and photosynthesis, thus resulting in disease. While symptoms of virus infection vary depending on the particular virus and plant, they generally involve stunted and distorted growth, changes in leaf coloration and shape, and poor flower and/or fruit yield and quality. Most plant viruses are not stable outside of living plant cells and so need a way to spread from infected plants to uninfected plants. Plant-feeding insects are ideal agents for spreading plant viruses because of their high rates of reproduction, dispersal abilities, and obligate use of particular plants as food. Indeed, intricate relationships have co-evolved among vectors, viruses and the plant hosts they share, resulting sometimes in remarkably efficient spread of viruses from plant to plant.

Moroccan farmers are already faced with a plethora of insect-transmitted viruses that can limit crop production, and for which there are few effective management options. While the viral diseases Moroccan growers currently face can be severe, the new introduced viruses could constitute a challenge for certain production systems.

It is important to mention that the development of IPM in greenhouse crops in Morocco has been surprisingly quick during the last five years. In some situations, farmers were able to manage tomato with only one insecticide application instead of 8 during the whole crop cycle. Unfortunately, the introduction of TYLCV has boosted the number of insecticide applications never registered before (28 per crop cycle). No doubt, that new introductions of pests and diseases will complicate further the implementation of pest management in Morocco.

In this article, we will review the current status of epidemics of TYLCV transmitted by a strain of whitefly that was also introduced to Morocco in the past.

Tomato yellow leaf curl virus

In contrast to many diseases caused by whitefly-transmitted geminiviruses, tomato yellow leaf curl is not a new problem. This disease was first described in Israel around 1940, and was associated with outbreaks of the sweet-potato whitefly. Tomato yellow leaf curl was subsequently described in parts of the Middle East, Africa, Southern Europe, India and Asia. The causal agent of tomato yellow leaf curl was identified as a geminivirus in 1988 and it is now known that a number of distinct geminiviruses cause tomato yellow leaf curl-like symptoms in different parts of the world.

Tomato yellow leaf curl geminivirus (TYLCV) may be the most damaging virus to tomatoes because plants infected at an early stage of growth fail to produce fruit. While TYLCV-infected tomato plants produce abundant flowers, the virus causes flowers to fall off (abscise) long before fruit setting. In the field, yield losses can reach 100%. TYLCV gets its name from the fact that infected leaves are yellow except for the veins, show strong upward curling of the outer leaf margins and are small and crumpled. Plants infected at a young age are severely stunted and new shoots grow straight up, resulting in small, compact plants with bushy tops that are commonly referred to as "bonsai" plants. In general, the younger the plant at time of infection, the more severe the stunting. The relative age at which a plant was

infected can be determined because only the new growth will show symptoms.

TYLCV is a geminivirus (Geminiviridae family), a group of plant viruses characterised by twinned icosahedral virus particles, and from which the family name is derived (the Latin word *geminus* means twin). Geminiviruses can be carried by either leafhoppers or whiteflies, and whitefly-transmitted geminiviruses are one of the major emerging groups of plant viruses world wide.

Status of TYLCV epidemics in Morocco

There are at least two possible ways new whitefly-transmitted geminiviruses could enter/appear in Morocco: (1) introduction via infected plant material or viruliferous whiteflies on plant material and (2) possible introduction via passive flights of viruliferous whiteflies from Spain. Spain is only 14 km from the Moroccan borders and it is possible for whiteflies to be carried by winds for longer distances. However, the most likely way TYLCV entered Morocco is via infected plant material. Consequently, measures were taken to prevent movement of plant material, especially tomato transplants, from Spain to Morocco.

The vector (*B. tabaci*) of TYLCV, had previously been reported in the Mediterranean region and had probably been also inadvertently introduced in the past into Morocco from Europe, through infested plantlets (such as strawberries or tomato transplants).

The first identification of TYLCV in Morocco was in the region of Casablanca. In the fall of 1998, unusual virus-like symptoms were noticed on tomato plants in some tomato farms, and during the 1998 growing season entire seed beds and fields were lost. Unfortunately, measures taken were not sufficient to limit its spread to other tomato production areas. Consequently, the virus has spread from these regions, possibly via long-distance movement of viruliferous whiteflies (those carrying the virus), to neighbouring regions and then to the Souss Valley. The latter was the last major tomato production area to be hit by this dangerous virus disease. The Souss Valley had a flourishing tomato industry. Almost 4000 ha of tomatoes were grown to supply the local market but also the continuously growing export market.

TYLCV has become officially established in the Souss Valley of Morocco since spring 1999. By the fall of 1999, virus incidence exceeded 50% in most field grown tomatoes and more than 10% of greenhouse tomatoes in Morocco. Many farmers had to destroy completely their tomato crops in open fields or in greenhouses when virus incidence had reached high levels. Exact estimates are not available, but no doubt that direct and indirect damage of TYLCV will cost the Moroccan tomato industry millions of dollars. Never, in the past, we have witnessed such a high number of acreage of tomatoes destroyed at an advanced stage of the crop. Small farmers who depended on tomato production for much of their living are devastated and some tomato production areas mainly open field tomatoes became economically depressed.

Until the winter 1999, TYLCV has not been reported in other tomato production areas such as the Tadla perimeter in central Morocco or the Saiss Valley in north of Morocco. However, whether the virus will become established in Central Morocco or in the Saiss remains to be seen. These regions have a very harsh winter which eliminate the whiteflies and make it impossible for farmers to grow tomatoes in the winter, especially in the open field. This will provide a period long enough to break down virus reservoir in these regions.

TYLCV is clearly a potential threat to tomato industries in the South and along the Atlantic coast, particularly because one of its vectors (the silverleaf whitefly) is well established in these areas. Thus, the introduction of TYLCV into these areas with tomato production and high whitefly numbers resulted in the rapid spread and establishment of the

virus. Now that it is established, TYLCV would probably be difficult if not impossible to eradicate from the Souss Valley, due to the diversity of crops grown and the wide plant host range of the silverleaf whitefly. These factors would make it difficult to implement solutions that have helped elsewhere, such as the host-free period in some parts of the World.

Management of TYLCV and its vector

Once the identity and distribution of the virus infecting the tomatoes was known, the Plant Protection Service of the Moroccan Ministry of Agriculture formulated a strategy to limit the risks of TYLCV epidemics. Some legislative measures were also taken, these include, prohibiting whitefly host crops (such as tomato, melon and pepper transplants) from being imported from Europe.

Now, that the virus is established in Morocco, it becomes imperative to develop TYLCV-resistant tomato varieties. This is being approached by commercial seed companies, using conventional breeding as well as biotechnology methods. Any resistant variety to be used whether in open field or greenhouse should bear the production and post harvest qualities of the commonly used cultivars.

Insect screening can prevent unwanted guests from entering the greenhouse. Researchers in the USA have found that different pests require different size screens for exclusion. Interestingly, some aphids require a finer mesh screen than do some species of whiteflies, even though the aphids are larger. This may be due to difference in wing placement or behaviour. Installing a screened chamber "SAS" at the entrance to the greenhouse is one way to prevent whiteflies from gaining access to the crops.

Now that the vector TYLCV are established in all important vegetable production areas, all is not lost, we have three choices for control programs.

Application of chemicals (including those that are not harmful to beneficials)

Traditional approach to whitefly control has been to use insecticides in the greenhouse. These insecticides are classified in the organophosphate, carbamate, synthetic pyrethroid, or chlorinated hydrocarbon classes. Some chemicals kill the insect by affecting its digestive system. These stomach pesticides are useless against whiteflies, since the pests' proboscis can penetrate through droplets of stomach poison into the plant tissue below without ingesting any of the chemical. A contact pesticide kills the insect by disrupting its outer membranes or affecting its internal systems on absorption. The spray must come in contact with the insect to be effective, so thorough coverage is necessary. Currently, the pesticides most commonly used for whitefly control are Endosulfan, methomyl, Pyriproxyfen, Thiamethoxam and Novaluron. Some growers prefer to mix two chemicals with two modes of action in the same application. The reasoning is sound, because if the population includes whiteflies resistant to one of the chemicals, the other will kill them.

Using biological controls

Predators and parasitoids have been registered for use in biological control in Morocco since 1992. Koppert Biological Systems and its partner in Morocco (CASEM) have been behind much of the efforts done to get permission for importation and use of natural enemies in biological control in Morocco. Nowadays, Koppert continues its development efforts in Morocco in collaboration with IAV Hassan II, on providing an IPM package adapted to local conditions. A new company Biobest Maroc has been established in Morocco and natural enemies are produced locally. No doubt that this latest development will contribute to a wider application of biological control in Morocco.

The chief natural enemy of the greenhouse whitefly (*Trialeurodes vaporariorum*) is a small parasitic wasp called *Encarsia formosa*. Several companies supply *Encarsia*. The insects

are shipped in the pupal stage, because it is the most tolerant to environmental extremes and therefore most able to survive the rigors of shipping. The companies provide instructions for placing them among the plants.

The sweet potato whitefly is a much newer greenhouse foe, and fewer proven control measures are available. *Encarsia* has limited efficacy on this species. However, *Eretmocerus* sp. gives an excellent control of this pest. The parasitic wasp is being used successfully in Morocco for the control of *B. tabaci*.

In an integrated approach, some predators and parasites will be very sensitive to chemicals farmers may apply to control other pests and diseases. Koppert Biological Systems provides the farmers with information on the side effects of traditional pesticides on natural enemies. Small quantities of traditional pesticides that persist on foliage can be lethal to emerging predators and parasites. For certain chemicals, even sprays applied months before release can be toxic.

Integrated pest management (IPM)

Integrated pest management (IPM) is a culmination of the non-chemical, traditional pesticides, and biological control methods. It is a carefully blended combination of scouting/monitoring, traditional chemicals, and biological controls. While most of the individual components of an IPM program are not new, the increasing popularity of combining them into an integrated production and protection (IPP) systems is new. IPP is in the news in Morocco and for very good reasons. Regulations regarding pesticide residues in exported agricultural products are likely to increase in number and severity. Worker protection regulations, economics, the registration process, public opinion, and most of all advances in technology have brought this systems approach to the forefront. The basic components of an IPP program are:

- 1- Sanitation: Start with a clean greenhouse. Clean up weeds, nonessential plants, and crop debris.
- 2- Scouting/Monitoring: Begin a weekly check of your greenhouses to track pest problems. Use yellow sticky traps to monitor populations. Inspect the underside of foliage and use indicator plants to estimate the efficacy of your control program.
- 3- Record keeping: Maintain detailed records of pest counts, treatment information, pest fact sheets, and pest control recommendations.
- 4- Exclusion: Use screens or barriers to prevent insects from entering from outdoors or spreading among different areas in the greenhouse.
- 5- Inspection: Isolate and treat any incoming crops to prevent infestations.
- 6- Chemical: Traditional pesticides continue to be an important part of an IPM program. Biological. The use of parasitic and predator insects and beneficial fungi will continue to play a major role in IPM in the future.

Conclusion

The unprecedented movement of people, agricultural products and propagates (such as seeds, bulbs, tubers and transplants) in late of the twenty century, provides tremendous opportunities for plant viruses and the insects that spread them (vectors) to move between widely separated geographic regions. Clearly, an awareness of these threats and the actions needed to avoid introducing these exotic pests is critical to all involved in horticulture. Morocco must adapt its regulations, human and material resources, to the increasing exchange of horticultural products in order to protect its horticulture.

IPP should be seen by our farmers simply as a combination of the best management practices and common sense. It is a systems approach, not an isolated treatment. The options

today are many and there will be more. New chemistry and refinements in biological controls present a wide range of pest control strategies. Our challenge is to find the ones that work best in our local conditions.

Section IV
Whiteflies

Section IV
Aleurodes

Monitoring for the whitefly *Bemisia tabaci* Genn. on Ribatejo and Oeste region of Portugal

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Abstract: During Spring-Autumn, 1999, a monitoring scheme was set up for the whitefly *Bemisia tabaci* Genn. on the Ribatejo e Oeste region of Portugal. Entomological material was collected in 85 greenhouses, on tomato crop in the most representative production areas of this region. Sampled material was reared in laboratory up to adult whitefly emergence. Identification of the specimens was made based on the differential morphological characteristics or, in case of doubt, by diafanization and microscopic observation of relevant characters. Although the existence of *B. tabaci*, since 1992 in Algarve region, *Trialeurodes vaporariorum* West. was the only whitefly species found on this survey. No infected plant with TYLCV was found, also.

Key words: *Bemisia tabaci*, whiteflies, TYLCV, Portugal

Introduction

Whiteflies are considered key pests on horticultural protected crops in Portugal. *Trialeurodes vaporariorum* West. is already reported all over the country. In 1992 a new whitefly species was detected, the cotton or tobacco whitefly, *Bemisia tabaci* Genn., on the Algarve region (in the South of Portugal). This whitefly is an efficient vector of TYLCV (Tomato Yellow Leaf Curl Virus) which is responsible for serious losses on tomato. For that reason growers are deeply worried by its presence (Guimarães & Louro, 1995; Louro *et al*, 1996).

A monitoring scheme for *B. tabaci* was set up in the Ribatejo and Oeste region in order to evaluate its presence in this region related with the risk of TYLCV infection.

Material and methods

In 85 greenhouses of the seven more important boroughs with horticultural protected crops (Caldas da Rainha, Mafra, Montijo, Palmela, Peniche, Sintra, Torres Vedras) one to ten leaves/leaflets infested with "pupae" and nymphs of whitefly species were collected. In the same greenhouses, surveys for TYLCV infected plants were done. The fieldwork was set up to cover all the horticultural important areas through out the region.

Leaves were chosen depending on the presence of 3rd instar nymphs or "pupae" of whiteflies on the underside. In laboratory the leaves/leaflets were positioned up-side down inside laboratory boxes (22 x 14 x 6 cm³) with ventilation holes covered with a net. In one of the box sides a glass transparent tube was inserted horizontally. The boxes were involved with aluminium paper to avoid light in their interior. Adults were collected from the glass tubes as they emerge and put on Eppendorf tubes on 70% ethanol. Identification was made at

stereoscopic microscope using the differential morphological characteristics (Hill, 1969; Gill, 1993). For identification of doubtful specimens microscopic slides were made after decontamination.

Results and discussion

In the survey only *T. vaporariorum* was found in the Ribatejo and Oeste region. However, there was a notice of sporadic presence of *B. tabaci* at open field crops in this region.

Plants infected by TYLCV have not been found in this region by the official services from the Ministry of Agricultural (Louro, pers. com.). Plants suspected to be virotic were collected from the field in this survey but were not infected with TYLCV. However, since some growers in the Oeste region buy their plants in nurseries in the Algarve region, theoretically, it could be possible to find TYLCV without the presence of *B. tabaci*.

Bemisia tabaci found the favourable conditions of development on Algarve greenhouses, mainly during the period between May and October. This region has a typical Mediterranean climate. The range of developmental temperatures for this species is very broad, being most favourable between 16 and 24°C. Temperatures below 9°C and above 40°C are lethal to the insect. The optimum RH for the insect development is between 30-60%. Rain, extreme temperatures and low humidity can impair oviposition (Lacasa & Contreras, 1995). The increased importance of protected crops has encouraged the insect presence in less favourable climates, where it has been displacing the greenhouse whitefly *T. vaporariorum* West. (Picó *et al.*, 1996; Arsénio *et al.*, 1998), at least temporarily.

It is possible that the temperature and humidity conditions in greenhouses of the Oeste region are not the most favourable to the proliferation of the *B. tabaci*. In this area the climate is dependent on the Atlantic Ocean influence (temperatures are not so high and relative humidity is higher and mists are frequent during a great part of the morning and at the end of the afternoon). All the other factors, like greenhouses' structure, crops and cultural practices are similar to the Algarve greenhouse horticultural production. As consequence the different patterns shown by the *B. tabaci* outbreaks in both regions can not be explained by agronomic practices but most rely on other factors, like weather.

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Les parasitoïdes indigènes du biotype «B» de *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). Que peut-on en attendre pour le contrôle biologique de ce ravageur?

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Résumé: Quatre espèces de parasitoïdes indigènes du biotype «B» de *Bemisia tabaci* (Gennadius), *Encarsia formosa* Gahan, *Encarsia pergandiella* Howard, *Encarsia hispida* De Santis et *Eretmocerus mundus* Mercet ont été testées pour le contrôle biologique de ce ravageur en culture de tomate. *Encarsia formosa* donne un parasitisme moyen s'il est élevé au préalable sur *B. tabaci*. *E. pergandiella* fournit un parasitisme voisin de 45%, limité dès la seconde génération par un hyperparasitisme important. *E. hispida* présente un parasitisme intéressant sans manifestation d'hyperparasitisme. *E. mundus* montre une efficacité élevée.

Mots-clés: aleurode, *Bemisia tabaci*, parasitoïde, *Encarsia* sp., *Eretmocerus* sp., lutte biologique, culture protégée, tomate.

Introduction

Bemisia tabaci a été signalé en France il y a une dizaine d'années (Della Giustina *et al.*, 1989). Plus récemment, Guirao *et al.*, (1997a) ont mis en évidence que l'on était seulement en présence, au niveau du littoral azuréen, du biotype «B» de *B. tabaci*, déjà reconnu pour être responsable outre-atlantique de dégâts sévères sur cultures maraîchères et florales alors que les biotypes «B» et «Q» étaient présents en Espagne (Guirao *et al.*, 1997b). Une prospection minutieuse des plantes maraîchères et florales cultivées sous serre et des plantes adventices susceptibles de permettre le développement larvaire du biotype «B» de *B. tabaci*, a été réalisée sur plusieurs saisons et dans de très nombreux biotopes du littoral méditerranéen. Les résultats obtenus à partir de ces prélèvements exhaustifs ont montré (Onillon *et al.*, 1994) que ce ravageur abritait une entomofaune parasitaire diversifiée comprenant des espèces du genre *Encarsia* (*E. pergandiella* et *E. hispida*) et *Eretmocerus* (*E. mundus*). Cette faune parasitaire n'est absolument pas comparable à la très grande richesse et à l'extrême diversité observée dans l'archipel canarien (Hernández-Suárez, 1999) où pas moins de 11 espèces de parasitoïdes appartenant aux genres *Encarsia* et *Eretmocerus* ont été signalées. Il était donc indispensable de juger, dans des conditions expérimentales comparables, les aptitudes de ces espèces indigènes à assurer un contrôle biologique satisfaisant de ce ravageur. Cette étape était indispensable à franchir avant toute éventuelle introduction de parasitoïdes d'origine exogène.

Matériel et méthodes

Origine de la souche de *B. tabaci*

La souche de *B. tabaci*, qui a servi pour les contaminations artificielles sous serre, provient d'un élevage permanent sur chou, une génération préalable sur tomate ayant été réalisée avant les contaminations artificielles sous serre.

Origine des souches de parasitoïdes

Les souches de parasitoïdes, issues des prélèvements de terrain, sont maintenues en élevage en thermopériode sur le biotype «B» de *B. tabaci* élevé sur chou.

Structure physique de la serre

Les serres, dans lesquelles ont été réalisées les expérimentations sont en verre dépoli sur châssis métallique d'une surface unitaire de 120 m². La température minimale obtenue par chauffage central est de 15°C et la régulation thermique des températures estivales est obtenue par un système automatique d'ouvrants se déclenchant au dessus d'un seuil de 25°C.

Mode de culture

La culture est de type hors-sol. Des sacs (Motex-Orsol) d'une contenance unitaire de 35 litres sont percés de 3 trous afin d'y disposer les plants. Quatre rangées de 17 sacs sont disposés dans la serre et permettent la culture de 200 plants. La solution nutritive est distribuée à chaque plant de tomate à l'aide de goutteurs. La fréquence d'irrigation est de 3 à 5 arrosages journaliers en fonction de l'état d'avancement de la culture.

Le végétal

La plante hôte utilisée est la tomate. La variété de tomate utilisée a été conforme à l'évolution des variétés utilisées chez les professionnels, la variété «Cobra» en 1992, et la variété «Vitex» en 1993, 1994 et 1995.

Contamination par le ravageur

Les contaminations, artificielles, sont faites à partir d'adultes venant d'émerger. Les lâchers sont réalisés 4 à 10 jours après la plantation, dès que l'état sanitaire des plants a été vérifié et qu'ils aient été vus démunis de tout stade larvaire d'aleurode. La proportion de femelles dans la population d'adultes de *B. tabaci* lâchés est comprise entre 0,70 et 0,75 dans tous les essais.

Lâcher des parasitoïdes

Le nombre d'individus à libérer a été déterminé initialement comme étant celui préconisé pour les lâchers d'*Encarsia formosa* dans le contrôle de *Trialeurodes vaporariorum* (3 adultes du parasitoïde pour 1 adulte du ravageur observé). Toutefois, le manque de disponibilité en auxiliaires n'a pas permis de réaliser cet objectif de façon systématique et les doses inférieures à 3/1 ont pu être observées avec l'emploi d'*E. mundus* en 1994 (doses de 0,7/1) et en 1995 (1/1). Tous les lâchers de parasitoïdes ont été réalisés avec des adultes.

Nature des observations

De 4 à 6 comptages ont été faits sur des plants de tomate tirés au sort en fonction de la densité imaginaire initiale de *B. tabaci*. Les feuilles de tomate ont été ramenées au laboratoire et toutes les larves de *Bemisia* présentes sur ont été disséquées sous la loupe binoculaire pour connaître la composition de la population parasitaire à l'intérieur des stades larvaires de l'aleurode et l'existence et la nature d'un hyperparasitisme éventuel.

Les modalités des expérimentations sont précisées dans le tableau 1.

Résultats

Expérimentation avec E. formosa.

Lors des deux premiers échantillonnages des 23 et 30 avril, le taux maximum de parasitisme des larves de la première génération de *B. tabaci* par *E. formosa* n'a pas excédé 22 % sur un effectif de plus de 800 larves disséquées pour chaque comptage. La composition de la population parasitaire est alors essentiellement au stade prénymphe et nymphe. La seconde génération du parasitoïde, sur la seconde génération de *B. tabaci*, se caractérise au cours du mois de mai par une légère augmentation du taux de parasitisme (39,6% le 7 mai) qui plafonne cependant aux environs de 42 %, pourcentage obtenu sur la dissection hebdomadaire de 800 larves de *B. tabaci* le 14 mai. Tout au long du mois de mai, la composition de la population embryonnaire et larvaire a été suivie sans mortalité préimaginale anormale du parasitoïde.

Tableau 1. Modalités des expérimentations sur le contrôle biologique du biotype «B» de *B. tabaci* sur tomate au moyen de parasitoïdes des genres *Encarsia* et *Eretmocerus*.

Caractéristiques des essais d'emploi des parasitoïdes	ENCARSIA			ERETMOCERUS	
	<i>formosa</i>	<i>pergandiella</i>	<i>hispida</i>	<i>mundus</i>	<i>mundus</i>
Saison (printemps)	1992	1993	1993	1994	1995
Date de l'infestation	19 / 03	29 / 03	29 / 03	28 / 03	11 / 05
En adultes de <i>B. tabaci</i>					
Nombre d'adultes de <i>B. tabaci</i> lâchés par plant	5	10	10	5	5
Rapport parasitoïde (adultes) / Bemisia (adultes)	3 / 1	3 / 1	1 / 1	0,7 / 1	1 / 1
Délai lâcher Bemisia - Lâcher parasitoïdes (jours)	14 jours	21 jours	18 jours	20 jours	20 jours
Stade de <i>B. tabaci</i> lors Des lâchers de parasitoïdes	L ₂	L ₂ - L ₃	L ₃ - L ₄	L ₂ - L ₃	L ₂ - L ₃
Nombre de lâchers d'adultes de parasitoïdes	1	3	2	6	6
Fréquence d'introduction Des parasitoïdes (jours)	1	8	8	3	3
Taux de parasitisme en fin de culture	41 %	46 %	43 %	41 %	85 %
Présence de dégâts sur la culture	OUI	NON	NON	NON	NON

Expérimentation avec E. pergandiella

Deux dénombrements exhaustifs ont été réalisés. Lors du premier prélèvement de la mi-mai (12/5) correspondant à la première génération du parasitoïde, et qui a porté sur la dissection de plus de 1.530 larves de *B. tabaci*, la population du ravageur est principalement au stade L₄ (55 %). Les larves mortes de *B. tabaci* représentent un pourcentage assez élevé (29,1%). Le taux

de parasitisme moyen des larves de l'aleurode par *E. pergandiella* est de l'ordre de 48,3 %. La composition de la population parasitaire est représentée presque exclusivement par des vieilles larves du parasitoïde (42,6 %) et des nymphes (46,8 %). Les premiers trous de sortie qui ont été notés (6 %) attestent bien que l'on est bien en présence de la totalité de la première génération du parasitoïde. Quelques nymphes mortes d'*E. pergandiella* sont également observées (3,5 %).

Lors du second dénombrement du début juin (2/6) correspondant à la deuxième génération du parasitoïde, et qui a porté sur la dissection de plus de 1.500 larves de l'aleurode, la composition de la population de l'hôte et du parasitoïde a été évaluée en tenant compte de la position des feuilles sur le plant de tomate. Sur la strate haute du plant de tomate, la structure de la population de l'aleurode est dominée par les L₄ et les nymphes (50,3 %). Le taux de parasitisme est de 36,6 %. Sur les 219 larves parasitées, le parasitoïde est au stade larve (73,1 %) et nymphe (21,5 %). L'hyperparasitisme est faible, de l'ordre de 1,8 %. Sur la partie basse du plant, la structure de la population est différente avec un fort pourcentage de L₄ et nymphes (81,0 %). Le taux de parasitisme est de 46 %. Sur les 437 larves parasitées, le parasitoïde est au stade larve (22,4 %) et nymphe (30,4 %). Un hyperparasitisme important d'*E. pergandiella* sur sa propre espèce est observé de l'ordre de 32 %.

Expérimentation avec *E. hispida*

Deux prélèvements ont été réalisés. Le premier à la mi-mai sur la première génération de l'aleurode. Le pourcentage de larves saines de *B. tabaci* est de 40,1% et le pourcentage de larves mortes de l'aleurode est de 24%. Le taux de parasitisme total est de 35,9 % et la population parasitaire est presque exclusivement (97,7 %) composée de larves et de nymphes du parasitoïde. Un faible pourcentage de nymphes mortes d'*E. hispida* (2,1%) est également noté. Sur un total de 507 larves parasitées de *B. tabaci*, un seul trou de sortie a été observé, attestant que l'échantillonnage a bien été focalisé sur la première génération du parasitoïde.

Le second prélèvement a été réalisé à la mi-juin, sur la seconde génération de l'aleurode. Le pourcentage de larves saines de *B. tabaci* est de 46% et le pourcentage de larves mortes de l'aleurode avoisine 11,1%. Le taux de parasitisme total est de 42,9 %, peu supérieur à celui obtenu lors du premier comptage. Un très faible pourcentage de nymphes mortes d'*E. hispida* (0,1%) est également noté. La structure de la population parasitaire montre qu'*E. hispida* est présent au niveau des larves (64 %) et des nymphes (32,4 %). Une faible implantation d'*E. pergandiella* est notée 28 larves sur un total de 1.216 larves parasitées.

Expérimentation avec *E. mundus*

Deux expérimentations ont été réalisées pour suivre l'action d'*E. mundus*. La première au printemps 1994, avec un ratio de 0,7/1 (tab.1). Le premier prélèvement, début mai, donne un taux de parasitisme de 28 % sur la première génération de *B. tabaci* avec une population parasitaire composée de jeunes larves (36 %), de prénymphe (30 %) et de nymphes (17 %). Seuls les œufs du parasitoïde sont trouvés sous les larves des trois premiers stades larvaires de *B. tabaci*. Le second prélèvement, réalisé début juin, montre un taux de parasitisme supérieur à 41 % avec une population parasitaire composée essentiellement d'œufs (46 %).

La seconde expérimentation réalisée au printemps 1995 avec un ratio de 1/1, a donné des résultats beaucoup plus spectaculaires. Sur la première génération de l'aleurode le taux de parasitisme observé est voisin de 40%, donc comparable à celui observé l'année antérieure au bout de 2 générations. Sur la seconde génération de *B. tabaci* début juillet, un taux de parasitisme de 85 % est observé.

Discussion

Les travaux, qui avaient porté sur l'efficacité des parasitoïdes indigènes utilisés dans le contrôle biologique de *T. vaporariorum* en zone de climat méditerranéen avec *E. formosa* (Argyriou, 1987; Nucifora et Vacante, 1989; Morosetti *et al.*, 1994) ou *E. tricolor* (Onillon *et al.*, 1989; Avilla *et al.*, 1990) avaient montré que l'on pouvait s'attendre à une croissance régulière du taux de parasitisme dans le temps en fonction de la dose initiale apportée en auxiliaires et du nombre de générations du ravageur. Les observations faites sur l'évolution du parasitisme des larves du biotype «B» de *B. tabaci*, par les parasitoïdes du genre *Encarsia*, montrent qu'un schéma, différent de celui élaboré entre *T. vaporariorum* et *E. formosa*, émerge dans la dynamique des relations entre *B. tabaci* et les parasitoïdes indigènes.

La faible efficacité d'*E. formosa*, même élevée sur *B. tabaci*, montre que cette espèce ne peut être utilisée avec sécurité contre les populations de ce ravageur. Il a été mis en évidence que ce parasitoïde manifestait une nette préférence pour les larves de *T. vaporariorum* (Boisclair *et al.*, 1990). Son utilisation a pu même être envisagée dans le contrôle biologique de *B. tabaci* par suite de son aptitude à parasiter et à prédateur les jeunes stades larvaires (Enkegaard, 1993). Mais cette utilisation impose, notamment sur poinsettia (Benuzzi *et al.*, 1990), un très grand nombre d'auxiliaires. La réalité du parasitisme d'*E. formosa* en présence de populations isolées de *B. tabaci* ne doit pas occulter les modifications comportementales que pourrait engendrer, chez ce parasitoïde, la rencontre avec des populations de *T. vaporariorum* et de *B. tabaci* en mélange.

L'on ne peut hélas revenir sur l'acclimatation d'*E. pergandiella* au niveau du Bassin Méditerranéen. Cette espèce est désormais présente partout et intervient soit en parasitoïde primaire, ce qui est intéressant, soit en parasitoïde secondaire sur sa propre espèce ou sur d'autres espèces indigènes, ce qui peut présenter un frein à l'extension et à la crédibilité du contrôle biologique des aleurodes sous serre. *E. pergandiella* peut présenter un certain intérêt sur le plan appliqué dans la mesure où cette espèce peut également parasiter *T. vaporariorum* (Maignet, 1995) mais son aptitude à parasiter les derniers stades préimaginaux de sa propre espèce ou d'autres espèces du genre *Encarsia* la rend très délicate d'emploi dès lors que l'on ne peut maîtriser au préalable la sex-ratio de l'espèce. Mais c'est surtout sur un plan fondamental que cette espèce peut constituer un modèle intéressant pour l'étude des relations hôte - parasitoïde primaire - parasitoïde primaire. Les travaux récents de Hunter (1989a et 1989b) sur la réceptivité de larves parasitées pour la production de mâles en hyperparasites et de Videllet *et al.* (1997) sur la prédation effectuée par les femelles d'*E. pergandiella* devraient permettre de lever une partie du voile sur la possibilité de son utilisation en lutte biologique ou sur les moyens de réduire son action destructrice sur l'entomofaune parasitaire.

La souche monoparentale d'*E. hispida* montre une certaine efficacité dans le contrôle biologique des populations de *B. tabaci*, due en grande partie aux particularités de son potentiel biotique (Maignet & Onillon, 1997). En effet le taux de parasitisme observé, avec 43% est voisin de ceux notés avec *E. formosa* (41%) et *E. pergandiella* (46%), mais avec un apport initial de l'entomophage trois fois plus faible. Il est très vraisemblable qu'un apport de 3/1 apporté sur les jeunes stades larvaires aurait singulièrement augmenté le pourcentage de parasitisme final. Il est bon de noter que ce phénomène d'hyperparasitisme observé chez *E. pergandiella* peut très bien se retrouver chez *E. transvena* et chez la souche biparentale d'*E. hispida* (Hernandez-Suarez, 1999).

Eretmocerus mundus a montré une excellente efficacité à la dose de 1/1, surtout lorsque les températures estivales sont élevées, ce qui est confirmé par les observations de terrain où des populations très abondantes de larves de *B. tabaci* parasitées par *E. mundus* ont été observées au mois de juillet et août sur Lantana.

Conclusions

Des quatre espèces de parasitoïdes testées en serre de tomate sur les populations du biotype «B» de *B. tabaci*, seules deux espèces montrent un intérêt certain pour une utilisation ultérieure: *E. hispida*, espèce parthénogénétique qui peut également intervenir sur *T. vaporariorum* et *E. mundus*, espèce bisexuée, très fortement inféodée à *B. tabaci*.

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Native parasitoids of the B-biotype of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). What can be expected from them for the biological control of the pest?

Abstract: Four native species of parasitoids of the biotype « B »-*Bemisia tabaci*, (Gennadius), *Encarsia formosa* Gahan, *Encarsia pergandiella* Howard, *Encarsia hispida* De Santis, and *Eretmocerus mundus* Mercet, were experimented for biological control of this pest, in tomato cultivation. A mean parasitism by *E. formosa* was obtained if it was previously reared on *B. tabaci*. *E. pergandiella* reached near of 45% parasitism which started to be restrained by an heavy hyperparasitism at its second generation. *Encarsia hispida* showed an interesting parasitism (more than 40%) and without hyperparasitism. *Eretmocerus mundus* was highly effective.

Key words: whitefly, *Bemisia tabaci*, parasitoid, *Encarsia sp.*, *Eretmocerus sp.*, biological control, protected crop, tomato.

E. hispida - pantheon.!

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

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Abstract: A comparative evaluation of *Encarsia formosa* Gahan and *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) as biological control agents of *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) was carried out on tomato under greenhouse in southern Italy (Campania). In a structure of 1330 m² (35 x 38 m), divided in 5 tunnels tomato plants cv Arletta were transplanted on 23.2.1999. Parasitoids were released weekly from 19.5.99 (harvest beginning) to 16.6.99 in 3 tunnels (tunnel 1, 3 and 5), each one alternated with a control tunnel (tunnel 2 and 4) where no parasitoids were released. Three combinations of releasing were performed: *E. pergandiella* (tunnel 1), *E. pergandiella* + *E. formosa* (tunnel 3) and *E. formosa* (tunnel 5). Pupal case density increased progressively into the five tunnels from 0.02 to 5.86 pupal cases/leaflet throughout the harvest time without reaching the intervention threshold. No significant damage on fruits caused by honeydew and sooty mould was observed. The percentage of parasitization increased regularly in all tunnels. On 7.7.99, after 1.5 months from the first release, it ranged from 17.73% to 36.75%, doubling after another 21 days (on 28.7.99) when the parasitization ranged from 57.06% to 70.63%. Between the two species inoculated, *E. pergandiella* was the most abundant and spread in each tunnel. *E. formosa* substantially spread into the tunnels where it was inoculated, suffering the competition from *E. pergandiella*. Into the tunnel 5, *E. formosa* reached the 49.63% of total parasitization on 28.7.99. Into the tunnel 3, where the parasitoids were released together, the competition performed by *E. pergandiella* was strong; *E. formosa* activity decreased from 13.04% (30.6.99) to 0% (28.7.99) of the total parasitization. Results achieved in the present work point out that, at least in greenhouse areas of southern Italy where natural populations of *E. pergandiella* are widespread, an effective control of the whitefly can be obtained reinforcing the activity of natural *E. pergandiella* populations by means of multiple inoculations of the same parasitoid as soon as whiteflies infest the crop. The dominance of *E. pergandiella* on *E. formosa* seems linked not only to the male hyperparasitism but also to a better adaptation of the first species to the climatic and growing conditions.

Keywords: aphelinid, parasitoid, whitefly.

Introduction

The biological control of *Trialeurodes vaporariorum* (Westwood) and in the last years also of the *Bemisia tabaci* (Gennadius) complex, has been attempted in several countries under greenhouses by using mainly *Encarsia formosa* Gahan (van Lenteren *et al.*, 1997; Hoddle *et al.*, 1998). Not taking into account the economy of the method, successful applications of *T.*

vaporariorum biocontrol has been achieved in Central and North Europe, but it failed for several reasons to become widely used in several countries, mainly Mediterraneans. In some cases the failure has been attributed to the competition with *Encarsia pergandiella* Howard (Gabarra *et al.*, 1999). This species was introduced into Europe (Italy) (Viggiani & Mazzone, 1980a) firstly to reinforce in open field the uneffective natural control of *T. vaporariorum* by the indigenous parasitoids and at the same time to evaluate an alternative to the unsatisfactory performances of *E. formosa* under the plastic greenhouses in the conditions of Central and South Italy (Viggiani & Mazzone, 1980b; Giorgini & Viggiani, 1994). *E. pergandiella* became in the subsequent years more and more widely distributed in Italy (Mazzone & Viggiani, 1985) and in other Mediterranean countries (Onillon *et al.*, 1994; Gabarra *et al.*, 1999). The parasitoid is also naturally occurring under greenhouses (Giorgini & Viggiani, 1994; Gabarra *et al.*, 1999), where it can play a role in the biocontrol of *T. vaporariorum*.

In the present paper multiple inoculations of *E. pergandiella*, *E. formosa* and a combination of both parasitoids have been evaluated.

Material and methods

The trial was carried out in a commercial plastic cold greenhouse of 1330 m² (35 x 38 m) divided into 5 tunnels, located in Eboli (SA) - Campania - Italy, an agricultural area where *E. pergandiella* is permanently established. 738 tomato plants cv Arletta were transplanted on 23.2.1999 in each tunnel. Tunnels consisted of 6 rows spaced on 110 cm, with plants spaced 30 cm apart within rows. Harvest started on second ten days of May and finished on first week of July, but the crop was maintained until the end of July to allow data recording on parasitoid - *T. vaporariorum* populations.

Sampling started on 17.3.99 (beginning of blossom time) and finished on 28.7.99. Up to 5.5.99, when first adults of *T. vaporariorum* appeared, 3 plants/row were observed weekly. From 12.5.99 the sampling plan was changed as follows: 35 plants/tunnel (about 5% of the plants) were randomly chosen and 3 leaflets/plant (respectively at the bottom, at the middle and at the top of the plant, excluding the first 4 leaves on the top) were sampled. Until 30.6.99 the total number of both apparently (no dissection was made) healthy and parasitized *T. vaporariorum* pupal cases (living + empty) were counted on each leaflet. From 7.7.99, the counts of unparasitized and parasitized pupal cases (living + empty), because of the high density, were made only onto 2 areas of 1 cm² on each leaflet. These two sampling areas were chosen respectively where the pupal case density was at minimum and at maximum rate. *T. vaporariorum* density (pupal case/cm²) was calculated as average of the pupal cases counted into the two areas with different density. From 12.5.99 samplings were carried out every 7-14 days.

Parasitoids were released starting from 19.5.99 (harvest beginning), when the minimum temperature rose around 15°C (Fig. 1), in three tunnels (tunnel 1, 3 and 5), each one alternated with a control tunnel (tunnel 2 and 4) where no parasitoids were released. Three combinations of releasing were performed: *E. pergandiella* (tunnel 1), *E. pergandiella* + *E. formosa* (tunnel 3) and *E. formosa* (tunnel 5) (Tab. 1). 10-20 groups of 10 females each were distributed randomly on the cultivation at each release. Females of *E. pergandiella* were mated before the distribution and released together males at a ratio of 2:1. Totally 3 females/m² (1.08 females/plant) were inoculated. At the same time natural occurrence of *E. pergandiella* was observed in adjacent greenhouses.

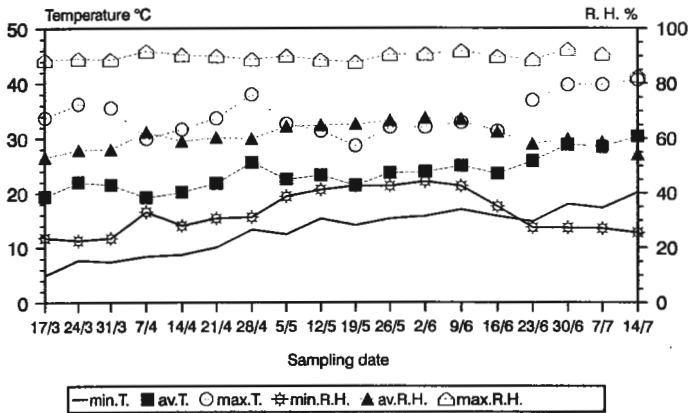


Fig. 1. Weekly averages of temperature and relative humidity (R. H.)

Parasitoids were obtained from a culture permanently kept at the Centro di Studio CNR sulle Tecniche di Lotta Biologica in Portici on *T. vaporariorum* infesting bean plants cv Borlotto nano at $25 \pm 1^\circ\text{C}$, 60-70% R.H. and 14 L/10 D photoperiod.

Tab. 1. Number of parasitoids released in the experimental tunnels.

Date	Tunnel 1 <i>E. pergandiella</i>	Tunnel 3 <i>E. formosa</i> + <i>E. pergandiella</i>	Tunnel 5 <i>E. formosa</i>
19.5	100	50+50	100
26.5	100	50+50	100
2.6	200	100+100	200
9.6	200	100+100	200
16.6	200	100+100	200

No pesticides were sprayed during the cultivation. Pollination was made by means of bumble bees.

Temperature and R.H. were recorded under greenhouse during the trial (Fig. 1).

Statistical analysis was performed on data recorded on 7.7.99 (harvest end) and on 28.7.99 (end of the sampling plan). Pupal cases/cm² were analyzed by ANOVA and the means separated by planned comparisons. The following comparisons were planned: control tunnels 2+4 vs. inoculated tunnels 1+3+5, tunnel 3 vs. tunnels 1+5 and tunnel 1 vs. tunnel 5. Because the comparisons planned on data recorded on 7.7.99 were not orthogonal the experimentwise error rate was adjusted by the Dunn-Sidak method. Percentages of parasitizations (parasitized pupal cases/healthy and parasitized pupal cases) were analyzed by R x C test of independence using G-test (Sokal & Rohlf, 1981).

Results and discussion

First adults of *T. vaporariorum* appeared on the crop rather late, at the beginning of May. From 26.5.99 pupal case density increased progressively into the five tunnels varying from 0.02 to 5.86 pupal cases/leaflet throughout the harvest time (Tab. 2) without reaching the intervention threshold of 7 nymphs/cm² (Hussey *et al.*, 1969) or of 3-4 healthy pupal cases/cm² (Giorgini & Viggiani, 1994). No significant damage on fruits caused by honeydew and sooty mould was observed.

Tab. 2. *T. vaporariorum* pupal case density. Standard deviation between brackets.

Sampling date	Tunnel 1	Tunnel 2	Tunnel 3	Tunnel 4	Tunnel 5
Pupal cases/leaflet					
12/5	0	0	0	0	0
26/5	0.12 (0.63)	0.02 (0.20)	0	0.04 (0.24)	0.09 (0.40)
9/6	0.41 (1.40)	0.44 (1.94)	0.03 (0.29)	0.15 (0.57)	0.67 (2.23)
16/6	1.48 (2.89)	1.4 (5.35)	0.33 (1.05)	0.07 (0.29)	0.58 (1.76)
30/6	4.63 (5.26)	2.72 (4.87)	3.49 (6.01)	4.10 (5.40)	5.86 (6.31)
Pupal cases/cm²					
7/7	2.60 (4.04)	1.58 (3.03)	1.81 (2.77)	1.85 (2.64)	3.66 (4.43)
14/7	3.05 (3.94)	1.76 (2.50)	2.22 (3.16)	3.27 (3.70)	3.13 (3.24)
21/7	2.39 (2.60)	4.49 (4.36)	2.22 (2.46)	4.06 (3.10)	5.30 (4.24)
28/7	3.65 (2.74)	5.55 (3.64)	3.33 (2.73)	5.63 (4.18)	4.52 (2.30)

At the harvest end (7.7.99) pupal case density (Tab. 2) was significantly different among all treatments ($F=6.25$, $Df=4$, $P=0.0001$) and lower in the control tunnels vs inoculated tunnels ($F=9.758$, $Df=1$, $P<0.01$). Parasitized pupal cases/cm² (Tab. 3) were significantly different among all treatments ($F=3.02$, $Df=4$, $P=0.0178$) and lower in the control tunnels vs inoculated tunnels ($F=10.856$, $Df=1$, $P<0.01$). Among inoculated tunnels, pupal case density was significantly lower in the tunnel 3 vs tunnels 1+5 ($F=10.065$, $Df=1$, $P<0.01$) but it was not different between tunnel 1 vs tunnel 5 ($F=4.925$, $Df=1$, $P>0.05$). Parasitized pupal cases/cm² did not differ statistically between inoculated tunnels (tunnel 3 vs tunnel 1+5: $F=0.528$, $Df=1$, $P>0.05$; tunnel 1 vs tunnel 5: $F=0.669$, $Df=1$, $P>0.05$).

Tab. 3. *T. vaporariorum* parasitized pupal case density. Standard deviation between brackets.

Sampling date	Tunnel 1	Tunnel 2	Tunnel 3	Tunnel 4	Tunnel 5
Parasitized pupal cases/leaflet					
12/5	0	0	0	0	0
26/5	0	0	0	0	0
9/6	0	0.10 (0.65)	0	0	0.03 (0.22)
16/6	0.14 (1.10)	0.06 (0.41)	0.05 (0.35)	0	0.05 (0.32)
30/6	0.27 (0.71)	0.21 (0.69)	0.22 (0.78)	0.22 (0.64)	0.36 (1.10)
Parasitized pupal cases/cm²					
7/7	0.71 (1.64)	0.36 (0.97)	0.67 (1.29)	0.33 (0.95)	0.86 (1.60)
14/7	1.26 (1.72)	0.71 (1.41)	0.89 (1.58)	1.48 (2.14)	1.29 (1.65)
21/7	1.03 (1.50)	1.77 (2.07)	0.83 (1.33)	1.19 (1.44)	1.49 (2.03)
28/7	2.57 (1.95)	3.43 (2.57)	2.06 (1.61)	3.21 (2.39)	3.18 (1.96)

At the end of the sampling period (28.7.99), the pupal case density (Tab. 2) was significantly different among all treatments ($F=11.46$, $Df=4$, $P=0$) and higher in the control tunnels vs inoculated tunnels ($F=38.054$, $Df=1$, $P<0.001$). Parasitized pupal case density (Tab. 3) was significantly different among all treatments ($F=7.40$, $Df=4$, $P=0$) and higher in the control tunnels vs inoculated tunnels ($F=14.448$, $Df=1$, $P<0.001$). Among inoculated tunnels, pupal case density was significantly lower in the tunnel 3 vs tunnels 1+5 ($F=3.894$, $Df=1$, $P<0.05$) and in the tunnel 1 vs tunnel 5 ($F=3.960$, $Df=1$, $P<0.05$). Parasitized pupal cases/cm² was significantly lower in the tunnel 3 vs tunnel 1+5 ($F=10.294$, $Df=1$, $P<0.01$) and in the tunnel 1 vs tunnel 5 ($F=4.325$, $Df=1$, $P<0.05$).

The percentage of parasitization increased regularly in all tunnels (Tab. 4). On 7.7.99, after 1.5 months from the first release, it ranged from 17.73% to 36.75%, doubling after another 21 days (on 28.7.99) when the parasitization ranged from 57.06% to 70.63%.

On 7.7.99 the percentage of parasitization resulted significantly different among all tunnels ($G_H = 38.397$, $Df = 4$, $p<0.001$) and that recorded in the tunnel 3 was the highest. No significant difference was recorded between tunnel 1 vs tunnel 5. Data collected on 28.7.99 confirmed the significance of differences among all tunnels ($G_H = 32.989$, $Df = 4$, $p<0.001$), but the parasitization was higher into the tunnel 1 and 5 (Tab. 5).

Tab. 4. Data on apparent parasitization of *T. vaporariorum* by *E. formosa* and *E. pergandiella*. % PAR = percentage of pupal cases parasitized; % EP = percentage of parasitization caused by *E. pergandiella*; % EF = percentage of parasitization caused by *E. formosa*.

Sampling date	Tunnel 1			Tunnel 2			Tunnel 3			Tunnel 4			Tunnel 5		
	% PAR	% EP	% EF	% PAR	% EP	% EF	% PAR	% EP	% EF	% PAR	% EP	% EF	% PAR	% EP	% EF
12/5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26/5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9/6	0	0	0	23.91	45.45	54.55	0	0	0	0	0	0	2.86	0	100
16/6	3.68	100	0	4.08	100	0	14.29	100	0	0	0	0	8.20	100	0
30/6	5.97	100	0	7.69	100	0	6.41	86.96	13.04	5.33	100	0	6.18	28.95	71.05
7/7	27.22	100	0	23.60	100	0	36.75	95.35	4.65	17.73	98.59	1.41	23.41	70.20	29.80
14/7	41.24	100	0	40.65	100	0	40.13	98.32	1.68	45.34	98.03	1.97	41.49	60.38	39.62
21/7	43.03	100	0	38.92	100	0	36.91	97.66	2.34	29.46	98.35	1.65	27.83	67.18	32.82
28/7	70.52	100	0	61.80	99.72	0.28	61.71	100	0	57.06	96.38	3.62	70.63	50.37	49.63

Tab. 5. Statistical comparisons between percentages of parasitization recorded in the experimental tunnels. T = tunnel

Comparison	7.7.1999		28.7.1999	
	G _H	P	G _H	P
T1 vs T2	1.387	>0.05	14.99	<0.01
T1 vs T3	9.365	>0.05	12.721	<0.05
T1 vs T4	12.782	<0.05	36.524	<0.001
T1 vs T5	2.446	>0.05	0.002	>0.05
T2 vs T3	14.315	<0.01	0.003	>0.05
T2 vs T4	3.503	>0.05	5.48	>0.05
T2 vs T5	0.005	>0.05	18.221	<0.01
T3 vs T4	33.317	<0.001	3.947	>0.05
T3 vs T5	21.972	<0.001	14.386	<0.01
T4 vs T5	4.628	>0.05	42.087	<0.001

Between the two species, *E. pergandiella* was the most abundant and spread in each tunnel (Tab. 4). *E. formosa* substantially spread into the tunnels where it was inoculated, suffering the competition from *E. pergandiella*. Into the tunnel 5, *E. formosa* reached the 49.63% of total parasitization on 28.7.99. Into the tunnel 3, where the parasitoids were released together, the competition performed by *E. pergandiella* was strong; *E. formosa* activity decreased from 13.04% (30.6.99) to 0% (28.7.99) of the total parasitization.

Parasitic activity of *E. pergandiella* was regular in both inoculated and control tunnels showing high dispersion of the wasp among the tunnels and from outside into the greenhouse.

In conclusion, the obtained results showed that under the experimental conditions the natural occurrence of *E. pergandiella* can cause a rather high percentage of parasitism of *T. vaporariorum*. An improvement of *E. pergandiella* performance can be achieved by means of multiple inoculations of the same parasitoid as soon as whiteflies infest the tomato plants. The dominance of *E. pergandiella* on *E. formosa* appeared not only linked to its heteronomous development, as considered by some authors (Mills & Gutierrez, 1996; van Lenteren *et al.*, 1997), but also determined by other biological characteristics, as a better adaptation to the relatively low (during winter) and high (late spring and summer) temperatures in both greenhouses and open field. In fact, *E. formosa*, which is an exotic species as *E. pergandiella*, despite the several releases made in Italy and other Mediterranean countries, mainly under greenhouses, did not become naturally established as the latter species.

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Biological studies with *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) in Israel.

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Abstract: Biological characteristics of *Eretmocerus mundus* have been studied under laboratory conditions. Females laid between 81.1 and 247.5 eggs each, during a life time of 10-16 days. They also killed hosts through feeding and mutilation. Whitefly mortality amounted to ca. 10% more than natural nymphal mortality. Older parasitoids (4-day old) killed more hosts and oviposited in less hosts than newly emerged or 2-day old females. Sex ratio of the progeny is about 60% females. However, progeny of 10 day old mothers was dominantly male (less than 20% females), possibly due to sperm depletion.

Key words: whiteflies, *Bemisia*, *Eretmocerus*, killing capacity, sex ratio, host feeding..

Introduction

The whitefly species *Bemisia tabaci* and *B. argentifolii* have been plaguing field crop growers for several years in many of the tropical and subtropical regions of the world (Gerling & Mayer 1996). *Eretmocerus mundus* Mercet is among the parasitoids attacking this pest in the Middle Eastern countries (Viggiani 1965, Gerling *et al.*, 1980). Its population dynamics in cotton fields have been studied (Gerling *et al.*, 1980), and its occurrence and life history reported in several countries (Gameel 1969, Hafez *et al.*, 1978, Sharaf & Batta 1985, Tawfik *et al.*, 1978 and Foltyn & Gerling 1985). In reviewing these studies, we found that information about several biological characteristics, which could assist in the exploitation of this parasitoid for whitefly control are lacking.

The lacking information pertains mainly to the host-killing capacity of the parasitoid; a characteristic that takes into account the parasitization, host feeding-induced mortality, and other parasitoid-caused host mortality. As discussed by van Lenteren (1986), it is this total killing capacity that one should consider, rather than only the number of produced progeny when estimating the potential value of a parasitoid as a controlling mechanism for greenhouse attacking hosts. Since both *Bemisia* species are well known as greenhouse pests, we studied host related parasitoid behaviors. We hope that understanding these behaviors can improve parasitoid utilization and serve as a decision tool whether or not to utilize *E. mundus* for whitefly control.

In order to establish the parasitoid's killing capacity, it was first necessary to standardize our experimental conditions in relation to parasitoid fecundity and longevity. This was especially pertinent since different writers produced different results pertaining to these parameters. These, no doubt were related to the rearing and other experimental conditions that differed in the various studies. Consequently, our studies included determination of parasitoid longevity and fecundity. We then continued to study the developmental duration, % emergence the sex ratio produced and the induction of host mortality through parasitoid

activity. Finally, searching behavior on host-infested patches and their relationship with parasitoid aging were examined.

Materials and methods

26°C

Parasitoids were collected as pupae on *Bemisia* hosts in the field, reared out in the laboratory and then kept in culture on whitefly-infested cotton seedlings (Acala SJ-2 variety). Parasitoids were collected from the culture by placing leaves with whitefly pupae in emergence bottles where the emerging adults congregated in a scintillation vial that was placed upon a black plastic bottle (Gerling & Fried 1997). They were collected from the vials within 24 hours from emergence and either placed on hosts (for experienced parasitoids) or used as is (for naive ones). Whitefly-infested cotton plants were obtained by releasing several hundreds of adult whiteflies onto potted cotton seedlings that had 4 true leaves. The whitefly adults were removed 24 hours later and the plants were kept ca. 10 days until a mixture of instar 2 and 3 of whiteflies was present on the leaves. Parasitoids were released upon these plants and the numbers of parasitized individuals was determined by counting the developing *Eretmocerus* pupae.

Determination of daily oviposition is contingent upon the presence of sufficient hosts on the patch that is presented to the female. Moreover, parasitoid confinement may lead to their ovipositing more eggs per patch than they would normally do (van Lenteren 1981). These eggs may be laid either in unparasitized hosts, or in already parasitized hosts, causing experimentally induced superparasitism. Therefore, we started our experiments with preliminary tests to determine the necessary patch size for oviposition tests and also tested whether confinement to one leaf in our experiments caused abnormal parasitism. We exposed single cotton plants each bearing one leaf with whitefly patches of different sizes each, to one ovipositing female. Prior to exposure, each leaf was cut to circular shape around the patch, so that its area could be readily calculated.

Twenty-four hour old parasitoid females were placed on a leaf within a cage for 24hrs after which they were removed to a new plant using a delicate aspirator. This was continued until the parasitoid was either found dead or not found anymore and presumed to have died. The same experiment was also used for the daily oviposition studies. For this purpose, the plants were kept for 11 days after the female had been removed. At that time each leaf was examined for live and dead whitefly nymphs and for whitefly nymphs that contained pupae of *Eretmocerus*. The nymphs were counted, whereas the pupae were cut out with a piece of leaf surrounding them, and placed in a gelatin capsule waiting for adult emergence.

Sex ratio, parasitoid emergence and parasitoid-induced host mortality were all calculated from the same material.

Observations on parasitoid behavior were made in order to recognize the behavioral sequences of parasitoid host-related activity on the leaf. Once these were known, we examined how they varied with female age giving us information on the relative investment of the female in different activities as she grows older. Our observations also informed us as to the need to host feed by *E. mundus* females immediately after emergence from the pupa, and the length of the preoviposition period.

Results

Generally, no correlation was found between the number of hosts on the leaf patch and the number of hosts that were parasitized. The only exception were the very small patch sizes from with 13-43 hosts per leaf, on which the number of nymphs per leaf was directly

correlated with that of those parasitized (Fig. 1). Seven of the females from the longevity and oviposition experiments lived 8 days, five lived more than 10 days and the last two died at the age of 16 days. During that time they laid between 81.1 and 247.5 eggs each. The three longest living females also laid most eggs, but oviposition by the other four females was not correlated with longevity. The overall daily oviposition pattern shows a rather stable level of about 10 eggs per female per day for the first nine days and a sharp decline in the tenth (Fig. 2).

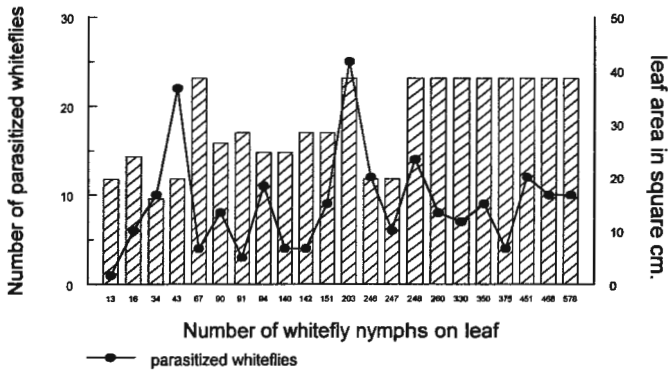


Figure 1. The relationship between the number of parasitized whiteflies per leaf and the number of hosts present

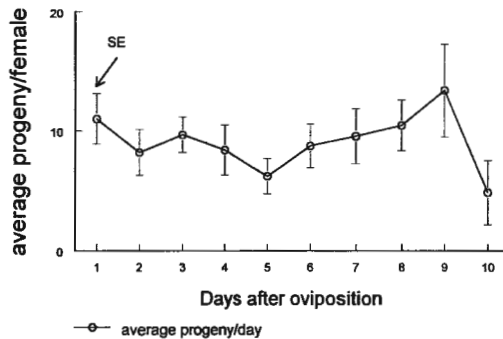


Figure 2. Number of eggs laid each day by females of *Eretmocerus mundus*.

The numbers of hosts attacked was followed and attacks for host feeding were counted separately from those for oviposition. This was done for three ages: Naive, 3-hour old females, 2 days old and 4 day old experiences females. The results (Fig. 3) showed that

oviposition attacks decreased with age whereas host feedings remained the same. Oviposition attacks (insertions of the ovipositor under the host) resulted in one of the three: emergence of an adult whitefly, emergence of a parasitoid, or no emergence at all (death). The proportions of these three events differed for the three parasitoid ages tested (Fig. 4), they were significant only for the differences between events for 4-day old females. However a clear tendency was observed that ovipositor insertion by older females resulted in more deaths and less successful developments of parasitoid. Additional immature mortality (i.e. host killing without parasitoid development) was also measured by comparing the mortality of hosts kept with parasitoids for 24hr with ones that were left free of parasitoids. The total immature mortality in the unparasitized lab population averaged 11.89% (n=38; range 0-37.84%). Mortality in the parasitized population averaged 23.02%. It varied daily and always exceeded that of the control, the difference was significant only on the 1st, 4th, 5th and 10th days (ANOVA p<0.05).

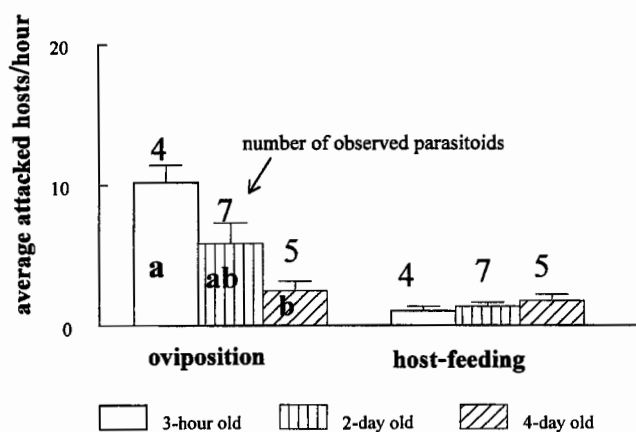


Figure 3. Age dependence of parasitoid attacks(oviposition vs. host feeding) on whitefly nymphs

Success of *Eretmocerus* emergence from the pupae was tested by observing each parasitoid, that had been collected at the pupal stage into a gelatin capsule, until it had either emerged or died. As controls we used identical pupae near which a pen mark was made. They were left on the leaf and allowed to emerge thereupon. The results gave 73.5% emergence from the parasitized material and 83.9% from the control (n=107; SE \pm 2.4, and n=8; SE \pm 9.1 respectively). The difference was not significant (t-test, p=0.18). The sex ratio of emerging parasitoids was examined on a total of 751 progeny that yielded an overall ratio of 62% females (283 males and 468 females). It varied greatly, with some leaves producing only males, and others only females. This pattern repeated itself almost daily and contributed to lack of significance in the overall average sex ratio among the days. However it is noteworthy that 10 day-old females, i.e. the oldest tested, were the only ones with a sex ratio that was lower than 20% females on the average (table 1).

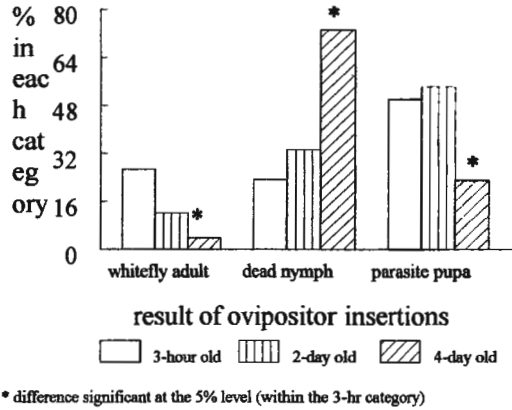


Figure 4. Age dependence of the results of parasitoid attacks on whitefly nymphs.

Developmental duration of the *751Eretmocerus* in our study lasted 15-22 days. The distribution of the emergence time is skewed with most adults emerging between days 15 and 18 (Fig. 5). A life table was prepared using the 7 females that were monitored throughout their lives. It produced the values of: Net reproductive rate $R_0=58.7$; innate capacity of increase $r_m=0.191$ and a generation time $T_c=21.3$.

Table 1. Percent females of *Eretmocerus mundus* progeny, sorted by age of the ovipositing female

Age of female (days)	Number observed	Average %	Standard error	Range
1	13	52.82	8.66	0-100
2	13	51.72	9.40	0-100
3	17	70.11	6.51	0-100
4	14	60.67	8.21	0-100
5	11	66.09	12	0-100
6	10	67.23	10.82	0-100
7	6	61.20	14.02	0-100
8	8	72.28	9.71	11.1-100
9	5	41.74	19.25	0-100
10	4	17.83	15.38	0-63.6

Discussion

Eretmocerus mundus has been considered the most important whitefly controlling agent in the plastic greenhouses of Almeria in Southern Spain (Rodriguez-Rodriguez *et al.*, 1994). It also had been noted already for a long time as a controlling factor of whiteflies in the Mediterranean vegetable growing systems (Avidov 1956). Recently, It has been introduced into several American states to control the pest there (Zolnerowich & Rose 1998). Therefore, information about its biology and host killing capacity is timely and can contribute to practical management of whiteflies in greenhouses.

The killing capacity of a parasitoid includes host mortality through the development of a parasitoid instead of a host. It also includes the killing of hosts through host mutilation and through oviposition an egg that will not develop into a parasitoid adult but result in the death of both host and parasitoid. Finally, host feeding also contributes to host mortality. In our studies all the factors aside from the development of healthy parasitoids, caused about 23% mortality, that was significantly higher than the ca. 12% that were reached when hosts were allowed to develop without a parasitoid attending them. Thus, the killing capacity of *E. mundus* includes about 10% of mortality that is added on direct parasitization.

The effective age of the parasitoids was about 10 days. Older parasitoids had diminished success in producing progeny, and produced has a higher ratio of males to females. The latter may be due to sperm depletion as observed for *Pachcrepoideus videmiae* by Nadel, & Luck (1985). On the other hand, females emerge from the pupa already with a substantial number of ripe eggs (Gerling & Fried 1997). Therefore, these parasitoids, although synovigenic, should be considered for utilization especially during the early part of their adult life.

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Étude comparative de la répartition spatio-temporelle de deux aleurodes des cultures légumières [*Trialeurodes vaporariorum* (Westwood) et *Bemisia tabaci* (Gennadius)] (Homoptera-Aleyrodidae) sur tomate

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Résumé: Les pullulations des aleurodes des cultures légumières (*Trialeurodes vaporariorum* et *Bemisia tabaci*) sont de plus en plus importantes dans toutes les régions à vocation maraîchère. Les travaux réalisés pendant l'année 1998 sur une culture de tomate sous abri serre dans le sub-littoral algérien montre que l'émergence des adultes diffère entre les deux espèces. En effet, l'émergence des adultes de *T. vaporariorum* précède de près de 3 semaines celle de *B. tabaci*. Le nombre de générations est fonction du cycle végétatif de la plante hôte. Il est de 4 générations chevauchantes pendant toute la durée de nos observations. La durée d'une génération est en moyenne de 20 jours pour *T. vaporariorum* et de 14 jours pour celle de *B. tabaci*. Les dénombrements effectués ont montré que la préférence des deux espèces va aux feuilles apicales avec une densité moyenne de 24,24 adultes/dm² et une ponte de 286.4 œufs/dm² pour *T. vaporariorum* et de 2.37 adultes/dm² avec une ponte de 5.69 œufs/dm² pour *B. tabaci*. Le développement des larves et plus particulièrement les larves du 4^{ème} stade se fait en général sur les feuilles basales. Les taux d'occupation des feuilles de tomate par les 2 espèces d'aleurodes sont nettement plus importants pour *T. vaporariorum* que pour *B. tabaci*.

Mots clés: Pullulations, Générations, compétition interspécifique, *Bemisia tabaci*, *Trialeurodes vaporariorum*, maraîchères.

Introduction.

Les aleurodes, ou mouches blanches, des cultures légumières, en occurrence *Bemisia tabaci* (Gennadius) et *Trialeurodes vaporariorum* (Westwood), constituent depuis une dizaine d'années des ravageurs importants en Algérie. Ce sont des homoptères caractérisés par des adultes ailés et des larves fixées sur la face inférieure des feuilles. Les dégâts causés principalement par les larves du 3^{ème} et du 4^{ème} stade résultent de la ponction excessive de la sève, le rejet du miellat qui favorise l'installation d'un champignon saprophyte : le *Fumago* et la transmission des maladies virales.

A partir de 1989, des recherches ont été effectuées sur la répartition géographique (Benmessaoud, 1991), le potentiel biotique et les fluctuations des populations sous abri serre et en plein champ. Les densités des populations enregistrées sont plus importantes pour une espèce que pour une autre et ceci d'une année à une autre.

L'objectif de cette étude est de comparer l'évolution spatio-temporelle des fluctuations des populations, connaître les densités moyennes et le taux d'occupation de ces deux aleurodes sur une même variété végétale.

Matériels et méthodes.

Nos observations ont été effectuées sur une culture de tomate sous abri serre dans la région de Staouéli, dans le Nord-ouest algérien. Cette région se situe dans le sub littoral algérien, caractérisé par un bioclimat sub-humide à hiver doux. La température moyenne annuelle est de 18°C et l'humidité relative moyenne de 66% (1997-1998) (Fig 1).

Le choix de la plante hôte a été dicté par son importance économique et l'incidence potentielle de ces deux aleurodes sur la culture de la tomate en Algérie. La culture a été mise en place le 22 décembre. Les plants de tomate sont répartis sur 18 lignes à raison de 13 plants par ligne soit un total de 234 plants échantillonnés. L'infestation de la culture par les aleurodes, s'est faite naturellement, soit par les mauvaises herbes avoisinantes soit par d'autres cultures déjà en place lors de nos prospections.

Les dénombrements ont débuté après l'opération de palissage des plants permettant ainsi à la culture d'acquérir une vigueur et une meilleure résistance aux infections. Les adultes et les larves du 4^{ème} stade représentent les stades les plus visibles à l'œil nu et sont par conséquent dénombrés sur le plant même en évitant les mouvements brusques qui pourraient les effrayer et les faire fuir. Le dénombrement consiste à recenser les adultes sur jeunes et vieilles feuilles avant de prélever les feuilles pour un autre dénombrement au laboratoire qui sera consacré aux stades fixés.

Les mensurations de la plus grande longueur et la plus grande largeur de chaque feuille examinée nous permettra de calculer la surface foliaire pour pouvoir estimer les densités d'individus par unité de surface, soit le dm².

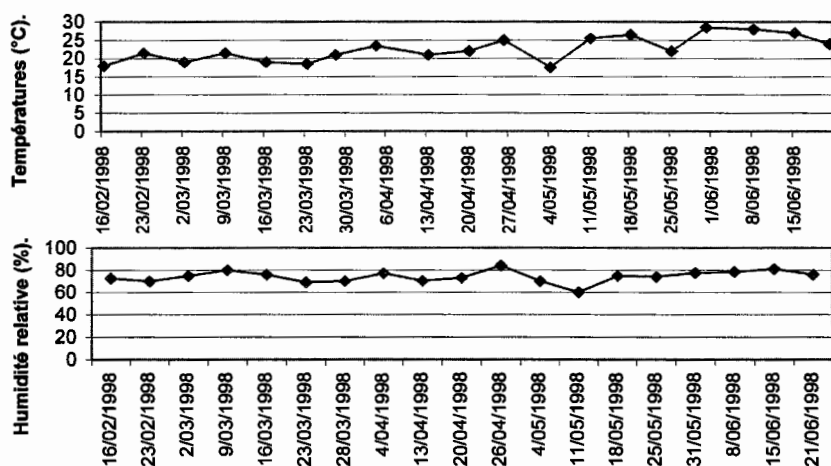


Figure 1. Variations de la température et de l'hygrométrie.

Résultats et discussions

1. Evolution des populations de *B. tabaci* et *T. vaporariorum* sur plant de tomate

Pour déterminer les générations, nous nous sommes basés sur le dernier stade de développement fixé, afin d'éviter les déperditions de comptages des adultes dont la mobilité fait moins ressortir les densités réelles.

Par ailleurs, pendant toute la période de nos observations qui correspondait au cycle végétatif de la plante, nous relevons 4 générations chevauchantes (Fig 2 et 3) avec des maxima représentant les pics des générations qui sont, pour le nombre de larves du 4^{ème} stade/plant de *T. vaporariorum*, de 6.6 le 20 avril, de 12.4 le 11 mai, de 8.2 le 1^{er} juin et de 4.7 le 15 juin. Les maxima enregistrés chez *B. tabaci* sont de 0.3 le 13 avril, de 0.5 le 27 avril, de 0.6 le 11 mai, et de 0.7 le 25 mai.

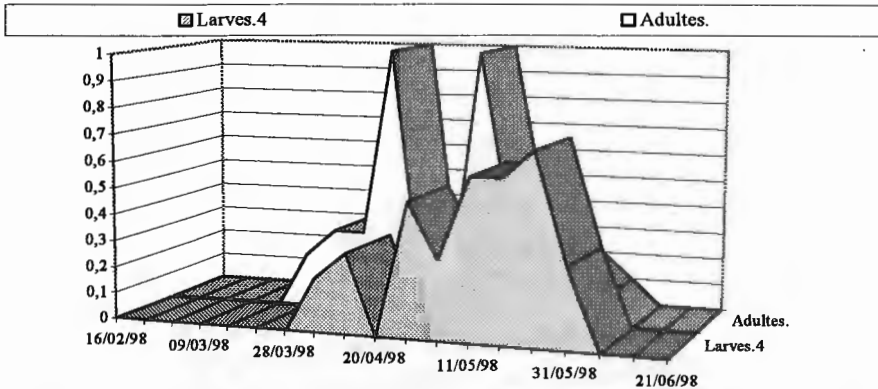


Figure 2. Evolution de la population imaginaire et larvaire du 4^{ème} stade de *B. tabaci* par plant.

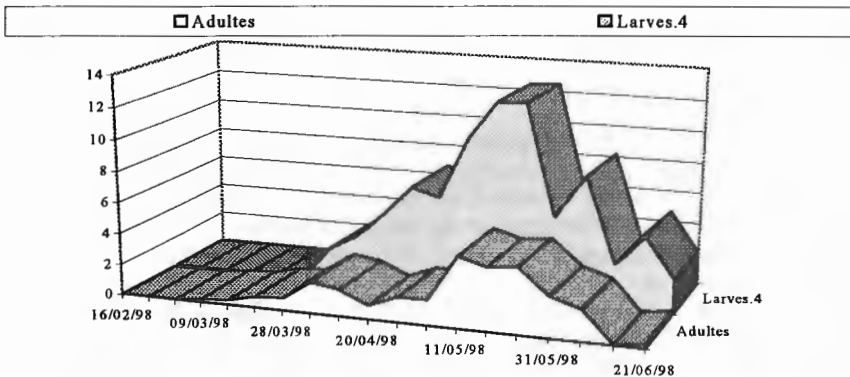


Figure 3. Evolution des populations imaginaires et larvaires du 4^{ème} stade de *T. vaporariorum* par plant.

L'évolution des populations des deux aleurodes montre que la première apparition des adultes, avec une densité moyenne de 0.2 adultes par plant, est celle de *T. vaporariorum* près de trois semaines avant l'apparition des premiers adultes de *B. tabaci*. Cette constatation serait due au fait que *T. vaporariorum* est moins exigeant à l'effet température que *B. tabaci*. En effet, la température optimale de développement pour *B. tabaci* est de 30°C (Hendi *et al.*, 1987) alors que celle de *T. vaporariorum* est comprise entre 17 et 20°C (Lebourgeois, 1985).

2. Evolution des densités moyennes de *B. tabaci* et *T. vaporariorum* sur les feuilles de tomate

Comme chez tous les aleurodes, le cycle évolutif de *B. tabaci* et de *T. vaporariorum* est en étroite relation avec la phénologie de la plante. A partir des comptages hebdomadaires effectués sur les feuilles apicales (J.F) et sur les feuilles basales (V.F), nous avons pu observer à quel niveau de la plante se situaient les différents stades de développement des 2 espèces d'aleurodes. Les adultes suivent la croissance de la plante pour se déplacer sur les feuilles étalées mais pas trop âgées.

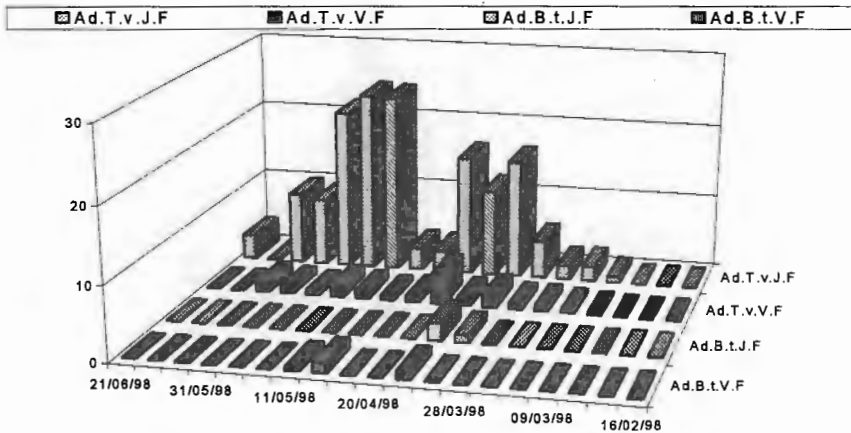


Figure 4. Evolution des populations imaginales de *B. tabaci* et de *T. vaporariorum* suivant l'âge des feuilles.

La figure 4 montre que les densités des adultes de *T. vaporariorum* sont plus importantes sur les jeunes feuilles avec 24.24 adultes/dm² le 18 mai contre 4.03 adultes/dm² le 20 avril sur les vieilles feuilles. La même remarque est relevée pour les adultes de *B. tabaci*, qui présentent des densités de 2.37 adultes/dm² sur les jeunes feuilles et 1.34 adultes/dm² sur les vieilles feuilles. Cette localisation des adultes d'aleurodes, peut s'expliquer par la qualité alimentaire de ces feuilles.

Par ailleurs, bien que les adultes migrent aussi vers les vieilles feuilles qui sont situées à la base du plant, ils préfèrent s'accoupler et pondre sur les jeunes feuilles tendres et turgescentes (Butler et al., 1983). C'est pour cela que les fortes pontes ont été enregistrées sur les jeunes feuilles (Fig.5) avec des densités maximales de 286.4 œuf/dm² pour *T. vaporariorum* et de 5.69 œufs/dm² pour *B. tabaci*.

Pour ce qui est des larves du quatrième stade (prépupe et pupa) leur répartition semble similaire aussi bien sur les jeunes que sur les vieilles feuilles pour *T. vaporariorum*. Ce stade, considéré comme le stade le plus nuisible, par la forte ponction de la sève et sa production de miellat, présente des maxima de 10.64 larves/dm² sur les jeunes feuilles et 10.49 larves/dm² sur les vieilles feuilles (Fig.6).

Par contre pour *B. tabaci*, le quatrième stade larvaire est représenté en densité plus importante sur les jeunes feuilles (1.6 larves/dm²) que sur les vieilles feuilles (0.99 larves/dm²).

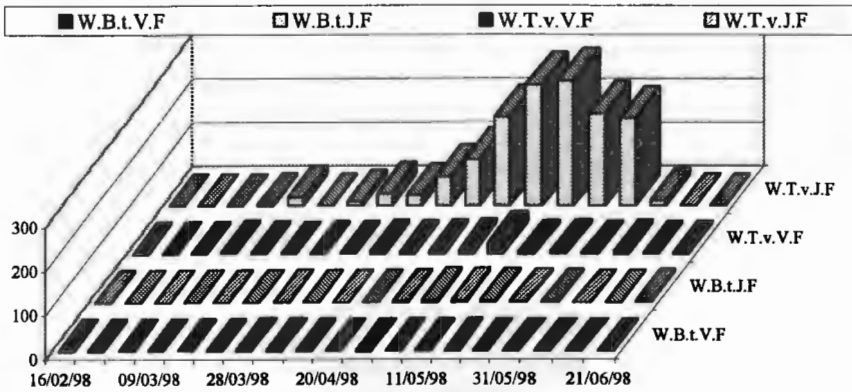


Figure 5. Evolution de la population embryonnaire chez *B. tabaci* et *T. vaporariorum* suivant l'âge des feuilles

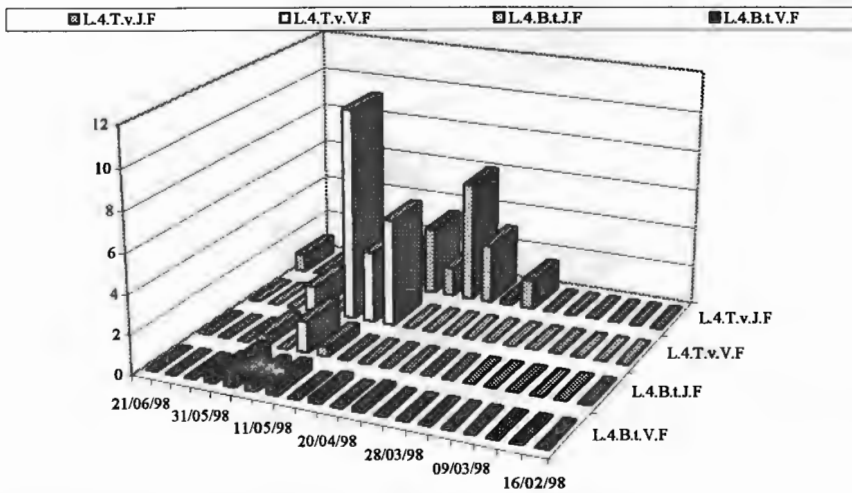


Figure 6. Evolution des populations larvaires du 4^{ème} stade de *B. tabaci* et *T. vaporariorum* suivant l'âge des feuilles.

L'analyse des résultats obtenus sur *T. vaporariorum* pour le quatrième stade larvaire montre qu'il a la même évolution quel que soit l'âge de la feuille de la plante hôte. Par ailleurs, ces résultats contredisent ceux obtenus en Algérie par Benmessaoud *et al.*, (1989) et (Benmessaoud, Boukhalfa., 1996) et en Tunisie par Chermiti (1991). Mais confirment les résultats concernant les populations embryonnaire et imaginalé.

3. La compétition interspécifique.

Elle s'explique par la concurrence qui s'établit entre les individus de différentes espèces pour une même source d'énergie. Lors de nos observations, nous avons remarqué que *T. vaporariorum* s'installe plus rapidement sur la culture de tomate que *B. tabaci*. Les adultes

émergeants 3 semaines avant ceux de *B. tabaci* commencent à pondre, passent par les différents stades de développement et occupent le maximum de surface vitale offerte par la plante hôte, empêchant ainsi l'installation, le développement voire même la multiplication de *B. tabaci*. C'est ainsi, que sur un même végétal et durant la même période d'observation, nous avons remarqué que les taux d'occupation des adultes, des œufs et des larves du quatrième stade se retrouvent en plus fortes proportions (Fig.7).

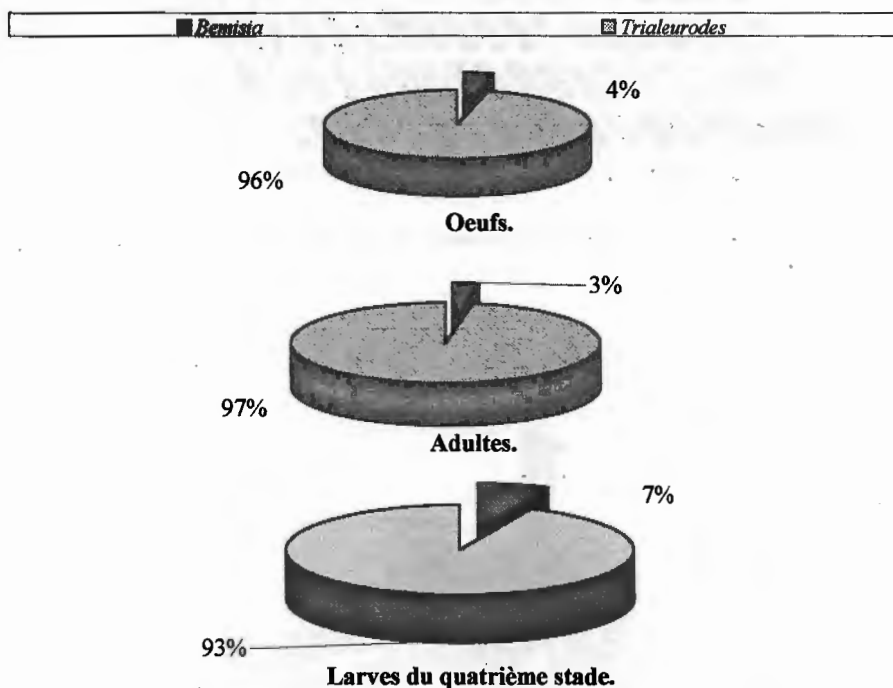


Figure 7. Taux d'occupation des populations embryonnaire, imaginaire et larvaire du quatrième stade de *B. tabaci* et *T. vaporariorum* sur plant de tomate.

Par ailleurs, le préférendum alimentaire, classe la culture de tomate en deuxième position après l'aubergine pour *T. vaporariorum* (Woets et Van Lenteren, 1976). Alors que Courdriet *et al.*, (1986) classe le choix de *B. tabaci* pour la tomate en 3^{ème} position après le concombre et l'aubergine.

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Abstract: Problems caused by whiteflies (*Trialeurodes vaporariorum* et *Bemisia tabaci*) on vegetable crops are becoming more and more important in all vegetable-growing areas. The work here presented, that was carried out in 1998 in a protected tomato crop in the Algerian sub-littoral area, shows that emergence of adults is different in the two whitefly species. Emergence of *T. vaporariorum* adults occurred by 3 weeks before that of *B. tabaci*. The number of whitefly generations depends on the crop cycling. We observed 4 overlapping generations during the study period. The mean generation duration was 20 days for *T. vaporariorum* and 14 days for *B. tabaci*. Adults of both species were found mostly on the upper leaves. Mean density and egg laying recorded in the study were 24,24 adult/dm² and 286,4 eggs/dm² for *T. vaporariorum* and 2,37 adult/dm² and 5,69 eggs/dm² for *B. tabaci*. Nymphal developed generally on lower leaves. *T. vaporariorum* was more abundant than *B. tabaci* along the study period.

Key words: Outbreaks, Generations, interspecific competition, *Bemisia tabaci*, *Trialeurodes vaporariorum*, vegetable.

Section V
Leafminers

Section V
Mineuses



Studies on the detection of the presence of *Liriomyza huidobrensis* in glasshouses in Poland

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Abstract: *Liriomyza huidobrensis* becomes in Poland a more and more dangerous pest of plants grown under cover. An effective control depends on the earliest detection of its presence in the plantation. For this purpose, there are sticky, colored and smelling traps. The most tempting traps for *Liriomyza huidobrensis* have proven to be yellow traps covered with 3 – phenylpropionaldehyde. This preparation increases the effectivity of the traps by more than 60 %.

Key words: *Liriomyza huidobrensis*, sticky and smelling trap

Introduction

Liriomyza huidobrensis, the South – American leafminer, next to other Middle – American miners such as: *Liriomyza trifolii*, *L. sativae* and *Amauromyza maculosa* is on the Polish list of organisms prohibited for transport, and in case of detection, it has to be obligatorily controlled. The first occurrence of *L. huidobrensis* in Poland was found in 1997 on gerbera plants in one glasshouse in the surroundings of Cracow. At that time, no radical control methods were undertaken and this species started to spread to other areas of Poland (Baranowski & Dankowska 1998 a). Until present, the occurrence of *L. huidobrensis* has been noted in Poland on such plant species as: gerbera, chrysanthemum, tomato, cucumber, primrose, daisy tagetes, salvia, verbena, petunia, eustoma, pink, pansy, gypsophila, galinsorga, bittersweet, small nettle, elder. The problem of glasshouse quarantine pests in Poland was signalled at EPPO Conference on Introduced Glasshouse Pests: Problems and Solutions – Pruchonice (2) 1998.10.13 – 15 (Baranowski & Dankowska 1998).

Chemical control of *L. huidobrensis* is difficult. As reported by Macdonald (1991), this species shows a high tolerance to plant protection agents. In order to prepare a precise program of *L. huidobrensis* control, important is the information referring to the effectivity of preparations in regard to the particular developmental stages of the pest. This problem is under studies.

Actually, for the control of *Liriomyza huidobrensis* in Poland, the following preparations are recommended: Vydate 240 SL (oxamyl) – 0,2 %; Vertimec 018 EC (abamectyn) – 0,05 %; Trigard 15 WP (cyromazin) – 0,1 %; Nogos 500 EC (dichlorfos) – 0,1 %; Confidor 200 SL (imidachloprid) – 0,1 %.

One of the important factors facilitating control is a quick detection of the pest's presence on the plants. This work presents the results of recent studies on the application of sticky, coloured and smelling traps in the detection of *L. huidobrensis* presence.

Material and methods

The studies were carried out in July 9 – 23, 1999, in production glasshouses with a plantation of gerbera strongly infected by *L. huidobrensis*. The glasshouse was divided into 4 chambers

of 9 x 30 m dimensions. The gerbera plants were grown on beds in bark – and – peat substrate, in a traditional method. The experiment was carried out in two stages. In the first period, the most effective colour attracting adult *L. huidobrensis* was identified. Yellow and blue traps were compared. In the second stage, the effects of different smell substances were compared; their characteristic are shown in table 1.

Table 1. Characteristic of smell substances

Smell substance	Smell	Chemical formula
3-phenylpropionaldehyde	cinnamon	C ₉ H ₁₀ O
Benzyl acetate	jasmine	C ₉ H ₁₀ O ₂
Benzaldehyde	almond	C ₆ H ₅ CHO

Method of the preparation of yellow traps covered with smelling substances. The lower part of the trap (the trap was in the form of a rectangular plate) was covered by an adhesive tape strip of 1 cm width. Then, the whole trap was covered with glue in aerosol. Subsequently, the adhesive tape was removed, and that place was covered with a smelling substance using a small paintbrush. In the experiment, the dimensions of the traps were 4 x 6 cm, and they were covered with an insecticidal glue. The traps were provided with a bit of string and they were hung on special thin wires. All traps were placed directly over the plant at height of 40 cm from the substrate. In each glasshouse, 10 traps of the investigated combinations were placed. The traps were in the glasshouse for 7 days and then they were transported to the laboratory to find the number of caught adult insects and to calculate their density per 1 cm². All results were statistically elaborated using the Duncan' test.

Results

The results of studies on the effect of colour on the number of caught adult *L. huidobrensis* are shown in table 2. It indicates that more adult insects were caught in yellow traps. The difference was 38,1 %.

The results of studies on the effect of smelling substance in combination with the yellow colour are shown in table 3. The greatest number of pests (62,4%) was caught in yellow sticky traps covered with 3 – phenylpropionaldehyde (cinnamon), slightly less individuals (34,6%) were caught in yellow traps covered with benzyl acetate (jasmine) in comparison to yellow traps without any smelling substances.

Table 2. Effect of colour on the number of caught of *L. huidobrensis*

Colour	Mean number per 1 cm ² of trap	Percentage
Yellow	2,1 a	100,0
Blue	1,3 b	61,9

Duncan's test $\alpha = 0,05$

Table 3. Effect of smell substances on the number of catches of *L. huidobrensis*

Smell substance	Mean number per 1 cm ² of trap	Percentage
3 – phenylpropionaldehyde	8,3 a	162,4
Benzyl acetate	6,9 a	134,6
Benzaldehyde	4,9 a	97,2
Control (yellow cards without smell)	5,1 a	100,0

Duncan's test $\alpha = 0,05$

Discussion

Liriomyza huidobrensis becomes in Poland an increasingly more dangerous pest of plants grown under cover. Particularly great are the damages on gerbera plantations, where it attacks the leaves and causes their drying. The plants become weaker and give poorer yields. The pest damages also the flowers depriving them of their commercial value.

Liriomyza huidobrensis is on the Polish quarantine list, and therefore, each new occurrence must be recorded. An early detection of the pest's presence on plants in the glasshouse permits an early application of control measures. For this reason, the precise determination of the moment of its appearance on the plants is of decisive importance. For this purpose, the use of coloured sticky traps are recommended (Berlinger 1980 & Brødsgaard 1989, Parrella & Jones 1985).

Important is the way of hanging the traps. Generally, it is advised to hang them just over the tops of the plants. (Baranowski *et al.*, 1992, Roditakis & Golfopoulou 1997). The most frequently recommended are yellow traps for the catching of whiteflies and for miners, and blue traps are effective for thrips. The presented studies have shown that the yellow traps were more attractive for adult *L. huidobrensis* than the blue ones. In order to increase the attractivity of coloured traps, combinations are made with smelling substances. The addition of such smelling substances as benzaldehyde or benzyl acetate to yellow traps will increase their attractivity for whiteflies *Trialeurodes vaporariorum* by 27 and 33 % respectively (Bant & Blaszk 1996), while the addition of 3 – phenylpropionaldehyde or 4 – methoxybenzaldehyde to blue traps will increase the trap's attractivity for Western Flower Thrips (*Frankliniella occidentalis*) by more than three times. The presented studies have shown that the addition of smelling substances to yellow traps increases their attractivity for *L. huidobrensis*. The most tempting smelling substance for this insect has proven to be 3 – phenylpropionaldehyde (cinnamon).

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Effect of different temperatures on development time and parasitization rate of *Diglyphus isaea* (Hymenoptera: Eulophidae) on *Liriomyza trifolii* (Diptera: Agromyzidae)

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Abstract: The effect of constant temperatures on development time and parasitization rate of *Diglyphus isaea* was examined in the laboratory. Parasitoid development was significantly effected by temperature. Adult emergence was about 3.4 times as fast at 35 °C than at 15 °C. Mean developmental times for *D. isaea* varied from 9.7 days at 35 °C to 32.7 days at 15 °C. The lower development threshold temperature estimated from the linear regression equation was 7.5 °C. Parasitization rate of *D. isaea* on *Liriomyza trifolii* increased with the temperature up to 30 °C, then decreased at 35 °C. Parasitization rates were 1.5, 41.0, 55.0, 64.0 and 56.0% at 15, 20, 25, 30, and 35 °C, respectively.

Key words: *Diglyphus isaea*, temperature, development time, parasitization

Introduction

Two species of leafminer, *Liriomyza trifolii* (Burgess) and *L. huidobrensis* (Blanchard) have been considered serious pest of greenhouse crops in Mediterranean region of Turkey (Yabas et al., 1994; Ulubilir & Yabas, 1996). Among the many parasitoids of above leafminers, *Diglyphus isaea* (Walker) naturally occurring in Turkey (Yabas & Ulubilir, 1993; Uygun et al., 1995), has become important in several European countries as a biological control agent (Baranowski, 1987; Nedstam et al., 1987, Van Lenteren & Woets 1988; Heinz & Parella, 1990, Minkenberg, 1990). Experimental use of *D. isaea* in integrated control programs on chrysanthemum and tomato indicate that it is an ineffective control agent in winter and spring, suggesting that low temperature may limit the development and reproduction of this parasitoid (Hendrikse et al., 1980, Woets & Van der Linden 1983, Wardlow 1984, 1985).

The main objective of the research reported here was to determine the developmental time of *D. isaea*, and its parasitization rate at five constant temperatures that correspond to greenhouse temperatures from winter to early summer in Mediterranean region of Turkey.

Materials and methods

Stock colonies

L. trifolii were reared at 25 ± 2 °C, 70 ± 10% RH, on bean *Phaseolus vulgaris* (L.). *D. isaea* was reared 25 ± 2 °C, at 70 ± 10% RH in insect rearing cages with *L. trifolii* as host (Minkenberg, 1989 & Beita et al., 1991). All rearing rooms were maintained at a photoperiod of LD 16:8.

Temperature regimes

All experiments were carried out in controlled environment rooms at 15, 20, 25, 30, and 35 °C. Temperatures were accurate to ± 1 °C, RH was 70 ± 10%, and L:D was 16:8.

Development/parasitization

Two bean plants placed in rearing cages, with 10-15 leaves, each infested with 40 third larval instars of the host, *L. trifolii*, were exposed to about 100 parasitoids (male+female) for 48 hrs (Minkenberg, 1989). The cages then were transferred to the environment rooms. Five cages (10 plants) were utilised for each temperature. Cages were examined daily until deace parasitoid emergence. Emerging adults were counted and removed from the cages. Thermal unit values were calculated with the following formula: thermal units (degree-days)= (constant temperature - development threshold) x development time. The development thresholds were predicted from the regression equations for the development rate (Leibe 1984). To determine the parasitization rate, leafminer larva on each leaf were examined under the stereomicroscope after emergence of all leafminer and parasitoid adults (Minkenberg, 1989). Calculations were made after correction of mortality of host due to feeding. Control cages with no parasitoid were used for comparison of natural mortality.

Statistical analysis

The effect of temperature on developmental time was determined by analysis of variance MSTAT-C, and means were compared by LSD (0.05).

Results and discussion

Development

Temperature strongly effected the developmental time of *D. isaea* (Table 1). Adult emergence was about 3.4 times as fast at 35 °C than at 15 °C. Mean developmental times for *D. isaea* varied from 9.7 days at 35 °C to 32.7 days at 15 °C. Minkenberg (1989) reported that development time of *D. isaea* was 26.0,16.6,and 10.5 days at 15, 20, and 25 °C, respectively for females, and 26.8, 15.8, and 10.3 days at 15, 20, and 25 °C, respectively for males. Minkenberg (1989) also reported that development time was 15.7 days for females, 14.7 for males at the alternating temperature of 18-22 °C, mean 20.3 °C. These values are much shorter than that of obtained here. These differences might be due to the host quality, since Minkenberg (1989) conducted his experiment on tomato, and bean plants were utilised here. *L. trifolii* was reported to exhibit differences for development rate when feeding on different host plants (Parrella, 1987). These differences might also due to differences among the ecotypes.

Table 1. Development time and thermal units in degree-days for *D. isaea* at five constant temperatures.

Temp °C	Development time*	Degree-days
15	32.7 a	243.7
20	22.9 b	286.2
25	17.9 c	313.3
30	14.4 d	326.2
35	9.7 e	266.7

*Means in the same column followed by a common letter are not significantly different at 5% level by Tukey-w test.

Equation of the rate of development against temperature was calculated, assuming that the mean developmental rates, i.e. the reciprocals of developmental times, were linearly related to

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temperature between 15 °C and 35 °C. The resulting line indicated that development rate was highly correlated with temperature ($y = .0034x - .026$, $R^2 = 0.94$). The estimated lower threshold temperature for development was approximately 7.5 °C. Minkenberg (1989) reported lower threshold temperature as 8.7 and 9.0 °C, for females and males, respectively. Again differences between the two studies might be due to the reasons given above. However, inaccuracy of X intercept method (Laudien, 1973; Minkenberg, 1989) may also be responsible for the differences.

Parasitization

The control larvae of *L. trifolii* in infested plants not exposed to *D. isaea* showed very low natural mortality (0.5 %) and therefore disregarded in the evaluation of parasitization rate. Parasitization rate of *D. isaea* on *L. trifolii* increased with the temperature up to 30 °C then decreased at 35 °C (Fig. 1), indicating that 35 °C was above the optimum and close to its upper threshold temperature for parasitization.

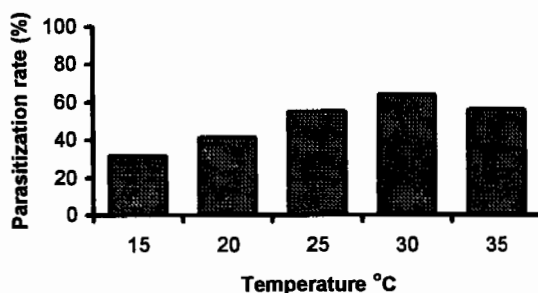


Figure 1. Parasitization rate of *D. isaea* on *L. trifolii* at five constant temperatures.

Statistical analysis showed that the differences among the parasitization rate from 15 to 30 °C were significant (F test, $P > 0.05$). Parasitization rate at 35 °C did not differ statistically than that of 25 °C, but was different from other temperatures. Although the total egg production in insects reaches its maximum at a temperature slightly lower than the optimum temperature for oviposition rate (Ratte, 1985), it was not found for *D. isaea* by Minkenberg (1989) for three temperatures 15, 20, and to 25 °C. The results for parasitization rate obtained in our study indicated that optimum temperature for fecundity is near 30 °C.

Decrease in development time and increase in parasitization rate with increasing temperature indicate that *D. isaea* can be considered suitable candidate for the biological control of leafminers in greenhouses along the Mediterranean belt of Turkey, since temperature regimes in growing season from October to May falls within the range studied here. Ulubilir (1999) studied parasitoid and predator ability of *D. isaea* by comparing life tables constructed at different temperatures, host stage preferences for oviposition, functional and numerical responses and also reported *D. isaea* as a suitable candidate for biological control of leafminers in greenhouses in Turkey. However, other criteria suggested by Van Lenteren (1986) such as generation synchrony and searching efficiency must be further examined to gain insight for the effectiveness of *D. isaea* for inoculative biological programs in Turkey.

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Effect of mass trapping by yellow sticky traps in controlling of leafminer, *Liriomyza* spp. (Diptera: Agromyzidae) injurious on vegetables in greenhouses in Icel

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Abstract: Studies were carried out to determine the effect of mass trapping by yellow sticky traps in the control of leaf miner, *Liriomyza* spp. injurious on the tomatoes in greenhouses in Icel in 1994-1995. One trap/10 m² were kept through out the growing season and placed 15-20 cm above from top point of the plants. Adult and larvae were observed at one week intervals. The adult were counted on eight yellow sticky traps, the larvae were counted on 25 leaves. It has concluded that leaf miner population could be controlled in IPM programmes by yellow sticky traps in protected vegetables.

Key words: *Liriomyza* spp., yellow sticky traps, mass trapping method, vegetables, IPM, greenhouses

Introduction

Growing vegetable in greenhouses is a kind of growing method which makes it possible to obtain more production than the others in a scale field. In Turkey greenhouse area is totally 35.000 ha. By this method mostly tomato, cucumber, pepper and eggplant are groved (Abak, 1993).

Important harmful insects in greenhouses are whitefly (*Bemisia tabaci* Genn.), spider mites (*Tetranychus cinnabarinus* Boisd., *T. urticae* Koch.), aphids (*Aphis gossypii* Glov., *Myzus persicae* Sulz.) and leafminer (*Liriomyza* spp.) in Mediterranean Region (Ulubilir & Yabas, 1996). In recent years *Liriomyza* spp. have became more important. Usually grovers use chemicals against leaf miners in greenhouses. However chemical control is not effective enough. Also chemical residues can be a problem especially on vegetables. In Holland, Germany & ABD in which covered growing method is very important IPM method are used against leaf miner (Lindquist *et al.*, 1980; Van de Veire & Vacante, 1984; Lenteren & Woets, 1988). In this method, using yellow sticky traps is given more importance.

This study was carried out In Icel in 1994-1995. The efficacy of using yellow sticky traps against adult and larvae of leaf miner was studied.

Material and methods

This study was carried out in two plastic houses near by near, infested by leaf miner (*Liriomyza* spp.) on tomatoes. The plastic houses are 600 and 800 m² respectively. In one of them yellow sticky traps were used and the other was used as control plot. Yellow sticky trap was made of fibre-glass and size is 20x25x03 cm. There is a special sticky substance (Trappit, Agrisence, BSC Ltd. Pontypridd, Cardiff CF 37-5 SU, UK) on it.

One trap was hanged at every 10 m² and it was 15-20 cm higher from the top of the plants (Nucifora *et al.*, 1983). Every week adult were counted on 8 traps which were determined before. In the control greenhouse, one trap was hanged at every 200 m². It was repeated in every week. In both greenhouses, larvae were counted on a leaf of 25 plants

selected at random. It was done by stereoscopic microscope. It was evaluated according to Abbott formula without percentage (Karman, 1971).

Result and discussion

In this study conducted to determine the efficacy of the yellow sticky traps, the population density of the adult and larvae determined in 1994 are shown in Figure 1, and the population density of the adult and larvae determined in 1995 are shown in Figure 2.

In the greenhouse where mass trapping method was applied, the population density of adult of leaf miner was found as 0-2 adult/trap in January and February, 5-41 adult/trap in May in 1994. The population density of larvae was found as 0-4 larvae/25 leaves during the trials in 1994. In the control greenhouse, the population density of adult of leaf miner was found as 0-15 adult/trap in January and February, 8-50 adult/trap in May. The population density of larvae was found as 0-4 larvae/25 leaves (Figure 1).

In the greenhouse where mass trapping method was applied, it was determined that there were many adult of leaf miner through out the trial in 1995 (11-930 adult/trap). The temperature of the greenhouses was higher in 1995 than in 1994, the leaf miner population density occurred higher in 1995. The population density of larvae was found as 0-56 larvae/25 leaves during the trials. In the control greenhouse, although the population density of adult of leaf miner was found as 0-1006 adult/trap, the population density of larvae was found as 0-92 larvae/25 leaves (Figure 2).

In both of the greenhouses with respect to the population density of larvae, there wasn't important difference in 1994-1995. Although there were enough amount of the adult population in the both greenhouses, the larvae was found low density. But the population density of larvae in the control greenhouse should have been higher than the other. The reason of this may be both of the characters (control and mass trapping method) couldn't be applied in the same greenhouse. Because climatic conditions can change in different greenhouses, despite of being next to each other.

Statistically the efficacy of yellow sticky traps against the larvae of leaf miner was found the highest (85%) in February 1995.

Yellow sticky trap is very important for IPM (Mc Clanahan, 1983; Nucifora *et al.*, 1983; Veire, 1991). Also it has no any harmful effect for both environment and human health. At the end of this study, it has been come to the conclusion that yellow sticky trap, especially with the other control methods, could be used against leaf miner in greenhouses.

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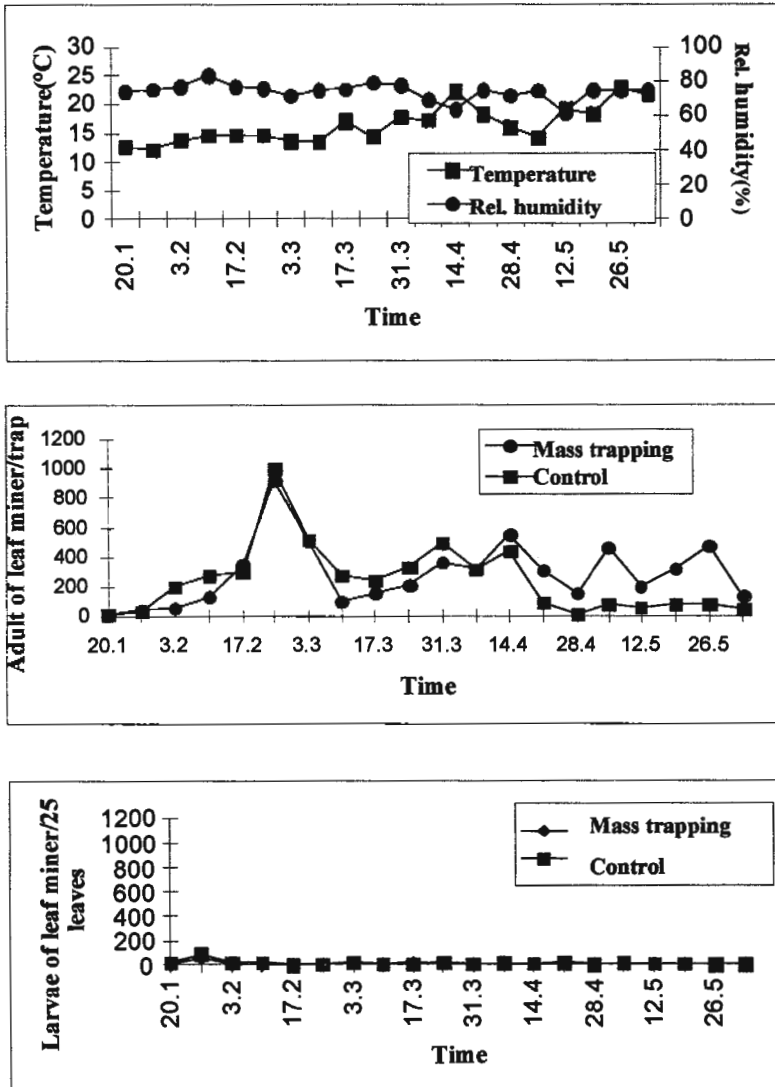


Figure 1. Population density of leaf miner in both greenhouses where mass trapping method was applied and for control in Icel in 1994

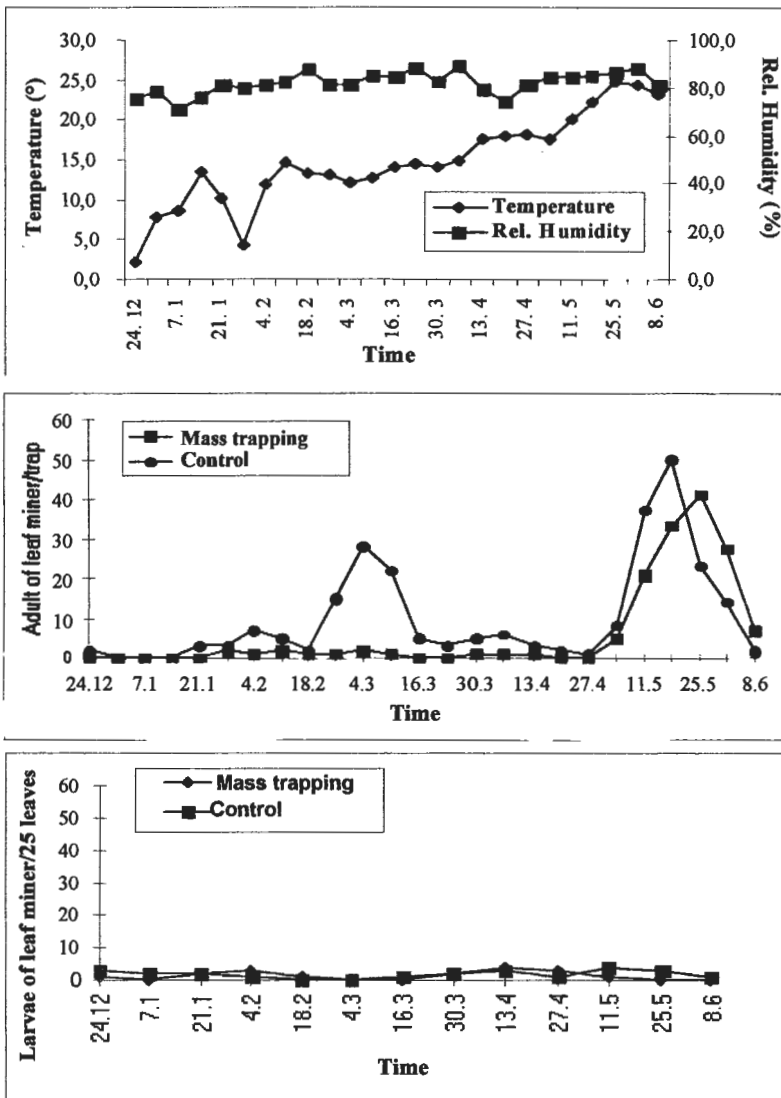


Figure 2. Population density of leaf miner in both greenhouses where mass trapping method was applied and for control in Icel in 1995

Studies on population development of leafminers (*Liriomyza* spp.) and parasitisation situation

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Abstract: One yellow sticky trap was placed every 200 m² and above the plants to observe the population development of leaf miner in the greenhouses in 1992. The traps were checked weekly and the number of the adults on it were recorded. To estimate the larval population, 25 leaves were collected. The situation of parasitisation were studied in three different greenhouses, the leaves with mine collected from these greenhouse each week, were cultured in the laboratory for the parasite emergence. The results showed that the adult population were high during April and May, and the population decreased in June in 1992. The adult population observed in Autumn indicated that population were high in November with the peak in mid November, then drop down at the end of December. In these two studies, larval population development were observed in April and May in Spring and again showed parallel development in Autumn, and declined to zero in December.

It was found that the parasitoids were *Chrysonotomyia chlorogaster* (Erdos), *C.formosa* (Westwood), *Diglyphus isaea* (Walker) (Hym.; Eulophidae) as a results of parasitisation studies. The rate of parasitisation were 8-52.5% on tomato and 5-68% on eggplant in 1993.

Key words: Population fluctuation, parasitoids, *Liriomyza* spp., vegetables, greenhouses

Introduction

In Turkey having suitable ecology for greenhouse growing method, there is totally 35.000 ha areas where mainly tomato, cucumber, pepper and egg plant are grown by this method.

According to the results of the survey carried out in the East Mediterranean Region in 1990 - 1991, white fly (*Bemisia tabaci* Genn.) and leaf miner [*Liriomyza trifolii* (Burgess)] have been determined as important harmful insects in this area. Especially in recent years, *L. trifolii* has become more important. In the world, there have been many studies conducted so far which show that it is very difficult to control leaf miner by using chemicals. That is why, its natural enemies have been investigated. (Parrella *et al.*, 1982; Lindquist, 1984; Chandler, 1985; Robb & Parrella, 1985; Parrella & Robb, 1988; Woets, 1983). In some studies, it has been indicated that yellow sticky traps are used to monitor the population dynamics of leaf miner. (Lindquist *et al.*, 1980; Van de Veire & Vacante, 1984; Van Lenteren & Woets, 1988).

In this study, the population densities of adult and larvae were observed and natural enemies were determined by using yellow sticky traps.

Material and methods

To determine the dynamic of the population of leaf miner (*Liriomyza trifolii* (Burgess)), one eggplant greenhouse (1.5 m²) and one tomato greenhouse (1.5 da) were chosen and at every 200 m² and 30 cm above of the plants, one traps sized 20x25x0.3 cm was placed in both of the greenhouses around Icel in 1992. Also in a pepper greenhouse, yellow sticky traps were placed in the same way in October of 1992. The traps were controlled and adults were

counted weekly. Since the first appearing of the adults, 25 leaves were collected from 25 plants selected randomly *and* the larvae on the leaves were counted by stereoscopic microscope.

To observe the possible natural enemies of leaf miner in eggplant greenhouse and tomato greenhouse, counting were carried out. Every week, larvae were counted on 50 leaves randomly selected. After that the leaves with larvae, were put in to the cages. The parasitoids emerging were counted and sent to Dr. Miklat Doganlar (Agricultural Faculty of Mustafa Kemal University, HATAY) for identification.

Result and discussion

The results of the studies carried out to determine the dynamics of the population of leaf miner are shown in Figures 1, 2, and 3.

In the eggplant greenhouse, the population of leaf miner increased at the beginning the trial and there was fluctuated in the density of population during the trial. After that, this decreased in June. The dynamic of the larvae population showed the same trend in this period

In the the tomato greenhouse, the population of adult was low at the beginning, but in April and May this increased. The larvae population increased in April and May (Figure 2.). In the pepper greenhouse, the adult population was observed very high and in the end of December decreased. The larvae population showed the same trend (Figure 3). According to the results of the study, leaf miner in vegetable greenhouses was seen in spring and autumn season. The number of the adults on a trap was observed as 13-1239. However it is difficult to say that there is a relationship between the numbers of adult and larvae. Although both population dynamics are similar to each other, the number of the larvae population did not increase as much as the number of the adult population. Parrella & Jones (1985) said that there is no linear relationship between the numbers of the adult and larvae.

The dynamics of leaf miners larvae and adult changed according to the crop in greenhouse for example, in the tomato greenhouse, the number of the adult/trap changed between 4 and 383. In the eggplant greenhouse this was 13-1230. In December and January, since the temperatures were low in the greenhouses, the population densities of leaf miner were low. This was seen in the end of pepper growing period and at the beginning of tomato and eggplant growing period.

In this study, *Chrysonotomyia chlorogaster* (Erdos), *C. formosa* (Westwood), *Diglyphus isae* (Walker) and *Sympies* sp. were identified as the parasitoids. The dynamics of these parasitoids and parasitization rate were given in Figure 4.

The parasitization rates were 8-52.5% on tomato, and 5-68% on eggplant. But parasitoids are reported to be affected by chemicals (Johnson *et al.*, 1980; Chambers & Kauskolekas, 1985; Johnson, 1987). The highest parasitization rate was seen in May on eggplant and in June on tomato. Veire & Vacante (1984) reported that *D. isaea* on *Dacnusa* spp. are the parasitoids of leaf miner. Although several chemical applications were done in these greenhouses, the activities of the parasitoids musn't be considered as little. To increase this activities, it is necessary to train the farmers, and unnecessary chemical applications must be avoided.

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The authors are grateful to Mahmut GUNES for his continuous support and writing the manuscript.

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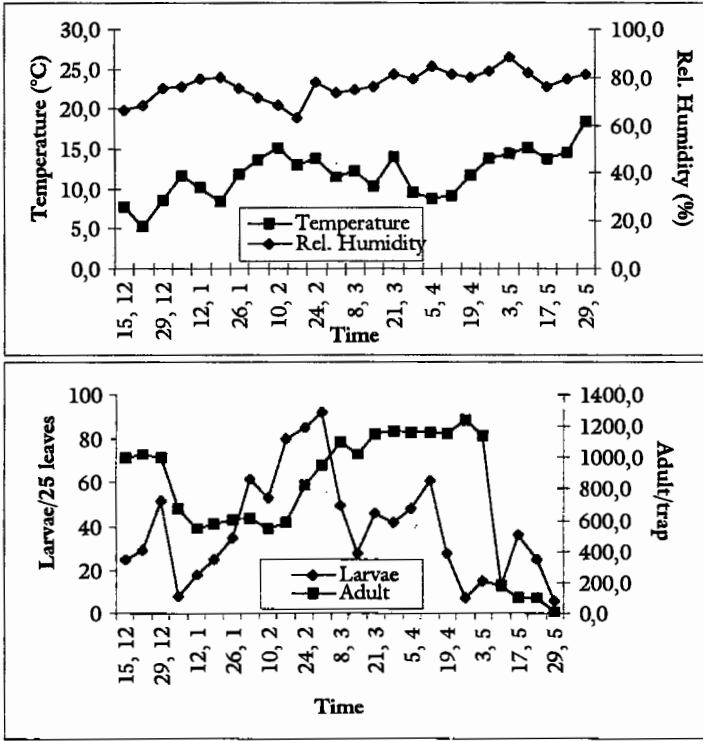


Figure 1. Population densities of leaf miner on eggplant in Icel in 1992

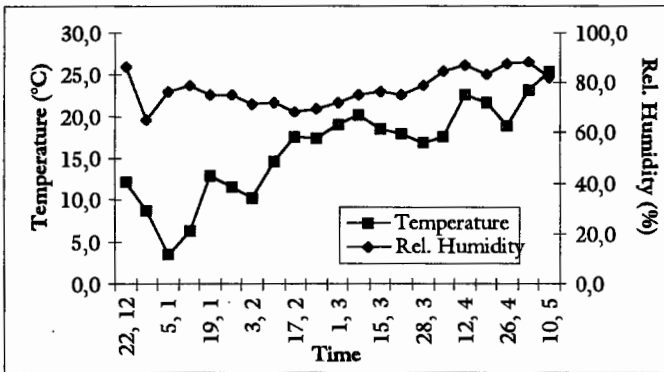


Figure 2. Population densities of leaf miner on tomatoes in Icel in 1992 (T and HR)

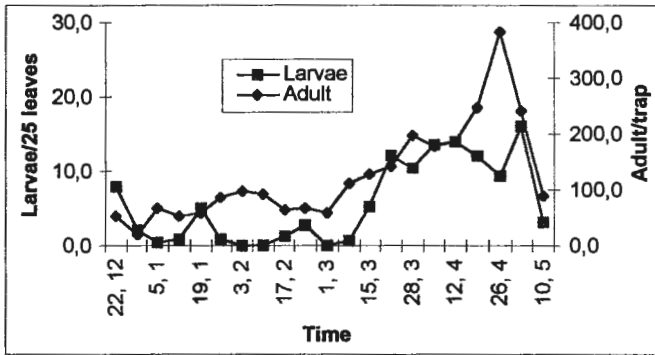


Figure 2 (cont.). Population densities of leaf miner on tomatoes in Icel in 1992

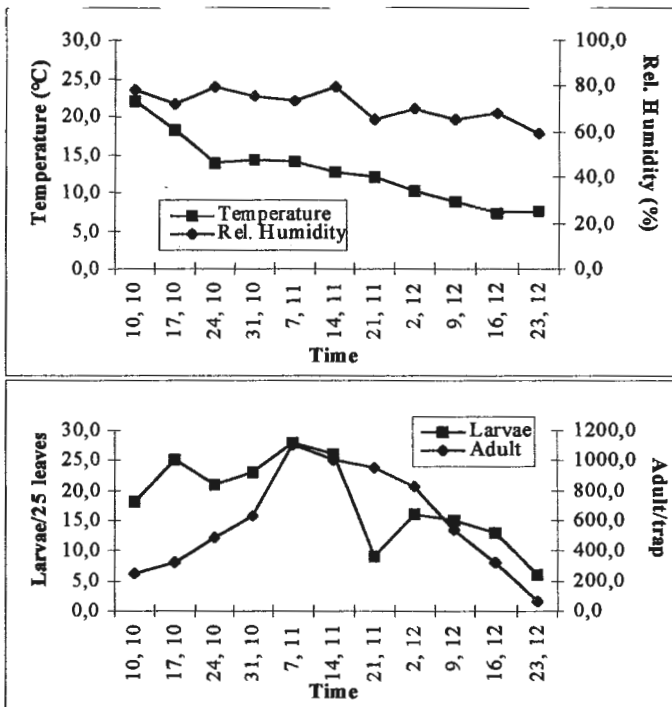


Figure 3. Population dynamics of leaf miner on pepper in Icel in 1992

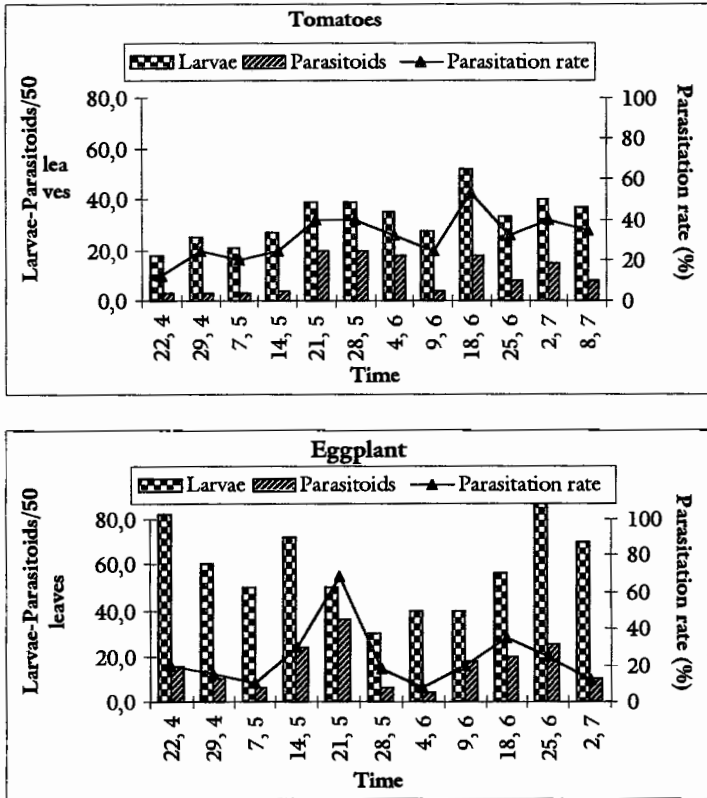


Figure 4. Parasitism rate of leaf miner in greenhouses in Icel in 1993

Leafminers (*Liriomyza* sp.) importance in greenhouses in the Oeste region of Portugal and its natural parasitoids as control agents in IPM programs

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Abstract: Leafminers species were considered as a key-pest on vegetable greenhouses in Portugal, particularly in the Oeste region of Portugal. *Liriomyza trifolii* (Burgess) and *Liriomyza huidobrensis* (Blanchard) are the two species, which were accidentally introduced before 1986 and 1990 respectively. The studies that were developed during five years are related with: (i) identification of the leafminer species; (ii) identification of the parasitoids complex species; (iii) population dynamics in different agroecosystems; (iv) monitoring; (v) evaluation of the parasitoids role as a natural control agents; (vi) and the development of an IPM program against leafminers in greenhouse vegetable crops.

The importance of the leafminers damages in the different crops, the leafminers species present and its parasitoids complex species are presented and discussed. Since 1991, *L. huidobrensis* dominates the leafminers species complex in greenhouse vegetable crops in all of the three "different" greenhouse agroecosystems that were surveyed. Yield losses occurred more frequently in green beans and lettuce crops. The damages on cucurbits and tomato seemed as being tolerable in general. The complex of the parasitoid species is very dynamic. In fact, the abundance and parasitism rates found varied during this period. In 1993 levels of parasitism of 100% were observed with *Diglyphus isaea* Walker + *Diglyphus poppoea* (Walker) and during 1994-95 the two most important species were *D. isaea* + *Diglyphus crassinervis* Erdős. The presence of *Dacnusa sibirica* Telenga was also registered as well as other species with less abundance.

Natural control is frequently enough to maintain populations on tolerable levels that is based on the activity of autochthonous fauna.

Key-words: vegetable greenhouses, leafminers, *Liriomyza huidobrensis*, natural control, *Diglyphus isaea*, *Diglyphus poppoea*, *Diglyphus crassinervis*, *Dacnusa sibirica*

Introduction

Mexia, in 1990, mentioned leafminers problems in greenhouses in Portugal as being responsible for the losses in bedding plants or in the beginning of the growing seasons. In 1991 *Liriomyza trifolii* (Burgess) was the most important species present in all surveyed greenhouses in the Oeste region (Godinho & Mexia, 1993). At the summer of 1992, another alien species, *Liriomyza huidobrensis* (Blanchard), almost substituted the previous species, at this Region.

In order to define IPM programs in this agroecosystem, biological control using parasitoids species available in the european market seemed possible and desirable. Studies with this goal were initiated in commercial greenhouses using *Diglyphus isaea* (Walker) and *Dacnusa sibirica* Telenga. The particular aspects of mediterranean greenhouses, which allows interactions between inside and outside of the structure, was the first constraint to the development of an inundative biological control method. Surveys in other greenhouses at the same region, revealed high levels of natural parasitism with the two species mentioned. Those

situations seemed to be related with the low pesticide pressure against leafminers or other pests such as caterpillars, aphids, trips, and whiteflies.

In this circumstances the studies of the IPM developing project were based mainly in order to evaluate the parasitoids role as natural control agents as well as the conditions that promotes their activity. The methods of sampling and the results are presented as also as a proposal of an IPM model to control leafminers populations in order to minimise pesticide applications.

Material and methods

The methodology of sampling pests and damaged crops was developed to the studies of the project PAMAF 2034- "Melhoria da produção hortícola em estufa na Região Oeste" carried on between 1994-1997 (Mexia *et al.*, 1999).

1. Six commercial greenhouses were sampled fortnightly. Growers had different attitudes to control the pests: (i) two organic farmers; (ii) two systems which farmers tolerate the presence of the pests; (iii) two systems where the pesticides applications were done by a previous established model of controlling pests and diseases using large spectrum active ingredients;

2. Thirty to sixty plants were observed per plot, depending on the total crop area. Yellow traps were also used mostly to whiteflies and leafminers adults. Vegetable damaged material sampled as well as insects (adults and pupae) were stored in plastic bags and in Eppendorf tubes, respectively, to be observed in the laboratory and parasitoids species were reared in a growth chamber;

3. Leafminers data in the greenhouses: (i) damaged plants with mines and feeding punctures; (ii) and presence of active larvae;

4. Leafminers data in the laboratory: (i) active larvae; (ii) dead larvae; (iii) parasitised larvae; (iv) pupae with endoparasitoids.

Results and Discussion

1. Leafminers species:

During 1994-1997, the results of the observation of *aedeagus* morphological aspects revealed the presence of *L. huidobrensis* in all of the six greenhouses surveyed.

2. Leafminers economic importance in the different crops/agroecosystems:

Green beans in all of the three agroecosystems seemed to be more damaged but without economical importance in organic farming.

At the tomato crop the damages are frequent but only until the first racemouse inflorescence in greenhouses with a resident population of parasitoids and where growers use selective pesticides. In those conditions, the leafminers' larvae are frequently almost 100% dead or parasitised. At the organic tomato crops sampled, the incidence of damaged plants was frequently 0%. At those two systems the presence of ectoparasitoids but also great levels of dead larvae were frequent.

Lettuce crop is more frequent, in the greenhouses, at the winter (November-December or December-January) during, only, about seven weeks. Damages on lettuce are more important in the beginning of the growing season but only when outbreaks occurred and when the farmers let damaged green beans or tomato crops stay inside the greenhouses at the end of the season. The population levels present and responsible for the losses on lettuce seemed to depend on the strategies used on the previous crops. Studies conducted in greenhouses on lettuce indicated that the population levels, which are responsible for yield losses, must be

very high. Only about nine adults/plant can damaged lettuce plants with economic importance (Godinho, 1997).

During this period, at the organic farming sampled systems and at the greenhouses with less pesticide pressure, leafminers are not economic important because the population levels seemed to be tolerable.

It was also verified, during this period, that farmers' knowledge about leafminers and its damages became better, which allowed them to avoid population outbreaks. They already can recognise better leafminers damages, which are mines and feeding punctures and they can relate the last ones with adults' presence. However, the growers' attitudes are different. The reasons on they make their decision of spraying are based on different aspects. This subject that is related with objective and subjective reasons is described in another paper at this Meeting.

3. Parasitoids species:

At all of the six greenhouses surveyed in this study were observed parasitoids present at the crops during the different growing seasons (Jan. to Dec.).

The more abundant species were *Diglyphus isaea*, *Diglyphus poppoea* and *Diglyphus crassinervis* as ectoparasitoids and *Dacnusa sibirica*, *Halticoptera* sp., *Chrysocharis* sp. and *Epiclerus* sp. as endoparasitoids (table 1).

Table 1 – Parasitoids list, present in the Oeste region of Portugal, on *Liriomyza huidobrensis* Blanchard

Parasitoids Families/Species	Crop		Agroecosystem	Season	Reference		
	Endo	Ecto					
Braconidae							
<i>Dacnusa sibirica</i> Telenga	•	•	•	Six systems	all	Christie & Parrella, 1987	
Diapriidae							
<i>Trichopria</i> sp.		•	•	-	-	Godinho <i>et al.</i> , 1994 ¹	
Eulophidae							
<i>Chrysocharis</i> sp. ²	•	•	•	Six systems	W	Grenouillet <i>et al.</i> , 1993	
<i>Diglyphus crassinervis</i>		•	•	•	Six systems	all	Grenouillet <i>et al.</i> , 1993
<i>Diglyphus isaea</i> (Walker)		•	•	•	Six systems	all	Grenouillet <i>et al.</i> , 1993
<i>Diglyphus poppoea</i> Walker		•	•	•	Six systems	W	Godinho <i>et al.</i> , 1994 ¹
Pteromalidae							
<i>Halticoptera</i> sp. ³	•	•	•	Organic system	Sp,Su	Grenouillet <i>et al.</i> , 1993	
Tetracampidae							
<i>Epiclerus</i> sp.	•	•	•	Six systems	Sp,Su	Godinho & Mexia, 1997 ¹	

² Grenouillet *et al.*, 1993 referred 5 species on *L. huidobrensis*;

³ Grenouillet *et al.*, 1993 referred 3 species on *L. huidobrensis*

g – green beans; t – tomato; W – Winter; Sp – Spring; Su – Summer.

3.1. *Diglyphus isaea* Walker

This species was the *Eulophidae* parasite more abundant comprised 77% of the total number, existing in 85% of the surveyed systems during 1992-93. This parasite species was recorded all over the year. *D. isaea* is present with other species.

3.2. *Diglyphus poppoea* (Walker).

This *Eulophidae* was referred as a biological control agent of leafminers species, for the first time, in Portugal (Godinho *et al.*, 1994). This species is not referred in the Grenouillet *et al.*, 1993 parasitoids list. This parasitoid was more abundant during 1992-93 and it was present in 39% of the samples with a 13% of the total of the specimens reared in laboratory. One important fact is its abundance, particularly on Autumn and Winter months.

3.3. *Diglyphus crassinervis* Erdős.

This species was detected in Oeste region during the period of 1994-95. Its present at the six greenhouses surveyed. It was detected during all year with *D. isaea*. At those samples did not appear *D. poppoea*.

3.4. *Dacnusa* sp.

Dacnusa sibirica Telenga was referred as a parasitoid of *L. huidobrensis* on tomato, for the first time during the period 1992-93 (Godinho & Mexia, 1994).

Dacnusa sp. was present in 15 % of the samples and specimens of *Dacnusi* tribus were present in 54% of the samples during 1992-93 (Godinho & Mexia, 1994). During 1994-95 specimens of *Dacnusa* sp. are very frequent being present at all six greenhouses especially on greenbeans.

4. Natural control or biological treatment?

The material observed at the laboratory from the six greenhouses surveyed had different levels of active larvae, parasitised larvae and dead larvae during the crop seasons. In fact, this data battery as well as other sporadic surveys carried on at that Region allows us to considered some hypothesis that must be tested. This problem is absolutely linked with the dichotomous approach for biological control along the Mediterranean area, where beneficial releases should be adopted as a complement to natural control agents and there richness.

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Section VI
Aphids, mites, thrips, Lepidoptera, and heteropteran pests

Section VI
Pucerons, acariens, thrips, lepidoptères et punaises

Pre-introductory evaluation of a coccinellid predator, *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) for biocontrol of cotton aphid, *Aphis gossypii* Glover (Aphididae: Hemiptera) in glasshouses

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Abstract: The present study was initiated to evaluate the potential of the coccinellid species, *C. sanguinea* L., for the biocontrol of cotton aphid, *Aphis gossypii* Glover, in laboratory. The studies focused on the aspects of development time, survival, fecundity, life-table parameters, food consumption and functional response of *C. sanguinea* at various temperatures. The data for development, survival and fecundity indicated that the most favourable temperature for *Cycloneda sanguinea* was found to be in the range of 20°C to 25°C. The intrinsic rate of increase (r_m) of *C. sanguinea* (0.16) was found to be optimum at 25°C. The highest fresh weight of aphids consumed by first, second, third and fourth larval instars and overall development of *C. sanguinea* was found to be at 25°C, 30°C, 25°C, 25°C and 25°C respectively. The fourth instar stage of the species was the most voracious, since about 81%-85% of the total food consumption were consumed during fourth larval instars of *C. sanguinea*. Functional response experiments indicated that the female adults of *C. sanguinea* which take less time to process the cotton aphids and are efficient searchers, required high number of cotton aphids to reach satiation, especially at high temperatures. These characteristics suggest that *C. sanguinea* can be a possible candidate for biological control of cotton aphid in glasshouses.

Key words: Coccinellid, cotton aphid, biological control, glasshouse, development, survival, fecundity, oviposition, intrinsic rate of increase, consumption, satiation, handling time

Introduction

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is a cosmopolitan species and widely distributed in tropical, subtropical and warm temperate regions (Blackman & Eastop, 1984). Cotton aphid has now colonised all zones with mild winters and occurs in the glasshouses in regions with cold winters. It is a world-wide pest of many plant species including cotton, cucurbits (melon, courgette, cucumber), citrus, coffee, vegetables (eggplant, okra, sweet pepper etc.) and ornamental plants (*Lantana*, *Hibiscus*, *Chrysanthemum*) (Blackman & Eastop, 1984).

The cotton aphid is an important aphid pest on cucumbers and chrysanthemums in glasshouses in Netherlands (van Schelt *et al.*, 1990) and Great Britain (Blackman & Eastop, 1984). Until a few years ago aphids were controlled using the selective insecticide pirimicarb which is widely used and compatible with biological control of insect pests in integrated pest management (IPM) systems. However, *A. gossypii* has developed resistance against this insecticide (Cross, *et al.*, 1983; Furk *et al.*, 1980). Therefore, cotton aphid is now usually controlled by non-selective chemicals, which inhibits the use of biological control of other pests in the glasshouse (van Steenis, 1992).

Some good results of aphid control in glasshouses have been obtained with the introduction of coccinellid larvae of *Coccinella septempunctata* L. and *Adalia bipunctata* (L.) on *Myzus persicae* (Sulz.) and *Macrosiphum rosae* L. (Hämäläinen, 1977), and *Adalia bipunctata* (L.), *Coccinella septempunctata* L., *Coelomegilla maculata* de G. and *Cycloneda sanguinea* (L.) on *Myzus persicae* (Sulz.) and *Aphis gossypii* Glover (Gurney & Hussey, 1970). A disadvantage of the use of coccinellids in aphid controls is that they are not able to form a self-perpetuating population in the glasshouse, so repeated introductions are necessary to suppress aphid populations (Hämäläinen, 1977). This can make the biological control of cotton aphids with coccinellids expensive (Hämäläinen, 1980). Coccinellids would be more suitable for one-time releases to control a heavy outbreak that escaped other means of control (Hodek & Honék, 1996).

Cycloneda sanguinea (L.) is a primarily aphidophagous coccinellid species and most diverse in temperate areas of Central and South America (Vandenberg & Gordon, 1988). It is also reported that it is an important coccinellid species feeding on aphids in several crops in Brazil (Santos & Pinto, 1981). It has been described as an efficient predator of aphids on cucumbers and chrysanthemums grown in the glasshouses (Gurney & Hussey, 1970).

Finding an effective natural enemy of cotton aphid will be an important contribution to IPM programmes for both glasshouse and outdoor crops. The occurrence of cotton aphid in a crop forces the grower to use broad spectrum insecticides, and abandon the successful biological control of other pests. Therefore, the present study was initiated to evaluate the potential of the coccinellid species, *C. sanguinea*, in laboratory for the biocontrol of cotton aphid with potential emphasis on the following aspects: (1) development time, (2) survival, (3) fecundity, (4) life-table parameters, (5) food consumption and (6) functional response of *C. sanguinea* at various temperatures.

Material and methods

The studies were conducted in incubator cabinets at three constant temperatures, namely 20°C, 25°C and 30°C ± 1°C, with a 16 L:8 D photo period and 60 % r.h. which was provided using a saturated salt solution of Magnesium Nitrate (Mg NO₃). The coccinellid predator, *Cycloneda sanguinea* L., was collected from the fields near Merida in Mexico by Mike Copland. The experimental studies were initiated with the mated female adults (< three weeks old) from the stock culture of the predators reared on the cotton aphids, *A. gossypii*, in a culture room at 26 ± 1-2°C. The cotton aphids were provided from the stock culture, rearing on the cotton and okra plants.

Development studies were conducted at seven constant temperatures, namely 17.5°C, 20°C, 22.5°C, 25°C, 27.5°C, 30°C and 32.5°C for *C. sanguinea* with a 16 L:8 D photo period and 60 ± 5% r.h. in incubator cabinets. Developmental studies were initiated with newly deposited eggs (<1 day old) from a colony of mated females on excised cotton leaves. An excised cotton leaf containing egg-cluster (8-12 eggs) for *C. sanguinea* was placed on the agar media in the Petri-dishes. They were replicated six times for each temperature. Each of two Petri-dishes containing the eggs deposited on an excised cotton leaf together with a saturated salt solution of Mg NO₃ in a small cup was placed into large transparent boxes (270 x 160 x 100 mm). They were observed three times a day for hatching. On the day of hatching the first instar larvae were transferred separately into the individual Petri-dishes with a sufficient number of the cotton aphid on excised cotton leaves. Larva and pupa were replicated twenty five times for each temperature. Larval and pupal development were also checked three times a day for ecdysis and mortality. Aphids were replenished for each observation as needed. Developmental data were analysed using one-way ANOVA and the means were separated

using the Tukey test at 1% level (Minitab Inc., 1995). Survival data were presented as a proportion but were analysed by using original (count) data. Survival data were analysed using the Chi-squared test (Minitab Inc., 1995).

For fecundity studies, newly emerged adults of *C. sanguinea* from the stock culture were sexed and placed, one male and one female, into plastic Petri-dishes containing excess cotton aphids on excised cotton leaves stuck to 8% agar medium. Each of two Petri-dishes containing a pair of newly emerged adults together with a small transparent cup with saturated salt solution of $Mg\ NO_3$ were placed into large transparent boxes (270 x 160 x 100 mm). The large transparent boxes then were placed in incubators at constant temperatures of 20°C, 25°C and 30°C, and at a photo-period of 16 L:8 D. The experiment on fecundity was replicated five times for *C. sanguinea*. The number of eggs laid by females was counted daily for a period of three weeks by checking Petri-dishes surface and excised cotton leaves. Food was replenished as needed. In a few cases, the male died and was replaced by another male to ensure the opportunity to mate. Oviposition rate (eggs/day) and total number of eggs laid (eggs/days) by individual females at differing temperatures were analysed using one-way analysis of variance (ANOVA). The means were separated using the LSD method (SAS Institute, 1985). The mean daily oviposition rate for each replicate was calculated by dividing the total number of eggs laid over a period of three weeks of the adult reproductive life. The life table statistics, described by Birch (1948), were estimated using a QBASIC program (Jervis & Copland, 1996). While the life table statistics were estimated, it was assumed that sex ratio equalled 50 % females and data on developmental time and mortality of immature stages were obtained from development and survival studies of *C. sanguinea*.

In order to determine the consumption of *C. sanguinea* at three constant temperatures, namely 20°C, 25°C and 30°C \pm 1°C, with a 16 L:8 D photoperiod, 20 newly-hatched larvae were transferred into individual Petri-dishes (15 x 60 mm) containing either 100, 200, 300 or 400 medium-size cotton aphids (third or fourth instar) for first, second, third and fourth instar larvae respectively on excised cotton leaves stuck to the agar medium. The Petri-dishes were placed in large transparent boxes (270 x 160 x 100 mm) with a saturated salt solution of $Mg\ NO_3$ in small cup. They were observed twice a day for moulting. Before and after each observation, the weights of aphids offered and remaining were recorded using a microbalance. Thus, numbers and fresh weights of aphids consumed for each observation were recorded. During larval development, the numbers of moulted skins were also recorded and removed at each observation. The total consumption and consumption rate were analysed using one-way ANOVA and the means were separated by LSD at 1% level (SAS Institute, 1985).

Functional response studies were conducted in incubator cabinets at three constant temperatures, namely 20°C, 25°C and 30°C \pm 1°C, with a 16 L:8 D photo period. Female adults were taken from the stock culture, starved for 24 h at 25°C in incubator cabinets in order to equalise their appetite. Thereafter, they were introduced individually in a 9 cm diameter petri-dish (approximately 64 cm²) together with 20, 40, 80, 120, 240, 480, 960 of aphid density on excised cotton leaves stuck to agar medium. Medium size aphids were selected (mean weight \pm standard error: 0.043 \pm 0.0014). Female adults of *C. sanguinea* were randomly assigned to one of eight and seven aphid density treatments respectively. Five to eight replicates at each aphid density were used. After 24 h, the number of aphids killed by the female adults was recorded by counting the aphids remaining in each Petri-dish. The number of aphids killed of *C. sanguinea* in 24 h at different aphid densities and temperatures were analysed separately using one-way analysis of variance (ANOVA). The means were separated using LSD method at 1% level (SAS Institute, 1985). The most widely used description of type II functional response of invertebrate predators to changes in prey density is the disc equation of Holling (1959):

$$(N_e = [aN_oT/(1 + aNTh)])$$

The model assumes that the number of prey attacked (N_e) is a function of prey density (N), the predator's rate of successful search (a), the length of time the predator and prey are exposed to one another (T), and the handling time per prey item (Th). The maximum number of prey attacked is limited by an upper asymptote defined by T/Th (Hassell, 1978). The number of aphids killed as a function of aphid density for each temperature was plotted, and an iterative non-linear least-squares regression (SAS Institute, 1985) was used to fit the disc equation to the means and to estimate the parameters for type II functional responses.

Results and discussion

Development time and survival rate of C. sanguinea at various temperatures

The mean development times for the immature stages and overall development of *C. sanguinea* are presented in Table 1. The developmental period was found to decrease with increasing temperature. However, the increasing temperature above 27.5°C resulted in a small decrease of developmental time at each developmental stage. There were no significant differences in developmental time of egg, larval, pupal and overall development at a temperature of 27.5°C, 30°C and 32.5°C whilst there were significant differences in developmental time of egg, larval, pupal, and overall development at temperatures ranging from 17.5°C to 25°C.

The developmental data of *C. sanguinea* on green peach aphid (Gurney & Hussey, 1970) showed that the relative duration of different immature stages was not affected by differing levels of temperature. However, overall development period (egg to adult) of *C. sanguinea* in this study was found shorter at each temperature regimes than that observed by Gurney & Hussey (1970). It is likely that difference in overall development period might be due to dietary factors and rearing procedures or maybe different strains of the beetle.

It appears that developmental time of overall development was optimal at 30°C since it gave fastest development (Table 1), but they had a low overall survival rate (0.56) at 30°C (Table 2). Therefore, data on development and survival (Table 1 and 2) indicated that optimum temperature for overall development was 27.5°C with 12.34 days of development time and 0.84 of survival. Obrycki & Tauber reported that optimal development for *Coccinella septempunctata* L., *Coccinella transversoguttata* Faldermann (1981), *Coleomegilla maculata* Lengi (1978) and *Hippodamia convergens* Guérin-Méneville (1982) on green peach aphid and pea aphid is at the temperatures between 26.7°C and 29.4°C.

Whilst aphidophagous predators belonging to family Chrysopidae and Syrphidae have a longer and similar development from egg to adult respectively than *C. sanguinea* under average glasshouse temperatures of 20°C to 25°C, aphidophagous predators belonging to the family of Cecidomyiidae have a shorter development than *C. sanguinea* (van Steenis, 1992). Both Chrysopidae and Syrphidae have a longer pupal, and a shorter larval and egg development (measured as a percentage of total development by Honêk & Kocourek (1990)) than these coccinellid species.

Table 1. Developmental times of various stages of *C. sanguinea* under constant temperature regimes. (Figures in brackets show the number of individuals as replication)

Developmental times (Days \pm standard error)								
Stage	17.5°C	20°C	22.5°C	Temperature 25°C	27.5°C	30°C	32.5°C	F and P value
Egg	6.18 \pm 0.03 a (6)	5.02 \pm 0.03 b (6)	3.76 \pm 0.04 c (6)	3.27 \pm 0.05 d (6)	2.47 \pm 0.06 e (6)	2.57 \pm 0.04 e (6)	2.63 \pm 0.02 e (6)	F= 190.1, df= 6, 35 P<0.01
1 st instar	5.79 \pm 0.13 a (25)	3.08 \pm 0.07 b (24)	2.49 \pm 0.04 c (23)	2.17 \pm 0.04 c (23)	1.58 \pm 0.05 d (25)	1.39 \pm 0.09 d (16)	1.52 \pm 0.08 cd (8)	F= 341.4, df= 6, 137 P<0.01
2 nd instar	3.43 \pm 0.14 a (22)	2.22 \pm 0.04 b (21)	1.66 \pm 0.06 c (21)	1.57 \pm 0.06 cd (23)	1.25 \pm 0.05 de (23)	1.03 \pm 0.03 e (16)	0.88 \pm 0.11 e (5)	F= 104.3, df= 6, 124 P<0.01
3 rd instar	4.3 \pm 0.18 a (21)	2.37 \pm 0.06 b (21)	1.79 \pm 0.05 c (20)	1.55 \pm 0.04 cd (22)	1.29 \pm 0.06 de (22)	1.03 \pm 0.10 de (16)	0.88 \pm 0.11 e (5)	F= 125.7, df= 6, 120 P<0.01
4 th instar	6.55 \pm 0.15 a (21)	3.76 \pm 0.03 b (21)	3.30 \pm 0.09 c (20)	2.67 \pm 0.03 d (22)	2.26 \pm 0.03 e (21)	1.83 \pm 0.05 e (16)	2.11 \pm 0.11 e (5)	F= 402.7, df= 6, 119 P<0.01
Prepupa	2.12 \pm 0.06 a (20)	1.39 \pm 0.03 b (20)	1.07 \pm 0.05 c (20)	0.93 \pm 0.02 cd (21)	0.79 \pm 0.03 d (21)	0.79 \pm 0.05 d (16)	0.66 \pm 0.006 d (5)	F= 127.2, df= 6, 116 P<0.01
Pupa	8.77 \pm 0.11 a (17)	5.27 \pm 0.04 b (19)	4.04 \pm 0.07 c (20)	3.48 \pm 0.05 d (20)	2.84 \pm 0.04 e (21)	2.79 \pm 0.07 e (14)	3.0 \pm 0.008 e (5)	F= 931.6, df= 6, 109 P<0.01
Total larva. ¹	19.63 \pm 0.41 a (21)	11.45 \pm 0.1 b (21)	9.22 \pm 0.17 c (20)	7.96 \pm 0.08 d (22)	6.27 \pm 0.09 e (21)	5.29 \pm 0.09 e (16)	5.43 \pm 0.11 e (5)	F= 575.2, df= 6, 119 P<0.01
Overall dev. ²	36.6 \pm 0.52 a (17)	23.15 \pm 0.1 b (19)	18.11 \pm 0.1 c (20)	15.64 \pm 0.1 d (20)	12.34 \pm 0.1 e (21)	11.33 \pm 0.1 e (14)	11.7 \pm 0.006 e (5)	F= 1247, df= 6, 109 P<0.01

Means within rows with the same letter are not significantly different (Tukey test at the 1% level). One way ANOVA was applied for data analysis. ¹ Total larval development time (1st, 2nd, 3rd and 4th instar larva); ² Overall developmental time (from oviposition to adult emergence)

The effect of temperature on the survival of immature stages and overall development of *C. sanguinea* is presented in Table 2. Overall survival was low at high temperatures (30°C and 32.5°C) with a survival rate of 0.56 and 0.2 respectively (Table 2). Whereas, overall survival was high at the temperatures ranging from 17.5°C to 27.5°C in 2.5 increments with an average of 0.78. Overall survival was significantly lower at 32.5°C than at any other of temperatures. The first instar larval stage suffered the highest mortality levels whilst the fourth instar larva had the highest survival rate. These results on survival are similar to that of *Coccinella transversoguttata*, *Coleomegilla maculata* and *Hippodamia convergens* studied by Obrycki & Tauber (1981), (1978) and (1982) respectively.

Table 2. Effect of temperature on survival rate of the various stages of *C. sanguinea*. (First and second values in the brackets show the numbers of surviving individuals at the start of each stage and numbers of individuals at the end of each stage respectively).

Stage	Temperature							χ ² test
	17.5°C	20°C	22.5°C	25°C	27.5°C	30°C	32.5°C	
1 st instar	1 (25/25)	0.96 (25/24)	0.92 (25/23)	0.92 (25/23)	1 (25/25)	0.64 (25/16)	0.32 (25/8)	-
2 nd instar	0.88 (25/22)	0.88 (24/21)	0.91 (23/21)	1 (23/23)	0.92 (25/23)	1 (16/16)	0.63 (8/5)	-
3 rd instar	0.954 (22/21)	1 (21/21)	0.952 (21/20)	0.96 (23/22)	0.96 (23/22)	1 (16/16)	1 (5/5)	-
4 th instar	1 (21/21)	1 (21/21)	1 (20/20)	1 (22/22)	0.95 (22/21)	1 (16/16)	1 (5/5)	-
Prepupa	0.95 (21/20)	0.95 (21/20)	1 (20/20)	0.95 (22/21)	1 (21/21)	1 (16/16)	1 (5/5)	-
Pupa	0.85 (20/17)	0.95 (20/19)	1 (20/20)	0.95 (21/20)	1 (21/21)	0.88 (16/14)	1 (5/5)	
Total larva. ¹	0.84 ab (25/21)	0.84 ab (25/21)	0.8 ab (25/20)	0.88 a (25/22)	0.84 ab (25/21)	0.64 b (25/16)	0.2 c (25/5)	χ ² =33.9 df = 6 P<0.01
Overall Surv. ²	0.68 ab (25/17)	0.76 ab (25/19)	0.8 ab (25/20)	0.8 ab (25/20)	0.84 a (25/21)	0.56 b (25/14)	0.2 c (25/5)	χ ² =43.6 df = 6 P<0.01

Survival rate followed by a different letter in the same row are significantly different.

¹: Total larval survival (1st, 2nd, 3rd and 4th instar larva).

²: Overall survival (Larva + Prepupa + Pupa stage).

Fecundity and life table of C. sanguinea at various temperatures

Oviposition rate (eggs/day) and total fecundity (eggs/days) of *C. sanguinea* for a period of three weeks are presented in Table 3. Oviposition rate and total fecundity were influenced by temperature ($P<0.01$). The total fecundity and mean daily oviposition rate were significantly higher at 25°C and 30°C than at 20°C whilst there was no significant difference in total fecundity and mean daily oviposition rate at temperature of 25°C and 30°C. There was a slight reduction in mean daily oviposition rate and total fecundity at 30°C compared with that at 25°C. The total fecundity of other species of the tribe *Coccinellini* have been reported in the literature. Blackman (1967) and Hämäläinen *et al.*, (1975) reported that the total fecundity of

Adalia bipunctata at 20°C and *Coccinella septempunctata* on *Myzus persicae* at 20°C were 676.2 and 654.4 eggs per female respectively. These results are in the range for that of *C. sanguinea* observed in this study.

Table 3. Oviposition rate (eggs/day) and total fecundity (eggs/days) of *C. sanguinea* for a period of three weeks at various constant temperatures

Temperature (°C)		Oviposition Rate (Eggs /Day) (Mean ± SE)	Total Fecundity (Eggs/Days) (Mean ± SE)
20	5 ¹	21.76 ± 0.54 a	492.2 ± 11.31 a
25	5 ¹	31.41 ± 1.51 b	659.8 ± 31.79 b
30	5 ¹	31.35 ± 0.61 b	658.4 ± 12.71 b
F and P value		F= 21.4, d.f. = 2, 12, P<0.01	F= 21.7, d.f. = 2, 12, P<0.01
LSD value		0.9923	89.72

Means within a column with the same letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied for data analysis.

¹ Number of replicates of pairs of adults for oviposition rate and total fecundity.

Van Steenis (1992) reported that three aphidophagous species, *Syrphus ribesii*, *Chrysoperla carnea* and *Aphidoletes aphidimyza*, belonging the families of Syrphidae, Chrysopidae and Cecidomyiidae respectively have lower average total fecundity than *Adalia bipunctata* and *Coccinella septempunctata*. However, the oviposition rates of *A. aphidimyza* and *C. carnea* are similar to that of these two coccinellids due to their short oviposition periods. The oviposition rate and total fecundity of *C. sanguinea* in this study seem to be almost similar to those of these three aphidophagous species reported by van Steenis (1992). However, some assessments of the role of coccinellids in controlling aphids have attributed lack of successful control to their slow reproductive rate, compared to that of the aphids, although coccinellids have a high reproductive capacity (Majerus, 1994).

Demographic statistics of *C. sanguinea* at various temperatures for a period of three weeks are presented in Table 4. The gross reproduction (GRR) increased with increasing temperature, but the increase of temperature from 25°C to 30°C resulted in only a small increase of GRR. The net reproductive rate (Ro) reached a maximum at 25°C. Ro declined at higher temperatures (30°C). The generation time (T) shortened with increasing temperature and was only 24.5 days at 30°C.

Van Steenis (1992) reported r_m value of 0.11 at 25°C for *Coleomegilla maculata* belonging to tribe Coccinelli. This is lower than value of *C. sanguinea* obtained in this study due to species and dietary differences and the assumption of constant reproduction during oviposition period of *C. maculata*. The intrinsic rate of increase of *Aphidoletes aphidimyza* (Rond.) ($r_m = 0.20$ at 21°C) (van Steenis, 1992) is higher than that of *C. sanguinea*, presumably due to the shorter developmental and oviposition period of *A. aphidimyza*.

An aphid/natural enemy system differs considerably from other pest/natural enemy systems. Pests like leafminers and thrips show initially a discrete population growth, but aphids show a non-discrete population growth, which is almost an exponential increase for a considerable period, especially in glasshouses with constant environmental factors and absence of natural enemies (van Steenis, 1992). The intrinsic rate of increase (r_m) of cotton

aphid, which is the parameter governing the pest population growth, is very high, varying from 0.35 to 0.5 at average glasshouse temperatures of 15°C to 25°C on cucumber (Wyatt & Brown, 1977) and cotton (Liu & Perng, 1987), mostly due to the short developmental period. In this study r_m values of *C. sanguinea* (0.16) was found to be optimum at 25°C. It seems that population growth rates of *C. sanguinea* are much lower than those of cotton aphid, which can result in poor control of cotton aphid. However, as pointed out by Huffaker *et al.*, (1977), it is a common error to conclude that a natural enemy having a lower r_m value than that of its host or prey would be a poor biological control agent. The predator need only possess an r_m high enough to offset that part of the prey's r_m that is not negated by predation.

Table 4. Demographic statistics of *C. sanguinea* at various constant temperatures for a period of three weeks.

PARAMETERS	20°C	25°C	30°C
Gross reproduction (GRR)	275.1	327.9	329.2
Net reproductive rate (Ro)	96.009	154.768	22.056
Capacity for increase (rc)	0.1074	0.1467	0.1169
Intrinsic rate of increase (r_m)	0.1129	0.1581	0.1262
Cohort generation time (Tc)	42.494	34.346	26.447
Generation time (T)	40.421	31.884	24.5049
Finite capacity of increase (λ)	1.1195	1.1713	1.1345
Doubling time (DT)	6.1383	4.3833	5.4905
Number of insects observed (n)	5	5	5

Optimum range of temperatures or humidities for development, reproduction and survival of a candidate biological control agent may be different from that of the pest, and natural enemies may either fail to establish or prove ineffective owing to the direct or indirect effects of climate in the area of introduction (Jervis & Kidd, 1996). In this study the data for development, survival and fecundity indicated that the most favourable temperature for *Cycloneda sanguinea* was found to be in the range of 20°C to 25°C. Therefore, this suggests that *C. sanguinea* may be well adapted to temperate glasshouses, since the glasshouse crops which the cotton aphid attacks are usually grown at temperatures ranging from 15°C to 25°C (Gurney & Hussey, 1970).

Consumption rate and total food consumption of larval instars of *C. sanguinea*

Table 5 gives the total consumption of the larvae in each instar as well as overall development of *C. sanguinea* at three different temperatures. Total consumption of fourth instar and overall development of *C. sanguinea* was not significantly different at 20°C from that at 25°C but they were significantly higher than at 30°C. However, there was a considerable decrease in total consumption of *C. sanguinea* with increasing temperature from 25°C to 30°C. The proportions of total food consumption of each larval stage of *C. sanguinea* at various temperatures are presented in Figure 1. It seems that the total consumption of each larval stage of *C. sanguinea* had slight differences at different temperatures. The fourth instar stage of the species was the most voracious. About 81%-85% of the total food consumption were consumed during the fourth larval instars of *C. sanguinea*. Gurney & Hussey (1970) noted a considerable increase in total food consumption during the entire larval development at lower

temperature by *Coleomegilla maculata* (De G.) and *Cycloneda sanguinea* on *Myzus persicae*. However, Hodek (1973) reported, based on the reports of various authors, that the total food consumption of coccinellid larvae is more or less stable, irrespective of temperature.

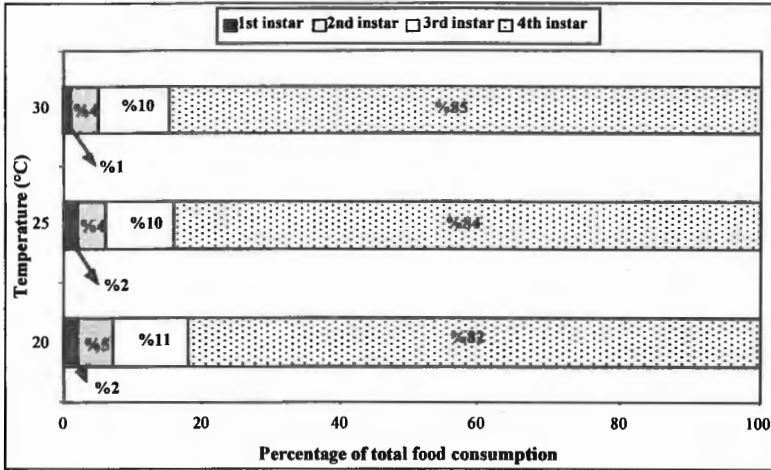


Figure 1. Proportion of food consumption in individual larval stages of *C. sanguinea* at various temperatures

Consumption rates (numbers of aphid consumed/day) of the larval instars as well as overall development of *C. sanguinea* at various temperatures are presented in Table 5. The consumption rate of each larval stage of *C. sanguinea* increased with increasing temperature. However, the increase in consumption rate of overall development of *C. sanguinea* was not significantly different at 25°C than at 30°C.

Fresh weight of aphids consumed by larval instars and overall development of *C. sanguinea* at various temperatures are presented in Table 6. The fresh weights of aphids consumed by fourth instars and overall development of the coccinellid species were significantly higher at 20°C and 25°C than at 30°C whilst they were not significantly different at 20°C than at 25°C. Although given a constant food level, the fresh weight of aphids consumed by larval instars of *C. sanguinea* varied with temperature. The highest fresh weight of aphids consumed by first, second, third and fourth instars and overall development of *C. sanguinea* was found to be at 25°C, 30°C, 25°C, 25°C and 25 °C respectively.

The literature contains a great amount of information on the numbers of prey insects consumed by the larvae of different coccinellid species. It is rather futile to compare the numerical data, because the results were obtained by different methods, and because different species of prey are concerned. Even if the same prey is used in similar conditions, the results may be totally different, depending on the developmental stage of the prey and its abundance. For instance, *C. sanguinea* larvae have been reported to consume an average of 276 cotton aphids at 21°C (Gurney & Hussey, 1970), but in this study it was 1824 at 20°C.

Table 5. Total consumption (numbers of aphid eaten/days) and consumption rate (numbers of aphid eaten/day) of larval instars and overall development of *C. sanguinea* at various temperatures. (Figures in brackets show the number of individuals as replicates)

Temperature	1 st instar		2 nd instar		3 rd instar		4 th instar		Overall development	
	Total consumption	Consumption rate	Total consumption	Consumption rate	Total consumption	Consumption rate	Total consumption	Consumption rate	Total consumption	Consumption rate
20°C COMPOSTIE	18.4±0.79 c (20)	7.72±0.33 b (20)	66.5±2.48 a (20)	38.14±2.54 c (20)	187.9±4.16 a (20)	104.6±5.91 c (20)	1551.4±58.2 a (20)	522.8±37.3 b (20)	1824.1±62.1 a (20)	673.3±42.1 b (20)
25°C COMPOSTIE	31.9±1.26 a (21)	24.3±1.45 a (21)	79.3±2.33 b (21)	67.67±3.34 b (21)	185.7±6.67 a (20)	143.6±9.84 b (20)	1525.3±47.9 a (19)	738.6±22.9 a (19)	1821.6±50.6 a (19)	975.1±27.4 a (19)
30°C COMPOSTIE	26.7±1.62 b (19)	25.17±1.81 a (19)	91.0±3.52 c (19)	115.6±11.1 a (19)	174.7±6.76 a (16)	178.84±14.1 a (16)	1277.2±80.1 b (15)	761.8±59.1 a (15)	1556.2±89.1 b (15)	1066±69.5 a (15)
F and P value	F= 30, d.f.= 2, 57, P<0.01	F= 53.5, d.f.= 2, 57, P<0.01	F= 18.9, d.f.= 2, 57, P<0.01	F= 34.3, d.f.= 2, 57, P<0.01	F= 1.33, d.f.= 2, 53, P=0.272	F= 13.4, d.f.= 2, 53, P<0.01	F= 5.57, d.f.= 2, 51, P<0.01	F= 11.5, d.f.= 2, 51, P<0.01	F= 4.84, d.f.= 2, 51, P<0.05	F= 20.2, d.f.= 2, 51, P<0.01
LSD value	3.5607	3.8004	7.8958	18.656	-	28.365	174.6	113.38	188.29	131.78

Means within a column with the same letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied for data

Table 6. Fresh weight of aphids consumed by larval instars and overall development of *C. sanguinea* at various temperatures. (Figures in brackets show the number of individuals as replicates)

Temperature	1 st instar	2 nd instar	3 rd instar	4 th instar	Overall development
	Fresh weight of aphid consumed (mg)	Fresh weight of aphid consumed (mg)	Fresh weight of aphid consumed (mg)	Fresh weight of aphid consumed (mg)	Fresh weight of aphid consumed (mg)
20°C	0.77±0.033 c (20)	2.79±0.1 c (20)	7.89±0.17 b (20)	62.1±2.33 ab (20)	72.8±2.49 a (20)
25°C	1.28±0.052 a (21)	3.17±0.09 b (21)	9.28±0.33 a (20)	68.6±2.15 a (19)	72.9±2.02 a (19)
30°C	1.12±0.068 b (19)	3.82±0.15 a (19)	9.08±0.35 a (16)	57.5±3.61 b (15)	62.72±3.35 b (15)
F and P Value	F= 25.1, d.f.= 2, 57, P<0.01	F= 20.5, d.f.= 2, 57, P<0.01	F= 7.04, d.f.= 2, 53, P<0.01	F= 4.3, d.f.= 2, 51, P<0.05	F= 4.7, d.f.= 2, 51, P<0.05
LSD value	0.149	0.3221	0.8287	7.5402	7.3794

Means within a column with the same letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied for data.

Functional response of female adults of *C. sanguinea* at various temperatures

Effect of the aphid density and temperature on number of aphids killed by the female adults of *C. sanguinea* for a period of 24 h are presented in Table 7. The number of aphids killed increased significantly with increasing aphid density at 25°C and 30°C whilst it increased significantly with increasing aphid density up to 240 aphids/64 cm² at 20°C. Thus, there was no significant difference between number of aphids killed at aphid densities of 240, 480 and 960 at 20°C whilst there was a significant difference in number of aphids killed at the aphid densities at 25°C (except first three aphid densities of 20, 40, and 80) and 30°C. The number of aphids killed was significantly higher at 25°C and 30°C than at 20°C at each aphid density (except at aphid density of 20 and 40) whilst there was significant difference between the number of aphids killed at 25°C and 30°C at the last two aphid densities (aphid density of 480 and 960). At the lowest aphid densities (aphid densities of 20 and 40 for *C. sanguinea*), all predators killed all the aphids offered.

We simply fit type II model (disc equation) at 20°C, 25°C, and 30°C. Thereafter, the parameters were estimated for type II model (disc equation). The **Th** (handling time) from type II disc equation decreased with increasing temperature (Table 8). Whereas, the **a** (attack constant) estimated from disc equation increased with increasing temperature from 25°C to 30°C whilst it decreased with increasing temperature from 20°C to 25°C. They had a higher **Th** and **a** estimated from the disc equation at low temperature (20°C) than at high temperatures (25°C and 30°C). *C. sanguinea* had the low handling times (**Th**) estimated from disc equation at each temperature, but *C. sanguinea* had the high attack constants (**a**). *C. sanguinea* was shown to have a high number of aphids eaten at each temperature, apparently due to its great voracity. Morales & Burandt (1985) reported that the number of the brown citrus aphids, *Toxoptera citricida*, killed by the female of *C. sanguinea* increased with greater prey density at 25°C and 42.6 brown citrus aphids were killed at the highest density (aphid density of 50). This result is much lower than that found in this study for *C. sanguinea*. This could be due to differences in aphid species used in the experiment since the brown citrus aphid is a much bigger species than the cotton aphid used in this study.

The graphic presentation of observed and predicted values fitted to the type II model (Figure 2) indicated that the number of aphids killed by this coccinellid species increased more rapidly with increasing number aphids at the higher temperatures (25°C and 30°C) than at the lower temperature (20°C). It also suggested that the asymptotic numbers of prey killed by both coccinellid species at high aphid numbers were greater at the higher temperatures (25°C and 30°C) than at the lower temperature (20°C).

Cycloneda sanguinea showed significantly short handling time and low search rate. The female adults of *C. sanguinea* which take less time to process the cotton aphids and are efficient searchers, required high number of cotton aphids to reach satiation, especially at high temperatures. Therefore, *C. sanguinea* can be effective at suppressing the cotton aphid populations when the cotton aphids are greater and the predator is likely to be limited primarily by handling time. On the other hand, the functional responses of predator tend to level off due to satiation, causing the maximum predation rate to settle at much lower values than expected from the predator's time budget for handling and searching (Sabelis, 1992). Therefore, this can limit the success of cotton aphid control by these coccinellid species.

Mills (1982) reported that having long periods of inactivity due to satiation can be a reason why most aphidophagous coccinellids are unsuccessful in biological control compared with a number of coccidophagous coccinellids that are generally small and feed almost continuously. *C. sanguinea* is more likely to spend a long period in inactivity (digestion and

cleaning) due to satiation. Therefore, having long periods of inactivity due to satiation can also limit the success of cotton aphid control by these coccinellid species.

Table 7. Effect of aphid density and temperature on the number of aphids killed for a period of 24 h by the female adults of *C. sanguinea* at various temperatures. (Figures in brackets show the number of individuals as replicate)

Aphid density/64 cm ²	20°C	25°C	30°C	F and P Value	LSD value
20	20.0 ± 0 cA (7)	20.0 ± 0 fA (5)	20.0 ± 0 gA (8)	-	-
40	40.0 ± 0 cA (4)	40.0 ± 0 efA (8)	40.0 ± 0 fA (7)	-	-
80	78.0 ± 1.3 bA (5)	80.0 ± 0 deA (6)	80.0 ± 0 eA (7)	F = 3.4, d.f. = 2, 15, P = 0.0612	-
120	94.4 ± 10.1 bB (5)	119.7 ± 0.3 dA (6)	118.3 ± 1.0 dA (8)	F = 8.41, d.f. = 2, 16, P < 0.01	13.91
240	166.6 ± 18.1 aB (5)	220.4 ± 12.3 cA (5)	235.0 ± 7.6 cA (7)	F = 8.4, d.f. = 2, 14, P < 0.01	37.68
480	161.6 ± 14.3 aC (5)	354.4 ± 45.4 bB (5)	457.8 ± 10.1 bA (5)	F = 28.7, d.f. = 2, 12, P < 0.01	86.48
960	165.0 ± 11.0 aC (5)	406.4 ± 12.9 aB (5)	534.8 ± 17.5 aA (5)	F = 177, d.f. = 2, 12, P < 0.01	43.38
F and P Value	F = 39.7, d. f. = 6, 29, P < 0.01	F = 89.3, d. f. = 6, 33, P < 0.01	F = 929, d. f. = 6, 40, P < 0.01	-	-
LSD value	29.429	46.299	18.274	-	-

Means within a column with the same lower-case letter and a row with the same upper-case letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied to data.

Table 8. Estimated type II response parameters for the female adults of *C. sanguinea* for a period of 24 h at various temperatures. The type II functional response equations was disc equation ($Ne = [aN_0T/(1 + aNTh)]$). ¹ 95% confidence interval. Time units are hours.

Temperature (°C)	Parameters			r ²
	a ± SE (95% C.I.) ¹	Th ± SE (95% C.I.) ¹	Maximum number of prey attacked (T/Th)	
20°C	0.071 ± 0.016 (0.028-0.11)	0.12 ± 0.012 (0.087-0.15)	200	0.984
25°C	0.058 ± 0.0067 (0.041-0.075)	0.038 ± 0.0037 (0.029-0.048)	631.58	0.994
30°C	0.059 ± 0.009 (0.034-0.083)	0.025 ± 0.0044 (0.014-0.036)	960	0.989

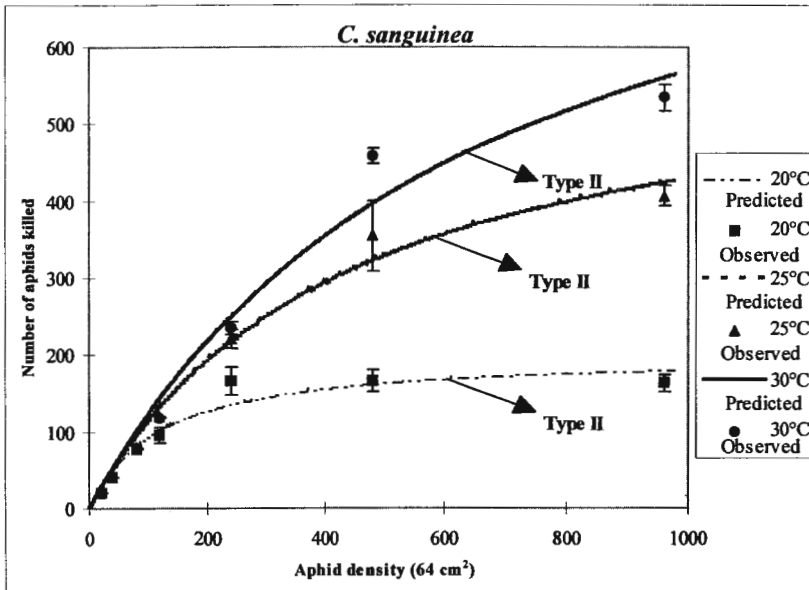


Figure 2. Observed number of aphids killed by *C. sanguinea* on different aphid densities for a period of 24 h, and the type II functional response disc equation curve fit by non-linear least squares at various temperatures. Five to eight individuals as replicates were used for *C. sanguinea*. \bar{x} = standard error of mean number of aphids killed

In conclusion, *C. sanguinea* provides some desirable criteria for pre-introductory evaluation for biological control of cotton aphid in glasshouses in temperate regions: (1) good climatic adaptation, (2) high reproductive potential, (3) high voracity, and (4) no negative effects (mainly aphidophagous species). These characteristics suggest that *C. sanguinea* can be a possible candidate for biological control of cotton aphid in glasshouses. However, even if this species seems to be suitable according to the criteria, this does not necessarily imply that it is able to control cotton aphid under glasshouse conditions. Therefore, studies should be conducted on the evaluation of this coccinellid species under practical conditions for its use in glasshouse biological control of cotton aphid. Moreover, the problem with using coccinellids for augmentative biological control is that most coccinellids are not cheap to mass rear in captivity. Therefore, finding cheap and easy methods for mass rearing of this species would be essential to make biological control of cotton aphid with this coccinellid economically viable.

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The use of open rearing units or «banker plants» against *Aphis gossypii* Glover in protected courgette and melon crops in Roussillon (South of France)

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Abstract: The introduction of aphid parasitoids in courgette (*Cucurbita pepo*) and melon crops by repeated inoculative releases is in general not very satisfactory. The efficiency of this method is not always sure, caused especially by the extreme rapid development of *Aphis gossypii*. Moreover, the need of several introductions of the parasitoids makes this method often too expensive for the growers. A method for introducing *Aphidius colemani* against *A. gossypii* by means of banker plants is proposed.

Keywords: IPM, bankerplants, *Aphis gossypii*, *Aphidius colemani*, melon, courgette

Introduction

One of the main insect pests in cucurbits like cucumber, melon and courgette in southern France is the aphid *Aphis gossypii*. Chemical control gets more and more difficult caused by the important tolerance of *A. gossypii* against many insecticides (Gubran *et al.*, 1990). Another important reason is the consumer's demand for less pesticide residues.

Traditionally, biological control against this aphid is carried out with the introduction of the hymenopteran parasitoid *Aphidius colemani*. One method consists of the introduction of the parasitoids after the first aphids have been detected in the culture. Several introductions take place until a stabilisation of the aphid population has been reached. The results obtained by using this method are often not very satisfactory caused by the extreme rapid development of the aphid population and moreover the number of introductions of the parasitoid makes this method generally too expensive for the growers. Since 1992 different authors have demonstrated the efficiency of a banker plant (open rearing) system in cucumber (Bennison 1992, Steenis 1995, Fischer 1997).

In 1995 this system has been introduced in the Roussillon (South France) (Schoen *et al.*, 1997) in melon and courgette cultures, at first in trial greenhouses but since 1997 in regular production greenhouses. The rearing of banker plants is now in the hands of commercial plant growers and can be considered like a normal commercial biological control system.

A natural millet *Eleusine coracana* originally growing in warmer regions has been chosen as host plant for the cereal aphid *Rhopalosiphum padi*. This millet endures very well the high spring and summer temperatures in plastic tunnels contrary to cereals like wheat or oat (Fisher 1997). *R. padi*, which is not able to infest cucurbits, is used like substitution host for *A. colemani*. Once *R. padi* has infested the millet, *A. colemani* is released. When the first mummies of the parasitoid appear, the banker plant is ready for use and can be transplanted in the production greenhouse.

The advantages of this system are multiple: (1) less parasitoids to introduce, (2) presence of the beneficials before the arrival of the aphids, and (3) permanent release of parasitoids.

Material and methods

The introduction of the banker plant in the production greenhouse is carried out 15 days after plantation of the concerned crop, and repeated 15 days later. The number of introduced bankerplants is 1 per 100m².

In these greenhouses 15 plants are randomly chosen. On every plant 5 old leaves and 5 young leaves arbitrary chosen at the moment of the counting and are observed on the presence of pest insects and beneficials every week.

The number of aphids is counted following a class system (Table 1) *A. colemani* is counted in real numbers. An example of this counting system is given in Fig. 1

For extension service purposes an easier and quicker way of counting is used. This counting method is based on the former described one. In this method control zones are established, at least 3 zones per greenhouse. In every determined zone, 3 observations are carried out at every counting just by looking at the underside if a whole tendril (for melon) or the all the leaves of one plant (for courgette).

For the aphids the same classes are used, for the *A. colemani* mummies we use the classes described in table 2.

Some examples are showed for melon (Fig. 2 and 3) and courgette (Fig. 4 and 5)

Table. 1 Counting classes of *A. gossypii*

Class	
0	0 aphids
1	some isolated aphids
2	2 or 3 small isolated colonies or many isolated aphids
3	many colonies and honeydew (less than 50% of the leaf surface)
4	many important colonies,

Table 2. Counting classes of *A. colemani* mummies

Class	
0	0 mummies
1	some mummies on one leaf
2	mummies on several leaves

Results and discussion

These results shows a classical development in courgette. The first aphids arrive at 05/05/98 and the number increases rapidly (class 2 observations gets very important on 19/05/99) The first *A. colemani* mummies show up the 14th of Mai and reach an important level 15 days later (29/05/98). From that moment on, certain equilibrium installs, and the number of aphids decreases to establish an acceptable level.

The below graphs show the very good efficiency of the banker plants introduction system. From the moment that the aphid population is stable or decreases the introduction of *A. colemani* is considered as successful and no chemical interventions are needed. The bankerplant introduction method seems to be a very helpful tool in the biological control of

aphids in melon and courgette. The "rapid counting method" seems good compromise for practical use by extension services.

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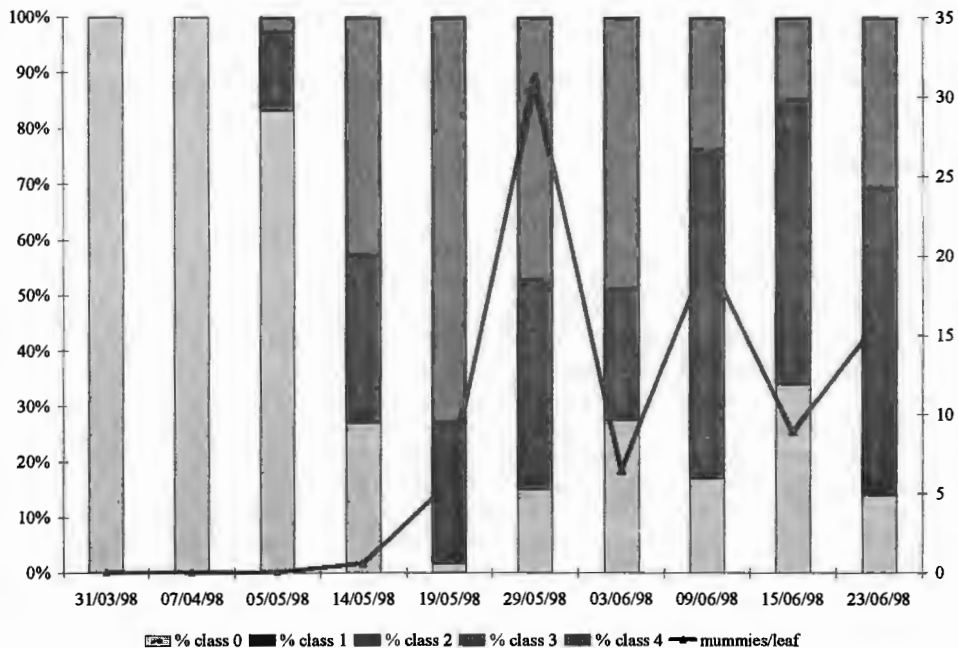


Figure 1. Percentage of different classes for *A. gossypii* and number of *A. colemani* in courgette

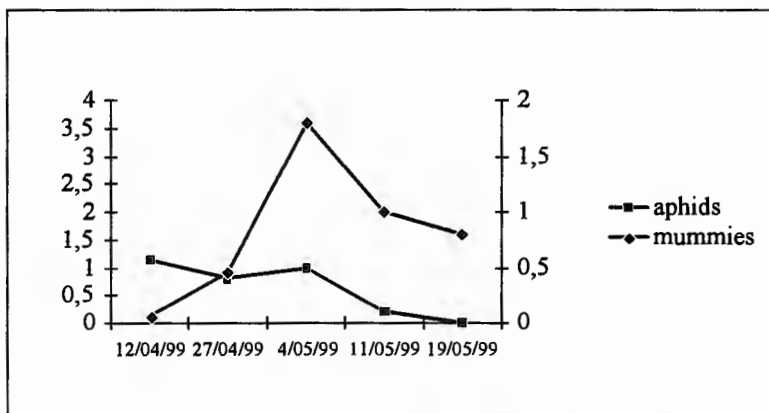


Figure 2. Development of populations of *A. gossypii* and *A. colemani* in a 2000 m² greenhouse of melon (average of 3 observations zones)

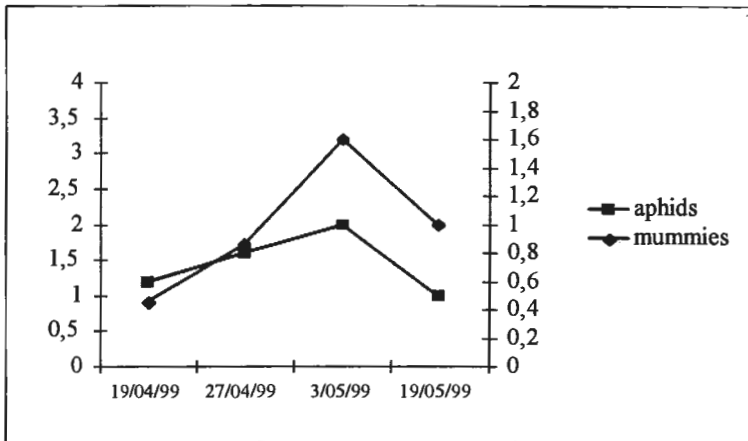


Figure 3. Development of populations of *A. gossypii* and *A. colemani* in a 8000 m² greenhouse of courgette (average of 5 observations zones)

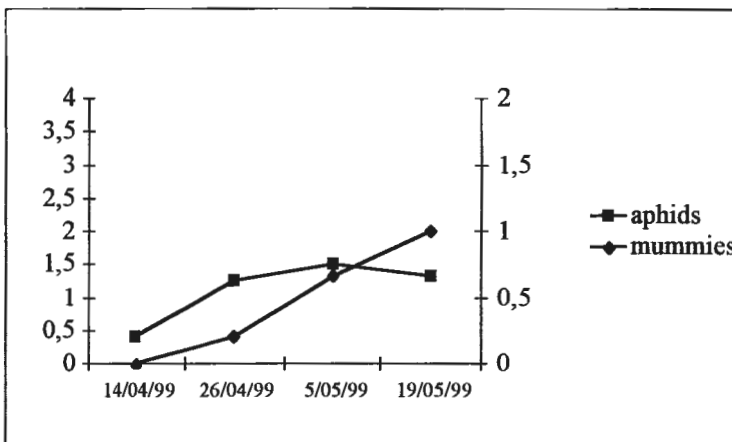


Figure 4. Development of populations of *A. gossypii* and *A. colemani* in a 12000 m² greenhouse of courgette (average of 6 observations zones)

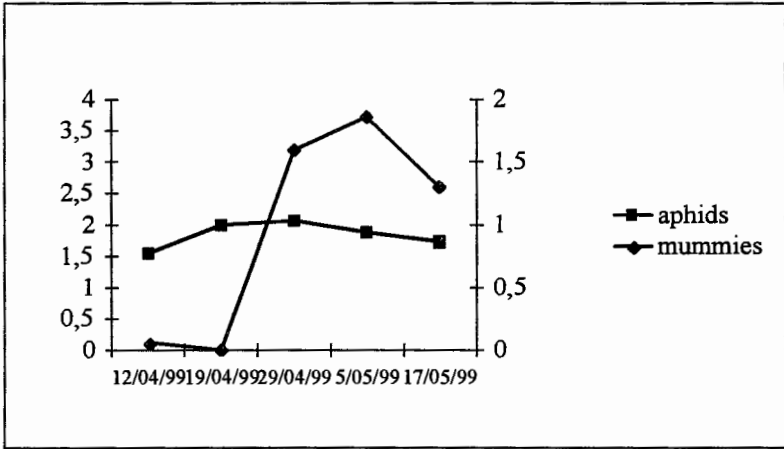


Figure 5. Development of populations of *A. gossypii* and *A. colemani* in a 6000 m² greenhouse of courgette (average of 4 observations zones)

The effect of *Amblyseius longispinosus* Evans (Acarina: Phytoseiidae) on *Tetranychus cinnabarinus* Boisd. (Acarina: Tetranychidae) on different cucumber cultivars

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Abstract: Effectiveness of *Amblyseius longispinosus* to control *Tetranychus cinnabarinus* was studied in greenhouses for two years. The experiments were conducted during spring and fall periods in 1992, and only in spring in 1993. Two cvs. Sahara F₁ and Alfons F₁ were used in 1992, only one, Sahara F₁ in 1993. Completely randomised block design with four treatments including the control was set up. Each plot consisted of thirty two plants, and with release rates of (prey:predator) 5:1, 15:1 and 30:1 per leaf. Each plot consisted of four rows with 8 plants. A row of plants was left as a block to prevent spreading of predator and prey from one plot to the other. Counts were made on 4-cm² area from the bottom side of each leaf. Twenty-five leaves from each plot were sampled randomly. Experiments were carried out for 5-7 weeks and then terminated due to the death of all plants in control plots. Experiments were carried out for 5-7 weeks and then terminated due to the death of all plants in control plots. *A. longispinosus* controlled *T. cinnabarinus* populations at 5:1 ratio on Sahara F₁ cv in spring of 1992 and 1993. But prey populations were much higher on Alfons F₁ cv in spring of 1992 compared to Sahara F₁ cv. Although predator populations were low and similar in all predator release plots in fall of 1992, there was some indication of the predator pressure on prey at 5:1 ratio on both cultivars. The differences between spring and fall experiments showed that the ability of *A. longispinosus* to regulate prey populations was influenced by temperature.

Key words: *Amblyseius longispinosus*, *Tetranychus cinnabarinus*, greenhouse, predator: prey ratio.

Introduction

Amblyseius longispinosus Evans is one of the important phytoseiid mite and its predatory ability to control mites tetranychid were studied under laboratory, greenhouses and open conditions (Mallik, 1975; Sengonca & Lababidi, 1987; Nakagawa 1991).

In Eastern Mediterranean area of Turkey, the Carmine spider mite, *Tetranychus cinnabarinus* Boisd. is one of the main pests in both greenhouses and open fields (Kazak 1991; Yigit & Erkilic, 1992). Due to its ability to develop resistance to a wide range of pesticides, biological control programs have been initiated in greenhouses (Kazak *et al.*, 1992a Kilincer *et al.*, 1992). *A. longispinosus* was imported from Bonn University, Bonn, Germany and included in these programs (Zaman *et al.*, 1990; Kazak *et al.*, 1992b; Colkesen *et al.*, 1994).

Main objective of the research reported here was to determine the effectiveness of *A. longispinosus* to regulate *T. cinnabarinus* populations at different predator:prey ratios on different greenhouse cucumber cultivars in spring and fall period.

Material and methods

Effectiveness of *A. longispinosus* to control *T. cinnabarinus* on different cucumber cultivars was studied in greenhouse condition for two years. The experiments were conducted during spring and fall periods in 1992, and only in spring in 1993. Two cvs. Sahara F₁ and Alfons F₁ were used in 1992, only Sahara F₁ in 1993. Three ratios of predator:prey; 5:1, 15:1 and 30:1 were established during release. Completely randomised block design with four treatments including the control was set up. Each plot consisted of four rows with 8 plants. A row of plants was left as a block to prevent contamination of predator and prey from one plot to the other.

In order to obtain the certain predator and prey ratios, 30 mated females of prey were released on each plant at six-leaf stage. Six, 2 and 1 mated female of *A. longispinosus* were released on each plant to obtain 5:1, 15:1 and 30:1 prey:predator ratios, respectively. Prey and predator were released at the same time on April 9, 1992; November 5, 1992, and April 14, 1993, respectively. One plot without predator release was left as control. Counts were initiated one week after the release. Twenty-five leaves from each plot were sampled randomly by taking three leaves from top, middle and bottom strata of the plants. All stages of the predator and the prey were counted on only on 4 cm² areas on bottom side of each leaf by hand-lens. Experiments were carried out for 5-7 weeks and then terminated due to the death of all plants in control plots. All predators (originated from Bonn University) and prey in these experiments were taken from laboratory cultures maintained for more than one year at Department of Plant protection, Agricultural Faculty, Cukurova University, Adana, Turkey, under the conditions of 25±1°C temperature and %60±10 RH with L:D of 16:8.

Results and discussion

Spring 1992 and 1993

The population development of *A. longispinosus* and *T. cinnabarinus* on Sahara F₁ cv. is given in Figure 1. The population density of prey in control plot was very similar for the first 21 days to the predator release plots then increased gradually after 28th day and reached to a peak of 92.6/4 cm² on the 49th day. The predators were not observed in 30:1 ratio during the first two weeks and remained at low levels during the rest of the experiment, and seemed inefficient to suppress the prey population. In 15:1 ratio the population of predator increased after the increase of prey population on the 28th day and then reached to a peak on 49th day. The population development of predator was not affected by Chlorpyrifos-ethyl 480g/l, which was applied locally for *A. gossypii* control. In 5:1 ratio, the population development was faster compared to other predator release plots but reached to a peak at the same time with 15:1 plot. At 5:1 and 15:1 ratios, predator was able to respond to the increase of prey populations. But decrease in prey populations was only observed 5:1 ratio.

The population development of prey and predators is given Figure 1 for Alfons F₁ cv. Prey populations were much higher on this cultivar compared to Sahara F₁ cv. Although prey density was comparatively lower at 5:1 ratio from other plots, predator were not able to suppress prey populations even at this ratio. The number of prey in control plot reached to 200.1/4 cm² and 311.7/4 cm² at 35th and 42nd days, respectively.

The population development of predator and prey in all plots on Sahara F₁ cv. is given in Figure 2 for spring 1993. The prey in control plot reached to 110.7/4 cm² on the 37th day. The predator seemed inefficient to suppress the prey populations at 30:1 ratio. At 15:1 ratio the predator population kept prey populations at low levels on 41st day. The predator was highly effective to control prey populations at 5:1 plot.

Mallik *et al.*, (1998) reported that *Tetranychus urticae* on rose could be effectively controlled with the predator *A. longispinosus*. Manjunatha & Puttaswamy (1993) found that *Oligonychus indicus* could be eliminated with the predator *A. longispinosus* in the ratios 10:1, 20:1, 30:1, 40:1 and 50:1 in 12, 18, 20, 24 and 30 days, respectively, under laboratory conditions at 24.5 to 28.7°C. The critical initial ratio was given 43:1 and 32:1 at 25°C and 20°C, respectively, for *A. longispinosus* to suppress the *Tetranychus kanzawai* populations on bean plants (Hamamura, 1986). But our results indicated that *A. longispinosus* could suppress the prey populations only at low prey densities. Petrova & Hramejeva (1989), also reported that *A. longispinosus* was effective at low prey densities. These differences might also be reflected from the host plants, since the ability of *A. longispinosus* to control the *T. cinnabarinus* was even different between cultivars utilised in this study.

Fall 1992

The population development of predator and prey in all plots on Sahara F₁ cv. is given in Figure 3. Tridimefon at a dosage of 75-100gr/100lt was applied against powdery mildew of cucurbits to all plots on the first week. The prey population in control plot increased steadily and then showed erratic changes until 40th day and then continued to increase again reaching a peak of 50.8/4 cm². Although predator populations were low and similar in all predator release plots, there was some indication of the predator slightly being effective to suppress the prey at 5:1 ratio for Sahara F₁ plot.

The population development of predator and prey in all plots for Alfons F₁ cv. is given in Figure 2. The prey populations in control plot increased rapidly and reached to high levels on 40th and 47th days. The predator populations were similar with that of Sahara F₁ plots.

The reason for the low population densities in all plots on two cultivars might be the lower temperature conditions since experimental units were not heated. Nakagawa (1991) found that *A. longispinosus* was able to suppress *T. kanzawai* population at 30°C at 30:1 initial prey:predator ratio, but was not effective on prey population at 25 and 20°C. Mori & Saito (1979) reported that *A. longispinosus* could not suppress *T. urticae* population in fall. Hamamura (1986) found that *A. longispinosus* was not able to regulate *T. kanzawai* population at 15°C even at 8:1 initial prey:predator ratio. Colkesen *et al.*, (1994) showed that populations of *A. longispinosus* reached to peak in relatively shorter time at 25°C than 20°C in laboratory.

Our results and above mentioned studies indicate that ability of *A. longispinosus* to regulate prey populations is highly influenced by temperature. Host plant may also play important role on its effectiveness.

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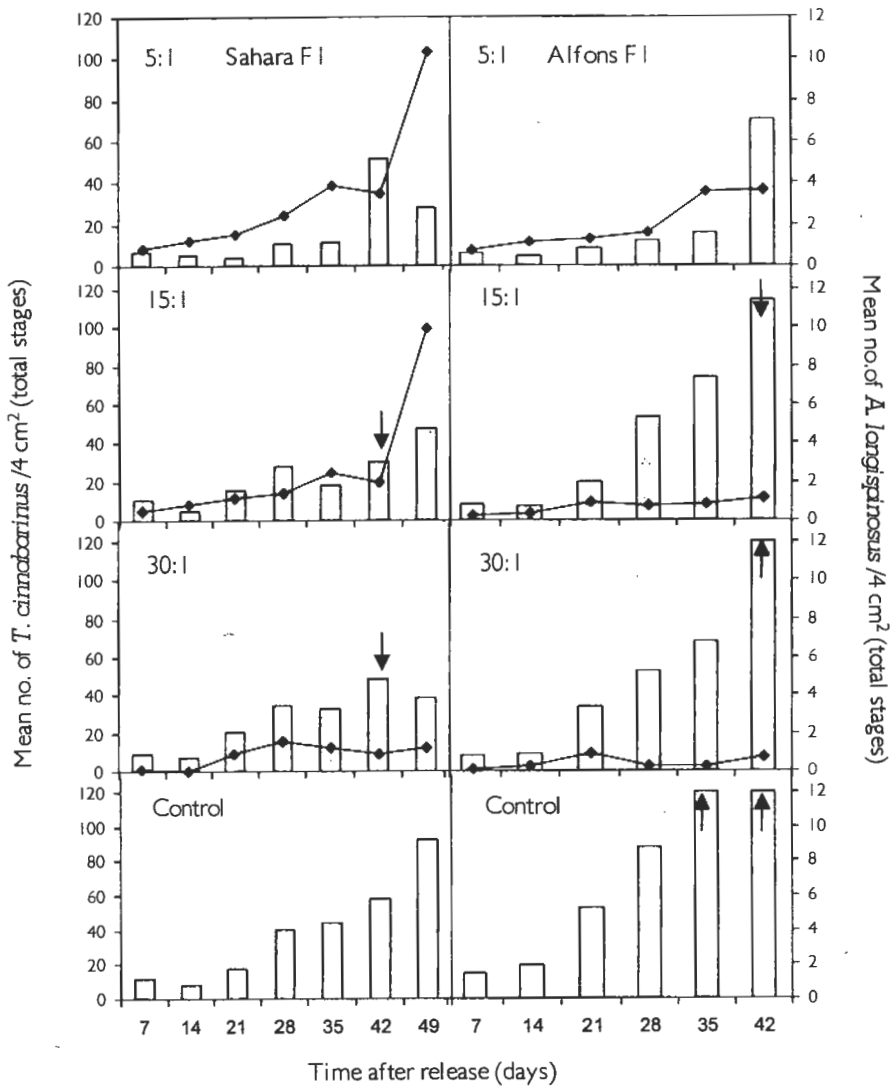


Figure 1. Mean number of *Tetranychus cinnabarinus* (▨) and *Amblyseius longispinosus* (—◆) at different initial prey-predator ratios on Sahara F₁ and Alfons F₁ cvs. during spring of 1992

↓ Chlorpyrifos-ethyl 480g/l applied against *Aphis gossypii*

↑ Mean number of prey was 181.00/4 cm² at 30:1 (Alfons F₁)

↑ Mean number of prey were 200.08/4cm² and 311.72/4cm² in control (Alfons F₁)

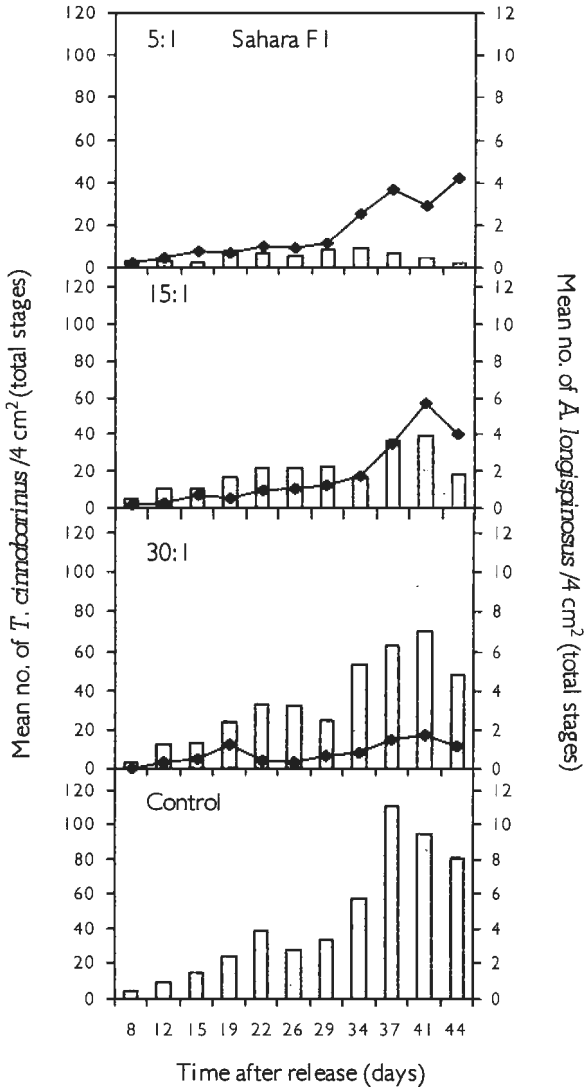


Figure 2. Mean number of *Tetranychus cinnabarinus* (▨) and *Amblyseius longispinosus* (—◆) at different initial prey-predator ratios on Sahara F₁ cv. during spring of 1993

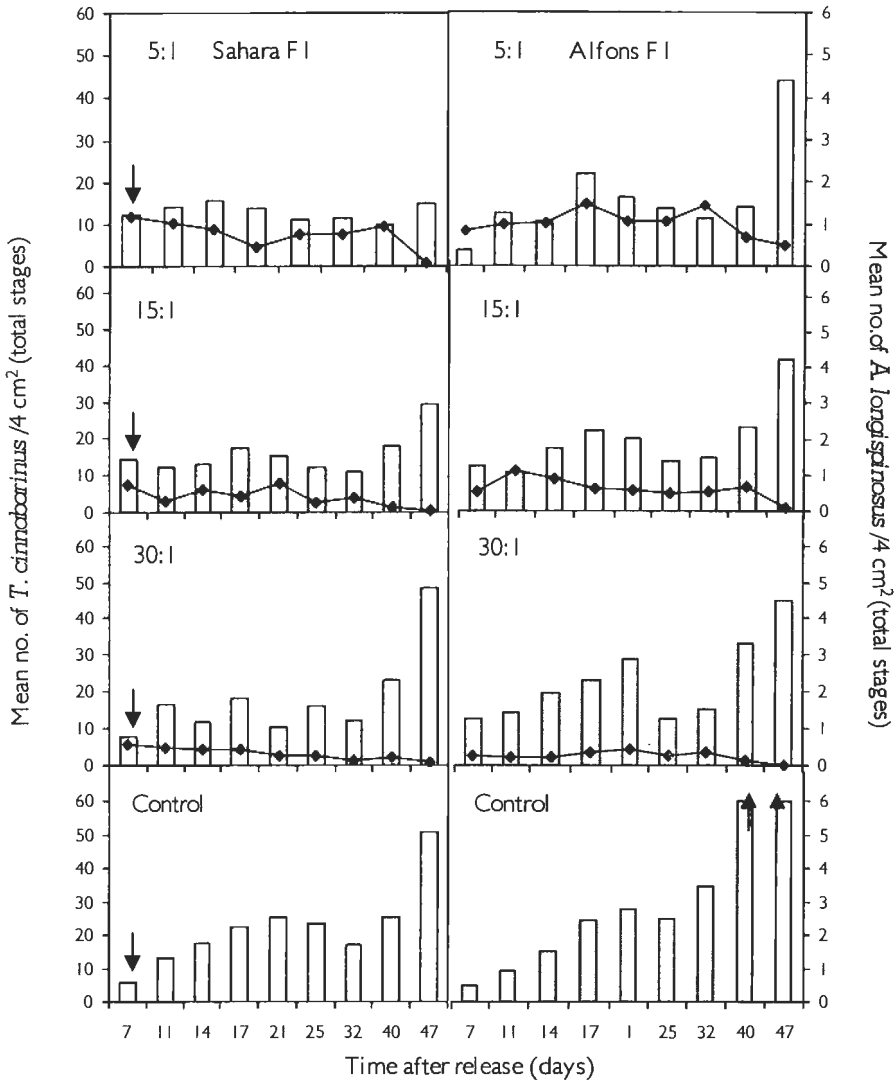


Figure 3. Mean number of *Tetranychus cinnabarinus* (▨) and *Amblyseius longispinosus* (—●—) at different initial prey-predator ratios on Sahara F₁ and Alfons F₁ cvs. during fall of 1992.

↓ Tridimefon 75-100gr/100lt applied against powdery mildew of cucurbits
 ↑ Mean number of prey were 74.00 /4 cm² and 98.72/4 cm² in control (Alfons F₁).

The population dynamics and predation of Hatay strain of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) on the prey *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae); effects of different initial prey and predator ratios on greenhouse cucumbers

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Abstract: The effectiveness of Hatay strain of *Phytoseiulus persimilis* on *Tetranychus cinnabarinus* was studied at three different initial prey and predator ratios (10:1; 20:1; 30:1) in comparison with sprayed and unsprayed control plots on greenhouse cucumbers. The lowest *T. cinnabarinus* population occurred in abamectin sprayed control plot, followed by 10:1, 20:1, 30:1 initial prey and predator ratios and unsprayed control, respectively. Throughout the experiment, the total average numbers of *T. cinnabarinus* were 49.00, 5.66, 8.20, 10.20 and 1.45 per cm² for unsprayed, 10:1, 20:1, 30:1 prey : predator ratios and sprayed control plot, respectively. According to given order above, total average numbers of *P. persimilis* were found to be 0.05, 0.36, 1.02, 0.77 and 0.02 per cm². Results showed that Hatay strain of *P. persimilis* was as effective as the other strains tested on different crops in earlier experiments to control *T. cinnabarinus* on greenhouse-grown cucumbers.

Key words: *Phytoseiulus persimilis*, strain, biological control, Turkey

Introduction

Phytoseiulus persimilis Athias-Henriot was first described by Athias-Henriot (1957) from Algerian specimen in 1957. Afterwards, *P. persimilis* has been found in other Mediterranean countries such as Italy-Sicily (Lombardini 1959), Lebanon (Dosse, 1967), Israel (Swirski & Amitai, 1968), Southern France (Rambier, 1972), and Greece (Swirski & Ragusa, 1977). But the surveys on phytoseiids of Turkey showed no indication of presence of *P. persimilis* until 1989 (Sekeroglu & Kazak, 1993).

In Turkey, *P. persimilis* was first detected on *Malva neglecta* Wallr. associated with *Tetranychus cinnabarinus* in 1989 in Kaledran, a coastal settlement in the center of Mediterranean region of Turkey, in small patchy habitat (10 m²) by pebbled sea shore. In following surveys *P. persimilis* was encountered in Alanya, 80 km west of Kaledran along Mediterranean coast on *Solanum melongena* L. in association with *T. cinnabarinus* in the same year. But the following surveys aimed to find *P. persimilis* at the mentioned two place above were unsuccessful due to habitat destruction (Sekeroglu & Kazak, 1993).

The third place where *P. persimilis* occurred was in Antakya situated at far east section of Mediterranean region of Turkey bordered with Syria. First detection of *P. persimilis* in this region was in 1991 on *S. melongena*. But following surveys clarified that in contrast the other two locations *P. persimilis* had very wide distribution within an area of 20 km radius, with well established colonies on both natural and agricultural plants; e.g. *M. neglecta*, *S. melongena*, *S. nigrum* L., *Lycopersicon lycopersicum* (L.) Karst. & Farw, *Phaseolus vulgaris*

L. associated with *T. cinnabarinus*. The colonies were present all year long (Sekeroglu & Kazak, 1993).

In order to determine the efficiency of *P. persimilis* in greenhouse crops in Eastern Mediterranean of Turkey, imported strains of this predatory mite was used in experimental basis until 1997. Imported strain of *P. persimilis* showed very promising results on different greenhouse-grown vegetables but the continuous rearing of the same strain sometimes reduced the effectiveness of released predator due to genetic selection.

For this reason, studies initiated to determine the performance of field collected wild population of *P. persimilis*. The population development and effectiveness of naturally occurring *P. persimilis* on *T. cinnabarinus* were studied at three different initial prey-predator ratios in comparison with sprayed and unsprayed control plots on greenhouse grown cucumber in Adana in 1998.

Material and methods

In order to establish predator mite culture, adults of *P. persimilis* were collected from *Tetranychus* spp. infected bean plants in the area of Samandag-Hatay during October 1998. Predatory mite was reared in a climate room on bean plants (cv. Barbunia) infested with *T. cinnabarinus*.

The experiments were carried out in 1999, in an experimental greenhouse at Plant Protection Department of Cukurova University in Adana; cucumber plants (cv. Afrodit) were grown on regular soil medium. Pruning, irrigation, fertilization and harvesting were conducted according to general practices among commercial cucumber growers.

Plots consisted of five 6-plant rows, with 75 cm between rows and plants with at least 40 plants for each initial prey-predator ratio (10:1; 20:1 and 30:1), unsprayed and sprayed control. Plots were separated from each other with 2 non-released rows as barrier. When cucumber plants reached to 3-leaf stage, required number of gravid females of *T. cinnabarinus* and *P. persimilis* were introduced on to each plant using a 000 camel-hair brush. Twenty gravid females of *T. cinnabarinus* were also released to unsprayed control. Sprayed control plot not received any prey or predator release but treated once with abamectin (25 ml/100 lt.) at the beginning of the experiment. No additional release of prey and predator were made throughout the experiment, and all the plots were sprayed with selective fungicide when needed to keep cucumber plants disease free [penconazole (25 cc/100 lt), dimethomorp+mancozep (200 gr/100 lt.)].

Sampling was started week after the prey and predator release. Randomly selected 15 cucumber leaves from each plot were collected at different height of the plants then wrapped by paper towel in order to prevent moisture build-up; put into plastic ice bags and returned to laboratory. Each leaf was separately brushed on to 15 cm diameter sub-sectioned glass plate by using Mite Brushing Machine (Leedom Engineering, CA-USA). Counts were made under binocular microscope for 10 one-cm² areas. The average number of total (including egg and motile stages) prey and predator obtained for one-cm² area were used for the presentation of the data.

Analyses of variance were conducted on the total average number of *T. cinnabarinus* and *P. persimilis* using a One-Way Anova after log(x+1) transformations. Means that differed at the 0.05 level of significance were separated by Duncan's multiple range test.

Results and discussion

Total mean number of *T. cinnabarinus* and *P. persimilis* are given in Table 1 for different initial prey-predator ratio, unsprayed and sprayed control plots.

Throughout the experiment, the highest mean number of *T. cinnabarinus* was determined to be 49/cm² in unsprayed control plot, which was statistically different from all other applications (Table 1). Total mean numbers of *T. cinnabarinus* were 10.20 and 8.20/cm² at 30:1 and 20:1 ratios, respectively. No statistical differences were found between these two ratios. At 10:1 initial prey-predator ratio, the total mean number of *T. cinnabarinus* was found as 5.66/cm² which was statistically lower than that of all but sprayed plot. Throughout the experiment, the lowest total mean number of spider mite was observed in abamectin sprayed control plot with 1.45 total stages/1 cm² (Table 1).

The total mean numbers of *P. persimilis* were 0.05, 0.02, 0.36, 1.02 and 0.77 total stages per cm² area for unsprayed, sprayed, 10:1, 20:1 and 30:1 ratios, respectively (Table 1). Statistical analyses indicated that total mean number of *P. persimilis* were significantly different from each other for all plots except 20:1 and 30:1 ratios; there was not any differences between these plots (Table 1).

Table 1. Total mean number of *Tetranychus cinnabarinus* and *Phytoseiulus persimilis* at different initial prey and predator ratios, unsprayed and sprayed control plots in greenhouse grown cucumber in Adana (Mean±SEM in one cm² leaf area)*.

Treatments	<i>T. cinnabarinus</i>	<i>P. persimilis</i>
unsprayed control	49.00±1.85 a	0.05±0.01 c
10:1 (prey:predator)	5.66±0.37 c	0.36±0.02 b
20:1 (prey:predator)	8.20±0.47 b	1.02±0.07 a
30:1 (prey:predator)	10.20±0.74 b	0.77±0.04 a
sprayed control	1.45±0.11 d	0.02±0.01 c

*Means within columns followed by the different letter are significantly different (P=0.05).

Weekly average population developments of *T. cinnabarinus* and *P. persimilis* are given in Figure 1 for different prey-predator ratios, unsprayed and sprayed control plots. Similar to total average numbers of *T. cinnabarinus*, the highest weekly population development of pest occurred in unsprayed control plot and reached to a peak on June 7 with 120 total stages per cm² (6th week after first release) and followed by 30:1, 20:1 and 10:1 prey and predator release plots with 36.99, 18.78 and 15.48 total stages per cm², respectively on the same date. The numbers of *T. cinnabarinus* was very low in sprayed control plot with a peak of 4.28 total stages/cm² on 7th week after the release (Figure 1).

According to different initial prey and predator ratios, the highest weekly average densities of *P. persimilis* were found to be 0.78, 3.43 and 1.97 total stages per cm², at 10:1, 20:1 and 30:1 ratios, respectively on 6th week after release (Figure 1).

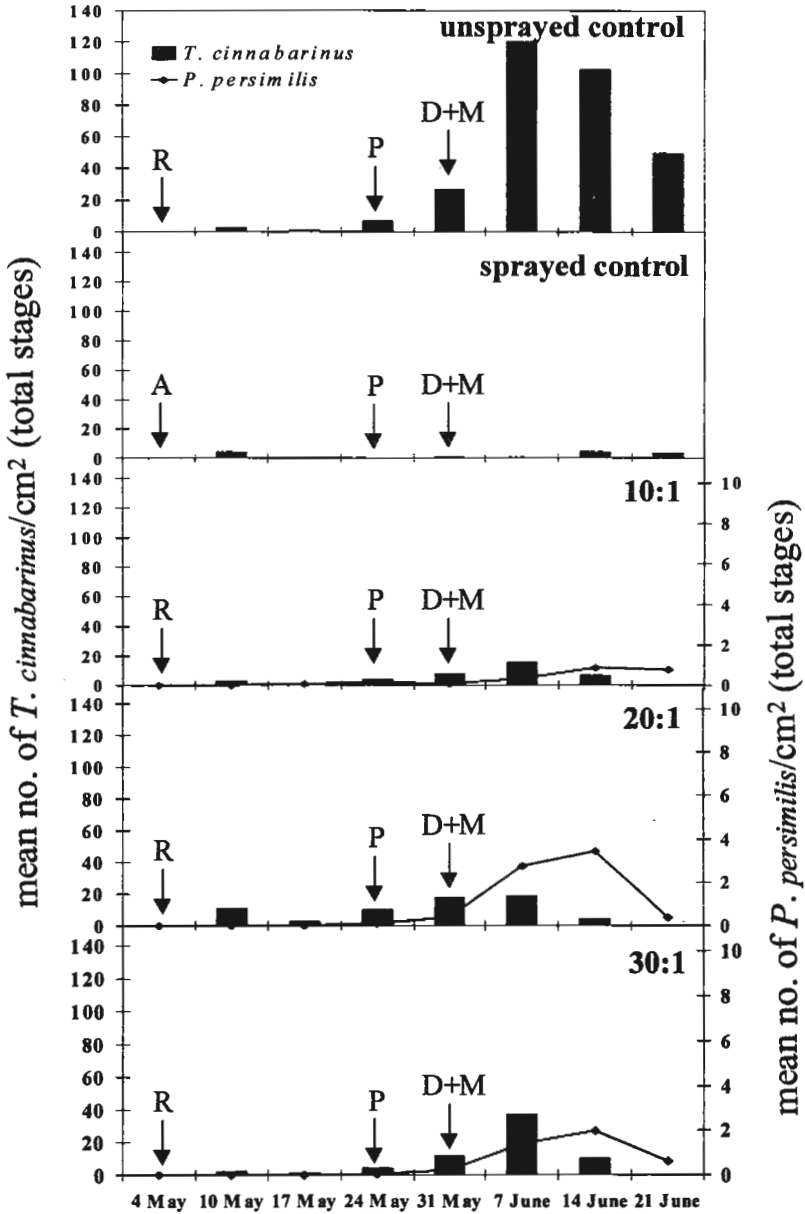


Figure 1. Weekly population dynamics and predation of Hatay strain of *Phytoseiulus persimilis* on *Tetranychus cinnabarinus* at different initial prey-predator ratios (10:1, 20:1, 30:1), unsprayed and sprayed control plots on greenhouse grown cucumber (R: prey and predator release, A: abamectin, P: penconazole, D+M: dimethomorp+mancozep spray).

The results indicated that effective control of *T. cinnabarinus* on cucumbers was achieved within six weeks after release of prey and predator. In addition, existence of the *P. persimilis* continued at least three weeks after elimination of the prey. However, a few number of cucumber plants showed *T. cinnabarinus* damage particularly at 20:1 and 30:1 prey-predator ratios. Former studies showed that early release of *P. persimilis* after first detection of spider mites on host plant was inevitable for successful biological control (Cross, 1984; Rasmy & Ellaihy, 1988; Kazak *et al.*, 1997). In this study, the lowest average number of prey density occurred in 10:1 prey-predator ratio compared to other ratios which indicated that predator was more effective on lower initial ratios.

The effectiveness of different strains of *P. persimilis* on tetranychids was stated by several authors (Hassan, 1982; Vacante & Firullo, 1983; Kazak, 1991; Galazzi & Nicoli, 1996). Hassan (1982) found that "Koppert", "Littlehampton" and "Hohhenheim" strains of *P. persimilis* showed no marked differences in the ability to control *T. urticae* under greenhouse conditions. Similarly, Kazak (1991) reported that Hohenheim strain of *P. persimilis* was successful to keep *T. cinnabarinus* under control at 5:1 prey-predator ratio in greenhouse grown cucumber. Cucumber plants sprayed once with two different fungicides (penconazole and dimethomorp+mancozeb), showed no harmful effect on predator mite during the experiment. *Aphis* spp. and *Thrips tabaci* L. were also encountered in the experiment but no control measures were taken because of their very low population densities. Minimum and maximum average temperature and relative humidity changed between 25 and 35 °C while the relative humidity was 34 to 70 % respectively in greenhouse during the experiment.

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Development of a sequential sampling program for *Frankliniella occidentalis* Pergande (Thysanoptera) on strawberry in plastic tunnels in southern Italy

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Abstract: Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is considered a key pest in tunnel-grown strawberries in southern Italy. We present data about the distribution of the population of WFT and develop a sequential sampling scheme based on Wald's procedure. The thrips population density was monitored in commercial plastic tunnels in two areas of Campania (southern Italy). The series of aggregated data were confronted with three theoretical distributions, i.e. normal, Poisson and negative binomial. Since for densities >1 thrips/flower the negative binomial distribution described better the pest population, we calculated the corresponding dispersion parameter (k). Single k 's were used to calculate a common k ($k_c = 1.56$). We constructed a sequential chart under the condition of negative binomial distribution by choosing 7 and 15 thrips/flower as the tolerance and the intervention thresholds respectively. The maximum average sample number (ASN) required to obtain maximum allowable risks fixed at $\alpha = \beta = 0.05$ is 11.1 flowers.

Key Words: Western flower thrips, negative binomial distribution, dispersion parameter.

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is considered a key pest in tunnel-grown strawberries in southern Italy (Laudonia & Viggiani, 1998 a and b; 1999). Sampling efficiency and accuracy are essential to have information to be used in pest management decision models, to improve the timing of control and to check the effectiveness of the measures used on the basis of economic threshold values.

In the present study, we report data about the distribution of the population of WFT in tunnel-grown strawberries and develop a sequential sampling scheme based on Wald's procedure.

Material and methods

Sampling plans

The study was carried out on the commercial cultivar Chandler in two areas by using a different sampling method in each of them. The first method (*sampling 1*) was performed in a commercial plastic tunnel (30 x 5 m in size) located in Eboli (SA), during the growing season from April to June 1996. The population density of thrips was monitored weekly, by using the shaking flower method (Laudonia & Viggiani, 1998 b; 1999). We chose at random a single flower out of ten plants) along one planting in double rows. The second one (*sampling 2*) was carried out in four commercial plastic tunnels (36 x 5 m in size) located in Capua (CE), during

the growing season from April to May 1999. The thrips population density was monitored according to the same procedures as described above, but we chose randomly 3 flowers i.e. 1 at the bottom, 1 in the middle and 1 at the top on 10% of the plants, randomly selected. In both cases strawberries were grown according to commercial practices, except that no insecticide was applied.

Data analysis

Since data of both the sampling methods were not enough to test whether they fit with any known theoretical distribution, we aggregated them, after having performed an ANOVA test. Data of *sampling 1* were compared by 2 consecutive weeks while data of *sampling 2* were compared by flower position and tunnel.

Both series of aggregated data were confronted with three theoretical distributions, i.e. normal, Poisson and negative binomial. Since all data of 1996 fitted better to the negative binomial (n. b.) distribution (Tab. 1), we calculated the corresponding dispersion parameter (k) with the formula (3) reported by Southwood (1966) for high population density.

Only 2 sets of 1999 fitted to n. b. distribution and formula (2) by Southwood (1966) was used to calculate the relative k , since the population density was low and about one third of the samples was blank. Single k 's of *sampling 1* were used to calculate a common k (k_c) by the formula reported by Bliss & Owen (1958), after having applied the graphical methods suggested by the same authors to detect and exclude gross outliers.

Table 1. Test of goodness of fitness to negative binomial distribution of *sampling 1* and 2 data and dispersion parameter (k) for *F. occidentalis* on strawberry.
(n.s.= not significant; *= significant at the specified probability level)

Sampling 1996	mean	s.d.	χ^2 (p-value)	k
April (1 st -2 nd weeks)	6.62	5.244	5.25 (≥ 0.05) n.s.	1.50
April (3 rd -4 th weeks)	10.63	8.961	2.57 (≥ 0.05) n.s.	1.51
May (1 st -2 nd weeks)	7.55	6.851	5.59 (≥ 0.05) n.s.	1.12
May (3 rd -4 th weeks)	6.58	7.610	2.61 (≥ 0.05) n.s.	0.72
June (1 st -2 nd weeks)	20.72	14.766	1.87 (≥ 0.05) n.s.	2.01
June (3 rd -4 th weeks)	14.88	10.933	7.47 (≥ 0.05) n.s.	1.88
Sampling 1999	mean	s.d.	χ^2 (p-value)	k
29 th April	0.91	1.617	9.59 (≤ 0.05) *	-
6 th May	1.20	1.809	2.21 (≥ 0.05) n.s.	0.64
13 th May	0.99	1.894	11.46 (≥ 0.05) n.s.	0.44
20 th May	0.30	0.773	5.93 (≤ 0.05) *	-
27 th May	0.50	1.166	9.46 (≤ 0.05) *	-

Formulae to apply for constructing the sequential chart under the condition of n. b. distribution as well as for the estimation of the average sample number (ASN) curve were derived by Onsager (1976). For the relative computation we set the values of type I (α) and type II (β) errors at 0.05.

Results and discussion

Since no significant difference emerged by comparing the values of 2 consecutive weeks in *sampling 1*, the relative data were pooled. All the available data were used to calculate a k_c , resulted 1.56. The relative test for agreement by Bliss & Owen (1958) did not detect any source of heterogeneity (tab. 2).

Since in *sampling 2* there was no significative difference by comparing the data of flower and tunnel, the relative data were pooled. However, we calculated the relative k for the two data sets following the n. b. distribution only.

Population density in the *sampling 1* ranged around values that included both the tolerance (7 thrips/flower) and the intervention (15 thrips/flower) thresholds as proposed by Viggiani (1997) and Laudonia & Viggiani (1999) and were much higher than those recorded in *sampling 2* (tab. 1)

Table 2. Test of homogeneity for k_c for *sampling 1* data.

Effect of $k_c = 1.56$	d.f.	Mean square	F	Probability level
Slope $1/k_c$	1	79.32	10.641	$P \leq 0.05$ *
Computed intercept against 0	1	4.66	0.626	$P \geq 0.50$ n.s.
Error	3	7.451		
Confidence limits for $k_c = 1.28$ and 2.00				

The sequential plan has been based on the forementioned tolerance and intervention thresholds. The maximum ASN, corresponding to a population mean of 10.08 (b =slope of the stop lines) resulted 11.1 (fig. 1).

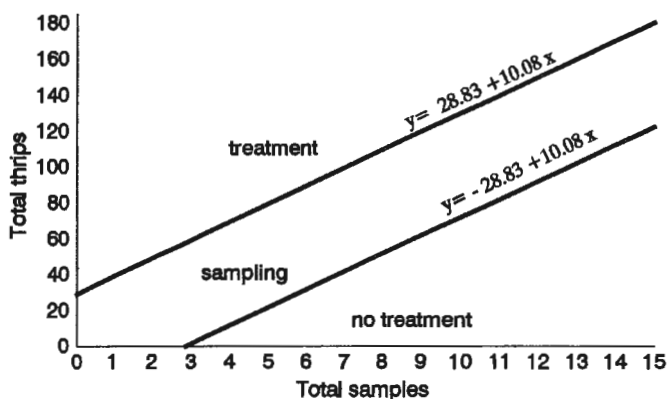


Figure 1. Sequential sampling lines for *F. occidentalis* on strawberry.

Several studies have revealed an aggregated spatial pattern of populations for *F. occidentalis* on numerous crops; furthermore, when was attempted to fit the sampling data with a known theoretical distribution the n. b. described better the thrips population (García-Marí *et al.*, 1994, Sanchez *et al.*, 1997). In our study the n. b. distribution seemed an adequate model to describe the thrips population though only at densities of at least 1 thrips/flower.

García-Marí *et al.* (1994) developed several sampling methods of *F. occidentalis* on outdoor strawberry growing and suggested that a binomial sampling with a tally threshold of 6 thrips/flowers is the most efficient plan, requiring 30 flowers to take a decision with an intervention threshold of 10 thrips/flowers and a precision level of 25%.

It would be interesting to validate our method in practice and comparing it with a sampling plan based on the binomial count as proposed by García-Marí *et al.* (1994).

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Parasitoid complex associated with lepidoptera on horticultural protected crops in the Oeste region of Portugal

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Abstract: Periodic surveys of eggs, larvae and pupa of lepidopteran pest species during 4 years, on several greenhouses at the Oeste region, on the most important horticultural crops, followed by laboratory rearing, until adult, of the entomological material captured allowed to the identification of the parasitoid complex species of lepidopteran pests. A list of the 14 parasitoid species found during this survey is presented indicating their hosts as well as the host plant and the period of the year. The most important are the larval parasitoids *Hyposoter didymator* and *Cotesia kazak* / *C. plutellae* (responsible for about 80-90% of the parasitism) and, in 1998, egg parasitoids *Telenomus laeviceps* and *Trichogramma* sp.

Key words: lepidopteran pests, noctuids, greenhouse crops, parasitoid complex, natural control, *Hyposoter didymator*, *Cotesia kazak*, *plutellae*, *Telenomus laeviceps*, *Trichogramma*

Introduction

Lepidopteran larvae, mainly noctuids, has been considered as a key pest on horticultural protected crops in the Oeste region, specially when outbreaks did occur like in 1996, 1998 and 1999. Although natural control is not always enough to maintain these pest populations on tolerable levels, on some special circumstances the parasitism rate of can be as high as 100% (ex. sweet pepper or tomato, IPM greenhouses, Summer, *Helicoverpa armigera* being the dominant species on noctuid complex) and the total parasitism rate on a commercial crop can be close to 30% in some greenhouses and crops (Figueiredo & Mexia, 1998, 1999; Mexia *et al.*, 1999).

Material and methods

Surveys of eggs and larvae of lepidoptera and plusinae pupae were made, fortnightly, from 1996 to 1999, observing 1plant/30m² with a minimum of 30 plants in each plot, in six greenhouses at Torres Vedras and Mafra sub-regions, on tomato, cucumber, sweet pepper, lettuce and green beans. Sporadic surveys were also made between 1993 and 1995 in some of these six greenhouses and from 1996 to 1999 in different greenhouses of the Oeste region. Pupae from other lepidopteran subfamilies could not be captured because it would be necessary to remove soil in the plant roots area with the consequent plant damage.

The eggs were stored in Eppendorf tubes with the leaf portion where they were located; the larvae were handled to plastic Petri dishes and the plusinean pupae to glass Petri dishes together with the leaf portion that the larvae had rolled around them.

The entomological material captured was reared in a growth chamber at 25±1°C, 75±5% RH and a photoperiod of 16L:8D, until the adult emergence of either from the lepidoteran or from the parasitoid species. Larvae were feed with an artificial corn based diet.

Results and discussion

During the four years of periodical surveys from about 400 to almost 1000 eggs, larvae and pupae of lepidopteran species were collected annually. They were mainly noctuids and the most frequent species were the Plusinae *Autographa gamma* (L.), *Chrysodeixis chalcites* (Esper) and *Thysanoplusia orichalcea* (Fab.), and the Heliothinae *Helicoverpa armigera* (Hbn.). The unique non noctuid species was the piralid *Udea ferrugalis* (Hbn.). It was found parasitism in all these species. Although not so frequent, other lepidopteran species where parasitism was also found are indicated in Table 1.

Parasitoids from eggs, larvae and pupae were found. They were all hymenopteran species, and almost all Ichneumonoidea, with the exception of two specimens of probably only one Tachinidae species reared from two plusinean larvae. The egg parasitoids found were *Telenomus laeviceps* Foerster (Hym.: Scelionidae) and *Trichogramma* sp. (Hym.: Trichogrammatidae). Larval parasitoids, presented in Table 1, were the most important in three of the four years of the periodic surveys. *Hyposoter didymator* Thunberg (average of 50% of total parasitism) and the complex *C. kazak* / *C. plutellae* (20-40% of total parasitism) are the most frequent parasitoid species. In this study *C. kazak* and *C. plutellae* are considered as a complex because they revealed to be very difficult to distinguish after being preserved in 70% ethanol. Egg parasitism represented about 8%-10% of total parasitism. However, this parasitism was unexpected high in 1998. The unique pupal parasitoid found was *Ctenocharaxes bicolorus* (L.), also indicated in Table 1.

Almost all these parasitoids have been already mentioned from field crops at the mediterranean region (ex. Cabello, 1989; Meierrose *et al.*, 1989; Caballero *et al.*, 1990; Izquierdo *et al.*, 1994; Oballe *et al.*, 1994) on these or other noctuid species, but not yet from protected crops.

However, it is important to mention that, for the best of our knowledge, it is the first report of *H. didymator* collected from *Udea ferrugalis* and that, as part of this research work, Madeira *et al.*, (1997) did also report for the first time the same ichneumonid and *Cotesia plutellae* collected from *Peridroma saucia* (Hbn.).

Acknowledgements

The authors thank to the formers students Alexandrina Madeira, Helder Gonçalves, Clarisse Marques and Sandra Branco to their collaboration in this research line and to the growers that allowed the surveys in their greenhouses. We wish to thank also to Eng. Passos de Carvalho (EAN, Portugal) for the identification of some of the lepidopteran specimens, Prof. Sedivý (Museum Praga) and Dr. C. Karl (CABI, Switzerland) for the identification of *H. didymator*. The others parasitoids were identified by A. Walker, J. LaSalle and A. Polaszek from CABI (London). This study was supported by the national research projects PAMAF 2034 and PRAXIS XXI 3/3.2/Hort/2164/95.

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Table 1. List of the larval and pupal parasitoids species, and their hosts, found on lepidopteran specimens collected in horticultural greenhouses in the Oeste region from 1993 to 1999.

parasitoid species	lepidopteran host ¹								crop host ²				
	Ag	Cc	Ha	Mb	Ps	To	Tn	Uf	cu	gb	le	sp	to
larval parasitoids													
Diptera: Tachinidae	?	?				?			Aug., Nov.		x		x
Hym.: Eulophidae													
<i>Euplectrus flavipes</i> Fonscolombe	x	?	x			x			Jun.-Jul., Sep.-Oct.			x	x
Hym.: Ichneumonidae													
<i>Hyposoter didymator</i> Thunberg	x	x	x	x?	x ⁴	x		x ³	Apr.-Nov.	x	x	x	x
Hym.: Braconidae													
<i>Aleiodes</i> sp. (2 sp.)	?	?				x			Jan., Jun., Nov.			x	
<i>Cotesia</i> sp. (gregarious sp.)					x	x			Jan., Jul., Sep.			x	x
<i>Cotesia kazak</i> (Telenga)			x						Jul., Aug.				x
<i>Cotesia plutellae</i> (Kurdyumov)	x?	x?			x ⁴	x?			May, Jul.	x			x
<i>Cotesia kazak/C. plutellae</i>	x	x	x		x	x		x	Jan.-Nov.	x	x	x	x
<i>Macrocentrus</i> sp. near <i>collaris</i> (Spinola)						x			Jan.			x	
<i>Meteorus pulchricornis</i> (Wesmael)			x?		x?				Oct.			x	x
<i>Microplitis mediator</i> (Haliday)	x?	x?				x			May-Oct.	x	x		x
pupal parasitoids													
Hym.: Ichneumonidae													
<i>Ctenochares bicolorus</i> (L.)	x?	x?				x?			Jul., Nov.		x		x

(1) Ag = *Autographa gamma* (L.); Cc = *Chrysodeixis chalcites* (Esper); Ha = *Helicoverpa armigera* (Hbn.); Mb = *Mamestra brassicae* (L.);

Ps = *Peridroma saucia* (Hbn.); To = *Thysanoplusia orichalcea* (Fab.); Tn = *Trichoplusia ni* (Hbn.); Uf = *Udea ferrugalis* (Hbn.);

(2) cu = cucumber; gb = green beans; le = lettuce; sp = sweet pepper; to = tomato;

(3) first host reference;

(4) first host reference (Madeira *et al.*, 1997).

Use of sexual pheromone trapping on risk assessment for noctuids on protected crops – a preliminary study in the Oeste region

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Abstract: Pheromone traps were used for monitoring of *Autographa gamma*, *Chrysodeixis chalcites* and *Helicoverpa armigera* in two “parral” greenhouses (≈ 5000 m²) and one traditional “capela” greenhouse (≈ 300 -400 m²) in the Oeste region of Portugal, during late Spring and Summer 1999. Simultaneously, egg and larvae of these species were searched on crop plants. Captures were almost nil with the exception of *C. chalcites* in one “parral” greenhouse after the unexpected removal of the plastic cover. However, egg, larvae and pupae were collected on plants.

Key words: noctuids, monitoring, pheromone traps, funnel traps, greenhouse crops

Introduction

Noctuids has been considered as a key pest on greenhouse crops. For risk assessment it would be important to have a quick and precise method to detect when adults are flying, specially when the use of IGR or Bt is foreseen or to release parasitoids such as trichogramma. The use of pheromone trapping in greenhouses either to monitorize or to control noctuids is being investigated world-wide (Linden, 1996; Szöcs *et al.*, 1996) with inconclusive, often disappointing results (Lenteren, 1995).

Material and methods

At the end of May 1999, in Torres Vedras sub-region, six dry funnel traps were installed in two “parral” type greenhouses (≈ 5000 m²) with pheromone capsules for *Autographa gamma* (L.), *Chrysodeixis chalcites* (Esper) and *Helicoverpa armigera* (Hbn.) (three traps in each greenhouse randomly, one for each studied noctuid species). The traps were installed at an height of 1,5m suspended from the wires which support the training system and with a distance between them of, at least, 150 m either on sweet pepper crop (in one greenhouse) or a tomato crop (in the other one). In both cases the remaining crops in the same greenhouse, apart from the experimental plot, was tomato. Greenhouses are surrounded by pine forest.

Dry funnel traps for *C. chalcites* and *A. gamma* were totally green. The one for *H. armigera* was a tricolour funnel (white receptacle, yellow funnel and green top) because this one revealed to be the more efficient in open field in the same region (Figueiredo *et al.*, in press). The pheromones dispensers used were from AgriSense. The pheromone capsules were changed every six weeks, according to the instructions of the producer. Dichlorvos impregnated bars were used as insecticide at the bottom of the receptacle. Captured adults were collected and counted weekly until the end of August.

Simultaneously, egg and larvae surveys were made on the plants.

Dry funnel traps for each studied noctuid species were also located in a greenhouse of the “capela” type ($\approx 300\text{-}400\text{ m}^2$) with tomato at the Mafra sub-region, following the methodology described above.

Results and discussion

The number of adults of each species weekly captured is indicated on Fig. 1, 2 and 3. In one of the “parral” greenhouses (“Parral” 2) the grower took off the plastic cover, unexpectedly, on the week 3rd-12th August and so, after that date, the tomato crop was not a protected crop any longer but should be considered as an open field one.

Adults of *C. chalcites* were captured at low levels all over the experimental period in the two “parral” structures until the uncover of the “Parral 2”. After that, specifically in this last “parral” (Fig.2), the number of captured adults of *C. chalcites* increased highly. On the “capela” greenhouse type the number of *C. chalcites* captured was relatively high during all the experiment, although the captures period was shorter (Fig. 3).

In all the three experimental greenhouses, larvae from this species were collected from the plants during all the experimental period.

Autographa gamma was not captured in the “parral” greenhouses excepting in “Parral” 2 during the week of 3rd-12th August when only two adults were captured. In the “capela” greenhouse *A. gamma* was also captured at low levels. No egg, larvae or pupae of this species was found in all cases.

Helicoverpa armigera, the most important lepidopteran pest, was not captured in all greenhouses, excepting “Parral” 2 when the plastic cover was taken off. However, since the beginning of this study, numerous eggs and larvae of this species were found on leaves and in fruits of the tomato and sweet pepper crops. Pheromone traps did prove to be an efficient tool for *H. armigera* monitoring in outdoor crops (Izquierdo *et al.*, 1992; Figueiredo *et al.*, in press) and captures have been related with egg counts on tomato crop (Izquierdo, 1996), making this tool an interesting monitoring device for risk periods definition. However, in greenhouse conditions it did not seem to be the case. Lenteren (1995) refers that, probably, in greenhouse conditions air current patterns are very different and, as a consequence, the pheromone distribution patterns are unnatural and the target insects can not react properly. This may very well be an explanation for the poor results achieved for *A. gamma*, *H. armigera* and *C. chalcites*, with the exception of *C. chalcites* when the plastic was taken off unexpectedly.

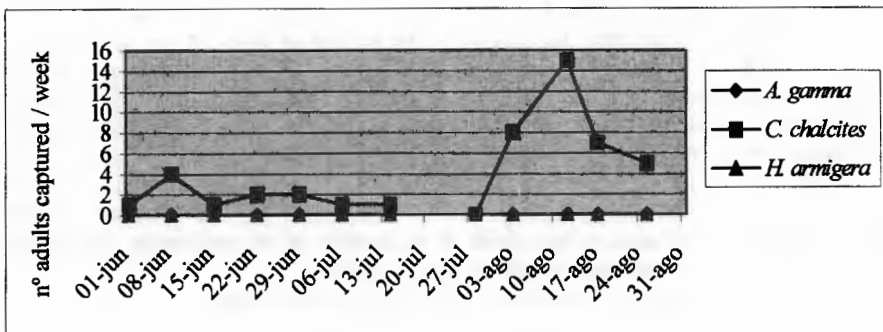


Figure 1. Weekly captures of adults of *Autographa gamma*, *Chrysodeixis chalcites* and *Helicoverpa armigera* by pheromone funnel trap at Santa Cruz – “Parral” 1.

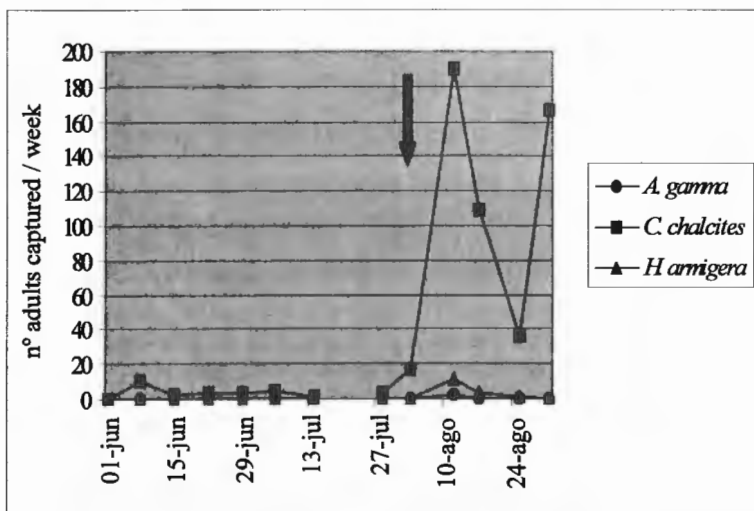


Figure 2. Weekly captures of adults of *Autographa gamma*, *Chrysodeixis chalcites* and *Helicoverpa armigera* by pheromone funnel trap at Santa Cruz - "Parral" 2. The arrow indicates the take off of the greenhouse plastic cover

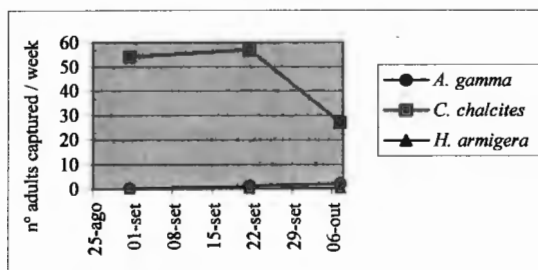


Fig. 3. Weekly captures of adults of *Autographa gamma*, *Chrysodeixis chalcites* and *Helicoverpa armigera* by pheromone funnel trap at Mafra - "capela" greenhouse.

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Biological control of *Nezara viridula* on egg plant, with an egg parasitoid *Trissolcus basalis* Wollaston

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Abstract: *Nezara viridula* (Heteroptera, Pentatomidae), also called Southern Green Stink Bug (SGSB), is one of the most important secondary pests on egg plant under IPM. Nowadays, there is no registered active ingredient to control it. These investigations consisted of several trials, using an egg parasitoid, *Trissolcus basalis* (Hymenoptera, Scelionidae). Three types of release were conducted, in commercial plastic tunnels in South of France: two rates of releases (0.1 *T. basalis* / m², two times, every two weeks, the higher one was 0.5 *T. basalis*/ m², three times, every two weeks) and one based on a banker device. Because of a large variability between pest populations, the results, relative to beneficial releases, didn't show conclusive effects on pest population reduction. However, information about population dynamic of SGSB in plastic tunnels was obtained.

Key words: *Nezara viridula*, *Trissolcus basalis*, Biological control, egg plant

Introduction

IPM on egg plant has been used for several years in the South of France. Major pests can be controlled in releasing large range of beneficials. In recent years, predatory bug *Podisus maculiventris* has been observed to be a predator of the southern green stink bug (SGSB), but this idea won't be developed more in this study. But secondary pest populations are increasing, because of reduction of chemicals. The SGSB, *Nezara viridula* (Linneus), is a very polyphagous secondary pest. It stings fruits and apex of cucumber, tomato, pepper, soya, rapeseed (Volkoff, 1990).

The damage due to this bug is economically important. However the tolerance thresholds are not clearly established. SGSB can be tolerated but the risk is that a sudden increase is difficult to control, using methods compatible with natural enemies. Thus, in an IPM program, a biological solution is considered. *Trissolcus basalis* (Hymenoptera, Scelionidae) is the main natural factor of reduction in SGSB populations (W.A Jones, 1988). This oophagous parasitoid, introduced in Australia, Fidji islands (1941), New Zealand, Hawaï and other islands, established and contributed to control SGSB populations (W.A Jones, 1988). Moreover, trials carried out to control SGSB, by inoculative releases on soya crop, were successful. (Corrêa-Ferreira, 1996). In France, *T. basalis* occurs naturally in spring, especially in the South (Sagliocco, pers. com.).

This study aimed at showing the effect of this parasitoid on the pest populations, on egg plant crop, under plastic tunnels. So different rates were tested, and also banker devices. This last mode of release is done in order to multiply egg parasitoid on the crop, in order to decrease the economic cost. To determine a tolerance threshold, a trial using pheromon traps was carried out ; netting systems were also used to decrease the SGSB populations. These two studies won't be dealt with here.

Material and methods

The trials were led from april to august 1999, in four farms, on egg plant crop (cv «telar» and «diva»), under commercial plastic tunnels, oriented North-South . This crop was planted on 6 rows, with a density of plantation from 1,5 to 2 plants /m² . The tunnel surface varied from 560 m² to 1200 m² .

Biological material

The biological material came from Koppert BV (The Netherland). *T. basalis* was reared on SGSB under laboratory conditions. Adults were shipped and stored at 10 °C during 3 days. SGSB was naturally present in the tunnel. Egg masses, used for detection and banker devices, came from mass rearing. Eggs were laid on paper, and then frozen until the shipment.

Counting and observations

They were realized in the morning, in the East row, twice a week. Nymphs, adults and natural egg masses were counted especially on fruits, apex and on the upper leaves.

To detect the establishment of *T. basalis* on the crop, 5 egg masses were put in the plant on central row, every two weeks. After one week, each egg mass was collected and parasitism rate was determined.

Experimental design

Two rates of *T. basalis* treatment were tested. The lower one was 0.1 *T. basalis* / m², two times, every two weeks, the higher one was 0.5 *T. basalis*/ m², three times, every two weeks. Two separate releases were conducted, depending on the presence of *N. viridula*. First release of earlier serie began at 5/4/99 in 4 tunnels, and at 6/15/99 for the latest serie, in another 4 tunnels.

Beneficials were spread all over the crop. They were released on the middle heigh of the plant. Egg masses were stapled on the lower side of the leaves.

At the same time, the banker devices were tested, by using banker egg masses: a 4-plant-area was noticed in each tunnel. On these plants, in each area, 3 egg masses were put, four times: the second a week later, and the followings, every two weeks. The *T. basalis* releases were 0.1 Tb/m², two times related to the two first egg masses bringing in. They were done close to these egg masses

Results and discussion

Biology of SGSB

The repartition of SGSB population referring to different generations was observed during the experiment. As there was a big variability between the tunnels, results were not so clear. The first generation was mostly shared out all over the tunnel. That may correspond to the generation descended from the adults overwintering in the tunnels. During the second part of the season, after the middle of july, corresponding to the second generation, population was mostly found in the entries. But these results couldn't allow us to conclude.

Results obtained with the two rates of *T. basalis* releases

Figure 1a and 1b show the results obtained with the earlier series of release, figures 2a and 2b show the results obtained with the later ones. Letters a and b are related to the farmer's plots.

Because of the lower number of adults and natural egg masses detected on the crop, the following results were expressed in cumulative number of nymphs, detected on the eastern row. The results showed a large variability of the evolution of SGSB population, in the different plots and also depending on the tunnels in the same plot. The results obtained shown in figures 1b and 2b indicate that a higher rate of release seemed to have an effect of decrease of SGSB population: for example, figure 2b shows that in the tunnel where a higher rate was

realized, the cumulative number of nymphs reached 150 individuals, whereas in the tunnel where the rate was 0.5 Tb/m^2 , the population level was nearly 3 times more important (400 ind.). However, it couldn't be confirmed by the results obtained in the farmer «a»'s tunnels, where the SGSB population reached a higher level when a higher rate was used: for example, the level in the higher rate tunnel of farmer «a» reached 400 individuals, whereas it reached 150 in the tunnel with a lower rate. But in these tunnels, the population curve leveled off before increasing again, and that could be due to the beneficial parasitism effect. These results couldn't allow us to conclude about the efficient use of the beneficial.

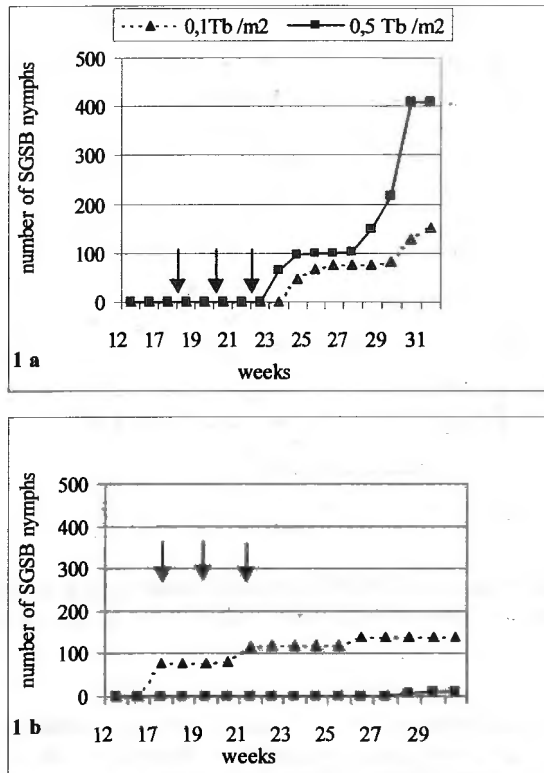


Figure 1: Cumulative number of SGSB nymphs detected on the eastern row of the tunnels, where earlier releases were done (early arrival of SGSB in the season) 1a: farmer «a», 1b: farmer «b»

Results obtained using egg masses as banker devices

The two releases close to the two first stapled egg masses were efficient, with a parasitism rate from 17.6% to 55.6%. But, due to bad quality of frozen egg masses, the two last ones didn't show any parasitism, then it was not possible to prove the multiplication of *T. basalis*. Thus, the banker device efficiency, by using frozen egg masses, couldn't be evidenced. Because of the large variability observed in the different plot, no significant results were obtained.

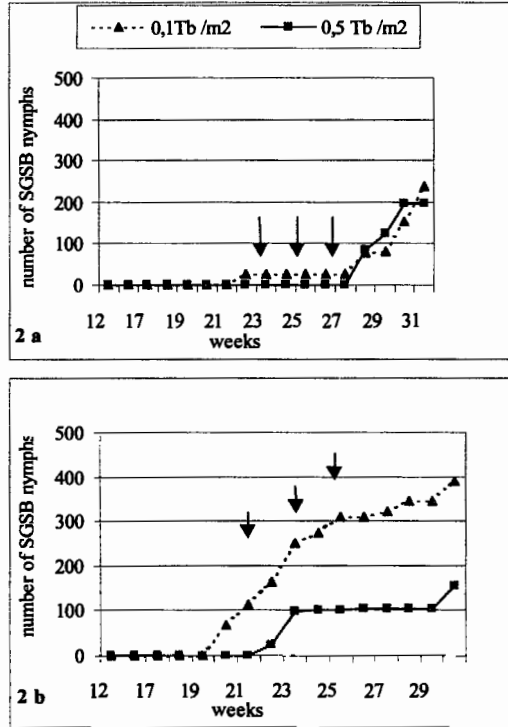


Figure 2: Cumulative number of SGSB nymphs detected on the eastern row of the tunnels, where later releases were done (late arrival of SGSB in the season) 1a: farmer «a», 1b: farmer «b»

However, the establishment of *T. basalis* has been determined, with detection egg masses, on which significant parasitism rate was observed 45 days after the last releases, corresponding to the end of June. This is much earlier than in previous year when egg parasitoids were first observed in August, indicating the parasitism in the test plots can not be due to natural parasitism.

These results, relative to different beneficial rates, or banker device, didn't indicate an efficient control of SGSB population. Even if economic damage is not determined, in some cases, growers decided to make treatment. But, some important points were noticed and could be interesting for next studies.

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Section VII
Polyphagous predators

Section VII
Prédateurs polyphages

Natural populations of *Macrolophus caliginosus* and *Dicyphus tamaninii* in the control of the greenhouse whitefly in tomato crops

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Abstract: Biological control of greenhouse whitefly by inoculative release of *Encarsia formosa* in spring tomato greenhouses is hampered by occasional immigrations of whitefly populations from outdoors. At the same time *Dicyphus tamaninii* and *Macrolophus caliginosus*, which are abundant polyphagous predators in the area, colonise these greenhouses where *E. formosa* is released. The role of these predators in the control of greenhouse whitefly was evaluated in 14 greenhouses at the end of the cropping season. They preyed on parasitized and unparasitized pupae according to their density in the crop. In the laboratory they consumed more greenhouse whitefly than *E. formosa* pupae when these were offered in the same proportion. Their effect on the regulation of greenhouse whitefly populations is complementary to that of *E. formosa* and contributes to the control of this pest in the Mediterranean area.

Key words: *M. caliginosus*, *D. tamaninii*, *E. formosa*, *T. vaporariorum*, biological control.

Introduction

In the Mediterranean area, the biological control of greenhouse pests by inoculative release of natural enemies is hampered by occasional immigrations of pest populations from outdoors since greenhouses have semi-open structures for ventilation. In the Catalan coast of Spain the control of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) by the parasitoid *Encarsia formosa* Gahan in spring tomatoes has to deal with whitefly invasions that limit the parasitoid effectiveness (Albajes *et al.*, 1994).

On the other hand, *Dicyphus tamaninii* Wagner and *Macrolophus caliginosus* Wagner are abundant in the area and colonise greenhouse crops, such as tomatoes or cucumbers, especially when biological control is applied, and no toxic side effects of pesticides interfere with their action (Gabarra *et al.*, 1988; Alomar *et al.*, 1991; Castañé *et al.*, 1997).

Here we study the role of these spontaneous predators in the biological control of the greenhouse whitefly. Do they interfere with the control by the whitefly parasitoid or do they improve it?

Material and methods

Fourteen greenhouses, located in the coastal area around Barcelona, were monitored at the end of the cropping seasons of 1993 and 1994. Crops lasted from mid February to mid July. Inoculative releases of *E. formosa* were made for whitefly control. Fifty plants per greenhouse were sampled to evaluate the density of whitefly and *E. formosa* pupae alive or predated by mirid bugs. Three leaflets per plant were at random taken from the leaves where whitefly

pupae are located and isolated in paper bags. In the laboratory they were examined for live or predated white (unparasitized and parasitized still white) and black (parasitized) pupae with a dissecting microscope. Ten leaflets were at random selected along the plant and the number of mirid bug adults and nymphs were annotated (Castañé *et al.*, 1996).

Predation of black and white pupae by *M. caliginosus* and *D. tamaninii* was studied in the laboratory ($25\pm 1^\circ\text{C}$, 16:8 light:dark photoperiod and $70\pm 10\%$ relative humidity). A ventilated plastic cage (7.5cm Ø) with a tobacco leaf disc (5cm Ø) upside down on a 4mm agar layer (0.5%) was used as a search arena in which 40 *E. formosa* and 40 *T. vaporariorum* pupae were deposited. A fifteen-day-old female, starved for 24 hours, was placed in the centre of the arena, and after 48 hours the number and type of prey fed upon were recorded. Fifteen replicates were made for each predator species.

Results and discussion

In mid May some mirid bugs, mainly adults, were already recorded in greenhouse samplings. From this time onwards their numbers increased until the end of the crop, at the beginning of July, when they were well established with a large proportion of nymphs in the population (Figure 1). Therefore, these Heteroptera produced a new generation feeding on greenhouse tomato pests, mainly whitefly.

Macrolophus caliginosus and *D. tamaninii* were the species that colonised these greenhouses, the former being more abundant than the latter (Figure 1): *M. caliginosus* nymphs and adults formed the majority of the mirid bug population, with $74.1 \pm 6.65\%$ of the total. The species composition found in 1993-1994 differed from that found in 1990, when up to 80% of the mirid bugs recorded in 16 greenhouses were *D. tamaninii* (Alomar *et al.*, 1991). In a survey done in 1999 in open field tomatoes in the same area, *M. caliginosus* formed more than 90% of the population (author's unpublished results). These data seem to indicate that *M. caliginosus* is becoming the predominant mirid species in our area, in which *D. tamaninii* was more abundant some years ago. Natural colonisation of *Macrolophus* species and other predatory mirid bugs on tomato crops without broad spectrum insecticide applications has also been reported from other Mediterranean areas such as the South of France (Malausa *et al.*, 1987), Italy (Delrio *et al.*, 1992, Calabrò 1992 and Tavella *et al.*, 1997), and Greece (Perdikis & Lykouressis 1996).

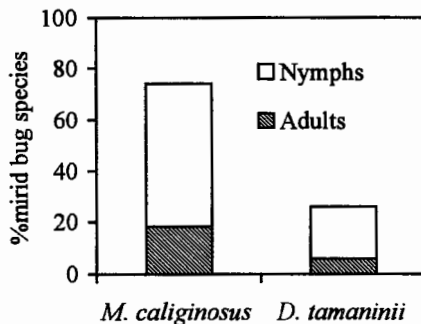


Figure 1. Mean percentage of *M. caliginosus* and *D. tananinii*, adults and nymphs, at the end of the spring tomato crop in 1993 and 1994 (n=14 greenhouses).

These predators have an impact on the whitefly population. At the end of the crop and in relation with their abundance in the greenhouse they produce high mortality of whitefly pupae (Figure 2). In fifty percent of the greenhouses examined more than forty five percent of white pupae were predated, and this figure reached almost 100% in greenhouses n° 12, 13 and 14. They also cause mortality of the black pupae. The proportion of mortality of each type of pupa varied according to mirid density and in relation to the efficacy of the control due to *E. formosa*. In greenhouses where the control with *E. formosa* was successful, at the end of the crop black pupae were the majority of the prey present in the plants and mirid bugs fed on them, as was the case of greenhouses n° 8, 12, 13 and 14. In greenhouses where the control with the parasitoid was low, mirid bugs consumed a large proportion of white pupae. In 12 of the 14 greenhouse sampled the percentage of white pupae consumed was higher than that of black pupae.

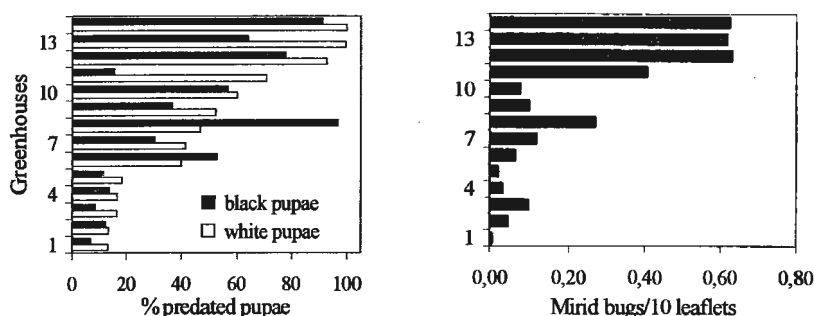


Figure 2. Predation on white (unparasitized and parasitized but still white) and black pupae (parasitized) and mirid bug abundance on spring tomato crops in 1993 and 1994 (n=14 greenhouses).

In the laboratory, *D. tamaninii* consumed significantly more prey (44.7 ± 1.93) than *M. caliginosus* (26.3 ± 2.65) in 48 hours, when a total of 80 pupae (half black and half white) were offered. Both species preyed more on *T. vaporariorum* than on *E. formosa* pupae, with *D. tamaninii* eating 2.1 times and *M. caliginosus* eating 3.99 times more greenhouse whitefly pupae. It can be assumed that it is harder to feed on black pupae because the predator has to penetrate the parasitoid pupal case and the host cuticle (Viggiani 1984).

We have repeatedly observed that whitefly control by *E. formosa* is complemented on greenhouses that are colonised by mirid bugs. Early releases of the parasitoid maintain the whitefly population under control and allow the establishment of natural populations of mirids. Once *M. caliginosus* is established in the crop, predation of parasitized whitefly pupae poses no risk to biocontrol because the predator substitutes the action of *E. formosa*. Current strategies for whitefly control in greenhouse tomatoes of Northern Europe reproduce this combination of both entomophagous (Malezieux *et al.*, 1995).

Conclusions

Spring greenhouse tomato crops in the Catalan coast that apply a biological control program with the parasitoid *E. formosa* become colonised with mirid bugs populations that are composed primarily by *M. caliginosus* from the middle of the crop onwards.

These predators feed on white and black pupae, and this action depends on their density in the crop. When offered in the same proportion they consumed more greenhouse whitefly than *E. formosa* pupae.

Their action may be complementary to that of the parasitoid *E. formosa* and may help to control the greenhouse whitefly in this crop. The concurrent use of *E. formosa* and *M. caliginosus* is a control strategy recommended by companies producing natural enemies (Gabarra & Besri, in press).

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Susceptibilité de *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) à la prédation intraguilde

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Résumé: Le miride *Macrolophus caliginosus* s'attaque entre autres aux pucerons et aux mouches blanches et de ce fait interagit avec les ennemis naturels de ces proies. La susceptibilité de *M. caliginosus* à différents prédateurs intraguilides a été évaluée en laboratoire. En présence du miride *Dicyphus tamaninii*, le niveau de prédation intraguilde était toujours inférieur à 20%, les nymphes de 2ème stade étaient les plus vulnérables, les adultes et les nymphes de 5ème stade étaient très peu susceptibles (<6%). Au contraire, en présence de prédateurs aphidiphages, les niveaux de prédation enregistrés étaient dans la majorité des cas beaucoup plus élevés tant sur les adultes que sur les nymphes de 2ème stade de *M. caliginosus*.

Mots-clés: IGP, prédation intraguilde, interaction, *Macrolophus caliginosus*, *Dicyphus tamaninii*, aphidiphage, zoophytophagie

Introduction

La tendance actuelle au niveau de la lutte biologique considère l'utilisation de combinaisons de plusieurs espèces d'ennemis naturels (Murphy *et al.*, 1999, Sher & Parella 1999, Walzer & Blümel 1999) ainsi que l'utilisation de prédateurs généralistes (Albajes & Alomar 1999). Il importe donc de prendre en compte les risques éventuels d'interactions négatives entre les différents agents de lutte ou encore entre les agents de lutte et les espèces indigènes. À ce titre, la prédation intraguilde (IGP) survient lorsqu'un ennemi naturel tue et dévore (ou parasite) un compétiteur (Polis *et al.*, 1989). La prédation intraguilde est responsable notamment de l'échec d'un programme de lutte biologique aux États-Unis en vue du contrôle d'*Aphis gossypii* en champs de coton (Rosenheim *et al.*, 1993). Ajoutons que les serres méditerranéennes sont caractérisées par un flux continu d'espèces provenant de l'extérieur (Albajes & Alomar 1999). La prédation intraguilde peut donc soit affecter le contrôle biologique au niveau de la serre elle-même, soit affecter les communautés environnantes et de ce fait la migration vers la serre.

Le miride *Macrolophus caliginosus* Wagner est utilisé avec succès en Europe pour le contrôle de la mouche blanche (Malézieux *et al.*, 1995). Le prédateur s'attaque en outre aux pucerons (Alvarado *et al.*, 1997), acariens (Foglar *et al.*, 1990), mineuses (Nedstam & Johansson-Kron 1999), noctuelles (Salamero *et al.*, 1987) et aux thrips (Gabarra *et al.*, 1995; Castañe *et al.*, 1996). Il partage ces proies avec une gamme très étendue d'ennemis naturels avec lesquels il interagit.

Le but de ce travail était de mesurer la susceptibilité de *M. caliginosus* à divers ennemis naturels qu'il est susceptible de rencontrer. Ont été testés, d'une part, le miride prédateur *Dicyphus tamaninii* Wagner qui constitue avec *M. caliginosus* le prédateur le plus abondant en culture de tomate en Catalogne, et d'autre part plusieurs autres espèces aphidiphages communes dans les cultures de pommes de terre environnant les systèmes serres.

Matériel et méthodes

Les essais ont été réalisés en chambre de croissance en conditions contrôlées ($25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ RH, photoperiode 16:8). *M. caliginosus* et *D. tamaninii* venaient d'élevages permanents à la station sur plants de tabac avec *Trialeurodes vaporariorum* Westwood et oeufs d'*Ephestia kuehniella* Zeller. Les prédateurs aphidiphages ont été collectés dans des cultures de pommes de terre près de Cabrils. Le dispositif expérimental était constitué d'une cage transparente (\varnothing : 75 mm x H: 32 mm) avec mousseline et une couche d'agar sur le fond (20 ml, 5 %).

En premier lieu, la susceptibilité des différents stades de *M. caliginosus* à la prédation par *D. tamaninii* a été évaluée. Les nymphes de 2ème et 5ème stades, les mâles et les femelles de *M. caliginosus* ont été testés face aux mâles et femelles de *D. tamaninii*. Un individu de chaque espèce était placé dans la cage. Après 24 heures, la mortalité de *M. caliginosus* était évaluée à la loupe binoculaire. Un témoin évaluait la mortalité naturelle (en absence de prédateur) des nymphes et des adultes de *M. caliginosus*.

En second lieu, nous avons évalué la prédation intraguilde par les prédateurs aphidiphages indigènes. Un adulte (ou une larve de stade avancé si l'adulte n'est pas prédateur) du prédateur aphidiphage était placé avec un individu de *M. caliginosus* (soit une nymphe de 2ème stade, soit une femelle adulte). Ont été testés: *Nabis mirmecoides* Costa, *N. fesus* (L.) (Het: Nabidae), *Orius* spp. (Het: Anthocoridae), *Chrysoperla* sp. (Neu: Chrysopidae), *Sphaerophoria* spp. (Dip: Syrphidae), *Aphidoletes aphidimyza* Rondani (Dip: Cecidomyiidae), *Scymnus* sp., *Coccinella septempunctata* L. (Col: Coccinellidae) et un forficule (Derm: Forficulidae). Les conditions étaient similaires à celles de l'expérience précédente. L'occurrence de l'IGP était comparée dans les deux expériences à l'aide du test de G (rapport de vraisemblance) (Scherrer 1984).

Résultats et discussion

Les nymphes de 2ème stade, les femelles et les mâles de *M. caliginosus* ont démontré une certaine vulnérabilité face à *D. tamaninii* (Fig. 1). Au contraire, les nymphes de 5ème stade, malgré les conditions extrêmes n'ont jamais été attaquées. Les niveaux de prédation enregistrés sur les différents stades de *M. caliginosus* étaient dans tous les cas faibles (max < 20%) ($G=5,56$, $df=166$, $P=0,1351$), beaucoup plus faibles que ceux présentés dans la littérature avec des prédateurs aphidiphages (Lucas *et al.*, 1998). Les nymphes de 2ème stade étaient plus attaquées que les autres stades, néanmoins non significativement. La vulnérabilité des plus jeunes stades à la prédation se retrouve dans la littérature tant au niveau de la prédation intraguilde, du cannibalisme que de la prédation classique (Lucas *et al.*, 1997, 1998; Polis 1981). Le niveau de prédation était similaire pour les mâles et les femelles de *D. tamaninii* ($G=0,10$, $df=166$, $P=0,752$). En ce qui concerne la prédation de *D. tamaninii* par *M. caliginosus*, seuls les adultes sont parvenus à tuer les adultes de *D. tamaninii* (max < 6%). Dans les cultures de tomate de Catalogne, les deux espèces de mirides cohabitent et constituent les prédateurs les plus communs (Alomar *et al.*, 1991). En fin de saison, leurs populations peuvent atteindre des densités relativement élevées (jusqu'à 4-5 individus par feuille) (Alomar 1994), tandis que les populations de proies sont très basses. Dans ces conditions, il est probable que la prédation intraguilde puisse apporter un supplément protéinique à l'alimentation végétale de ces zoophytophages. Des observations complémentaires ont montré que la prédation intraguilde survient fréquemment au moment de la mue. La vulnérabilité semble associée à l'immobilité des individus en mue, vulnérabilité qui

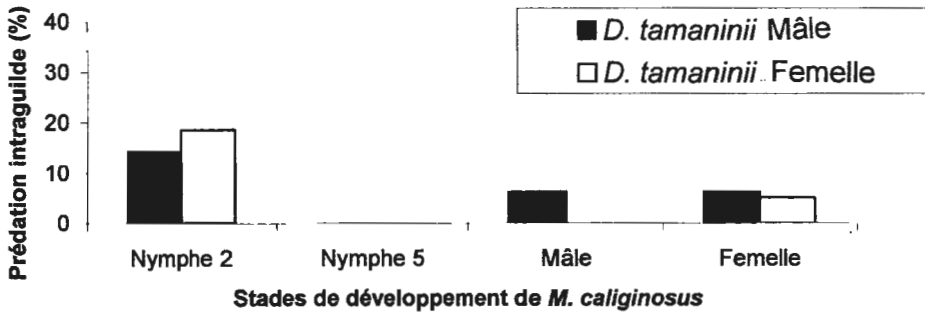


Figure 1. Susceptibilité de *Macrolophus caliginosus* à la prédation intraguilde de *Dicyphus tamaninii*

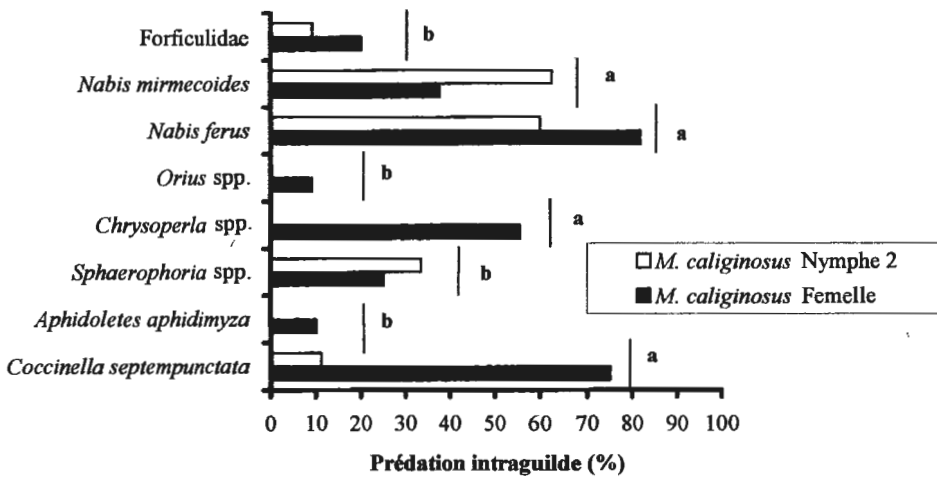


Figure 2. Susceptibilité de *Macrolophus caliginosus* à la prédation par la guildé aphidiphage

Lors de la seconde partie de l'étude, le niveau moyen de prédation intraguilde sur les adultes de *M. caliginosus* dépassait 30% soit quatre fois plus qu'avec *D. tamaninii* (Fig. 2). Sur les larves, le niveau de prédation moyen était de 41% soit trois fois plus qu'avec *D. tamaninii* ce qui confirme le niveau très bas d'IGP de *D. tamaninii* sur *M. caliginosus*. Ceci confirme également la propension élevée de la plupart des prédateurs aphidiphages à pratiquer la prédation intraguilde (Lucas *et al.*, 1998, Rosenheim *et al.*, 1993). Les prédateurs les plus dangereux pour *M. caliginosus* étaient les Nabidae, les syrphes et la coccinelle à 7 points ($G=33,76$, $df=156$, $p<0,0001$). Il est intéressant notamment de constater la vulnérabilité des femelles de *M. caliginosus* aux deux espèces de Nabidae ainsi qu'aux Syrphidae. La différence obtenue entre *D. tamaninii* et les prédateurs aphidiphages pourrait

femelles de *M. caliginosus* aux deux espèces de Nabidae ainsi qu'aux Syrphidae. La différence obtenue entre *D. tamaninii* et les prédateurs aphidiphages pourrait être due en partie à son régime alimentaire zoophytophage, qui permet au prédateur de survivre sur plante seule (Lucas & Alomar in prep.). *M. caliginosus* a lui-même pratiqué la prédation intraguilde sur ses compétiteurs, mais en de rares occasions et semble donc plus être une proie qu'un prédateur intraguilde. Il s'attaque cependant au parasitoïde *Encarsia formosa* Gahan membre de la guilde des ennemis naturels de la mouche blanche (Castañé *et al.*, 1999). Finalement, dans le système de cultures protégées méditerranéen, la communauté est caractérisée par un flux continu d'espèces provenant de l'extérieur (Alomar *et al.*, 1991). Ces résultats préliminaires montrent que la prédation intraguilde peut affecter le contrôle biologique en serre de deux façons, soit au niveau de la serre elle-même, soit au niveau des cultures environnantes sources de migration vers la serre.

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Susceptibility of *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) to intraguild predation.

Abstract: The mirid *Macrolophus caliginosus* attacks several prey including aphids and whiteflies, and then interacts with natural enemies of these prey. The susceptibility of *M. caliginosus* to different intraguild predators was evaluated in the laboratory. When confronted to the mirid *Dicyphus tamaninii*, the level of intraguild predation was always lower than 20%. Young nymphs (N2) were the more vulnerable stages. Adults and older nymphs (N5) showed a very low susceptibility (<6%). At the opposite, when aphidophagous predators were tested, the levels of intraguild predation were much more higher, both on adults and nymphs of *M. caliginosus*.

Key-words: IGP, intraguild predation, interaction, *Macrolophus caliginosus*, *Dicyphus tamaninii*, aphidophagous, zoophytophagy

Predatory activity of two *Orius* species on the western flower thrips in protected pepper crops (Ligurian Riviera, Italy)

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Abstract: The colonisation ability and the predatory activity on *Frankliniella occidentalis* of *Orius laevigatus* and *O. insidiosus* were studied in IPM pepper crops of the plain of Albenga (Ligurian Riviera, north-western Italy). The trials were carried out in three plastic tunnels in 1993-1994; releases of *O. laevigatus* and of *O. insidiosus* were made in tunnel 1 and tunnel 3, respectively, whereas no release was made in tunnel 2. From late April to early September, fortnightly samplings were conducted to monitor the thrips and anthocorid populations. In both years the thrips populations peaked in June; there was a higher infestation level in 1993, due to the transplanting of non thrips-free plants. In both years the highest and lowest predator numbers were sampled in tunnel 1 and in tunnel 3 respectively. Moreover, the first anthocorid captures occurred in tunnel 1 in late May, about a month before the natural dispersal of *Orius* as assessed in tunnel 2. *O. laevigatus* was always the most abundant species, whereas *O. insidiosus* was collected only at the beginning of the season. Thus *O. laevigatus* proved to be the most suitable thrips predator in protected pepper crops of the Mediterranean area. The effects of host plant and temperature on biology and behaviour of the two species are discussed.

Key words: *Frankliniella occidentalis*, *Orius laevigatus*, *O. insidiosus*, colonisation ability, IPM sweet pepper.

Introduction

Since its first detection in Italy in 1987, the western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) has spread rapidly throughout the country, becoming one of the major pests of protected crops (Tommasini & Maini, 1995). In Liguria (north-western Italy), this species infested at first the floral crops, such as chrysanthemum (Arzone *et al.*, 1989), and then vegetables, mainly sweet pepper, on which it causes a great yield loss both for silvering and subsequent necrosis on leaves and fruits, and above all for the tospovirus transmission (Lisa *et al.*, 1990). Previous research showed that damage was more severe in the chemically sprayed crops than in the IPM ones, because a local anthocorid, *Orius laevigatus* (Fieber), used to enter the greenhouses and, when it was not disturbed by insecticide treatments, it colonised the crops and carried out an effective pest control (Tavella *et al.*, 1991).

Therefore, the predatory activity of this anthocorid has been considered immediately as a possible alternative to the chemicals so not to vanish IPM programmes already adopted in the crops of the area. Furthermore, insecticide treatments had to be repeated several times and did not always give satisfactory results in controlling the outbreaks of *F. occidentalis*, also for its ability to develop quickly a pesticide resistance. Unfortunately, adults of *O. laevigatus* often entered pepper greenhouses too late in the growing season; thus releases of commercially produced predators were necessary to anticipate the thrips control.

The first anthocorid commercially available in Italy was *O. insidiosus* (Say), a nearctic species successfully used in North America. It was released in the Ligurian crops in 1991-1992, but samplings carried out in pepper greenhouses at the end of summer showed that the most abundant species was always *O. laevigatus* (Tavella *et al.*, 1994). So the aim of the present investigations was to check the colonisation capacity of the two species and assess the role played by them in the effective control of *F. occidentalis*.

Materials and methods

Experimental plots

Research was carried out in the plain of Albenga (province of Savona, north-western Italy) during 1993-1994. For the trials, an IPM horticultural farm was chosen, where sweet peppers were grown in plastic tunnels from spring to early autumn; in particular in five neighbouring tunnels of the same surface (120 m²), having an east-western orientation. For the experiments, three tunnels (1, 2, 3) were chosen in which three sectors (A, B, C) were distinguished to simplify samplings. Furthermore, a hygrothermograph was placed in the middle of each tunnel to record temperature and relative humidity during the trials.

In 1993 and 1994, 300 young pepper plants per tunnel were transplanted on March 25 and April 6, respectively. Firstly the tunnels were closed, then starting from late May they were opened at the ends; only a net (6×7 mm) was used to prevent the entrance of noctuids. During the cultivation the usual cultural practices were made. The pest management included one treatment with endosulfan before planting, localised sprayings with dicofol and pirimicarb in the case of infestations of tarsonemids and aphids respectively; releases of *Phytoseiulus persimilis* Athias-Henriot against tetranychids in both years and of *Chrysoperla carnea* (Stephens) against aphids in 1993.

Introduction of *O. laevigatus* and *O. insidiosus*

Releases of *O. laevigatus* and of *O. insidiosus*, coming from an Italian biofactory (Biolab of Cesena, province of Forlì), were made in tunnel 1 and in tunnel 3, respectively; no *Orius* specimens were introduced in tunnel 2, which was considered as a control plot. In 1993, three releases of 150 adults per each species were carried out on April 23, May 7 and June 3; while in 1994, two releases of the same number of adults were made on May 18 and June 9.

Field samplings and laboratory analyses

In both years, samplings were carried out every two weeks from April 28 to September 1. To monitor thrips and *Orius* populations, samples of 10 flowers and 10 leaves were collected in each sector of the three tunnels, and put into tubes and into pots containing alcohol 70%, respectively. In 1994, thrips were sampled also by means of light blue sticky traps (125×200 mm), which were placed over the pepper vegetation in the sectors A and C of each tunnel and changed every two weeks from April 14 to September 1. Moreover, in 1994 during every survey starting from June 26, visual checks of 50 flowers per tunnel were made to count *Orius* specimens, the adults of which were identified directly in the field. Means (\pm standard deviation) of anthocorids observed per flower were calculated by Kruskal-Wallis analysis and separated by Student-Newman-Keuls test ($P \leq 0.05$).

In the laboratory, the samples of flowers and leaves were examined to separate and count prey and predator specimens. Then the adults were classified: thrips were observed and identified according to Palmer *et al.* (1989); anthocorids were dissected to extract the genital clasper in males and the copulatory tube in females and were determined according to Herring (1966) and Péricart (1972). The traps were observed at a microscope to check the number and determine the species of thrips captured.

Results

Thrips infestations

In 1993 thrips populations were more consistent than in 1994, because the plants coming from the nursery were not thrips-free. Therefore, the insects could reproduce and spread in the crop more rapidly, in particular in tunnel 1, where an average of 3.6 larvae per flower was observed already during the first sampling, a month after transplanting (table 1). However, the most severe infestation was checked in tunnel 2 at the end of June when an average of 40.9 thrips per flower was sampled. In 1994 thrips-free plants were transplanted; as a consequence the first insects were found in mid May and the population level was always lower.

Table 1. Thrips population monitored on pepper vegetation.

Date	Tunnel 1			Tunnel 2			Tunnel 3		
	Larvae	Adults		Larvae	Adults		Larvae	Adults	
		WFT	OT		WFT	OT		WFT	OT
29 April 1993	108	24	0	1	3	0	1	9	0
12 May 1993	146	10	1	1	0	0	15	3	0
26 May 1993	156	50	1	1	0	0	32	20	1
9 June 1993	580	130	0	119	48	1	89	51	1
23 June 1993	28	28	0	1053	205	0	93	28	1
7 July 1993	8	4	0	108	101	0	40	14	0
21 July 1993	3	0	0	1	6	0	6	2	0
4 August 1993	0	0	0	0	0	0	0	0	0
18 August 1993	0	0	0	0	0	0	0	0	0
1 September 1993	0	0	0	0	0	0	0	0	0
29 April 1994	0	0	0	0	0	0	0	0	0
12 May 1994	0	0	1	0	0	0	0	0	2
26 May 1994	5	0	8	18	0	12	5	0	5
9 June 1994	16	2	16	28	0	44	37	0	32
23 June 1994	56	0	14	59	1	27	17	0	9
7 July 1994	4	0	3	2	0	1	0	0	5
21 July 1994	0	0	0	0	0	0	0	0	3
4 August 1994	0	0	0	0	0	0	0	0	0
18 August 1994	0	0	0	0	0	0	0	0	0
1 September 1994	0	0	0	0	0	0	0	0	0

Another difference between the two years was related to the proportion of the thrips species. In both years two species were collected: *F. occidentalis* and the onion thrips (OT), *Thrips tabaci* Lindeman; in 1993 WFT was the predominant species while in 1994 OT prevailed as assessed also by the traps (table 2). No strict correlation was found between the adult numbers captured on vegetation and by traps using the Spearman correlation test (correlation coefficient = 0.562; $P < 0.01$; sample size = 60).

Orius populations

Data on the anthocorids monitored on pepper vegetation during the two-year research are given in table 3. In both years the highest predator numbers were sampled in tunnel 1, in which the first nymphs were captured about 20 days after the release effected in May. On the contrary, the lowest numbers were monitored in tunnel 3 in spite of the repeated introduction

of *O. insidiosus*. In this plot the first nymphs were collected about 20 days after the release of May in 1993, and only on June 23 in 1994. On the same date in both years anthocorid nymphs were found also in the tunnel 2 in which no release was made. Analysing data obtained by visual checks of pepper flowers in 1994, the highest mean number of predators per flower was observed in tunnel 1 in each sampling date (table 4). Statistical analysis showed that the values were significantly different between plot 1 and the other two plots throughout August.

Table 2. Thrips population monitored by blue sticky traps in 1994.

Date	Tunnel 1		Tunnel 2		Tunnel 3	
	WFT	OT	WFT	OT	WFT	OT
29 April	0	0	0	0	0	0
12 May	1	0	0	1	0	3
26 May	3	59	0	51	5	125
9 June	0	83	10	223	4	133
23 June	4	420	39	646	31	657
7 July	7	260	10	522	1	162
21 July	0	84	0	46	1	30
4 August	1	40	1	48	0	42
18 August	1	16	4	30	0	10
1 September	4	40	0	36	1	21

Table 3. Anthocorid population monitored on pepper vegetation. I = *Orius insidiosus* (Say); L = *O. laevigatus* (Fieber); M = *O. majusculus* (Reuter); N = *O. niger* Wolff.

Date	Tunnel 1		Tunnel 2		Tunnel 3	
	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults
29 April 1993	0	0	0	0	0	0
12 May 1993	0	0	0	0	0	0
26 May 1993	3	0	0	0	2	0
9 June 1993	0	2L	0	0	2	1I
23 June 1993	40	9L	1	1L	1	2I, 1L
7 July 1993	16	24L, 2N	5	6L	5	1I, 2L, 1M
21 July 1993	24	16L, 1N	27	5L, 3N	19	4I, 8L
4 August 1993	23	16L	28	8L, 1N	9	11L, 1N
18 August 1993	43	1I, 36L, 3M	12	6L	7	7L
1 September 1993	15	3I, 21L, 4M	6	6L	12	9L
29 April 1994	0	0	0	0	0	0
12 May 1994	0	0	0	0	0	0
26 May 1994	0	1L	0	0	0	0
9 June 1994	2	0	0	0	0	0
23 June 1994	3	5L	6	3L	2	1I, 2L, 1N
7 July 1994	10	7L, 1N	18	5L, 2M	14	2L
21 July 1994	16	14L, 1M, 1N	18	9L, 4N	7	4L
4 August 1994	28	21L, 1N	22	15L, 1N	15	4L, 2N
18 August 1994	17	26L	16	21L	8	20L, 2N
1 September 1994	25	11L	24	24L	19	23L

Besides *O. laevigatus* and *O. insidiosus*, other two anthocorid species were occasionally collected in all tunnels in both years: *O. niger* Wolff (4.6%) and *O. majusculus* (Reuter) (2.4%). However, the most abundant species was always *O. laevigatus*, which managed to settle in all tunnels independently from the releases and proved to be the most suitable predator in the investigated area, reaching 87% in 1993 and 93% in 1994 of the adults totally sampled. On the other hand, despite its introduction, *O. insidiosus* was unable to colonise the pepper crop; adults were found in low quantities (5% in 1993 and 0.4% in 1994), only during the first samplings and nearly exclusively in the tunnel where it was released.

Table 4. Anthocorids sampled by visual checks of pepper flowers during 1994. Means (\pm s.d.) within every sampling date followed by a different letter are significantly different (Student-Newman-Keuls test; $P \leq 0.05$).

Date	Tunnel 1			Tunnel 2			Tunnel 3		
	Mean \pm s.d. per flower	Total adults		Mean \pm s.d. per flower	Total adults		Mean \pm s.d. per flower	Total adults	
		I	L		I	L		I	L
23 June	0.44 \pm 1.13a	0	4	0.30 \pm 0.61a	0	5	0.24 \pm 0.52a	5	1
7 July	0.74 \pm 0.88a	0	13	0.86 \pm 1.01a	0	8	0.62 \pm 0.70a	6	4
21 July	0.94 \pm 0.82a	0	25	0.84 \pm 0.82a	0	17	0.46 \pm 0.65b	1	9
4 August	1.52 \pm 1.23a	0	34	1.06 \pm 1.19b	1	21	0.82 \pm 0.83b	4	10
18 August	2.14 \pm 1.43a	0	59	1.48 \pm 1.15b	0	40	1.24 \pm 1.17b	0	38
1 September	1.86 \pm 1.21a	0	42	1.66 \pm 1.08a	0	43	1.48 \pm 1.13a	0	42

Relationships between thrips and anthocorids

Population dynamics of thrips and anthocorids are given in figure 1. Data were expressed as the mean number of individuals per flower, since over 98% of both thrips and anthocorid populations was sampled in the flowers. In both years, independently of the infestation rate, thrips populations peaked in June, while *Orius* adults started to colonise the pepper greenhouses spontaneously only from late June. On the contrary, the artificial introduction of *O. laevigatus* allowed to anticipate the presence of the predator of about a month and to obtain a higher population level at the end of June. The action of predators was determinant because when they reached a density of 0.3 individuals per flower thrips populations decreased rapidly.

Climatic conditions

Temperature and relative humidity monitored during the growing season are shown in figure 2. Since the tunnels were closed in the first period, the highest maximum temperature, about 40°C, was recorded inside the tunnels at the beginning of May in 1993 and at the end of April in 1994. Also the lowest temperature, about 10°C, occurred in the same period when, consequently, the highest max-min range of about 30°C was reached. Starting from mid May the maximum temperature decreased, more evidently in 1994 than in 1993; on the contrary the minimum one rose, going up almost to 20°C.

The maximum relative humidity varied from 75% to 94% in 1993 and from 72% to 86% in 1994, while the minimum went from 34% to 58% in 1993 and from 33% to 61% in 1994. Also in this case the max-min range was the highest in early May in 1993 and in late April in 1994. More generally, in the two years the minimum temperature and relative humidity showed a similar trend, whereas the maximum ones were higher in 1993 than in 1994.

Discussion

In the Ligurian Riviera *F. occidentalis* has become the key pest of pepper crops because of its efficiency in transmitting tospoviruses (Tavella *et al.*, 1997). The intensive use of chemicals to control the thrips, often unsuccessfully, causes residual problems and makes IPM programmes not feasible. Therefore, to develop an effective and environmentally sustainable control method it is necessary to rely on prevention measures, releases of commercial beneficials and protection of spontaneous natural enemies. The present research confirmed the importance of prevention: the high thrips population level in 1993 was solely due to the presence of *F. occidentalis* on the plants from the beginning of the growing season, which made more difficult the control by the predators. Transplanting thrips-free plants is required to avoid early outbreaks. Then, accurate samplings are needed to monitor the first infestations; traps can be used to detect pest appearance. Since most of thrips and anthocorids were collected in the flowers, blossom samplings are recommended for checking their population density, according to previous studies (Shipp & Zariffa, 1991; Shipp *et al.*, 1992).

The palaeartic anthocorid *O. laevigatus* and the nearctic *O. insidiosus* have been reported as efficient predators of several pests (Riudavets, 1995). After the introduction in Europe of *F. occidentalis*, investigations have been carried out to find a solution to the outbreaks of this pest. Therefore, the natural presence of *O. laevigatus* preying on the thrips has been recorded in IPM crops of the Mediterranean area (Tavella *et al.*, 1991; Villeveille & Millot, 1991), whereas *O. insidiosus* was successfully released in greenhouses of Belgium and Holland (Riudavets, 1995). More recently, comparative experiments were conducted to assess the predatory activity of some *Orius* species in protected pepper crops. Compared to *O. insidiosus*, both the palaeartic species *O. niger* and *O. albidipennis* (Reuter) showed a better colonisation ability; they totally replaced the nearctic predator at the end of the growing season (Van de Veire & Degheele, 1992; Van de Veire, 1995).

In the Ligurian Riviera *O. laevigatus* proved to be the best thrips predator, being able to colonise the crops both after artificial release and by natural dispersal. Contrarily, *O. insidiosus* could not settle on pepper vegetation in spite of repeated releases. The reasons of this failure can be related to biotic and above all abiotic factors. It is known that the plant can exert an effect on biology and behaviour of predators. In laboratory tests *O. laevigatus* preferred to oviposit on pepper and cucumber rather than on tomato (Riudavets & Castañe, 1994). *O. insidiosus* had a low efficiency in prey searching and suffered high mortality on tomato, but in field trials it showed an inability to develop also on pepper, when the prey was scarce (Coll & Ridgway, 1995). Thus a negative effect of the local pepper cultivar could be hypothesized. However, the climatic conditions play probably a more important role. *O. laevigatus* is well-adapted to support the temperature typical of protected crops of Mediterranean area, as assessed by previous studies (Tommasini & Benuzzi, 1996). In laboratory tests at constant temperatures, *O. insidiosus* showed a shorter developmental time at 32°C (Insenhour & Yeorgan, 1981), but little information has been available about its biological performances under fluctuating thermoperiods similar to those recorded during our experiments.

In conclusion, the artificial introduction of *O. laevigatus* managed to anticipate the natural dispersal and, as a consequence, to control efficiently the pest outbreaks if thrips-free plants were transplanted and the releases were made in May. In fact, too early or too late releases were ineffective. On the contrary, *O. insidiosus* showed not to be adapted to the Mediterranean climate, as confirmed also by the fact that it has never been found in recent

surveys carried out in Sicily where it was released some years ago (Tommasini, personal communication).

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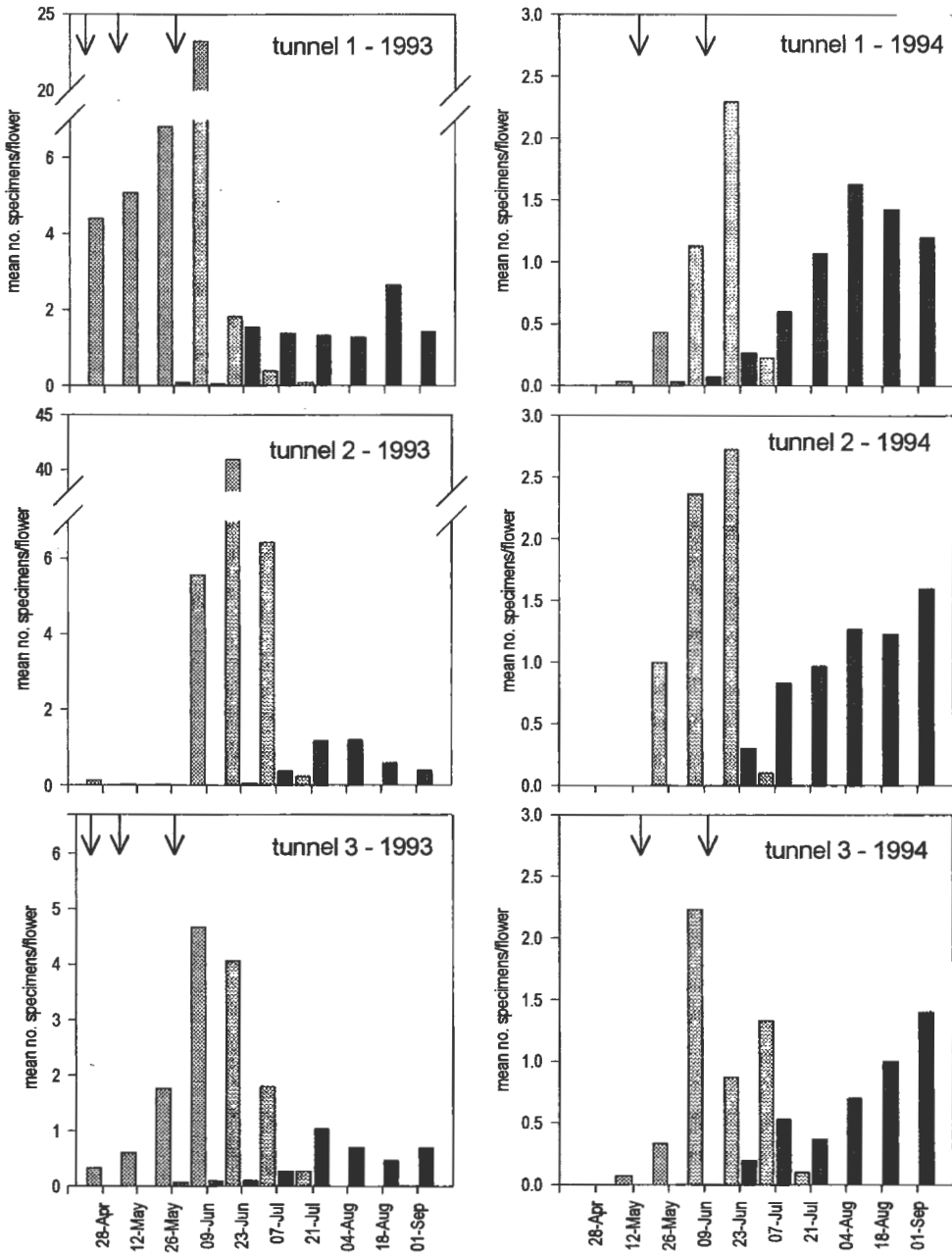


Figure 1. Population dynamics of thrips (▨) and anthocorids (■) checked during the two-year research. Arrows indicate release dates.

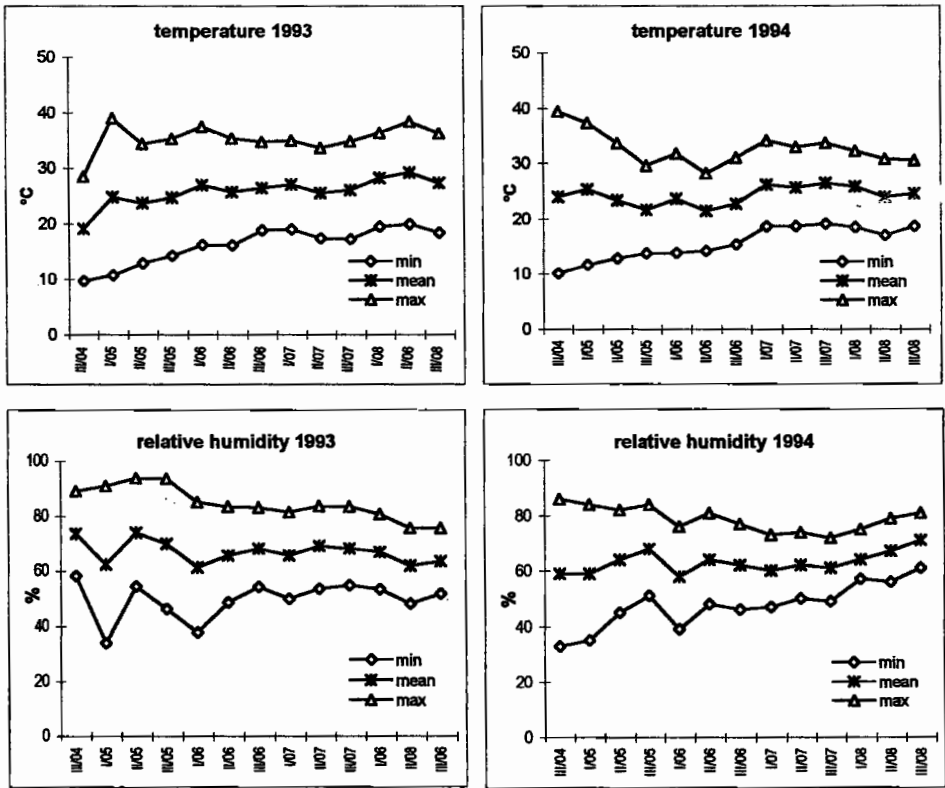


Figure 2. Temperature and relative humidity recorded inside the tunnels during the growing season in the two-year research.

Conservation of *Macrolophus caliginosus* Wagner (Het. Miridae) in commercial greenhouses during tomato crop-free periods

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Abstract: *Macrolophus caliginosus* is a native polyphagous predator that is abundant in field tomato crops. It colonizes greenhouse crops when no broad-spectrum insecticides are used. However, this colonization may occur too late to control whitefly. The use of banker plants as refuges for the predator during tomato-free periods is being studied. Preliminary results indicate that indigenous populations of the predator *M. caliginosus* can be conserved during winter crop-free periods in unheated plastic greenhouses, which ensures early colonization of the spring tomato crop.

Key words: conservation, greenhouses, predators, mirid bugs

Introduction

Integrated Pest Management (IPM) for spring tomato greenhouse crops in Catalonia is based on seasonal inoculative releases of *Encarsia formosa* Gahan. However, the efficiency of the program in the Mediterranean region has recently been challenged due to the spread of *Encarsia pergandiella* Howard (Gabarra *et al.*, 1999). On the other hand, whitefly control in greenhouses is aided by spontaneous colonization by predatory mirid bugs (e.g. *Macrolophus caliginosus* Wagner) (Alomar *et al.*, 1991, Castañé, Alomar, Goula & Gabarra in this volume). These predators are abundant and widely distributed in the Mediterranean Basin (Goula & Alomar 1994, Goula & Arnó 1994). However, greenhouse colonization may occur too late to control whitefly populations.

To enhance the establishment of natural enemies in the crop, the use of banker plants has been proposed in vegetable crops such as cucumber and tomato (Bennison & Corless 1993, Leger 1994, Bourgeois 1997, Maisonneuve *et al.*, 1997) or in field crops such as cotton (Parajulee *et al.*, 1997). The aim of these techniques is to provide a reservoir for the natural enemies when the target crop is not growing in the field. The objectives of our work were (1) to conserve *M. caliginosus* from summer crops during the winter inside commercial greenhouses, and (2) to study the influence of banker plants on spring tomato crop colonization by the mirid bug.

Material and methods

Two trials were conducted in El Maresme area during the crop season of 1998-1999.

Conservation of M. caliginosus in tobacco plants during winter periods

In 31 commercial greenhouses, tobacco plants (at a rate of 1 plant / 80 m²) were transplanted and placed together along one of the margins of each greenhouse during the first fortnight of October. Mirid bugs spontaneously established in the tobacco plants, but additional predators collected from open field tomato crops were also released. Five tobacco plants per greenhouse and 3 leaves per plant (an upper, a middle and a lower leaf) were sampled to evaluate mirid bug populations. Mirid bug species and stages (adults and nymphs) were recorded separately.

Colonization by M. caliginosus of spring greenhouse tomato crops

This was studied on two farms. On Farm 1 a single greenhouse was involved in the trial. Tobacco plants (n=30) were transplanted on July 10, 1998. They were planted together along a margin of the greenhouse and maintained there until May, 1999. An autumn tomato crop was transplanted on July 24, 1998 and lasted until the end of December. After harvest, tomato plants were left in the greenhouse until they become completely dry and they were then removed. Spring tomatoes were transplanted on March 5, 1999.

On Farm 2 two greenhouses were involved. In mid October 1998, 31 potted tobacco plants were introduced into a greenhouse with an autumn tomato crop (transplanted before the end of July). In mid December the harvest finished and the crop was left in the greenhouse until dry. Tobacco plants were kept there until April 24, 1999. Then, 15 plants were moved to a neighbouring greenhouse where spring tomatoes had been transplanted on February 19, 1999. Tobacco plants were individually covered with a mesh before the move, to prevent the mirids from flying away. Plants were not kept grouped but regularly dispersed in the greenhouse.

Ephestia kuehniella Zeller eggs were spread over the tobacco plants to feed mirid bugs (a minimum of 0.2 g/plant).

On Farm 1, mirid populations in tobacco plants were estimated by counting the number of adults and nymphs on 3 leaves of 5 plants and then transformed to the number of mirids per plant. On Farm 2, mirids were counted on all the leaves of all the plants. To assess the colonization of the tomato crop, one tomato plant was sampled every 10 plants (approx. 4 m) along each double row of tomatoes. Mirid nymphs were counted on 10 leaflets at random along the plant (Castañé *et al.*, 1996).

Results and discussion

Conservation of M. caliginosus in tobacco plants during winter periods

In the conditions of El Mareme area mirids can be maintained in unheated greenhouses throughout the winter period (Table 1). Although there was a reduction in mirid adult and nymph populations during December, numbers had recovered by March, when spring tomatoes were transplanted. However, *Nesidiocoris tenuis* (Reuter) was found in December in most of the greenhouses. In these greenhouses tobacco plants were removed in order to avoid the risk of injury by this predator (Vacante & Tropea Garzia 1994).

In March tobacco plants were still growing in 4 greenhouses. *M. caliginosus* was the main mirid species found on them, and accounted for 92% of all adult mirids. *Dicyphus tamaninii* Wagner was recorded in only one greenhouse (7% of total adults) and *N. tenuis* was found in 2 greenhouses (7% and 29% of total adult mirids). No damage was attributed to this mirid bug in the spring tomato crop.

Colonization by *M. caliginosus* of spring greenhouse tomato crops

As can be seen in Figure 1, *M. caliginosus* populations in tobacco plants were very low in December, but they recovered in March. In March populations on Farm 1 were higher than on Farm 2, and the proportion of nymphs was also higher (97% on Farm 1 and 64% on Farm 2).

Table 1. Average numbers of mirid bugs (\pm SE) per 3 leaves on tobacco plants in unheated greenhouses (n: number of greenhouses sampled).

Date	n	Adults	Nymphs	No. of leaves/plant
November 1998	27	3.1 \pm 0.68	1.5 \pm 0.30	8.9 \pm 0.43
December 1998	24	0.7 \pm 0.15	2.3 \pm 0.52	9.7 \pm 0.48
March 1999	4	2.4 \pm 0.63	5.9 \pm 0.63	6.5 \pm 0.06

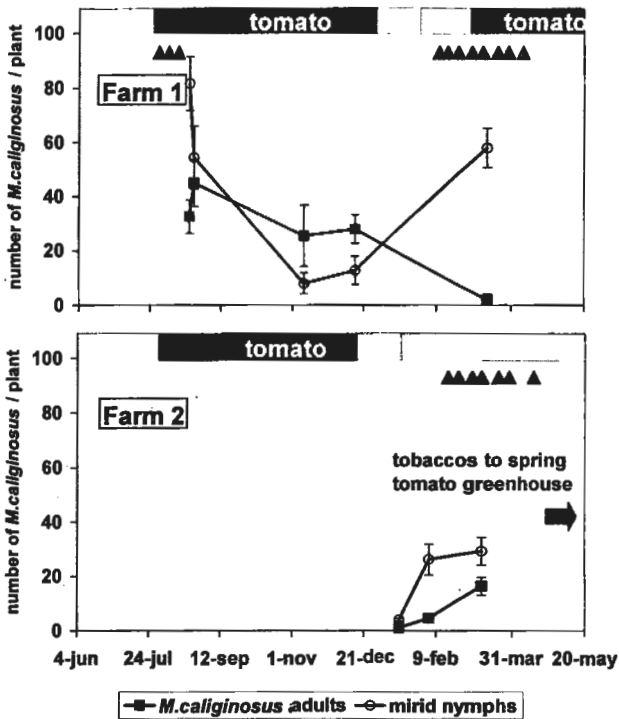


Figure 1. Conservation of predatory mirids on tobacco banker plants in two unheated greenhouses during winter. Solid bars indicate the tomato crop period in the greenhouse, hatched bars indicate the time when the crop was drying, and the white bars indicates the tomato-free period (indicates the spread of *E. kuehniella* eggs as food for *M. caliginosus*).

On Farms 1 and 2, the positive effect of tobacco banker plants ensured early colonization of spring tomato greenhouses by *M. caliginosus*. In Farm 1 (Figure 2), mirid nymphs were

found up to 50 plants (approx. 20 m) into the tomato crop, but still only part of the greenhouse had *M. caliginosus* nymphs 11 weeks after tomato transplant. From this initial source, mirids rapidly established in most of the greenhouse, but some minor whitefly foci developed on the opposite side. Note that Figure 2 reflects approximately half of the greenhouse in Farm 1.

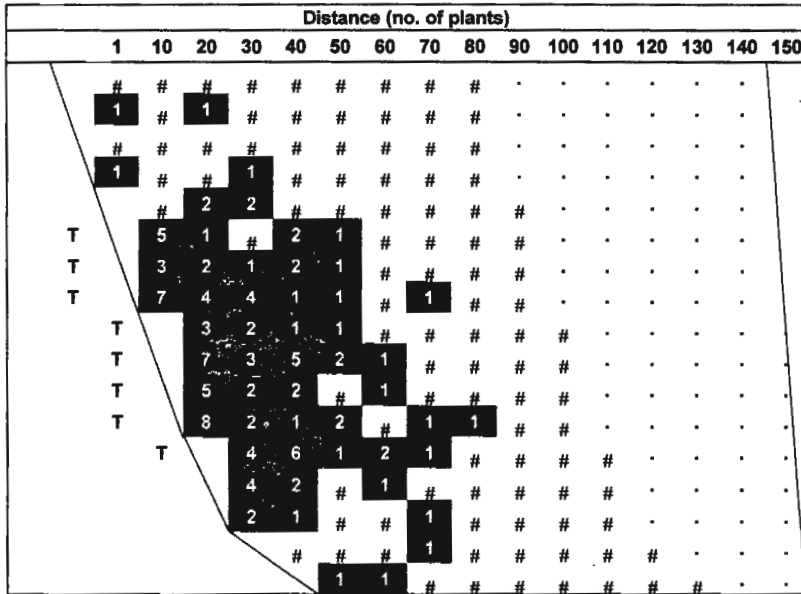


Figure 2. Distribution of *M. caliginosus* nymphs in the spring-tomato greenhouse on Farm 1 from the tobacco banker plants (T) placed together along a margin of the crop (n=30). Numbers in squares show the number of nymphs/tomato plant, (#) indicates tomato plants without nymphs and (•) the position of tomato plants that were not sampled.

The average number of nymphs on the spring tomato plants surrounding a tobacco banker plant on Farm 2 is displayed in Figure 3. There were 15 banker plants distributed throughout the greenhouse. Results from Farm 2 show that *M. caliginosus* nymphs were found up to 30 tomato plants (approx. 12 m) apart from the tobacco banker plants, even if tobacco plants had been in the spring-tomato greenhouse for only four weeks. This suggests that dispersing the banker plants within the greenhouse may result in a more uniform distribution of *M. caliginosus* within the crop. Since mirid nymphs were present on most of the 200 tomato plants (87 m² approx.) placed around a tobacco plant (average of 0.5 ± 0.2 nymphs/plant), the ratio of 1 tobacco plant per 80 m² seems sufficient to ensure the establishment of *M. caliginosus* in the greenhouse.

In conclusion our results indicate that *M. caliginosus* can be maintained in unheated greenhouses during winter months in banker tobacco plants and that they will establish in spring tomatoes in advance of spontaneous colonization from outside refuges. The rate of 1 tobacco plant per 80 m² may ensure the establishment of *M. caliginosus* in the greenhouse.

Further developments of this strategy may improve biocontrol in spring-tomato greenhouses by conservation and augmentation of native predators.

Distance (no. of plants)										
40	30	20	10	0	0	10	20	30	40	
#	0.5	#	0.2	#	0.2	#	#	0.4	#	#
#	#	#	#	0.7	T	0.9	0.2	0.2	0.4	#
#	#	0.2	0.6	4.4		4.0	0.7	0.4	#	#
#	#	#	0.1	1.2		1.0	0.2	#	#	#
#	0.1	0.2	0.2	#		0.1	#	#	0.4	#

Figure 3. Average abundance of *M. caliginosus* nymphs on tomato plants surrounding a tobacco banker plant (T) in Farm 2 (n= 5-10). Numbers in squares indicate the mean number of nymphs per plant and (#) indicates the tomato plants without nymphs.

Acknowledgements

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Biopropagation of *Macrolophus caliginosus* Wagner for a quicker establishment in southern tomato greenhouses

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Abstract: *Macrolophus caliginosus* is the major beneficial used in an IPM program in tomato greenhouse against greenhouse whitefly (*Trialeurodes vaporariorum*) and *Bemisia tabaci*. In nursery, on 20 days old plants, 0,8 adults per plant are released and food based on *Ephestia kuehniella* eggs is supplied. This biopropagation method is efficient to get a quicker establishment of *M. caliginosus* all over the greenhouse one month after plant dispersal than the regular introduction in the production greenhouse. Nevertheless, several key parameters are requested as the use of netting, extra food supply, time needed for sufficient egg laying and the influence of chemical insecticides. This study was conducted in a two years program in order to optimise all these parameters. In this paper, only data concerning the use of netting are detailed, and a method is proposed for a successful biopropagation of *M. caliginosus*.

Key words: whitefly, biological control, *Macrolophus caliginosus*, tomato, nursery

Introduction

The predatory bug, *Macrolophus caliginosus*, is known to be efficient for biological control of whitefly in tomato glasshouses. It is now used everywhere in France. Unfortunately, it takes about 10 to 12 weeks to establish and to reach a significant level of population able to control whitefly.

In southern area, we can notice three major periods for tomato planting. The earliest period starts in October. In autumn, whitefly population can increase rapidly and only a few weeks after planting we can observe high infestation levels. Furthermore, climatically conditions are not always favourable for establishment of beneficials such as *Encarsia formosa* and *M. caliginosus*. Corrective treatments are often requested and consequently the overall IPM program will be disturbed (Lenfant *et al.*, 1998).

For a good IPM program, several parameters are considered as "success key parameters": early establishment of beneficials, reduction of treatments before releasing, reduction of cost, make management of greenhouse easier (climate control, leaf cutting), keep virus problems (PVY, TSWV,) under control.

Taking into account above requests, we have carried trials out during two years in experimental glasshouses at the INRA station in Alenya. The feasibility of releasing *M. caliginosus* in nursery greenhouse was tested and specific conditions were identified. This method was studied earlier by Trotin-Caudal & al (1995) but under different conditions (no netting, no extra food, 1 adult *Macrolophus* per plant on December the 15th, planting on 12/29). In our case we feed *M. caliginosus* with alternative food, *E. kuehniella* eggs, in order to stimulate fecundity. In this way we obtain a maximum number of eggs in each plant and a high *M. caliginosus* population a short time after planting. We call this "biopropagation"

In 1998, 0,8 *M. caliginosus* adults per plant were released 20 days after sowing and fed with 20 gr. of *E kuehniella* eggs for 700 plants. This release was done under netting in order to maintain *M. caliginosus* on the plants. After planting, *M. caliginosus* was fed 5 times during the development of *M. caliginosus* larvae (Ridray *et al.*, 1998). The first emergence of *M. caliginosus* was observed 30 days after release with mean of 5 larvae per plant and 95% of occupied plants. In the control plot with normal release of *M. caliginosus* after plant dispersal, the same population level was observed ten weeks later.

In 1999, we applied this method in commercial greenhouse nurseries. In the experimental glasshouse we carried trials out for an optimisation of this method.. Two items were studied : reduction of feeding, introduction without netting and the compatibility with treatments against thrips and aphids.

Material and methods

Release of Macrolophus in nursery and alternative food with Ephestia eggs

Sowing was done on October the 21st. Two modes were tested : presence or absence of netting over the young plants. In each plot, 0,8 adults per plant were released on November the 10th. First feeding was carried out in the nursery greenhouse at the same time (0,06 gr. per square meter) The second feeding was done on December the 8th (week 50), 13 days after planting at dose 0,08 gr. per square meter. This date for feeding was chosen according to the first emergence of first instar larvae.

Planting and initial pest situation

Planting was done on November the 25th (week 48) in the experimental glasshouses of 300m² with a density of 2,4 plant per m². At that time, we observed 0,03 whitefly adults and 0,3 thrips per plant. Leafminers were observed since the nursery. No chemical treatments were carried out on the young plants. No additional releases of whitefly parasitoids were took place.

Counting and observations

Every week between week 49 to week 18 (first week of May), whitefly and *M. caliginosus* were counted on 20 plants ad random over the plot. For whitefly, adults and L4 were observed. All *M. caliginosus*, instars (young and old larvae, adults) were counted.

Results and discussion

The 99 results confirm that biopropagation is efficient. After only 4 weeks, *significant M. caliginosus* population is present. During the six weeks after hatching, young larvae are observed. Adults are present after the seventh week mixed with older larvae. The second offspring is two times more important even if food, like whitefly larvae or eggs, is hardly present.

The use of netting (graph 1A) during the release and the egg-laying period leads to a higher population level (6 larvae per plant) than without netting (3 larvae per plant) (graph 1B). *M. caliginosus* is known to be an efficient whitefly predator when its population reach about 5 individuals per plant. Furthermore, in case of use of netting, more than 90% plants are occupied by predatory bugs and less than 50% in the case without netting.

In certain periods, only larva stage is present. This is a risky situation when whitefly population is increasing. In fact each instar hasn't the same predatory capacity. This is why figure 1A and 1B show an increase of whitefly even if predatory bug is present. In the worst situation (figure 1B), a larvicide was requested.

In 98, 4 food supplies were carried out (Ridray *et al.*, 98), and population of *M. caliginosus* was higher. However, one feeding at the L4 instar seems to be enough for building up a reasonable *M. caliginosus* population.

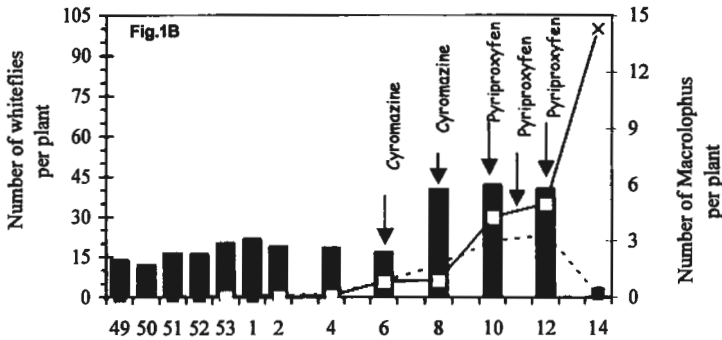
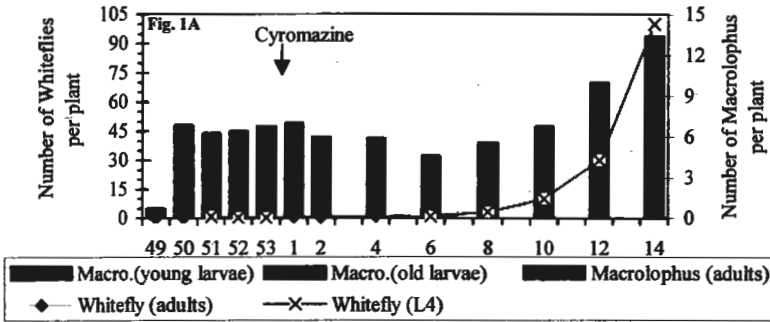


Figure 1. *Trialeurodes vaporariorum* and *M. caliginosus* population in tomato. *M. caliginosus* is released on week 46, in nursery on 20 days old plants, at 0,8 adults per plants with *Ephestia* as extra food. 1A : nursery under netting, 1B : without netting. Second extra feeding is done on week 50, then 13 days after planting.

As presented in Schoen *et al.*, (1999), most insecticide treatments carried out in nurseries are harmful for *M. caliginosus*. During this critical period several pests as leafminer, whitefly, aphids and thrips can contaminate plants. Beneficials should be released to control leafminer, whitefly and aphids under nursery conditions. The major problem is still virus transmitted by aphids or thrips. Use of insect-proof nurseries seems to be the best way of vector control.

This biopropagation method is a improvement compared to the classical introduction method in which *M. caliginosus* establish itself rather slowly and much less homogenous in the crop. Nevertheless, to succeed in biopropagation several key parameters are requested. They are summarised in table 1.

Table 1: Method for a successful *M. caliginosus* biopropagation program in nursery greenhouses

Plant stages	Steps for biopropagation	Remarks
Sowing	Installation of netting above the plants	Start biological control with leafminer, aphid and eventually whitefly parasitoids. Insectproof.
Dispersal	Feeding <i>M. caliginosus</i> Release	<i>M. caliginosus</i> released must be adults No insecticides Use drip irrigation
Planting		Continue biological control No leafcutting
1 st month in production greenhouse	Check hatching of <i>M. caliginosus</i> Feeding when presence of young and older larvae.	Avoid leafcutting till the first adults appear

This *M. caliginosus* biopropagation is a good opportunity for Mediterranean countries where IPM is a problem early crops. Further trials will be conducted to verify if biological control of *Bemisia tabaci* is possible.

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***Dicyphus tamaninii* in the biological control of cucumber pests**

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Abstract: Our research on the use of the predatory bug *Dicyphus tamaninii* (Heteroptera, Miridae) for the biological control of greenhouse cucumber pests (the greenhouse whitefly *Trialeurodes vaporariorum*, the western flower thrips *Frankliniella occidentalis* and the cotton aphid *Aphis gossypii*) is reviewed. The predator was able to provide a good control of whiteflies and to greatly reduce the thrips populations when both pests infested the crop simultaneously. However, scarring appeared in cucumber varieties with long fruits. When the cotton aphid infested the crop, *D. tamaninii* greatly reduced their populations in comparison with a control treatment without predators, but did not prevent the exponential growth of aphids. Additional measures to complement the action of the predator should be applied for the control of thrips and aphids.

Key words: *Trialeurodes vaporariorum*, *Frankliniella occidentalis*, *Aphis gossypii*, *Dicyphus tamaninii*, mirid bugs, biological control, greenhouse cucumbers, polyphagous predators

Introduction

Cucumbers grown in greenhouses (mainly varieties with long fruits) have several pests, such as the western flower thrips *Frankliniella occidentalis*, the greenhouse whitefly *Trialeurodes vaporariorum* and the cotton aphid *Aphis gossypii*. Damage caused by these pests consists of loss of crop yield and fruit scarring in the case of the western flower thrips, and development of sooty mould on the honeydew excreted by whiteflies and aphids. Fruits become unmarketable or they produce a low quality yield with the corresponding loss of income by the grower.

The use of polyphagous predators for the control of greenhouse vegetable pests is increasing due to the coexistence of several pests in these crops. These natural enemies are usually released against one target prey, but growers recognise they have fewer problems with other pests present when they use predators. The mirid bug *Dicyphus tamaninii* feeds on thrips, whiteflies and aphids, among other potential preys, and reproduces in cucumber crops (Albajes *et al.*, 1996). It is an efficient whitefly predator in field tomatoes (Alomar and Albajes, 1996), and reduces *T. vaporariorum* or *F. occidentalis* in exclusion cages in cucumber (Gabarra *et al.*, 1995). In greenhouse experiments (Castañé *et al.*, 1996) a predator-to-prey ratio of 3:10 maintained the thrips population below 16 individuals/400 cm² of leaf area, a threshold that avoids damage in short fruit cucumber varieties.

In this paper we summarise our recent work on the use of *D. tamaninii* for the control of cucumber pests in inoculative strategies of biological control.

Simultaneous control of whiteflies and thrips

D. tamaninii was efficient in reducing greenhouse whitefly and western flower thrips populations in previous trials with cucumber varieties of short fruits but no data are available

of its control capacity when both prey coexist in long fruit varieties, more sensitive to fruit damage.

A predator:prey ratio of 3:10, considering both preys together, and a control treatment were tested in two greenhouse trials performed in 1995 and 1997 (Figure 1). *D. tamaninii* controlled the greenhouse whitefly. *T. vaporariorum* populations were significantly lower in the predator than in the control treatment during most of the crop season in both years, and no sooty mould appeared. On the other hand *D. tamaninii* reduced thrips densities. *F. occidentalis* populations were also kept significantly lower in the predator than in the control treatment during most of the crop cycle, especially in 1997, when thrips were more abundant. However, scarred fruits due to thrips appeared in both treatments. It does not seem that *D. tamaninii* alone can prevent thrips damage with simultaneous infestations of both pests in long cucumber varieties. A complementary control measure for thrips that is compatible with the release of the predator (releases of phytoseiid mites or application of entomopathogenic fungi) is needed.

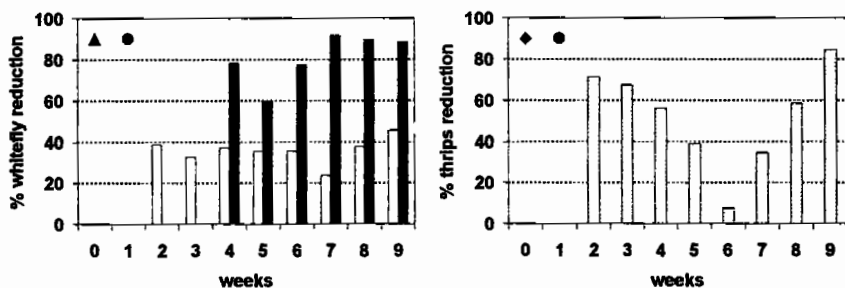


Figure 1. Percentage of population reduction of *T. vaporariorum* adults (white bars) and nymphs (shaded bars), and *F. occidentalis* (nymphs + adults) when comparing a 3:10 ratio of predator:prey with a control treatment without *D. tamaninii*. Release of *D. tamaninii* (●), *T. vaporariorum* (▲) and *F. occidentalis* (◆).

Aphid control

D. tamaninii was voracious in the laboratory, consuming up to 46.6 young nymphs per female (Alvarado *et al.*, 1997), but no information is available on its control capacity in greenhouse crops.

In 1997 a low (6:36) and a high (6:6) predator:prey ratio was tested and compared to a control treatment without predators. Adult mirids were released shortly after infesting the crop with aphids. The predator established in the crop and produced a new generation of nymphs, indicating that this aphid species allows the reproduction of the predator. *D. tamaninii* had a large impact on aphids, especially at the higher ratio tested (Figure 2). Although the exponential aphid growth was retarded by the action of the predator, its population eventually reached high levels and its control failed at the two ratios tested.

In the 1998 greenhouse trial the predator was allowed to establish before the pest was introduced. *Ephestia kuehniella* eggs were regularly sprinkled on the plants as prey before the aphid introduction. The same predator:prey ratio (6:6) as in the previous trial, two times (6:3), and six times (6:1) this ratio were tested and compared with a control treatment without

predators. Three weeks after aphid introduction up to 90% reduction of the pest population was observed in the highest predator:prey ratio. Nevertheless, the number of aphids per plant was again too high (a mean of 1438 ± 591 aphids per 5 leaves in the highest ratio) and aphid populations grew exponentially at all ratios tested.

D. tamaninii does not appear to prevent to avoid the exponential growth of *A. gossypii* populations, even at a high doses that would not be feasible commercially. In cucumbers other measures should be integrated for the control of this aphid species in order to protect the crop from yield loss.

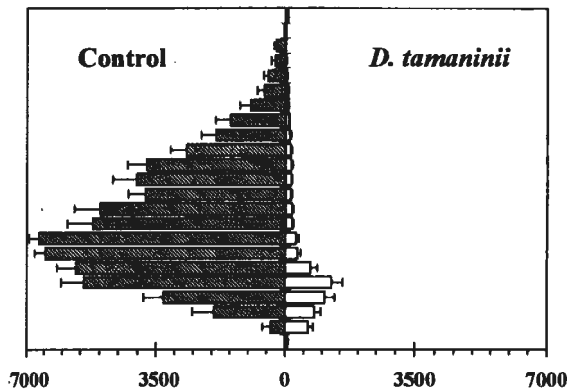


Figure 2. Mean number of *A. gossypii* per leaf in plants of the control and the predator treatment on week 4 after transplanting. Bars correspond to the successive leaves of the plant, with the oldest at the bottom of the figure.

Risk of damage due to mirid bugs

Round punctures different from fruit scars due to thrips have been found in some cages of these trials. *D. tamaninii* is known to injury tomato fruit when populations are high and prey is no longer available. A decision chart has been developed to manage predator/prey densities (Alomar & Albajes 1996). Similarly, the management of *Camptyloma verbasci* (Meyer) in European pome fruit orchards can take advantage of this mirid bug. Yield damage in susceptible varieties can be avoided by applying insecticides during the sensitive period according to a population threshold (Thistlewood & Smith 1996). Our current work is focusing in evaluating damage caused to cucumbers and ways to prevent it.

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Dicyphini collected on vegetable and wild plants in north-western Italy (Heteroptera Miridae)

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Abstract: Dicyphini (Heteroptera Miridae) comprise small, slender, long-legged species that may live on a large variety of host plants. They may colonise crops under IPM, either protected or open air. Attention has been focused on some Dicyphini species as they have revealed to be efficient pest predators in horticultural crops. In the Mediterranean Basin, these biological control agents have already been studied in Spain, France, Italy and Greece. Latest objective of the research was to check indigenous fauna for IPM purposes.

In north-western Italy, particularly in Liguria, previous investigations on these predators were performed only on tomato crops. To improve the knowledge, further samples were taken in IPM fields and their surroundings in the last ten years. Several localities in Piemonte and Liguria were prospected. Main crops surveyed were tomato, sweet pepper, eggplant and zucchini.

Macrolophus spp. and *Dicyphus errans* (Wolff, 1804) were the most common species found in the crops. *Nesidiocoris tenuis* (Reuter, 1895) was abundant but only in Liguria at the end of the summer. Other species were collected only on the wild plants near the crops.

Impact of *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) on whitefly populations in protected tomato crops

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Abstract: Different studies carried out in protected tomato crops in Tenerife (Canary Islands) have shown the effect of the mirid bug *Nesidiocoris tenuis* on mixed populations of the whiteflies *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius).

Nesidiocoris tenuis was first recorded for the Canary Islands by Horvath in 1909, and nowadays appears regularly on protected and open-air tomato crops of our archipelago. *N. tenuis* has been collected in Fuerteventura, Gran Canaria, Tenerife, Gomera and La Palma islands, where it is widely distributed. It is regularly found on tomato crops, but also on other horticultural crops like beans, cucumber, sweet pepper or potato, ornamentals such as *Poinsettia pulcherrima* or wild flora as *Hyoscyamus albus* or *Datura stramonium*. In the archipelago, it has been only recorded preying on the whiteflies *T. vaporariorum* and *B. tabaci*. Although *N. tenuis* has been initially considered a cosmopolitan tomato pest (CAB, 1971; Dessoukki *et al.*, 1976), more recently its role as whitefly predator has been recorded in several areas (Vacante & Garzia, 1994).

Field observations in tomato crops of the Canary Islands, along more than five years, have shown its effects on whitefly populations, as well as on other tomato pests. In the light of these satisfactory results, we have carried out different trials to verify *N. tenuis*-efficacy as predator of whiteflies and its after-effects on tomato plants. The objective of this work was to confirm the possibility of using *N. tenuis* for whitefly control in IPM programmes in tomato crops in the Canary Islands.

All field trials were carried out during 1996 in commercial tomato greenhouses developing organic agriculture. The assessment of the natural control of mixed populations of *T. vaporariorum* and *B. tabaci* by *N. tenuis* consisted of visual observations on randomly taken plants where the number of whiteflies and mobile stages of *N. tenuis* were registered. To assess the effect of *N. tenuis* on the whitefly parasitism, leaves were picked up and the number of whitefly preyed nymphs registered, distinguishing between previously parasitized or not. *N. tenuis* feeding damages on tomato plants was evaluated by visual observations.

Laboratory trials were carried out during 1999. Both *B. tabaci* and *N. tenuis* individuals used in the experiments came from laboratory rearings and were collected originally on tomato. *N. tenuis* was reared on *Nicotiana tabacum* potted plants under 26,5°C in 16L:8D and *B. tabaci* was supplied as prey.

Our results show that natural populations of *N. tenuis*, regularly present on tomato, reduce whitefly populations and therefore reduce their damage on this crop. Moreover, it has been confirmed that *N. tenuis* is harmless to tomato plants and that it does not meaningfully affect natural parasitism. Along its postembryonic development, *N. tenuis* consume 335 whitefly nymphs, last nymphal instars showed the highest *B. tabaci* consumption.

Key words: *Nesidiocoris tenuis*, *Trialeurodes vaporariorum*, *Bemisia tabaci*, protected tomato crops.

First approach on the potential role of *Dicyphus cerastii* Wagner (Hemiptera: Miridae), as natural control agent in Portuguese greenhouses

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Abstract: A first set of data concerning the most common mirid bug in the Portuguese greenhouses of the Oeste region, the Dicyphinae *Dicyphus cerastii* Wagner 1951, (Hemiptera: Miridae) is presented. Along with seasonal evolution of the population on protected tomato, a laboratorial trial showed the potential to predate the leafminer *Liriomyza huidobrensis*. In the laboratory it was also possible to demonstrate that the necrophagy occasionally observed in the field, is an adaptative advantage for this facultative phytophagous and predator, since feeding cadavers from entomological origin increases the fertility.

Key words: *Dicyphus cerastii*, mirid, greenhouse crop, tomato, *Liriomyza huidobrensis*, facultative predator

Introduction

From the hundreds of mirid bugs (Heteroptera: Miridae) known in the Mediterranean basin (Goula, 1986), only a very small group has been studied as potential agents for biological pest control. In Europe, most IPM relevant data about mirid bugs concern two species: *Macrolophus caliginosus* Wagner and *Dicyphus tamaninii* Wagner. Both belong to the sub-family Dicyphinae (Wagner, 1970) and have zoophytophagous feeding habits although but with different consequences, as Alomar *et al.*, (1994) referred: from the phytophagous part of the diet of *M. caliginosus* no damages with economic impact seems to appear, in opposition to *D. tamaninii* which under certain conditions (low mirid bug/prey ratio) could be nocive and therefore needed to be controlled with a chemical spray (Alomar, 1995).

In Portugal, the survey of greenhouses in Oeste region, carried out in 1997, showed that, in those structures under less chemical pressure, another mirid bug *Dicyphus cerastii* Wagner was the most common. In the absente of informations about this mirid, field data had to be sampled, to analyse its potential as agent for biological control in the future.

In Catalonia, Alomar (1994) showed that *D. tamaninii* could be a efficient natural control agent of the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). There are also references showing the suitability of *M. caliginosus* to predate not only this aleyrodide but also aphids (Foglar *et al.*, 1990). In Portugal, since the mortality of the leafminer *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae), due to parasitoids, could not explain the total mortality observed in greenhouses of the Oeste region, the common presence of *D. cerastii* suported the idea that the predatory action of the mirid could be involved (Godinho, com.pess.).

Material and methods

Since the beginning of 1997 a group of greenhouses of "capela" type, in the surroundings of Mafra, Portugal, belonging to growers which worked following the rules of a local organic growers association, has been surveyed, trying to register the moment when the colonisation by mirids starts and the evolution of the population through the tomato growing season. 30 plants were observed in each greenhouse ($\pm 300\text{-}400\text{m}^2$) and the number of adults and nymphs found, and ethological data were registered. Periodically adult mirids were sampled and later were sent for identification to the University of Marburg, in Germany, to prevent possible mistakes due to the difficult systematic of the genus *Dicyphus* sp.

Tomato plants were grown kept in growing cages. When they were 50 cm high, adults of the leafminer *L. huidobrensis*, captured in greenhouses of the same region were released in the cages. So it was possible to have leaves with larvae all of the same age, free from the action of parasitoids. With a 1cm tall and 3cm wide micro-cage, it was possible to isolate every larvae of *L. huidobrensis* and, through a small window of the cage, put either one male or one female inside this light plastic device, which held itself close to the leaf, avoiding the attempts of the mirids to escape, but allowing the normal growth of the mine, even outside the range of the cage. After 15 days it was possible to see how many leafminers died inside the 40 cages with mirids, and how many pupated, and compare this number with the one resulting from the cages without mirid.

The advantages of the observed necrophagy, often seen in the greenhouses, were studied with 40 bags, 360cm^3 each. 20 of them, kept one couple of adult *D. cerastii* each, with no other supply than feeding from the tomato plant and in the other 20 bags, the same number of adult couples got every second day, one recently killed diptera. After 40 days the bags were opened and the number of new mirids, in all development stages, was counted.

Results and Discussion

The adults of *D. cerastii* start colonising the greenhouses in May. The population stays at very low levels until June, but it's in July that the number of mirids on tomato plants increases at higher levels. At the end of August, beginning of September, as the plants start drying from the bottom to the top, the nymphs concentrate in the higher parts of the plant, while the adults start leaving the crop. Although no specific chemical treatments were made against *T. vaporariorum* or against *L. huidobrensis* these two pests have never really established in the observed greenhouses during the season. Nevertheless, no damages on the fruits, caused by the mirids, were found despite their large number in July and August.

From the 20 larvae of the leafminer kept in the micro-cages with females of *D. cerastii* only six could pupate. Four from them escaped from the micro-cage and pupated outside (Fig.1). From the group of 20 micro-cages with one male *D. cerastii* each, the results are less expressive, since only 11 larvae died inside the cages (Fi.2). Nine leafminers achieved to pupate, seven of them, outside the cage. Nevertheless, there is a big difference between the two groups and the reference, where all the larvae pupated.

The results suggest that *D. cerastii* is able to predate on *L. huidobrensis*, and that the females are more effective and/or faster in action than the males. This should be investigated in the presence of other preys and confirmed under field conditions.

The results of the experiment about necrophagy were a much higher fertility of the mirids which had a necrophagous additive to the diet (Fig.3). It is possible that when predation is possible, the necrophagy is not any longer so important. This needs further studies, but if it is so, necrophagy can be relevant in periods of low prey availability and so, a important adaptation of the species for survival under difficult environmental conditions. In the perspective of biological control, since no damage was noticed, the increase of the population even with no major pests as preys available, can be positive because the answer to a possible newcoming from the outside would find a large number of predators already established in the greenhouse, and so a much more secure situation for the grower.

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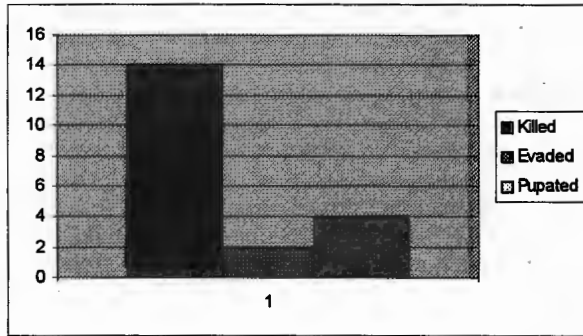


Fig. 1 - Predation of the females of *Dicyphus cerastii* on larvae of the leafminer *Liriomyza huidobrensis*.

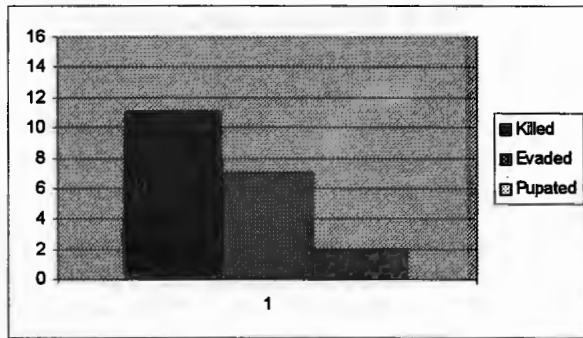


Fig. 2 - Predation of the males of *Dicyphus cerastii* on larvae of the leafminer *Liriomyza huidobrensis*.

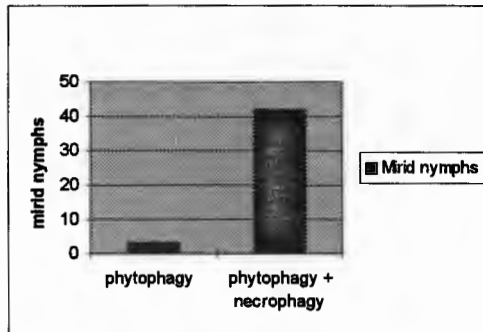


Fig. 3 - Effect of the necrophagy on the fertility of *Dicyphus cerastii*.

The role of Chrysopids as natural control agents in Portuguese greenhouses

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Abstract: Several species are among the native polyphagous predators of the entomofauna in Portugal. Although 28 species have been observed in Portugal, there is a big lack of information related with their potential use as pest control agents. In Portugal, the more relevant data concerning Chrysopids are, most of them, if not all, based on *Chrysoperla carnea* (Stephens) ignoring the real significance of all other species, some of them very common. In this work the most common species are listed and its relative importance is discussed, specifically in protected crops in the Oeste region, based either in ammonium dihydrogen phosphate and protein traps, or on yellow sticky traps, used isolated in some experimental plots or combined, in other plots.

Key words: Chrysopids, *Chrysoperla carnea*, natural limitation, greenhouse crops

Comparative behaviour of three predators used in Biological Control in greenhouse crops

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Abstract: The mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii* and the anthocorid bug *Orius majusculus* are abundant generalist predators in nonsprayed horticultural crops in the Spanish Mediterranean coast. Their behaviour when they were exposed to low and high densities of greenhouse whitefly pupae was evaluated, in order to understand their prey searching strategy, as well as the differences among predators. *M. caliginosus* was a resident species remaining on the patch independently from prey density, whereas *D. tamaninii* and *O. majusculus* left the plant when prey density was low. When prey density was high, all predators found and consumed the prey, but the presence of the prey affected the time budgets of only few activities. Rest was an important activity in *M. caliginosus*, which seems to be more energetically conservative than the other two predators, in which walk+tap was the main activity. The lack of prey intake when prey density was low didn't lead to an increase in plant consumption in any of the species tested. Mirid bugs fed on the plant more time than the anthocorid bug in both high and low prey conditions

Key words: *Dicyphus tamaninii*, *Macrolophus caliginosus*, *Orius majusculus*, miridae, anthocoridae, predatory behaviour, biological control.

Introduction

Vegetable crops on the Mediterranean coast support several pests that can reach high densities in the absence of their natural enemies, among which the mirid bugs *Dicyphus tamaninii* Wagner and *Macrolophus caliginosus* Wagner and the anthocorid bug *Orius majusculus* (Reuter) are particularly abundant in nonsprayed cucumber crops in the Spanish Mediterranean coast (Riudavets *et al.*, 1995). Generalist predators –like the cited species- are becoming increasingly appreciated for biological control purposes because their dynamics are less depending on a single prey than the more specific ones. Although some of these predators are currently being used as biological control agents, the relationship that they establish with their prey is not well known. The functional responses of the three predators to varying prey density has been studied (Montserrat *et al.*, submitted) but more detailed and continuous observations of the behavior underlying functional responses are needed to confirm or modulate conclusions reached from functional response experiments. Additionally, understanding the behaviour of predators when searching for their prey may be critical for improving their use in biological control programmes, in particular when predator can facultatively feed on plants, as it is the case of the three predators cited. Facultatively phytophagous predators have been regarded as 'dangerous' in biological

control because they can cause damage to crops, but also as 'useful' because they can maintain themselves on the crop at low prey densities or even in the absence of prey.

The aim of this study is to describe and compare the behaviour of these three predators when they are exposed to high and low densities of pupae of *Trialeurodes vaporariorum* (Westwood), a main pest on greenhouse cucumber crops.

Material and methods

Insects

Predators came from laboratory colonies maintained in controlled conditions (25 ± 1 °C, 16:8 h light:dark and $70 \pm 10\%$ RH); *D. tamaninii* and *M. caliginosus* were reared on tobacco plants with *Ephestia kuehniella* (Zeller) eggs as prey, and *O. majusculus* was reared on bean pods with *E. kuehniella* eggs as prey. *T. vaporariorum* was reared on tobacco plants in a heated greenhouse, and only the pupal stages were used in our experiments.

Behavioural observations

Predators used in the experiment were reproductive females (7-12d old) which were fed greenhouse whitefly pupae for 24 h and then starved for another 24 h. Direct and continuous observations of their behaviour were conducted at room temperature (25 ± 1 °C). The experimental set consisted on a 20cm cucumber plant with all leaves but one removed and with one whitefly pupa (low density) or 0.41 pupae per cm^2 (high density) glued to the leaf with carboxymethylcellulose. This is 50-60 whitefly pupae per leaf, the maximum predation observed in six hours in a previous experiment by these predators. Predators were placed at the bottom of the stem and their behaviour was observed continuously for approximately 6 hours or until they left the plant. Five replicates per predator and treatment were done with one single individual observed each time. Their activities were defined and the time spent on each activity on the patch was recorded.

Data analysis

Two two-factor analysis of variance (ANOVA) tests were used to compare the time spent in each activity among species at the same prey density, and between prey densities for each predator. Data were log-transformed before analysing. To analyse time allocated per patch, a two-factor ANOVA was performed. When needed, LSD was used to compare means. (SAS Institute 1989).

Results and discussion

When comparing the number of individuals that left the patch before ending the experiment, fewer *M. caliginosus* than *D. tamaninii* or *O. majusculus* left the cucumber plant at low and high prey density conditions (Table 1). Correspondingly, significantly ($P < 0.05$) more time per patch was allocated by the first predator than by the other two independently of the patch prey density. The response of time allocation to patch prey density was different in the three predators studied. Whereas *M. caliginosus* did not devote significantly ($P > 0.05$) more time to high density patches, *D. tamaninii* and *O. majusculus* spent more time in patches with more prey (Table 1). This may indicate that *M. caliginosus* is a more resident species than the other predators tested and it is not so dependent on prey density as the other two species to stay on the plant. This could explain why this species is found consistently on crops where prey is scarce (Arnó. J., personal

communication). The other two predators seem to be more dependent on the prey, searching more actively among patches.

Six activities performed by all predators were identified as components of their behaviour in the patch. They are: (1) **feed on the prey**; the insect inserts the proboscis into the prey, (2) **walk+tap**; this step includes two concurrent activities as the insect taps the plant with the stylet and the antennae while walking, (3) **rest**; the insect remains motionless with the antennae forwards and the stylet backwards, (4) **feed on the plant**; the insect inserts the proboscis into the plant, (5) **oviposition**; the insect unfolds the ovipositor, inserts it into the plant and folds it again, (6) **groom**; the insect rubs its body with its forelegs and/or the stylet.

Table 1: Number of predators that left the patch before ending the experiment ($\approx 6h=360min$) and, between brackets, mean time devoted by predator on low and high density patches. *D. tamaninii* and *O. majusculus* spent significantly ($P<0.05$) more time in high prey density patches and significantly ($P<0.05$) less time than *M. caliginosus* independently of the patch prey density.

PREDATOR	LOW PREY DENSITY	HIGH PREY DENSITY
<i>M. caliginosus</i>	1 (346.2 min)	0 (354.4 min)
<i>D. tamaninii</i>	5 (167.2 min)	2 (262.0 min)
<i>O. majusculus</i>	4 (132.2 min)	1 (298.4min)

The predators did not show big differences in their time activity budgets when prey was abundant or scarce, except for the time spent feeding on the prey that significantly ($P<0.05$) increased at high prey density in the three predator species (Figure 1). In *M. caliginosus*, mean time devoted to walk and tap was significantly ($P<0.05$) reduced in high prey density patches, although only two out of five females fed on the prey. In the other two predators studied, mean time devoted to activities other than prey consumption did not significantly ($P>0.05$) increased in the high prey density patch –excepting grooming activity in *D. tamaninii*– (Figure 1) in spite of the total time per patch was higher at high prey densities (Table 1) and four out of five females of *D. tamaninii* and *O. majusculus* successfully attacked and consumed the prey. The considerable time (more than half the total) spent by *O. majusculus* feeding on the prey could indicate that preoral digestion is more complete in this species than in the mirids, also because they rest less than at low prey density (Figure 1).

Walk+tap was the main activity displayed by *D. tamaninii* and *O. majusculus* at both high and low prey density conditions, whereas in *M. caliginosus* it was remarkable only when prey density was low. *M. caliginosus* was less active when searching for the prey than *D. tamaninii* or *O. majusculus*, since their females stood still moving only their antennae for an important part of the time recorded as walk+tap at low prey density. Moreover, *M. caliginosus* spent significantly ($P<0.05$) more time resting (motionless) than the other two predators, at both prey densities (Figure 1). This species may need prey ingestion for reproduction but may not be essential for its survival in the short range, as has been described for another zoophytophagous heteroptera, *Podisus maculiventris* (Say) by Wiedenmann et al (1996). It is probably optimal for *M. caliginosus* to minimise its energetic output (i.e. searching for prey within or between patches) by remaining motionless during an important period of its life. This strategy may also

result in lowering risk mortality from predation, parasitism or adverse weather. Nevertheless, further studies are needed in longer time period and more complex plant structures in order to confirm this hypothetical strategy.

The time spent **feeding on the plant** by the three predators was not dependent on prey density as it did not change at the two prey densities tested within each predator (Figure 1). Three models have been described for facultative phytophagy displayed by several heteropteran bugs: positive prey density-dependence, negative prey-density dependence and independence from prey density (Gillespie et al 1999). The mirids and the anthocorid studied seem to follow the last model since the lack of prey consumption at low prey density was not counterbalanced by an increase or decrease in plant (leaf or stem) consumption. This conclusion is particularly relevant for use of the three predators in biological control as increasing prey consumption is not accompanied by increasing plant feeding and thus risk of plant damage. This pattern, however, cannot be extended to the situations in which plant tissue exposed to predator feeding is the fruit, as *D. tamaninii* switches to feed on green tomato fruits when the greenhouse whitefly populations are low causing yield damage (Salamero *et al.*, 1987; Alomar & Albajes, 1996). When comparing among predators, mirid bugs fed on the plant during significantly ($P < 0.05$) more time than the anthocorid bug (Figure 1). The family Miridae contain plant feeders, zoophytophagous and some obligate predators, while anthocorids are mainly predators (Fauvel, 1999) and probably prefer to feed on other plant tissues as pollen although they also feed on green tissues to take water (Armer *et al.*, 1998).

Although conclusions reached from the present results should be confirmed in other plant-prey-predator situations –f. i. by varying the host-plant and predator species and growth stage, or the predator development stage–, general traits of the behavior of the three predators studied agree with that concluded in previous functional response studies. In these, greenhouse whitefly pupae were also used as prey and *M. caliginosus* was observed to have higher handling times and lower attack coefficients than *D. tamaninii* and *O. majusculus* (Montserrat *et al.*, submitted).

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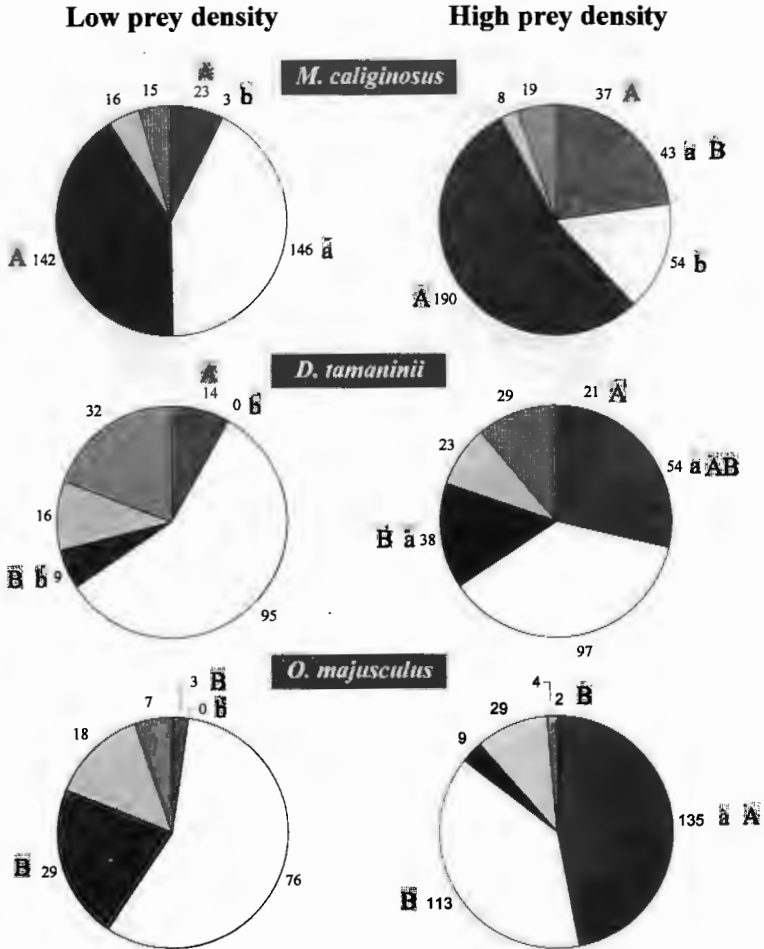


Figure 1. Mean time (minutes) spent in each activity by predatory bugs at low and high prey density: Activities: ■ feed on plant; ■ feed on prey; □ walk + tap; ■ rest; ■ oviposition; ■ groom. Times followed by different lower case letters are significantly (P<0.05) different when compared between densities and within predators, and times followed by different capital letters are significantly different when compared among predators and within densities.

Section VIII
Selective use of pesticides

Section VI
Utilisation selective de pesticides

Growth enhancement of some plants and effects of fungicides on mycorrhizal colonisation

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Abstract: Mycorrhizal fungus *Glomus etunicatum* colonization rate of plants changed between 27.4%-67.3%. The highest colonisation rates were obtained from tomato, pepper and common bean as 67.3%, 63.9% and 63.1%, respectively. In tomato which had the highest colonisation rate, the length of plant and root and dry weight of plant were found significantly high compared to control. However, in onion and eggplant with the lowest colonisation rate, dry weight of the plant was found higher than the control as 28% and 44%, respectively. Fungicides reduced *G. etunicatum* colonisation relatively more than *G. mosseae*. Only fentinacetate, chlorothalonil and oxadixyl+mancozeb effected at similar rate. *G. etunicatum* did not effected by fosectly-Al, *G. mosseae* colonisation ratio was decreased as 23%. Benomyl had an effect with the highest rate as 75.9% on *G. etunicatum* colonisation. The effect of the other fungicides on *G. etunicatum* colonisation were between 51.7%-65.8%. Fentinacetate effected the *G. mosseae* colonisation with the highest ratio (61.2%) and this was followed by benomyl (52%). Both mycorrhizal fungi colonisation were effected by NPK with relatively lower ratio. The effect of NPK on colonisation were found as 29.2% for *G. etunicatum* and 27.2 for *G. mosseae*.

Keywords: mycorrhizal fungi, fungicides

Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi are beneficial fungi that are associated with the roots of most crops and provides some benefits to plants (Agrios, 1997). Due to enhanced nutrient uptake, mycorrhizal fungi improve the plant growth and plants appear healthier than nonmycorrhizal ones. Also, according to the results, they increase the yield. The most important thing should be considered is protecting the agricultural crops against plant pathogens.

Diverse group of fungicides are widely and extensively used in the control of plant disease. However, application of these fungicides in open and protected areas result in effecting of both pathogenic as well as beneficial organisms including vesicular-arbuscular mycorrhizal (VAM) fungi which is nonpathogenic to plants. Shortly, balance established between mycorrhizal fungi and plants is destroyed by these applications.

The objective of this study was to determined the mycorrhizal colonisation of some plants and the effect of some fungicides on *G. etunicatum* and *G. mosseae*.

Materials and methods

Root colonisation of G. etunicatum and its effects on plant development

Glomus etunicatum produced on *Zea mays* L. were used as mycorrhizal fungi and mycorrhizal inoculum was consisted of infested soil mixed with root fragment.

In the experiment, most common agricultural plants including cabbage, onion, eggplant, cucumber, common bean, tomato, peanut and pepper used as the plants materials for evaluating colonisation and growth enhancement.

50g (600 spores/10g soil) soil inoculum mixed with root fragments was incorporated 2-3 cm below the seeds before sowing (Menge & Timmer, 1982), then five seeds of each plants were sown in each pot with 5 replicates per plant including control no mycorrhizal inoculum applied.

Experiment was conducted under controlled conditions at 25°C with a photoperiod of 16 hours plants were watered with distilled water during the experiment.

After 6 weeks, the length of plant, dry weight of plant were measured. Dry weight of plant was determined after drying plant material at 80°C for 48 hours. The differences between mycorrhizal plants and control were analysed by t test.

For determining the mycorrhizal colonisation, clearing and staining were done as describe by Koske & Gemma (1989). After harvesting, roots of plants were washed with tap water and then distilled water carefully and then cut into 1 cm pieces; they were cleared by heating in 10% KOH at 65°C for one hour. After rinsing and acidifying with dil.HCL, the root segments were stained with 0.05% trypan blue. Endomycorrhizal colonisation was estimated using the gridline intersect method (Giovannetti & Mosse, 1980). Roots placed on a grid of 10 mm divisions and examined under dissecting microscope (40x). Number of roots and colonized roots by *G. etunicatum* were counted and infection percentage was determined.

The effect of fungicides on mycorrhizal colonisation

In this part of the study, maize was used as the test plant because of good colonizing the roots by *G. etunicatum* and *G. mosseae* (Khaliq *et al.*, 1997). In soil application, nine fungicides and one commercial fertilizer were used in the experiment. They were: Oxadixyl+mancozeb, thiram, benomyl, chlorothalonil, fentinacetate, captan, fosetyl-Al, quintozene, mancozeb and N-P-K.

Application of two mycorrhizal inoculum was done in the same way as describe above and three seeds were sown in each pot. Each fungicide and commercial fertilizer were drenched two times at the recommended dose (50 ml in each pot) one week interval after emerging when the fungal endophyt had just begun to infect maize roots. 50 ml distilled water applied to control plants at the same time. Each treatment was replicated five times for both mycorrhizal fungi. The assessment of mycorrhizal development was made six weeks of plant growth as describe above. The differences between applications were done according to the LSD test.

Results

Root colonisation of G. etunicatum and its effects on plant development

Mycorrhizal fungus *G. etunicatum* colonized on eight the most common crops at changing ratio between 27.4%-67.3% (Table 1). The highest colonisation rate (67.3%) was obtained from tomato plants. Colonisation rate was also found high on pepper and common bean as 63.9% and 63.1%, respectively. The plants with the lowest root colonisation were onion as 24.4% and eggplant as 29.5%.

In tomato which had the highest colonisation rate, as the length of plant was increased 11% (48.0-42.8cm), the length of root was increased 38% (20.8-13.0cm) also, compared to control.

Similarly, in peanut with 45% of root colonisation ratio, both length of plants (23%) and length of roots (45%) were found significantly higher than the control.

Table 1. The effect of *G. etunicatum* on development of plant.

Plants	Colonization %	Plant length (cm)		Root length (cm)		Dry weight (g)	
		GE	Control	GE	Control	GE	Control
Cabbage	47.1 c	31.2*	26.3	9.4	7.1	0.342	0.300
Onion	27.4 d	37.9	44.9	19.7*	14.0	0.501*	0.360
Eggplant	29.5 d	22.8*	18.0	11.7	10.6	0.219*	0.123
Cucumber	49.7 bc	43.0	46.0	20.4	17.6	0.728	0.785
Bean	63.1 ab	131.8*	114.8	20.9	19.0	1.934	1.885
Tomato	67.3 a	48.0*	42.8	20.8*	13.0	1.111*	0.866
Peanut	45.0 c	49.3*	38.2	23.3*	12.8	0.621	0.790
Pepper	63.9 ab	37.4	36.4	16.6*	14.2	0.905	0.867

*Mean values within a line are significantly different based on t test

** Mean values within a column are significantly different based on LSD (0.05) test

However, on pepper roots in which high *G. etunicatum* were obtained, only length of the roots were increased (16.6-14.2cm), and only length of the plants (131.8-114.8cm) were higher in common bean).

Dry weight of the plant was found to have in tomato plants (1.110-0.866g) and the value was increased 22%. However, in onion (27.4%) and eggplant (29.5%) with the lowest colonisation rate, dry weight of the plant was found higher than the control as 28% and 44%, respectively. Colonisation of *G. etunicatum* had no significant effect on dry weight of the other plants.

The effect of fungicides on mycorrhizal colonisation

The effects of most common nine fungicides and one commercial fertilizer that contented NPK+microelement used in agricultural crops on mycorrhizal fungi *G. etunicatum* and *G. mosseae* are shown in Table 2.

Fungicides reduced *G. etunicatum* colonisation relatively more than *G. mosseae*. Only fentinacetate, chlorothalonil and oxadixyl+mancozeb effected at similar rate. Whereas, *G. etunicatum* did not effected by fosetyl-Al, *G. mosseae* colonisation ratio was decreased as 23%.

Benomyl had an effect with the highest rate as 75.9% on *G. etunicatum* colonisation.

While fosetyl-Al had no significant effect on colonisation of *G. etunicatum*, oxadixyl+mancozeb decreased the colonisation ratio as 38.6%. The effect of the other fungicides on *G. etunicatum* colonisation were between 51.7%-65.8%.

Fentinacetate effected the *G. mosseae* colonisation with the highest ratio (61.2%) and this was followed by benomyl (52%). While PCNB had no significant effect on *G. mosseae* colonisation, fosetyl-Al prevented the colonisation at the ratio of 23%.

G. mosseae was effected with lower degree by the other fungicides and the decrease of the colonisation was found between 33.8%-48.5%.

Both mycorrhizal fungi colonisation were effected by NPK with relatively lower ratio. The effect of NPK on colonisation were found as 29.2% for *G. etunicatum* and 27.2% for *G. mosseae*.

Discussion

Wide host range of *Glomus* species among vesicular-arbuscular mycorrhizal fungi and its ease adaptation to various ecosystems were reported by many researchers (Bhatia *et al.*, 1996;

Bonfante-Fasolo & Scannerini, 1992). *G. etunicatum* is one of the most common species with high colonisation ratio. Ozgonen *et al.*, (1999) reported the colonisation of *G. etunicatum* on tomato roots as 62%. In this study, colonisation of *G. etunicatum* was found more than 60% in tomato, bean and pepper. However, 30% colonisation were found on eggplant. Matsubara *et al.*, (1995) reported that colonisation of *G. etunicatum* on eggplant reached to 40.8% in the 10th weeks. Whereas, in this study, the colonisation was calculated in the 6th weeks.

Table 2. The effect of fungicides on colonisation of mycorrhizal fungus.

Fungicides	Colonization (S)		% Effect	
	GE	GM	GE	GM
PCNB	22.9 de	52.9 ab	62.2 bc	10.3 bc
Fosetyl-Al	52.7 a	45.4 bc	13.1 e	23.0 c
Benomyl	14.6 f	28.3 de	75.9 a	52.0 ab
Fentinacetate	22.0 de	22.8 e	63.7 bc	61.2 a
Captan	24.4 de	36.9 cd	59.7 bc	37.4 bc
Chlorothalonil	28.7 de	30.3 de	52.7 c	48.5 ab
Thiram	29.3 cd	38.2 cd	51.7 c	35.1 bc
Mancozeb	20.7 ef	31.8 de	65.8 b	46.1 ab
Oxadixl+mancozeb	37.2 bc	39.0 cd	38.6 d	33.8 bc
N-P-K	42.9 b	42.9 cd	29.2 d	27.2 bc
Control	60.7 a	59.8 a		

*Mean values within a column are significantly different based on LSD (0.05) test.

As the colonisation of *G. etunicatum* was at the highest level on tomato, the length of plant and root and dry weight of the plant were increased also. It was reported by other researchers that after mycorrhizal colonisation, growth and dry weight of tomato plants were increased (Al Momany & Al Raddad, 1988; Edathil *et al.*, 1995; Caron *et al.*, 1986). Although colonisation on eggplant was lower, length of plant and number of leaf increased after 8th weeks compared to the control (Matsubara *et al.*, 1995). Length of eggplant was increased by *G. etunicatum* as 21% in this study. As a result, weight of dry material was found 30% higher than the control.

It was reported in many studies that, mycorrhizal colonisation was reduced by fungicides applications (Sieverding, 1991; Menge, 1982). Especially, foliar applications of systemic fungicides even could have an effect on colonisation (Jalali & Domsch, 1975). It was reported that, relatively significant reduction rate of mycorrhizal colonisation was observed after a few days of application of systemic fungicides benomyl and ethirimol, But thiabendazole could induce VAM colonisation (Sieverding, 1991; Jalali & Domsch, 1975; Menge, 1982).

In this study, oxadixyl+mancozeb reduced the colonisation of *G. etunicatum* and *G. mosseae* as 38.6% and 33.8%, respectively. However, metalaxyl which is effective and specific to Oomycetes fungi could also induce VAM fungal development (Sieverding, 1991). In this study, the mycorrhizal colonisation may be effected by mancozeb not oxadixyl.

In our study, fosetyl-Al had no significant effect on mycorrhizal colonisation. It was reported that, fosetyl-Al have not effect on VAM fungi growth and in some cases even induced the development of VAM (Sieverding, 1991). However, it thought to be reduced the colonisation with a little ratio through inducing the defense mechanism of plants by fosetyl-Al.

On the other hand, in N-P-K application, colonisation reduction was because of inert substrate of the formulation. This study showed that, the effect of not only fungicides but also pesticides on mycorrhizal fungi in plant should be considered. In addition, this study will be helpful for further studies. These should be focused on the performance of two mycorrhizal fungi on plants showed the best colonisation under greenhouse and field conditions including yield parameters and using the mycorrhizal fungi against soilborne plant pathogens.

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Laboratory investigations on some natural pesticides for use against pests in vegetable greenhouses

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Abstract: Some natural pesticides (namely Garlic barrier, Hot pepper wax, Organica neem oil, Neem Azal T/S, Savona, Soft soap, Alum, Starch, Sodium bicarbonate, Stinging nettle and Tobacco) which could be used against pests in greenhouses were tested in laboratory for insecticidal effects in 1999. The most effective materials were Organica neem oil, Neem Azal T/S, Savona, Soft soap and Tobacco. Sodium bicarbonate also gave promising results. These materials need to be investigated further in the greenhouse, and also the repellent effects of Garlic barrier and Hot pepper wax.

Key words: natural pesticides, spider mites, whiteflies, aphids

Introduction

Greenhouse conditions provide a favourable climate for the growth of pests and diseases, with intensive pesticide application causing contamination of the environment, residue problems on crops for the consumers, phytotoxic plant reactions and possible resistance development to pesticides in pest organisms. Because of this, natural pesticides as alternatives to conventional chemicals have been investigated in ecological farming in recent years. Some of these natural pesticides include propolis, kiesलगur, sodium bicarbonate, soft soap, sodium silicate, some vegetable and animal oils, extracts of some plants, paraffin oils, etc. (Ertem, 1993; Onogur, 1996).

Successful biological control practices were carried out in protected vegetable and strawberry cultivation against whiteflies and red spider mites from 1992-1997 in Izmir (Öncüer *et al.*, 1994; Yoldas *et al.*, 1996; 1997; Kismali *et al.*, 1997). However, biological control is still not used on a commercial basis for greenhouses in Turkey. This study therefore aimed to prove the suitability of some natural pesticides against aphids, whiteflies and red spider mites (these being the most harmful pests in protected cultivation) and to decrease the use of conventional pesticides in greenhouses.

Material and methods

This study was carried out in the laboratories of E.U. Faculty of Agriculture, Department of Plant Protection in Izmir in 1999.

Mass rearing of pests

The greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera, Aleyrodidae) was reared on tomato plants. The pea aphid *Acyrtosiphon pisum* Harris (Homoptera, Aphididae) was reproduced on broad bean plants. The carmine spider mite *Tetranychus cinnabarinus* (Boisduval) (Acari, Tetranychidae) was reared on bean plants. All were under controlled conditions in climate rooms (22 ± 1 °C temperature, 60-70 % relative humidity and 16 hour light + 8 hour dark).

Natural pesticides which were treated

Insecticidal effects of 11 natural or alternative materials were tested in laboratory conditions. The commercial bio-rational products namely Garlic Barrier, Hot pepper wax and Organica neem oil were bought from the firm Biocontrol Network. The products Savona and Neem Azal T/S were supplied respectively by Turkish representatives of the firms Koppert and Trifolio M. The other materials Soft soap, Alum, Starch, Sodium bicarbonate, Tobacco (*Nicotiana tabacum*) and Stinging nettle (*Urtica urens*) were sold in the markets for different aims, and found easily. The commercial products were used according to the recommended doses, while the others were applied according to their references and the doses which ecological farming companies were already applying.

The commercial products: **Garlic barrier** is made only from pure garlic (*Allium sativum*) juice concentrate at a rate of 93 %. It is absorbed through stomata and it travels systemically throughout the entire plant. Because this product has a repellent effect on many pests, it is recommended to apply it before there is a pest problem. The sulfur-containing compounds in garlic, especially allicin, allin, cycloalitin and diallyldisulphide, are the most active pesticidal ingredients. So, it is claimed that even insect larvae are often killed from contact with this product. **Hot pepper wax** is derived from hot cayenne pepper extract blended with food grade wax. It includes capsaicin and other capsaicinoids at a rate of 0.00018 % as active ingredient and repels many pests, according to the prospectus. **Organica neem oil** is produced from the neem tree *Azadirachta indica* whose effects are well known as a natural pesticide and have been well studied in recent years (Schmutterer & Ascher, 1984; Kleeberg & Zebitz, 1997). This product includes potassium salts of fatty acids of neem seeds at a rate of % 25. **NeemAzal T/S** is neem seed extract with 1% azadirachtin A. **Savona** includes potassium salts of fatty acids at a rate of 49 %.

The application doses for these commercial materials are for Garlic barrier 1:100; Hot pepper wax 1:16; Organica neem oil 1:50 (preparate : water), Neem-Azal T/S 0.5 %; Savona 2 % (for red spider mites and aphids) and 1 % (for whiteflies).

The others: The cleaning material **Soft soap** (or potassium soap) was applied at the rate of 3 %. So, 3 gram of soft soap was melted with 1 ml of ethyl alcohol (% 96) and then mixed with water. It was used in the same rate of alcohol for control units. **Alum** (Potassium aluminium sulphate-12-hydrate) which is recommended for aphids was dissolved in hot water and was applied after cooling. Food materials **Starch** and **Sodium bicarbonate** were applied at the rates of 1.5 % and 1 %, respectively, and the spreader was added (0.03 %) to both materials. Control units were also applied in the same rate of spreader. **Stinging nettle** (burning nettle) which is recommended as a plant growth stimulant in ecological farming was applied at the rate of 2 %. So, 100 g of fresh plant was added to 1 liter of boiling water and then 10 ml of this extract was mixed with 40 ml water. **Tobacco** was used at the rate of 10 %. So, 1 kg of dry tobacco dust was left in 10 liters of water for one night and then applied after filtering.

Application methods for natural pesticides on the pests

Bean leaves infested with sufficient amounts of carmine spider mite at certain stages were cut in the 6-7 cm² area. The natural or alternative materials were sprayed directly on these individuals. The tests were conducted in three different stages on egg, nymph and adult female. The nymphs and adults were observed on the first and fourth day. After the fourth day the individuals tended to disperse. The eggs were evaluated on the eighth day.

Whiteflies were tested on the egg, second and third nymph and the pupal stages. The whole tomato leaflets in the 6-7 cm² area infested with sufficient amounts at particular stages were

sprayed directly. The individuals on the leaflets were identified by marker pen. The treatments lasted until the 8th day inclusive.

The tests on aphids were made on whole broad bean leaves in the area of 6-7 cm² infested with sufficient amounts of young nymph and mature nymph at certain stages. The natural pesticides were sprayed on the individuals directly. To prevent the aphids escaping, micro cages were put on the leaves after spraying. The tests lasted through the first day due to the fact that the leaves began to spoil as from the second day and this caused food stress for aphids.

The tests concerning the three pests were conducted with 10 individuals on each leaf for each stage and set up according to randomized block design with 5 replications. The leaves were placed upside down on the wet cotton in the petri dishes. The natural pesticides were sprayed by an electric pulveriser at 1.5 atmospheric pressure and good spray coverage was attained. Distilled water was applied to control units. All the treatments were carried out under aforesaid controlled conditions. Results were evaluated by Abbott formula.

Results and discussion

The insecticidal effects of the repellents **Garlic barrier** and **Hot pepper wax** were tested in the laboratory and the results are shown in Table 1. Garlic barrier mostly affected the eggs of the spider mites and its insecticidal effect on the other pests was very low. Hot pepper wax was able to affect pests physically due to its wax content. Since these products are claimed as repellents, they need to be investigated in the greenhouse.

Table 1. The insecticidal effects of **Garlic barrier** and **Hot pepper wax** on main greenhouse pests

Natural pesticides	Pests	Biological stages	Effect (%)
Garlic barrier	Spider mite	Egg	78.0
		Nymph	0.0
		Adult	4.0
	Whitefly	Egg	4.0
		Nymph	2.0
		Pupa	0.0
	Aphid	Young nymph	18.0
		Mature nymph	18.0
	Hot pepper wax	Spider mite	Egg
Nymph			50.0
Adult			82.0
Whitefly		Egg	48.0
		Nymph	6.0
		Pupa	10.0
Aphid		Young nymph	77.5
		Mature nymph	96.0

The results of the tests of botanical pesticides **Organica neem oil** and **Neem Azal T/S** are given in Table 2. The insecticidal effects of these products were found to be high against

all the studied pests. It was observed that hatched larvae and the other individuals which remained alive of the spider mites and whiteflies were seen to be very unhealthy. It is reported by many researchers that neem derivatives have been found to possess pest control properties that include antifeedant, antiovipositional, growth inhibitor and lethal toxic activities against a wide variety of pests. Owing to their effectiveness against pests, biodegradation, considerable selectivity, harmlessness to beneficial and non target organisms, neem derivatives could play a potentially useful role in integrated pest management (Mansour & Ascher, 1984; Schmutterer, 1988; Schulz *et al.*, 1997; Awad *et al.*, 1998).

Table 2. The insecticidal effects of **Organica neem oil** and **Neem Azal T/S** on main greenhouse pests

Natural pesticides	Pests	Biological stages	Effect (%)
Organica neem oil	Spider mite	Egg	80.0
		Nymph	100.0
		Adult	100.0
	Whitefly	Egg	0.0
		Nymph	64.0
		Pupa	96.0
	Aphid	Young nymph	96.0
		Mature nymph	92.0
Neem Azal T/S	Spider mite	Egg	58.0
		Nymph	88.0
		Adult	70.0
	Whitefly	Egg	0.0
		Nymph	82.0
		Pupa	90.0
	Aphid	Young nymph	100.0
		Mature nymph	98.0

The results obtained from **Savona** and **Soft soap** tests are shown in Table 3. These materials killed the pests successfully in a physical way, but it is obvious that Savona had a poor effect on whitefly eggs and Soft soap had little or no effect on both whitefly and spider mite eggs. However, Yumruktepe & Uygun (1994) were reported that Savona did not have any toxic effect on the predator insects *Cryptolaemus montrouzieri* Mulsant. and *Nephus includens* Kirsch. (Coleoptera, Coccinellidae) in laboratory tests.

The results concerning **Alum**, **Starch** and **Sodium bicarbonate** are given in Table 4. Alum showed very poor effect on spider mites and whiteflies while the results were promising on aphids. However, because of the death of some aphid individuals in the control units, it was decided to repeat the aphid test again. Starch also had very little effect on the pests. Starch was tested due to its similarity with starch-dextrin which was recommended by Pickford & Mathieson (1990) against *Tetranychus urticae* Koch (Acari, Tetranychidae) and *T. vaporariorum* in greenhouses. These researchers tried sprays of household starch onto *T. vaporariorum* pupae to see if it could stop adult emergence. Household starch proved to be effective, in contrast with our results, but was phytotoxic on cucumber. Starch was not phytotoxic on broad bean, tomato and bean leaves in our study. However, they found a

particular starch-dextrin to be effective and non phytotoxic. The potential of starch-dextrin solution to reduce *T. urticae* infestations by up to 89 % has been shown, and it was suggested that this was caused by some physical mechanism. Starch-dextrin solution was also found to control eggs and pupae of *T. vaporariorum*. Pickford & Mathieson (1992) recorded that starch-dextrin solution had no adverse effect on the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae). Sodium bicarbonate affected the nymphs and adults of spider mites and aphids at the rate of approximately 50 %. These results are very promising due to the fact that Sodium bicarbonate is also recommended as an alternative material against powdery mildew in the integrated pest management programmes in vegetable greenhouses (Yoldas *et al.*, 1997). However, Yoldas *et al.*, (1998) recorded the negative effect of Sodium bicarbonate on *P. persimilis* when applied directly.

Table 3. The insecticidal effects of **Savona** and **Soft soap** on main greenhouse pests

Natural pesticides	Pests	Biological stages	Effect (%)
Savona	Spider mite	Egg	72.0
		Nymph	90.0
		Adult	100.0
	Whitefly	Egg	4.0
		Nymph	84.0
		Pupa	58.0
	Aphid	Young nymph	100.0
		Mature nymph	100.0
Soft soap	Spider mite	Egg	24.0
		Nymph	72.0
		Adult	100.0
	Whitefly	Egg	0.0
		Nymph	98.0
		Pupa	92.0
	Aphid	Young nymph	100.0
		Mature nymph	77.5

Stinging nettle and **Tobacco** results are shown in Table 5. Stinging nettle was found to be ineffectual on the pests while Tobacco was very effective on all three pests, especially spider mites and aphids.

As a result of this study, the most effective and promising materials which are going to be tested in greenhouse conditions are Organica neem oil, Neem-Azal T/S, Savona, Soft soap, Sodium bicarbonate and Tobacco. The repellent effects of Garlic barrier and Hot pepper wax should also undergo further greenhouse investigation. After further greenhouse tests we will have more alternatives to chemical pesticides for use in ecological farming or integrated pest management programmes.

Table 4. Insecticidal effects of Alum, Starch and Sodium bicarbonate on main greenhouse pests

Natural pesticides	Pests	Biological stages	Effect (%)
Alum	Spider mite	Egg	8.0
		Nymph	0.0
		Adult	24.0
	Whitefly	Egg	2.0
		Nymph	2.0
		Pupa	0.0
	Aphid	Young nymph	-
		Mature nymph	-
	Starch + spreader	Spider mite	Egg
Nymph			0.0
Adult			12.0
Whitefly		Egg	2.0
		Nymph	0.0
		Pupa	4.0
Aphid		Young nymph	27.5
		Mature nymph	7.5
Sodium bicarbonate + spreader		Spider mite	Egg
	Nymph		40.0
	Adult		58.0
	Whitefly	Egg	8.0
		Nymph	38.0
		Pupa	28.0
	Aphid	Young nymph	50.0
		Mature nymph	47.5

Table 5. The insecticidal effects of Stinging nettle and Tobacco on main greenhouse pests

Natural pesticides	Pests	Biological stages	Effect (%)
Stinging nettle	Spider mite	Egg	4.0
		Nymph	0.0
		Adult	6.0
	Whitefly	Egg	0.0
		Nymph	2.0
		Pupa	0.0
	Aphid	Young nymph	24.4
		Mature nymph	4.0
	Tobacco	Spider mite	Egg
Nymph			96.0
Adult			100.0
Whitefly		Egg	50.0
		Nymph	46.0
		Pupa	30.0
Aphid		Young nymph	100.0
		Mature nymph	100.0

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Resistance to tetradifon in the carmine spider mite *Tetranychus cinnabarinus* Boisd.

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Abstract: Resistance to tetradifon in carmine spider mite *Tetranychus cinnabarinus* Boisd. populations from different localities in Antalya was detected using a laboratory strain as reference. The tests were conducted by exposing the eggs to tetradifon in a Potter Spray Tower. Resistance ratios from 1.4 to 75.9 folds at LC_{95} were found in the populations from greenhouse grown vegetables and 1.9 and 12.0 folds in those from cotton. Resistance increased to 50 folds at LC_{95} in the laboratory strain selected by tetradifon and dicofol applied alternatively for 6 months. The resistance to tetradifon decreased to 2.2 folds after 5 months the selection pressure was ceased.

Key words: *Tetranychus cinnabarinus*, eggs, tetradifon, resistance, greenhouse, cotton, Antalya.

Introduction

Pest control failures due to pest resistance to pesticides is common. There are two significant economic consequences of resistance. Crop losses are faced before resistance is detected, and attempts to tackle resistance by increasing the dose and the frequency of pesticide applications lead to an increase in the cost of pest control operations (Georghiou & Lagunes-Tejeda, 1991). The development of resistance in greenhouses is enhanced by the limited chances for dilution of resistance by dispersing susceptibles in relatively isolated pest populations, numerous generations in an extended season and high frequency of spraying (Cranham & Helle, 1985). The incidence of resistance is expected to be further increased in situations where pest control overwhelmingly relies on pesticides, as it is the case with the protected crops in Turkey.

The carmine spider mite *Tetranychus cinnabarinus* Boisd. is a major pest of protected crops and cotton in the Mediterranean Region of Turkey. A number of acaricides have been in use for its control. Tetradifon was registered in 1963 in this country (Anonymous, 1997). It was one of the most commonly used acaricides along with dicofol and chlorobenzilate in Antalya until 1985. Although there was a decrease by the time tetradifon has been used in a considerable quantity in the province until recently (unpublished, Plant Protection Service, Antalya).

No information is available on the resistance of *T. cinnabarinus* against tetradifon in Turkey. However it was reported to be effective on the eggs of *T. cinnabarinus* but not to be able to control the pest on strawberry in the East Mediterranean Region of this country in the years 1987 and 1988 (Yigit & Erkilic, 1992). No records of resistance to tetradifon in *T. cinnabarinus* are also available for the rest of the world. A number of cases of resistance to tetradifon were, however, reported in the populations of closely related species *T. urticae* Koch in several parts of the world (Georghiou & Lagunes-Tejeda, 1991).

The aim of the present study was to document the resistance to tetradifon in *T. cinnabarinus* populations on protected crops from various districts of Antalya province, to

asses the potential of a laboratory strain of *T. cinnabarinus* to develop resistance through artificial selection and the stability of the resistance. For comparison resistance levels in two cotton populations were also detected.

Materials and methods

A laboratory strain from a culture of *T. cinnabarinus* maintained for approx. 3 years without exposure to pesticides at the Plant Protection Department of Akdeniz University was used as susceptible (reference) strain.

The origin of *T. cinnabarinus* populations used for resistance detection are shown in Table 1. The mites used in the tests were maintained on cowpea (*Vigna sinensis* (L.) Savi) at $26\pm 1^\circ\text{C}$, $70\pm 10\%$ r.h. and L:D 16:8.

Table 1. The origin of *T. cinnabarinus* populations used for detection resistance against tetradifon.

locality	host plant	date of collection
GREENHOUSE I		
Aksu	cucumber and strawberry	03.06.1996
Demre1	cucumber and pepper	01.05.1996
Kumluca1	bean and pepper	01.05.1996
Manavgat	cucumber	14.05.1996
Serik	cucumber and eggplant	14.05.1996
Topcular	cucumber and pepper	01.05.1996
GREENHOUSE II		
Alanya	cucumber and bean	05.06.1996
Demre2	cucumber and bean	04.07.1996
Gazipasa	cucumber and bean	05.06.1996
Kumluca2	cucumber and eggplant	04.07.1996
COTTON		
Manavgat	cotton	15.08.1996
Serik	cotton	15.08.1996

Only the egg stage of *T. cinnabarinus* was exposed to tetradifon. Eggs were obtained by placing 6-8 females on a cowpea leaf disc (dia. 2.6 cm) for 24 h. The total number of eggs (n) used for each test was over 300.

The tetradifon and dicofol used in the tests was provided by Hekta (Tetradifon 75.2 mg A.I./l E.C.; Hekthane 195 mg A.I./l E.C.).

Resistance detection

Doses of tetradifon used for resistance detection were established on the basis of recommended dose, 112.8 mg/l, on vegetables in Turkey (Anonymous, 1995) and ranged between 7-14336 mg/l. Tetradifon diluted in distilled water was sprayed on the eggs resting on cowpea leaf disc using a Potter Spray Tower (Burkhard, U.K.) under a pressure of 0.84 Atm. Only distilled water was sprayed on the control eggs. After treatment the leaf discs with eggs

were maintained at the same conditions mentioned above. Final mortality counts were done 6 days after exposure to tetradifon or water. The eggs did not hatch were counted as dead.

Table 2. Tetradifon resistance in *T. cinnabarinus* populations from different localities in Antalya

Location	Slope	LC ₅₀ mg(AI)/liter (%95 CL)	LC ₉₅ mg(AI)/liter (%95 CL)	Resistance ratio ^a	
				LC ₅₀	LC ₉₅
GREENHOUSE I					
Laboratory	1.31	18.8 (13.0-27.3)	338.8 (234.4-489.8)	-	-
Aksu	1.48	196.1 (46.5-827.5)	478.6 (120.2-1905.5)	10.4	1.4
Demre1	0.87	22.4 (12.6-40.0)	1584.9 (891.3-28.18.4)	1.2	4.7
Kumluca1	1.33	96.0 (55.8-165.2)	1659.6 (977.2-2818.4)	4.3	4.9
Manavgat	1.40	39.5 (26.2-59.7)	575.4 (380.2-871.0)	2.1	1.7
Serik	0.75	166.9 (56.4-494.6)	25703.9 (8709.6-75857.8)	8.9	75.9
Topcular	1.42	37.2 (24.9-55.7)	537.0 (363.1-794.3)	2.0	1.6
GREENHOUSE II					
Laboratory	1.69	20.2 (13.9-29.4)	186.2 (127.4-272.3)	-	-
Alanya	0.90	141.3 (42.5-470.5)	9120.1 (1202.3-13182.6)	7.0	49.0
Demre2	0.83	39.4 (13.3-116.97)	3715.4 (120.3-11220.2)	2.0	20.0
Gazipasa	1.03	133.7 (45.0-397.4)	5128.6 (1714.0-15346.2)	6.6	27.5
Kumluca2	1.49	34.3 (21.3-55.2)	426.6 (264.9-687.1)	1.7	2.3
COTTON					
Laboratory	1.83	46.6 (29.5-73.9)	371.5 (234.4588.8)	-	-
Manavgat	1.15	171.1 (56.8-515.3)	4466.8 (1479.1-13489.6)	3.7	12.0
Serik	2.33	138.5 (74.5-257.3)	691.8 (371.5-1288.2)	3.0	1.9

^aResistance ratio was determined by dividing the LC₅₀ and LC₉₅ value of field strains to LC₅₀ and LC₉₅ value of laboratory(susceptible) strain.

Selection studies

For determination of the resistance potential of *T. cinnabarinus* against tetradifon a population from the laboratory strain was subjected to selection by applying tetradifon and dicofol alternatively in order to simulate field situations where tetradifon and dicofol were usually used in mixture. Both acaricides were sprayed on to cowpea plants infested with *T. cinnabarinus* at a rate of 100 ppm using a hand sprayer. Selection was continued for 6 months and tetradifon and dicofol were applied 8 and 6 times, respectively, at intervals of 8-15 days. At the end of this period resistance detection was carried out as described above. For determination of the stability of tetradifon resistance the selected population was reared for 5 months free of pesticide exposure and at the end of this period the resistance level against tetradifon was measured.

Table 3. Tetradifon resistance in the laboratory strain of *T. cinnabarinus* selected with tetradifon and dicofol.

Strain	Slope	LC ₅₀ mg(AI)/liter (%95 CL)	LC ₉₅ mg(AI)/liter (%95 CL)	Resistance ratio ^a	
				LC ₅₀	LC ₉₅
Susceptible	1.81	51.90 (32.5-1387.2)	416.9 (251.2-691.8)	-	-
resistant	0.93	367.7 (97.5-1387.2)	20893.0 (5623.4-77624.7)	7.1	50.1

^aResistance ratio was determined by dividing the LC₅₀ and LC₉₅ value of field strains to LC₅₀ and LC₉₅ value of laboratory(susceptible) strain.

Table 4. Tetradifon resistance in the selected *T. cinnabarinus* strain after 5 months the selection ceased.

Strain	Slope	LC ₅₀ mg(AI)/liter (%95 CL)	LC ₉₅ mg(AI)/liter (%95 CL)	Resistance ratio ^a	
				LC ₅₀	LC ₉₅
Susceptible	0.73	41.50 (18.3-93.8)	724.4 (316.2-1659.6)	-	-
resistant	0.97	329.6 (137.3-791.3)	1621.8 (676.1-3890.5)	7.9	2.2

^aResistance ratio was determined by dividing the LC₅₀ and LC₉₅ value of field strains to LC₅₀ and LC₉₅ value of laboratory(susceptible) strain.

Statistical analysis

Mortality data were corrected for natural mortalities in control and were subjected to probit analysis to estimate LC₅₀ and LC₉₅ (Ecevit, 1977).

Results and discussion

Low resistance ratios, 1.4-4.9 folds at LC₉₅, for tetradifon were detected in the first group of greenhouse populations of *T. cinnabarinus* except that of Serik in which resistance ratio was approx. 76 folds (Table 2). However in the second group of greenhouse populations the resistance ratios detected were in the range of 20-49 folds at LC₉₅ except for the population Kumluca 2.

It may be assumed that tetradifon has not been in use for a lapse of time in greenhouses of some districts of Antalya where low resistance ratios were detected. It also seems that the growers in Antalya did not have the same tendency toward the use of tetradifon as exemplified by the significant differences, from low to very high, in resistance ratios in populations of *T. cinnabarinus* collected from different localities. Such large differences also show that aerial dispersal of the mite was very limited. Cotton populations detected are considered to exhibit low to moderate resistance levels. This may indicate that either tetradifon has not been in use for a relatively long period in that particular cotton fields or the resistance was diluted by susceptible strains drifted on to cotton from sites not exposed to tetradifon, or both.

After a selection by applying tetradifon and dicofol alternatively within a 6 month period resistance to tetradifon increased 50 folds at LC₉₅ in the laboratory strain of *T. cinnabarinus* (Table 3). The resistance decreased, however, to 2 folds after 5 months the selection was ceased (Table 4). These results suggest that *T. cinnabarinus* has a potential to develop resistance against tetradifon in a short time when repeatedly applied. The tetradifon resistance may, however, decrease to very low levels when the selection pressure is ceased, that is the resistance is not stable.

Based on the control failures in the field Gough (1990) reported that resistance to tetradifon occurred very quickly in *T. urticae* in Australia and that resistance was stable. Quick development of resistance against tetradifon is in accordance with our findings. The data over the stability of the resistance are, however, conflicting. The discrepancy may be due to different species involved and differences in the way the data collected e.g., field vs. laboratory tests.

Tetradifon is one of the acaricides with remarkably low mammalian toxicity. Its acute oral toxicity for male rats is LD₅₀: 14700 mg/kg and dermal toxicity for rabbits is LD₅₀: 10000 mg/kg (Worthing, 1987). All available measures should be taken to prolonge the useful life of such valuable selective pesticides which have been used with no known harmful effects for many years since newer ones are usually more expensive and may pose unforeseen social and environmental risks.

There is little room for resistance management to prolonge the efficacy of tetradifon in greenhouses due to their confined environments described above and the fact that spider mites have limited dispersal powers. However eliminating the selection pressure through less frequent use of tetradifon seems to be a valid option. It may be concluded that it may not take long the loss of resistance even in greenhouses where high levels of resistance to tetradifon were detected as *T. cinnabarinus* populations exhibited low resistance levels in a good number of greenhouses in Antalya and the resistance in a selected laboratory strain was not stable.

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