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Integrated Control in Citrus Fruit Crops

editor:

Ferran Garcia-Mari

IOBC / WPRS

Working Group "Integrated Control in Citrus Fruit Crops"

International Conference

on

Integrated Control in Citrus Fruit Crops

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International Conference on
Integrated Control in Citrus Fruit Crops

Universidad Politécnica de Valencia

Consellería de Agricultura de la Generalitat Valenciana

Preface

After six years since the last meeting, the Citrus Study Group started a new period of activity. This IOBC/WPRS group has a long tradition of accomplishments on biological and integrated control of citrus pests and diseases in the Mediterranean area. The Citrus Working group last met in Firenze in 1996. That year the pest situation in the Mediterranean was focused on the recently introduced citrus leafminer. During the last six years old and new phytosanitary problems keep raising challenges to researchers and technicians. New pests have appeared recently in several citrus producing countries, and old problems like the medfly are nowadays of great concern for farmers, and researchers are being asked urgently for solutions. Let's hope that this meeting will represent a right step in that direction.

The purpose of the meeting held in Valencia from 6 to 8 November, 2002, was gathering together researchers around the Mediterranean that work on citrus plant protection in order to promote exchange of information, results and strategies, and to encourage international cooperation and coordination of activities.

In all, 64 participants assisted to the meeting, presenting 41 communications (26 oral and 15 as poster). Countries represented were Spain, Italy, Morocco, Portugal, Algeria, Belgium and Germany. The two most reported single pests were the citrus leafminer and the medfly, with nine and eight presentations respectively. Most presentations on citrus leafminer dealt with biological control with native and exotic parasitoids in different areas of Spain, Italy and Morocco. Concerning the medfly, four presentations were based on field control (with chemosterilizants, nematodes, fungus and mass-trapping), and three on the comparison of traps and attractants. Coccid scales were presented in 10 communications dealing with different species like *Aonidiella aurantii* or *Planococcus citri*, and with different topics like parasitoids, population dynamics or sensibility to oil sprays. Finally, three communications dealt with identification and sampling of beneficials, two with thrips and two with the mite *Tetranychus urticae*.

I would like to emphasize that the scope of the Citrus study group is not only pests, but crop protection as a whole, including pests, diseases and weeds. The group should try to attract in the future not only citrus entomologists but also plant pathologists and weed experts as well.

Finally, the organizers thank the Spanish organisms that have supported this event, the Consellería d'Agricultura de la Generalitat Valenciana and the Universitat Politècnica de València.

Looking forward to see you again in the next meeting..

Valencia, January 2003

F. Garcia-Marí, Convenor

ii

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Table of Contents

CB

BENEFICIALS

THRIPS

MITES AND OTHERS

CITRUS LEAFMINER
(*Phyllocnistis citrella*)

Establishment of Exotic Parasites of Citrus Leafminer, *Phyllocnistis citrella*, in Citrus Groves in Morocco

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Abstract: The citrus leafminer, *Phyllocnistis citrella* stainton, was first detected in Morocco in 1994. During this year the insect population expanded and exploded, covering all the citrus areas of the country except the south, which was infested in 1995. The pest was only controlled by repeated applications of insecticides. Like many other Mediterranean countries dealing with citrus production, a classical biological control project was initiated in Morocco in order to import and establish in citrus groves the main parasites of this pest. The project was undertaken by the "Domaines Agricoles" since 1995. Five species of parasites, *Ageniaspis citricola, Cirrospilus ingenuus, Semielacher petiolatus, Quadrastichus sp.* and *Citrostichus phyllocnistoides*, were introduced, reared and released in the main citrus-growing regions in Morocco Souss, Gharb, Oriental, Tadla and Haouz. *A. citricola* was only recovered from the field in Souss in 1996 and in Rabat and Gharb in 1996 and 1997, but it didn't establish in those regions. *S. petiolatus* was established in Rabat and Gharb since 1997. *C. phyllocnistoides* was released in the field in 2000 and it was established in Souss, Haouz and Gharb. The introduced parasites and the predators are contributing to the control of CLM infestation during the summer and autumn. *Pnigalio* sp. and *Cirrospilus pictus*, the 2 major native parasites observed in the field, are showing a very low control of the pest, especially after the establishment of the introduced species.

Key words: *Phyllocnistis citrella*, citrus leafminer, biologicol control, parasites

Introduction

The citrus leaf miner (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae: Phyllocnistinae), has rapidly spread throughout many citrus-growing areas of the world during 1993 and 1994. California, which was considered one of the fewest citrus production areas still free of the pest, was infested in 2000.

Like many other Mediterranean countries, Morocco was invaded by CLM in 1994. The pest was reported for the first time in the east part of the country in august 1994 (Nia et al, 1996). Within a few months, it was present in all the citrus-growing areas. The south of the country (Souss region) was infested in 1995. Damage on leaves caused by the pest is generally low in spring and high in summer and autumn. In the south area the attacks start earlier in the spring.

Chemical application was not a good approach for the control of the pest. It was expensive and caused outbreaks of the other pests.

One way worth to be attempted is classical biological control methods based on the importation and establishment of host-specific parasites to restore the biological balance in the citrus ecosystem. At the end of 1995, the Domaines Agricoles, in coordination with the Citrus Growers Association in Morocco (GIRA), elaborated a classical biological project in order to introduce the main parasites of CLM. Those were imported from USA, Australia and

Spain, where similar projects were undertaken. Scientist who assisted us in this importation: Dr. F. Garcia-Mari, Dept. of Ecosystem and Forestry, Polytechnic University of Valencia, Dr. D. Smith, Dept. of Primary Industries, Hort. Station, Queensland, Australia and Dr. M.A. Hoy, University of Florida, Gainesville, Florida, USA.

Material and methods

Rearing facility
The infrastructure for rearing natural enemies was operative in December 1995 (Nia et al., 1997). A screen and a plastic houses were used for rearing the five introduced parasites, *Ageniaspis citricola*, *Semielacher petiolatus*, *Cirrospilus ingenuus*, *Quadrasticus* sp. and *Citrostichus phyllocnistoides*. The screen house had natural conditions of temperature and humidity and it was used for rearing *S. petiolatus* during spring and summer time. The plastic house was maintained under controlled temperature and relative humidity (respectively 18-30°C and 60 to 80%) and was used the whole year for rearing the other parasites.

The plastic house facility was divided into 3 compartments. Each compartment can hold up to 1500 plants. The first compartment was used for flush initiation, the second for CLM infestation and the third one for rearing the parasites. *A. citricola* was the only parasite reared inside the cages.

Introduction of the parasites and rearing methods
Ageniapis citricola Logvinovskaya (Hymenoptera: Encyrtidae) was the first species introduced from Florida in December 1995. The strain was collected in Thailand (Hoy and Nguyen, 1994) and was introduced in Florida. A modified Smith and Hoy rearing method (1995) described by Nia et al., (1996) was used during the first year. This method was improved in order to facilitate the rearing system and to increase the parasite production (Rizqi et al., 1997). This last method is based on the introduction of citrus leaves containing pupae of *A. citricola* approaching the adult emergence into the rearing cages containing plants infested with CLM at the first stages (eggs and L1, adequate stages for parasitism by *A. citricola*).

Semielacher petiolatus (Girault) (Hymenoptera: Eulophidae) was introduced from Australia in 1996. The first generations of the wasp were obtained in the plastic house facility where its multiplication was very low. The reason is probably the high temperature and humidity which was not supported by the parasite. We did the first release with a few individuals in 1996 and discovered that it multiplied and spread rapidly in natural conditions. So, for the following years, we used a screen house as rearing facility for this parasite. A stock of about 2000 plants was used to rear the parasite. During the winter, the plants infested with CLM were kept in warm conditions in the heated plastic house facility to maintain the pest multiplication. In the spring, they were moved to the screen house to induce CLM infestation on the young flushes induced in the stock plants. Flush growth was initiated by plant defoliation when needed. At the end of April, when L2 and L3 stages of CLM were abundant, adults of *S. petiotalus* were released in the screen house. Leaves containing the parasite were harvested from Mai to the beginning of the autumn. The parasite was sent to the growers as adults or pupae in the leaves.

Citrostichus phyllocnistoides (Narayanan) (Hymenoptera: Eulophidae) was introduced in 1999 from Spain. This parasite was reared in the plastic house in the same temperature and humidity conditions used for rearing *A. citricola*. The use of cages wasn't necessary. The parasite was reared in one compartment containing about one thousand plants infested with CLM. Introduction of newly infested plants in the rearing compartment was necessary to keep CLM infestation. When the parasite became abundant in the rearing compartment, adults were

directly collected from the leaves with an aspiration system. The parasite was sent to the field mainly as adults. The releases in the field of this species started in 2000.

Quadrastichus sp. *(=Tetrastichus* sp.)* (Hymenoptera: Eulophidae) was introduced from Spain in 1997. The rearing method is exactly similar to the one used for *C. phyllocnistoides*. The parasite was easily reared, but the releases in the field didn't start in the field until 1998.

Cirrospilus ingenuus (Gahan) (Hymenoptera: Encyrtidae) was introduced from Australia in 1996. A few individuals were collected from the sample of leaves received and it wasn't enough to initiate the parasite rearing.

Results and discussion

The number of individuals released from each parasite in the major citrus-growing regions are presented in table 1.

A. citricola was the first parasite released in the orchards in 1996, followed by *S. petiolatus* in the same year, *Quadrastichus sp.* in 1999 and *C. phyllocnistoides* in 2000.

About 135.000 individuals of *A. citricola* were released during 1996 and 1997. The first recovery of this parasite from the field was done during the autumn 1996 in Rabat. Its parasitism rate reached 38%. *Pnigalio sp.* and *Cirrospilus pictus*, the two main native parasites observed in Morocco were also contributing to the control of CLM in this region. Their parasitism rate didn't exceed 19% (Abbassi et al., unpublished work). *A. citricola* was also recovered from the Gharb and Souss. In this last region, the parasite was recovered from one site at the beginning of summer in 1996 and it disappeared in the next autumn. In 1997, more than 50.000 individuals were released in this region without recovering it from any of the sites of release.

Table 1: Releases of CLM parasites in citrus-growing regions in Morocco.

Parasites	Years	Regions of releases						
		Gharb	Rabat	Souss	Haouz	Tadla	Oriental	Total
A.citricola	1996	7.000	15.000	23.000	9.000	3.000	2.000	59.000
	1997	10.000	3.000	50.000	6.000	4.500	1.500	75.000
S.petiolatus	1997	2.700	1.000	8.500	600	200	1.900	14.900
	1998	-	-	3.000	1.000	1.000	800	5.800
	1999	-	-	8.700	4.500	2.100	-	15.300
Quadrastichus sp.	1999	400	500	600	-	500	-	2.000
C.phyllocnistoides	2000	7.000	-	9.500	18.700	1.700	-	36.900

A. citricola was not established in the Mediterranean areas, but it was established in Florida (Hoy et al., 1997) and in the Canary Islands (Garcia-Mari et al., 2000). It seems that the parasite needs humid climate and successive flush production to be able to maintain its multiplication in the field.

S. petiolatus was released in the citrus-growing regions from 1996 to 1999. The parasite was established in Rabat and Gharb since 1997. During 1998 and 1999, the parasite was

mainly released in the regions where it was not established (Souss, Haouz, Tadla and Oriental). It was never recovered from those regions. A total of about 35.000 individuals of *S. petiolatus* were released in the citrus-growing regions. The strain of *S. petiolatus* established in Gharb, was collected in 1998 and 1999 from this region and sent to the others without been able to recover it.

A survey was conducted in Gharb region in 1997, to measure the activity of *S. petiolatus* and *A. citricola* in the field. The data presented in table 2, shows that the two parasites were found in most of the orchards surrounding the sites of release. Their parasitism level reached 100% for *A. citricola* and 60% for *S. petiolatus*. The parasites were found at 40 to 50 Km away from the sites of releases.

Table 2. Situation of *A. citricola* and *S. petiolatus* in some orchards in Gharb region in 1997.

Orchards	No. CLM examined	Parasitism rate (%) A.citricola	Parasitism rate (%) S.petiolatus	Parasitism rate (%) local parasites
Souk El Tlet 1	401	52	-	-
Souk El Tlet 2	621	99	-	-
Kssiri 3	33	96	3	-
Kssiri 4	277	96	3	3
Jemaa 5	231	42	8	5
Souk El Tlet 6	184	41	4	12
Tazi 7	163	77	4	2
S. Slimane 8	60	5	63	17
S. Slimane 9	369	0	21	12
S. Slimane 10	134	0	58	2
S. Slimane 11	259	0	60	2

In 1998, *S. petiolatus* became the most important parasite in Rabat and Gharb. However, *A.citricola* was not recovered any more from those regions. A survey conducted from the summer to the beginning of autumn 2002 is presented in table 3. Samples of developed leaves infested with CLM were taken from some orchards in 4 regions; Souss, Gharb, Haouz and Rabat. Alive CLM pupae, emerged CLM adults and parasites pupae were recorded. The parasite larvae found were kept until pupation to identify the parasite specie.

C. phyllocnistoides was established in the south regions (Marrakech and Souss) where the climate is dry. In 2 years, it colonized most of the orchards. The parasite was also recovered from Gharb region where *S.petiolatus* was already established. However in this region, *C. phyllocnistoides* didn't spread away from the sites of releases. So, it was not found in Boudera which is about 30 Km far from the sites of releases (SA I and SA II).

In Rabat orchards (DES K and DES N), where pesticide applications were maintained, CLM population was high and *S. petiolatus* was less active comparing to Temara, a non treated orchard. The native parasites, *Pnigalio sp.* and *C. pictus*, were found in most of the orchards. But, they are less active in the regions where the introduced parasites were established.

Table 3. Situation of CLM parasites in some citrus-growing regions in Morocco during the summer and autumn 2002.

Regions	Orchards	Number of individuals per sample of 200 leaves / orchard				
		CLM Pupae	Emerged CLM Adults	*Citrostichus* Pupae	*Semielacher* pupae	*C. pictus* & P*nigalio* Pupae
Souss	Kedima 1	2	2	32	-	-
	Kedima 2	2	3	18	-	-
	Saouda	6	3	11	-	2
Marrakech	Agafay 1	6	7	37	-	7
	Agafay II	-	-	19	-	-
	Agafay I 1	5	-	29	-	2
	Agafay I 2	7	1	34	-	1
Rabat	Temara	34	14	-	230	2
	DES K	140	134	-	6	43
	DES N	55	62	-	8	23
Gharb	SA II 1	-	-	48	47	2
	Boudera	32	8	-	36	1
	SA II 2	5	5	6	5	-
	SA I	-	2	37	23	1

Similar results concerning the establishment of *C. phyllocnistoides* were obtained in other Mediterranean countries. The parasite expanded to many orchards in Valencia and became the more important parasite in the central area (Garcia-Mari et al., 2000). The same situation is found in Israel, where *C. phyllocnistoides* became the most important parasite of CLM (Argov, personal communication).

The experience in Morocco has been similar to that in other parts of the world where CLM has found its way into citrus. Initially it was a massive problem because it has few local natural enemies. Citrus growers, who are not used to the visual impact of distorted leaves caused by this pest, tend to over react with greatly increased use of insecticides. The effect of CLM attacks started to decrease with the development of native parasites activity and predators. The situation was improved considerably with the establishment of the introduced parasites and the reduction of pesticide use.

Actually, mature citrus trees tolerate the pest attack with no apparent effect on productivity. For the young trees newly planted in the field, chemical treatments are needed to save the new growths.

Acknowledgements

6

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Biological control of the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Spain: native parasitoids and establishment of *Citrostichus phyllocnistoides* (Hymenoptera: Eulophidae)

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Abstract: A survey of parasitoids of the Citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was carried out in more than 100 citrus orchards located within 60 km of Valencia. Orchards were sampled all along the citrus growing season from 1995 to 2001. Ten different species were found. The two main species, *Pnigalio* sp and *Cirrospilus brevis* Zhu, LaSalle and Huang represented more than 90% of the adults identified. The parasitoids originated from native leafminers living mostly on woody plants. The preferred stages for parasitism were third (27-38% of parasitism rate) and fourth instars (30-53%). Second instar and pupae were less parasitized (10 and 5%, respectively). A program of introduction of exotic parasitoids was carried out between 1996 and 1999. Through a total of 37 shipments coming from nine countries, six species were released in the field. *Ageniaspis citricola* (Logvinosvskaya) established temporarily but was unable to overwinter. Between 1999 and 2001 *Citrostichus phyllocnistoides* (Narayanan) expanded to all citrus orchards grown in continental Spain and the Balear Islands, becoming the most abundant parasitoid and displacing native and other introduced parasitoids. Parasitism on second instars increased to 65% and foliar surface damage by the citrus leafminer decreased by 56%.

Key words: Native parasitoids, *Ageniaspis citricola*, biological control, *Citrostichus phyllocnistoides*, citrus, introduction, parasitism, *Phyllocnistis citrella*.

Introduction

Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae) is a pest native of Eastern and Southern Asia that since 1993 invaded all citrus-growing regions in America and the Mediterranean basin. The citrus leafminer attacks developing leaves and causes problems mainly in young trees, nurseries and overgraftings. The first attempt to control the new pest was using broad spectrum insecticides, but chemical control appeared to be a costly and short-term solution (Knapp *et al.*, 1995; Argov and Rössler 1996).

Native parasitoids, in some environments, have been able to control host population. *Galeopsomyia fausta* Lasalle, in Mexico, Central and South American (Cave, 1996; Cano et al., 1996; Castaño et al., 1996; Martínez, 1996; Lasalle y Peña, 1997), *Semielacher petiolatus* (Girault) in Australia (Smith and Neale, 1996) and *Platocharis coffeae* (Ferrière) in South-Africa (Ware y Hattingh, 1996) have been reported as effective parasitoids for *P. citrella* control.

In USA, Australia and Mediterranean countries the effort was also made in Classical Biological Control programs with the introduction of exotic parasitoids. The first

introductions of parasitoids in the Mediterranean area were initiated in Israel in 1994-1995 with the introduction of six species (Argov and Rössler 1996; Argov *et al.*, 1998).

After the introduction of *P. citrella* in Spain, a research project was initiated in the eastern Spanish citrus belt of Valencia with two aims, to study the effect of native parasitoids on the introduced pest, and to develop a program of Classical Biological Control with the introduction of exotic parasitoids (Garcia -Marí et al.,1997; Vercher et al, 1997; Vercher et al., 2000). In this paper we document on the native parasitoid guild associated with citrus leafminer, the level of parasitism over the years and its effect on *P. citrella* populations. The results of the exotic parasitoid releases and the following establishment of self-perpetuating field populations are also reported.

Materials and methods

Samplings were made in full production commercial citrus orchards selected within 60 km of Valencia (situated in the middle of the Eastern-Spain citrus region). From 1995 to 2001, more than 1,000 samples were collected all along the citrus growing season in a total of 105 orchards. Each orchard was sampled between one and three times a year. To evaluate the level of parasitism and parasitoid guild associated with *P. citrella*, 100-150 developed new shoots, containing different development stages of the citrus leafminer, were taken to the laboratory. Fifty alive leafminers of each susceptible development stage (second, third, fourth instars and pupae) were checked under a stereomicroscope looking for the presence of parasitoids. Immature parasitoids found were isolated in glass vials and allowed to develop to adult stage for identification. The abundance of *P. citrella* was estimated by calculating the percentage of damaged leaf area.The adult leafminer population was estimated with a commercial leaf suction aspirator adapted to collect citrus foliage (Garcia -Marí et al, 2002).

A study of the parasitoids that attacked other species of leafminers was carried out in order to establish the primary hosts from whom some native parasitoids have happened to act on *P. citrella*. Herbaceous and woody plants attacked by leafminers and located in the proximities of the target citrus orchards were also sampled through the years 1996, 1997 and 1998 (Vercher, 2000).

Exotic parasitoids of the citrus leafminer were imported between 1996 and 1999 from different countries, usually as adults from laboratory colonies of countries with similar undergoing biological control programs (Garcia-Marí et al., 2000; Vercher et al., 2000). A total of 37 shipments were received during 1996-1999. Insects were reared with three trophic levels (citrus plants, leafminers and parasitoids) following the method of Smith and Hoy (1995), slightly modified as described in Serrano et al.(1996). Releases of parasitoids were made mostly in commercial groves selected from an area extending 60 km around Valencia. In general, releases consisted of 500-1,000 adults and were repeated several times over the season for each site. Field releases were carried out either with adults collected inside glass vials, or with pupae of the parasitoids developed on potted seedlings or detached citrus shoots. Ten to 20 release sites were sampled twice a month all year long between 1996 and 2001. Percent of parasitism and impact on *P. citrella* population were evaluated.

Identification of parasitoids was made by the authors and confirmed by J. LaSalle (British Museum Natural History, London, England) and by M. J. Verdú (Institut Valencià d'Investigacions Agràries, València, Spain).

Results

Native Parasitoid

Overall, 10,397 native parasitoids of *P. citrella* were identified during the five years of studies. Ten different species were found, belonging to two families, Eulophidae (with nine species) and Pteromalidae (only one species, *Pteromalus* sp). Two major species, *Pnigalio* sp. and *Cirrospilus brevis* Zhu, LaSalle and Huang (known before as *Cirrospilus* sp.*near lyncus,* (Zhu et al., 2002)) represented more than 90% of the parasitoids identified. *Sympiesis gregori* Boucek, *Cirrospilus pictus* (Nees), *C. vittatus* Walker and *Ratzeburgiola cristata* (Ratzeburg) represented 7% of the parasitoids (secondary species). N*eochrysocharis formosa* (Westwood), *Chrysocharis pentheus* (Walker), *Baryscapus* sp and *Pteromalus* sp. were found as minor species.

The relative abundance of native parasitoids changed between 1995 and 1999 (Fig. 1). In 1995, only *Pnigalio* sp. was predominant and *Cirrospilus brevis* represented only 3% of total native parasitoids. In 1996, *C. brevis* increased considerably up to 22 %, and increased even more during 1997, 1998 and 1999, becoming more abundant than *Pnigalio* sp. in many samples. Both species coexisted and were predominant between 1997 and 1999.
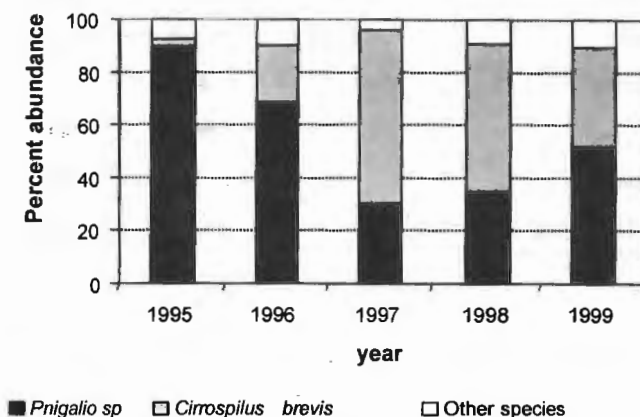
Figure 1. Relative abundance of native parasitoids of *P. citrella* between 1995 and 1999 in citrus orchards from Valencia (Spain).

The native parasitoids of *P. citrella* appeared parasiting leafminers in herbaceous and woody plants situated close to the target orchards (Vercher, 2000). However, common parasitoids of leafminers such as *Diglyphus minoes* (Walker) and *C. variegatus* were never found attacking *P. citrella* in Spain. The major native parasitoids of *P. citrella, Pnigalio* sp. and *Cirrospilus brevis,* represented 3 to 30% of total parasitoids in other hosts.

The parasitism level inflicted by native parasitoids changed with the stage of *P. citrella.* In general, the preferred stage for parasitism were third (27-38%) and fourth instars (30-53%). Second instar was less parasitized (10%). Pupae were even less affected by parasitoids (5%).

Introduction of Exotic Parasitoids

Six species of citrus leafminer parasitoids were released in the field between 1996 and 2000 (Table 1). Releases began in 1996 with *A. citricola* and ended up in 1999 with *C. phyllocnistoides*. A temporal establishment of *A. citricola* in 1996 was observed in 15 out of 17 release points. In seven orchards where parasitism was monitored periodically, the average percentage of parasitized pupal chambers increased significantly from 20 to 50% between July and November (Fig. 2). Parasitoid pupae were observed at 1 km from three release points and 2 km from one point. But during the winter season of 1996-1997 *A. citricola* disappeared, and was not recovered again during 1997 in any of the release sites where it had been previously released. New field releases in 1997 and 1998 produced similar negative results. Apparently, no *A. citricola* was able to overwinter after establishing temporarily the previous season.

Table 1. Number of *P. citreíla* exotic parasitoids released from 1996 to 2000 in Spain

SPECIES	YEAR			
	1996	1997	1998	1999
Ageniaspis citricola	71.500	16.500	23.800	
Quadrastichus sp.	2.500	34.300	10.400	3.100
Galeopsomyia fausta		80		
Semielacher petiolatus		800	700	470
Cirrospilus ingenuus			300	80
Citrostichus phyllocnistoides			7.000	700
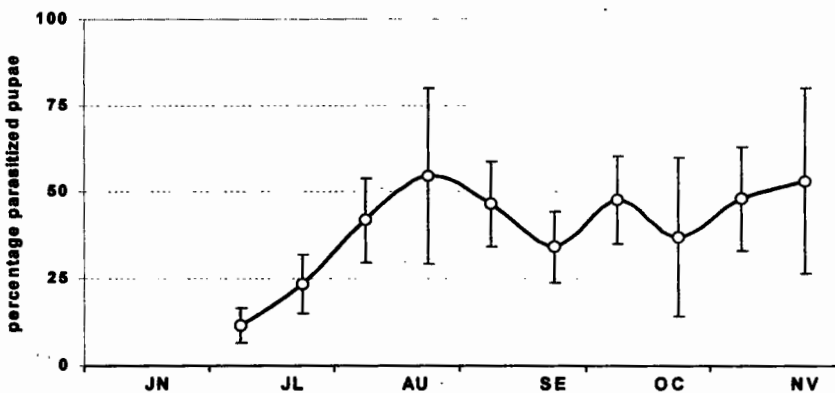
Figure 2. Temporary establishment and parasitism of *Ageniaspis citricola* in seven release sites during 1996 in citrus orchards from Valencia (Spain).

C. phyllocnistoides was first released in summer and autumn 1998 in 15 orchards, and it was recovered the same year in six of them. In 1999 parasitoids appeared in July in high numbers and between August and November the population dispersed rapidly (up to 30 km from initial release points). In 2000, *C. phyllocnistoides* was present in the central part of the Eastern Spain citrus belt, in an area extending 200–300 km from north to south. In 2001 the process of expansion was apparently completed in Spain as the parasitoid reached all citrus areas in the Iberian peninsula and the Balear Islands. Five years after the first releases of exotics parasitoids, the temporary establishment recorded so far in Spain is reported in table 2.

Table 2. Species of exotic parasitoids of *P. citrella* established in Spain

Species	Area established
Ageniaspis citricola	Canary Islands
Quadrastichus sp.	València
Semielacher petiolatus	Andalucia and Balear Islands
Citrostichus phyllocnistoides	Continental Spain and Balear Islands

The citrus leafminer parasitoid complex changed throughout the seven-year period 1995-2001 in the Valencia area. After the temporary establishment of *A. citricola* in 1996 and *Quadrastichus* sp. between 1997 and 1999, a dramatic and continuous increase in the proportion of *C. phyllocnistoides* was clearly observed between 1998 and 2001, with a corresponding decrease in the proportion of native species. In 2001 more than 90% of parasitoids were *C. phyllocnistoides*.

In 1999 and 2000, in coincidence with the quick build up and dispersal of the introduced parasitoid *C. phyllocnistoides*, parasitism on second instars increased from less than 16% in previous years to 65% (Figure 3). Overall, the mean percentage parasitism considering all susceptible development stages did not change in the period 1995 through 1998, when native parasitoid species predominated, reaching values of 20-25%, and increased significantly to values near 60% from 1999 to 2001 when the introduced parasitoid *C. phyllocnistoides* became predominant.

There were no significant differences in average annual abundance of the citrus leafminer population between 1995 and 1999, when parasitoid species were mostly native. In 2000–2001, as the introduced parasitoid *C. phyllocnistoides* established and dispersed, the citrus leafminer population density decreased significantly compared with previous years. Considering the average mean values between July and October, the comparison of the years 2000 and 2001 with levels found in previous years reveals a 44% decrease in number of eggs and young instars, a 56% reduction in foliar surface damage and a 72% reduction in number of citrus leafminer adults captured in suction samples.
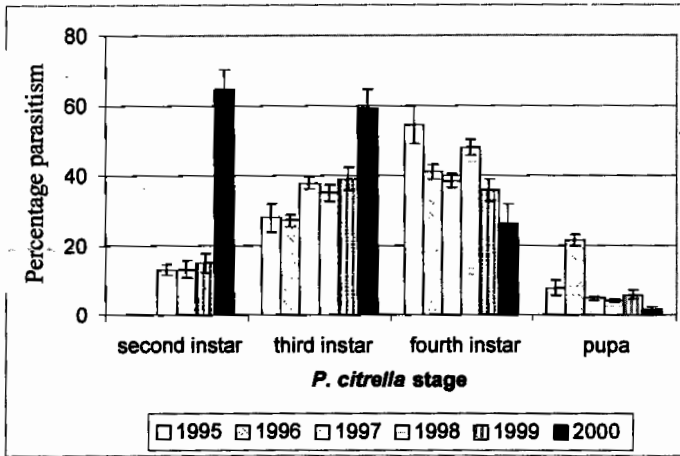
Figure 3. Percentage parasitism (± SE) on *P. citrella* for each of six sampled years (1995 to 2001) in Valencia, Spain. Average values of all the samples taken each year.

Discussion

Native parasitoids are important agents of biological control against introduced pests. In our study, we found in 1996 ten different species of native parasitoids attacking *P. citrella*. In other Mediterranean regions the number of natives parasitoids observed on citrus leafminer vary between 6 and 10 (Schauff et al., 1998).

The complex of native parasitoids changed during the study period. In 1995 more than 90% of the parasitoids were *Pnigalio* sp., which prefers fourth to third instars; during 1996 another native species, *Cirrospilus brevis*, started to increase and from 1997 to 1999 became one of the two prevailing native parasitoids, together with *Pnigalio* sp. Both species accounted for over 90% of the native parasitoids identified. Level of global parasitism inflicted by native parasitoids was low (20-25%) and stable over the years. These results are quite similar to other studies made in other Mediterranean areas (Malausa et al., 1996; Uygun et al., 1996; Argov et al., 1998).

We observed in this study that native parasitoids of *P. citrella* were present on other leafminers of herbaceous and woody plants. Native parasitoids represent an important biodiversity reserve and a source of potential biologic control agent against the occurrence of new pests (Waage, 1991; LaSalle, 1993; LaSalle y Gauld, 1993).

A. citricola, C. quadristriatus , C. phyllocnistoides , S. petiolatus and *Quadrastichus* sp., are the five species considered as more efficients (Ujiye 1988; Ujiye and Morakote 1992; Ujiye and Adachi 1995; BingLin and MingDu 1996; Ujiye *et al.*, 1996) and more frequently released in classical biological control programs developed in citrus producing countries of Australia, America and the Mediterranean region in recent years (Beattie and Smith, 1993; Garcia -Marí, 1996; Hoy et al, 1997; Argov et al., 1998; Vercher et al., 2000). In continental Spain *A. citricola* is able to survive and expand in the field during the summer months in areas with low humidity, but it is not able to survive the winter period typical of the Mediterranean climate, with cool mean temperatures (10-12°C) and lack of flushes during

several months. Other Mediterranean countries have also reported on this failure, as Israel (Argov *et al.,* 1998) or Greece (Tsagarakis *et al.,* 1999). Climate is the most commonly cited cause of failure in biological control programs, especially when introducing enemies from tropical to temperate areas (Hokkanen, 1985; Stiling 1993).

C. phyllocnistoides established all over the Spanish citrus region. This parasitoid causes over 50% reduction in leaf area damage, suggesting that this species is an effective biological control agent of the citrus leafminer in Mediterranean climates.

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14

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Native auxiliary complex of Citrus Leafminer *Phyllocnistis citrella* Stainton in Málaga province (Spain). Effects of competence with the introduced auxiliary species *Citrostichus phyllocnistoides* Narayanan

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Abstract: By improving the present knowledge about the population dynamics of native citrus leafminer (CLM) parasites, we can enhance the control of this pest and optimise its natural enemy introduction process. Our aims are to know the native auxiliary complex of CLM in Malaga province and their role on the development dynamic of the pest, and to evaluate the efficacy of the exotic auxiliary insect *Citrostichus phyllocnistoides* (Narayanan) as a parasite and its competence relationships with indigenous species. We prospected for parasites from 1999 to 2001 in three areas of Malaga province: Western Coast (Estepona), Guadalhorce Valley, and Axarquia (Benamargosa). In every parcel we randomly collected shoots infested by CLM larvae of different ages, determining the parasite abundance and its developmental stage. Leaves with CLM parasites were isolated in Petri dishes, where the larvae were reared to adults for their final identification. The CLM parasite complex in Malaga province basically consists of the following species: *Semielacher petiolatus* (Girault), *Cirrospilus pictus* (Ness), *Cirrospilus* nr. *lynchus* Walker and *Pnigalio pectinicornis* L. We have succeeded in the introduction of *C. phyllocnistoides* in all the areas with citrus orchards in Malaga. This parasite has shown great acclimation and dispersion capabilities. Our observations confirm a certain degree of displacement of the less efficacious native auxiliary species after *Citrostichus phyllocnistoides* has been introduced.

Key words:, Citrus leafminer, biological control, native parasites, *Citrostichus phyllocnistoides*

Introduction

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton, is a native pest from Southeast Asia and is now widely distributed where citrus is grown in Asia, Australia, Northern and Central Africa, Florida (USA), Central and Southern America and all over the Mediterranean basin. In Spain, it was first detected in Malaga province in 1993 (Garijo & García, 1994). Lately, it has invaded the whole Mediterranean basin, where it became a very important pest from an economic point of view. The damages caused by CLM affect all shoot sprouts from the end of Spring.

Along the first years after it appeared, the CLM populations increased controlled only by abiotic factors. In the beginning, the efforts for its control were driven to search an effective, economical and environmentally friendly chemical control method. However, the native fauna of parasite Himenoptera species (most of them of the Eulophidae family, usually polifagous and feeding from other leafminers), soon began to act on the new Lepidoptera species (Garrido, 1995). Since then, the native parasitoid list found on the Citrus Leafminer has increased gradually. The prospecting of indigenous parasites was one of the first objectives of the different research groups involved in the study of CLM (Costa-Comelles *et al.*, 1995; García-Marí *et al.*, 1997; Garrido, 1995; Llácer *et al.*, 1995; Vercher *et al.*, 1995; Verdú, 1996

and Verdú *et al.*, 1998). The later efforts concentrated on classical biological control methods, the establishment of exotic enemies to restore the biological balance in the citrus ecosystem, and the reduction of the pest below the economic injury threshold (Argov & Rössler, 1996; García-Marí *et al.*, 1997; García-Marí *et al.*, 2000 and Ripolles, 1995).

This work is framed in the research context described, and has the following objectives: 1) to set up the catalogue of CLM parasites in Malaga province; 2) to determine the role of the parasite complex on the development dynamics of the pest; 3) to study the dispersion and colonization processes of the exotic auxiliary insect *C. phyllocnistoides*, and 4) to evaluate its efficacy as a parasite and its competence relationships with the indigenous ones.

Material and methods

To build the catalogue of native CLM parasites we collected, from 1999 to 2001, infested shoots in 10 random trees per orchard, in the three main citrus areas of Malaga province (Western coast, Guadalhorce Valley and Axarquia, see Figure 1). In each area we took samples in lemon, orange and tangerine orchards. At the laboratory we examined 200 individuals of CLM, and noted down their larva stage and if they were parasitized. Pupae of parasitoids were reared to adults in Petri dishes. Then, we collected the adults and conserved them in alcohol, for its identification in the Dpto. Protección Vegetal de la Universidad Politécnica de Valencia.

Figure 1. Main citrus areas in Malaga province

Along 2000 we released 9,500 *C. phyllocnistoides* individuals in 17 spots in Malaga province. For this, we put shoots with CLM parasitized by *C. phyllocnistoides* in plastic glasses with water, which were fixed to the trunks and branches of about 30 trees per orchard. These shoots were located within the canopy to protect them from direct insolation, wind, and desiccation.

To study the dispersion process of the exotic parasitoids in the different citrus areas of Malaga province, we collected infested shoots in plots chosen at random next to the orchards where exotic parasites were released in 2000, within a radius of 3 to 10 km around it. Once in the laboratory, we analysed 200 CLM individuals per sample and distinguished among CLMs parasitized by native species and CLMs parasitized by *C. Phyllocnistoides*. The areas where we confirmed the presence of the exotic parasitoid were mapped. The sampling period lasted from April to September.

To analyse the role of the parasite species on the pest dynamic, we identified two variables on 600 leaves, collected always in the same parcels: the proportion of leaves with CLM and the mean damage index (MDI). This last variable is an estimation of the leaf surface damaged by CLM. For it we used the following scale: 1, for damages lower than 12% of the leaf surface; 2, for damages between 12 and 25%; 3, for damages between 25 and 50%, and 5, for damages higher than 75%. For every leaf, we always took into account the summed surface of both sides.

Results and discussion

The final catalogue consists on a list of five species which appeared along the three years: *Neochrysocharis sp., Pnigalio sp,. Cirrospilus prox. lynchus, Cirrospilus pictus* and *Semielacher petiolatus* (Figure 2). These are non-specific parasitoids of CLM.
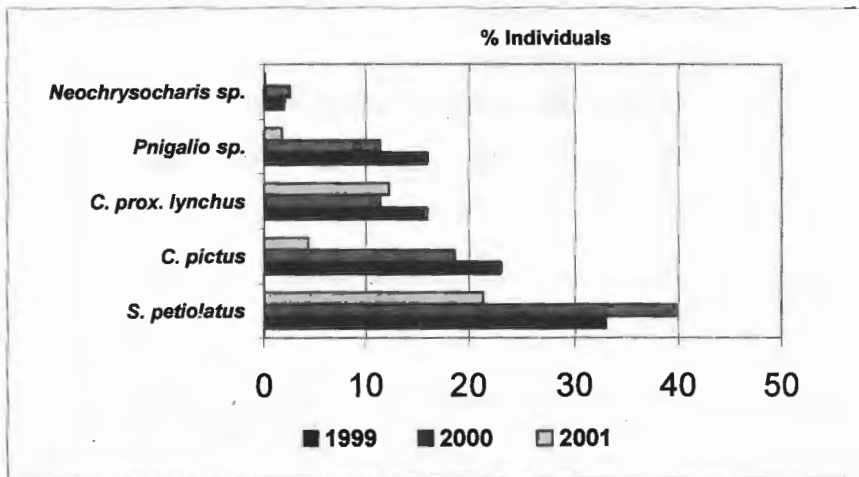
Figure 2. Individual percentage of each parasitoid species found in Malaga province during 1999-2001.

Two species, *Simpiesis sandanis* (Walker) and *Cirrospilus vittatus* Walker, that had been collected in 1994 in a previous study, did not appear in our catalogue. However, as the species identified were the same from 1999 to 2001, it seems that the auxiliary complex of CLM is quite stable. The most abundant species was always *S. petiolatus*, which is an exotic Hymenoptera probably introduced by natural factors from Morocco, and is at present well

acclimatised. The second most abundant species was *C. pictus*, whereas the least frequently found was *Neochrysocharis* sp., which was not detected in 2001.

Most of the species found in Malaga are present also in other citrus areas in Spain, though their relative abundances are different. Thus, in the Comunidad Valenciana, the most frequent species was *C.* nr. *lyncus*, followed by *P. pectinicornis* (Llácer *et al.*, 1998; Urbaneja *et al.*, 1998).

The introduction of *C. phyllocnistoides* in 2000 caused changes in the relative abundance of native parasitoids, after it became the most abundant species (see Figure 3). In the first samplings after the releases, the CLM parasitized by *C. phyllocnistoides* was lower than 10%. Instead, in 2001, after having survived the first winter, this percentage turned into 60%. Except for *C. prox. lynchus* (which stood on similar proportion levels), the relative abundance of the other native species suffered an important decrease. A similar behaviour of the relative proportions was observed in the Comunidad Valenciana (García-Marí et al., 2000).
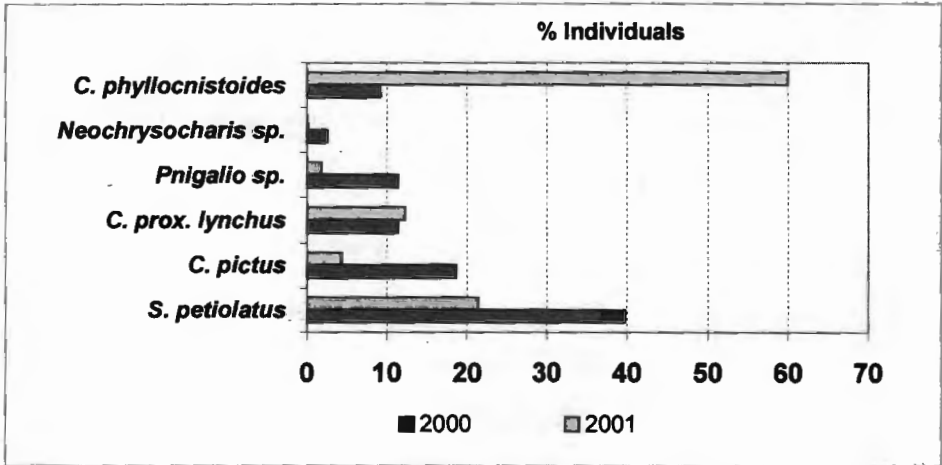
Figure 3. Individual percentage of each parasitoid species found in Malaga province during 1999-2001, including *C. phyllocnistoides*.

Figure 4 shows the evolution of parasitism percentages and of damages on leaves caused within the sampling period. The total parasite percentage includes *C. phyllocnistoides*. Both the percentage of leaves damaged and the DMI were higher in summer than in other seasons. However, although about 75% of leaves were damaged, only a small surface within them was really affected. As the parasitism percentage increased, the number of leaves with CLM and their surface damaged decreased. This happened from June to July, and more definitely in Autumn. Several authors (Costa et al., 1995) pointed out that the CLM Autumn decrease can be caused by the colder temperatures, and not only by the increase of parasitism.
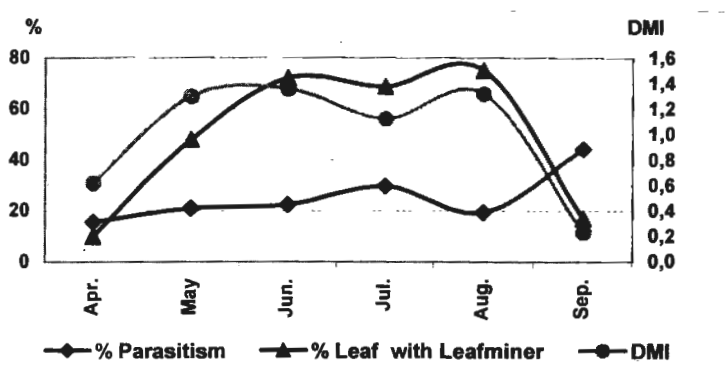
Figure 4. Evolution of the parasitism percentage, the percentage of leafs with Leafminer, and the damage mean index (DMI) along 2001.

Figure 5 shows that sometimes the parasitism due to *C. phyllocnistoides* reached 50% of total parasitism. García-Marí et al. (2000) demonstrated that this species parasites frequently on L2 larvae, which are barely used by any of the indigenous parasitoids. This means that *C. phyllocnistoides* not only increases the parasitism incidence, but takes advantage of a population segment of the Citrus Leafminer not exploded by the previous fauna, thus contributing to a more effective control of it.
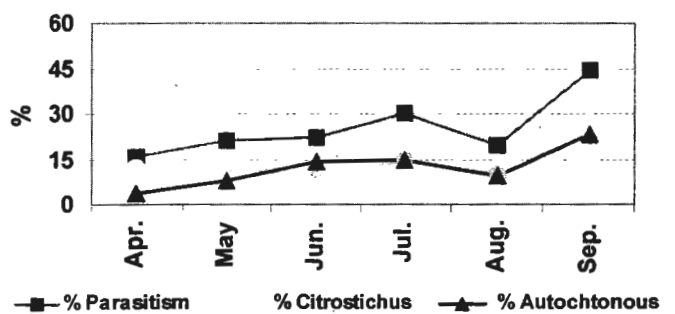
Figure 5. Evolution of the parasitism percentages due to all the species, to *C. phyllocnistoides*, and to the native species complex.

The dispersion process of *C. phyllocnistoides* has been quick and successful, as in only one year the introduced species has become dominant. In 60 of the 68 orchards sampled within a radius of 3 to 10 Km around the release spots, *C. phyllocnistoides* has been detected in 2001. This means that the exotic parasitoid has been able to survive in Malaga along the

22

winter, and to colonise other areas. The great capability of *C. phyllocnistoides* to settle down, reproduce and disperse had already been demonstrated in Valencia (García-Marí *et al.* 2000).

Acknowledgements

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Activity of Indigenous and Exotic Parasitoids of *Phyllocnistis citrella* Stainton (Lepidoptera : Gracillariidae) in Western Sicily

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Abstract: *Phyllocnistis citrella*, detected in Spain in 1993 and in Italy in 1994, appeared in Sicily in 1995 and spread rapidly in all citrus areas of Southern Italy. Some indigenous parasitoids, generally associated with a variety of leafmining hosts, were found on citrus leafminer. A number of species were found and they included : *Apotetrastichus sericothorax* (Srelènyi), *Cirrospilus diallus* (Walker) e *Teleopterus erxias* (Walker), *Pnigalio* sp.,*Ratzeburgiola incompleta* Boucek, *Cirrospilus diallus*(Walzer), *Cirrospilus pictus* (Nees), *Apotetrastichus sericothorax* (Szelènyi), *Apotetrastichus* sp., *Chrysocharis* sp., *Neochrysocharis* sp.,*Teleopterus erxias* (Walker) (G. Liotta et al., 1996). The species, which were found with high levels of parasitization, were *C. pictus*, *C. diallus*, *Pnigalio* spp. and *R. incompleta* (M. Lo Pinto e G. Salerno, 1996-97). From June 1995 to February 1997 the parasitizazion of *C. diallus* was on average between 45,9% and 100% in some citrus areas. Subsequently, from 1997 to 1999, new parasitoids were found: *Asecodes delucchii* (Boucek), *Baryscapus* sp., *Elasmus* sp. *Aprostocetus* sp (G. Mineo, 1999). The total level of parasitism, in some citrus areas, exceeded 50%, but this rate seems not to have been of enough effect to control CLM population. In order to stress the natural control against CLM these exotic parasitoids were introduced into Sicily: *Ageniaspis citricola* Longvinovskaya (Barbagallo et al., 1998), *Quadrastichus* sp. (Siscaro et al., 1999) and *Citrostichus phyllocnistoides* Narayanan (Mineo et al., 2001). Along with it, there was the recording of a new exotic parasitoid, in 1995, *Semilacher petiolatus* (Girault), Australian eulophid wasp. This ectoparasite wasp arrived in Sicily probably due to the accidental introduction of citrus material from North African countries, which has recently been introduced from Australia. At the moment the most active CLM parasitoids are *Semilacher petiolatus* and *Citrostichus phyllocnistoides,* these are also displacing indigenous parasitoids.

Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae: Phyllocnistinae) was first detected in some citrus orchards in Sardinia in autumn 1994 in Italy (Benfatto, 1995; Ortu et al., 1995). The pest arrived in Sicily in summer 1995 (Balzani et al., 1995; Liotta and Manzella, 1995; Longo and Siscaro, 1995) infesting all citrus orchards on the west coast of Sicily and subsequently spreading to all citrus areas in Sicily. The citrus leafminer probably arrived in Sicily from the Magrebine coasts (Morocco, Algeria) transported by low winds.

This spectacular invasion of CLM alarmed citrus growers and nurserymen who applied various offensives against the pest.

Indigenous natural enemies

At the same time, research programmes to identify indigenous parasitic wasps associated with this pest were started.

In Western Sicily, Liotta et al. (1996) recorded the following indigenous parasitoids on CLM in summer 1995: (Fig. 3) *Pnigalio* sp. A, *Pnigalio* sp. B, *Pnigalio* sp. C, *Ratzeburgiola incompleta* Boucek, *Cirrospilus diallus* (Walker), (Fig. 4) *Cirrospilus pictus* (Nees), (Fig. 1) *Apotetrastichus sericothorax* (Szelènyi), *Apotetrastichus* sp., *Chrysocharis* sp., (Fig. 2) *Neochrysocharis* sp., *Teleopterus erxias* (Walker). These native parasitoids are generally associated with a variety of leafmining hosts. The most commonly found were *Pnigalio* spp. and *C. pictus* with a parasitization rates exceeding 60% in some areas in Sicily (Maniglia et al.,1996).

Subsequently other new native parasitoids were found on CLM in Sicily: *Apotetrastichus postmarginalis* (Boucek), *Neochrysocharis formosa* (Westwood), *Pnigalio agraules* (Walker) (= *mediterraneus* Ferr. et Del.) (Caleca et al., 1996) and *Cirrospilus vittatus* Walker (Nucifora and Nucifora, 1996). Lo Pinto and Salerno (1998) reported that the percentage of parasitism by native parasitoids, in different areas of Western Sicily, from June 1996 to March 1997 ranged between 0 and 41,1% and never, on average, exceeded 20%. The most abundant parasitoid, in the same period, was *C. pictus* with 69,8% of the total species found on CLM, *C. diallus* with 14,1%, *C. vittatus* with 0,2%, *R. incompleta* with 10,3%, *N. formosa* with 3,9%, *Pnigalio* sp. with 1,3% and *Apotetrastichus sericothorax* with 0,4%. From December 1997 to March 1999 new species of parasitoid of CLM on several citrus groves near Palermo (Western Sicily) were found (Mineo, 1999): *Asecodes delucchii* (Boucek), *Baryscapus* sp., *Elasmus* sp. *Aprostocetus* sp. The total level of indigenous parasitoids, in some citrus area of Western Sicily, exceeded 50%, but this rate does not seem to be high enough to control CLM population.

Exotic enemies

Various exotic parasitoids were introduced and released in Sicily in order to increase the natural control against the pest. The first exotic parasitoids of CLM imported and released in Sicily were: *Ageniaspis citricola* Logvinovskaya and *Quadrastichus* sp. (Siscaro et al., 1997). *A. citricola* was imported from Florida in 1995 and *Quadrastichus* sp. from Israel in 1996 by the Institute of Agricultural Entomology of the University of Catania. These two parasitoids were released in citrus groves of Eastern Sicily.

The Encyrtid *A. citricola* has shown parasitization levels near to 80-90% only in very limited area in Eastern Sicily whereas *Quadrastichus* sp. did not become estabilished (Siscaro et al., 1999). In 1999, (Fig. 6) *Citrostichus phyllocnistoides* Narayanan, was introduced into Western Sicily from Jordan to control of CLM (Mineo and Mineo, 1999).

Along with it, there was the recording of a new exotic ectoparasitoid, (Fig. 5) *Semielacher petiolatus* (Girault) (Mineo et al., 1998), an australian Eulophid wasp, arrived in Sicily probably due to the accidental introduction on citrus material from North African countries or from Israel, where they had been introduced from Australia (Argov and Rössler, 1997; Nia et al. 1997). At the moment the most active CLM parasitoids are *Semielacher petiolatus* and *Citrostichus phyllocnistoides*, which are displacing indigenous parasitoids.

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State of the citrus leaf miner (*Phyllocnistis citrella* Stainton) parasitoid complex on lemon trees in the South East of Spain.

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Abstract: *Phyllocnistis citrella* was dectected for the first time in Spain in the coast of Andalucy on 1993. Next year, it spread all over the South East, where lemon tree is dominant among citrics. In this last area, native parasitoids belonging to the genus *Pnigalio* and *Cirrospillus* were found developing on the new pest. However, parasitim rates have a great seasonal variation and a satisfactory control of the leaf miner was not achieve.

Since *P. Citrella* was first reported in Spain, several species of exotic parasitoids have been introduced but only a few have successfully adapted to the new conditions. *Citrostichus phyllocnistoides* has been one of the species spreading from the releasing points over the areas of Levante and South East in the last few years.

Lemon trees were sampled on 2002 in two fields from Murcia and Alicante provinces to determine the distribution and abundance of *Phyllocnistis citrella* and its parasitoids. Every week shoots susceptible to the leaf miner attack were sampled. The following parameters were measured on each shoot: number of leaves, number of alive and death individuals and number of parasitized individuals. To determine the proportion of each parasitoid species, parasitized larvae were allowed to complete their development to adults.

Most of the parasitism was due to the exotic parasitoid *C. phyllocnistoides*. This parasitoid species was found only parasitizing second and third instar larvae. The parasitims of native parasitoids was also important. *Cirrospilus spp.* y *Pnigalio sp* were the mosts abundant.

Current status of the biological control of the citrus leafminer in Sicily

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Abstract: In Sicily, due to the low efficiency of indigenous entomophagous detected on *Phyllocnistis citrella* Stainton (Lep. Gracillariidae), a classical biological control program has been started in 1995 by introducing, rearing and releasing the exotic Hymenoptera parasitoids *Ageniaspis citricola* Logvinovskaya (Encyrtidae), *Quadrastichus* sp. and *Citrostichus phyllocnistoides* (Narayanan) (Eulophidae). The first one was recovered in some coastal lemon orchards where it overwintered in 1998, although without any following permanent establishment. *Quadrastichus* sp. was found only after releases (1996), but it has not overwintered. *C. phyllocnistoides* overwintered in all the release sites and showed a high dispersal rate. At the moment it represents one of the main natural enemies of the citrus leafminer together with *Semielacher petiolatus* (Girault) (Hym. Eulophidae), recovered for the first time in 1998 and naturally spread in all citrus areas in Southern Italy.

Key words: *Phyllocnistis citrella*, Hymenoptera parasitoids, citrus groves.

Introduction

Phyllocnistis citrella Stainton (Lep. Gracillariidae), native from South-East Asia, has spread throughout all citrus areas around the world in the last decades (Hoy & Nguyen, 1997); in Italy, the first record of citrus leafminer infestations was reported in 1994 (Benfatto, 1995).

Several predators, mainly spiders, bugs, lacewings and ants, have been detected on the phytophagous (Browning & Peña, 1995); besides, over 90 hymenopterous parasitoid species, belonging to the families Braconidae, Encyrtidae, Eulophidae, Euritomidae, Eupelmidae and Pteromalidae have been reported (Heppner, 1993; Hoy & Nguyen, 1997; Schauff *et al.*, 1998). Among these species, about 70 are considered as primary parasitoids and only 6 are classified as preferentially living on *P. citrella*.

Indigenous natural enemies, mainly parasitoids, detected on the CLM never reached an effective control in all new infested citrus areas, such as Florida (Hoy & Nguyen, 1997), Israel (Argov & Rössler, 1996), Spain (Garrido Vivas, 1995), Turkey (Uygun *et al.*, 1996) and Italy (Barbagallo *et al.*, 1998; Caleca & Lo Verde, 1998; Giorgini *et al.*, 1998).

Since in the native areas of the pest the host-specific enemies represent the main biological mortality factor of the phytophagous (Binglin & Mingdu, 1996; Morakote & Nanta, 1996; LianDe *et al.*, 1999), in Italy a classical biological control program has been started in 1995 by introducing exotic parasitoids. According to the multiparasitism strategy and considering previous and successful experiences carried out in Italy against other citrus pests, 3 exotic Hymenoptera parasitoids [*Ageniaspis citricola* Logvinovskaya (Encyrtidae), *Quadrastichus* sp. and *Citrostichus phyllocnistoides* (Narayanan) (Eulophidae)] have been introduced, reared and released.

Moreover, in 1998 the Australasian ectoparasitoid *Semielacher petiolatus* (Girault) was recorded for the first time in Italy. The species has probably naturally spread after its introduction in other countries of the Mediterranean basin (Mineo *et al.*, 1998; Siscaro, Longo *et al.*, 1999).

In 2002, periodical observations have been carried out in 10 citrus orchards in Sicily to verify the activity, the diffusion and the composition of *P. citrella* parasitic complex.

Exotic parasitoids

Ageniaspis citricola Logvinovskaya

It is a poliembryonic koinobiont endoparasitoid of *P. citrella* eggs and young larvae (Edwards & Hoy, 1998).

The species shows several biological features which characterize effective parasitoids, such as host-specificity, high reproductive rate (more than 180 eggs/female, female-biased sex ratio and short cycle), high dispersal and searching rate (Hoy & Nguyen, 1997). Therefore the encyrtid *A. citricola*, native of Taiwan, Thailand and Vietnam, has been used in biological control programs for the citrus leafminer in several citrus regions: Algeria, Argentina, Australia, Bahamas, Brazil, Cyprus, Colombia, Florida, Greece, Honduras, Israel, Louisiana, Morocco, Mexico, Peru, Syria, Spain, Texas, Tunisia, Turkey and Venezuela (Berkani & Mouats, 1998; Schauff *et al.*, 1998; Siscaro *et al.*, 2000). In Italy the encyrtid has been introduced and reared since 1995 (Siscaro *et al.*, 1997; Siscaro & Mazzeo, 1997). Nearly 15,000 adults have been released in Eastern Sicily and Calabria during 1996-2000 (Siscaro *et al.*, 2000). The species was recovered in some coastal lemon orchards where it overwintered in 1998 (Siscaro, Longo *et al.*, 1999) and this strain collected in the field has been reared and released in the following years, although without any permanent establishment (Siscaro, Barbagallo *et al.*, 1999).

The encyrtid is, however, one of the major CLM antagonist in several countries (Argentina, Australia, Bahamas, Brazil, Florida, Honduras, Canary Islands, Louisiana and Venezuela), where it has permanently established (see a summary of original references in Zappalà & Siscaro, 2002). Therefore *A. citricola* appears to be climatically adapted to humid tropical and subtropical climates (Hoy & Nguyen, 1997). Recent laboratory observations have shown that the species, at the pupal stage, has a great resistance to low temperatures (5, 10°C); adults survive longer at 10°C while individuals exposed to temperatures higher than 25°C die after less than 24 hours. High mortality of adults at 25-35°C induces to consider the parasitoid unsuitable to the biological control of *P. citrella* in Mediterranean citrus orchards (Zappalà & Siscaro, 2002).

Quadrastichus sp.

It is an ectoparasitoid of CLM second and third instar larvae. The eulophid biological cycle lasts about 20 days at 20°C and R.H.>80%; at the same temperature the adults survive up to 40 days (Argov & Rössler, 1998; Llacer *et al.*, 1998).

The parasitoid, native of China, Japan, Taiwan and Thailand, has been introduced in Morocco (Smaili *et al.*, 1999), Cyprus, Spain and Israel (Schauff *et al.*, 1998), with no evidence of establishment (Argov, 2000), except in Spain where the species overwintered in the Valencia area (García Marí *et al.*, 2000).

In 1996 *Quadrastichus* sp. has been introduced in Southern Italy and about 3,000 specimens have been released in more than 30 sites. Although preliminary observations indicated that *Quadrastichus* sp. seemed to have a good adaptability to Italian citrus areas (Longo & Siscaro, 1997), it has not overwintered in any release site (Barbagallo *et al.*, 2000).

Citrostichus phyllocnistoides (Narayanan)

The species is reported as larval ectoparasitoid of *P. citrella* (Subba Rao & Ramamani, 1965; Bouček, 1988; Neale *et al.*, 1995); nevertheless it has been recently recovered in India on *Trioza obsoleta* Buckton (Hom. Psyllidae) on *Diospyros melanoxylon* (Roxb.) (Dash & Das, 1997). Further studies (Massa *et al.*, 2001; Massa & Rizzo, 2001; Lo Duca *et al.*, 2002) have shown *C. phyllocnistoides* parasitization also on Lepidoptera Nepticulidae (*Acalyptris minimella* (Rebel) on *Pistacia lentiscus* L, *Stigmella* sp. on *Rubus ulmifolius* Schott and an unidentified nepticulid on *Salix alba* L.).

 C. phyllocnistoides prefers second and third instar CLM larvae both for ovipositing and host feeding, while first instar larvae are selected only for host feeding (Reina & Siscaro, 2002). The female lays one or more eggs (up to 5), but only one will complete its development (Subba Rao & Ramamani, 1965). Its cycle lasts 12-13 days at 22-26°C (Ding *et al.*, 1989). Sex ratio is female-biased, 80% of females is obtained from third instar larvae, while 70% of males from second instar larvae (Ujiye & Adachi, 1995).

 The eulophid is reported on *P. citrella* in Afghanistan, China, India, Indonesia, Japan, Oman, Pakistan, South Africa, Sudan Taiwan, Swaziland and Thailand (Schauff *et al.*, 1998).

 The parasitoid has been introduced in Australia, Cyprus, Greece, Israel (Schauff *et al.*, 1998) and Spain (García Marí *et al.*, 2000) and its permanent establishment has been recorded in all these areas with the exception of Australia (Argov, 2000; García Marí *et al.*, 2000).

 In Italy the parasitoid has been introduced in 1999 (Mineo & Mineo, 1999a) and in the same year about 600 specimens have been released in Western Sicily (Mineo *et al.*, 2001); in 2000-01 more than 3,000 specimens have been released in Eastern Sicily (Conti *et al.*, 2001). The eulophid was recovered in all the release sites (Conti *et al.*, 2001) and also overwintered far from them (Mineo *et al.*, 2001); moreover further observations, carried out in 2002, showed its permanent establishment in Sicily. In July–August 2002 it represented 36% of all parasitoids, while in September-October it reached 90% of them.

Semielacher petiolatus (Girault)

The entomophagous is a solitary ectoparasitic wasp and eggs are laid on *P. citrella* second and third instar larvae (Bouček, 1988), although it is frequently recovered also on prepupae (Mineo & Mineo, 1999b). *S. petiolatus* has been recovered on alternative hosts, such as Diptera Agromizidae *Agromyza hiemalis* Becker on *Urtica* spp., *Chromatomyia horticola* (Goureau) on *Sonchus* spp. and *Liriomyza* sp. on *Merculiaris annua* L. and Lepidoptera *Cosmopterix pulchrimella* Chambers (Cosmopterigidae) on *Parietaria diffusa* M. and K., *Stigmella aurella* (Fabr.) (Nepticulidae) on *Rubus ulmifolius* Schott and *Dialectica scalariella* Zeller (Gracillariidae) on *Echium* sp. (Massa & Rizzo, 2000; Massa *et al.*, 2001).

 The parasitoid pupates in host mine and parasitized CLM larvae will not pupate. The life cycle is completed in 10 days at 25°C. Host feeding was observed in laboratory (Argov & Rössler, 1998).

 S. petiolatus has been recovered on *P. citrella* in Australia (Bouček, 1988) and in Solomon Islands (Schauff *et al.*, 1998). The eulophid has been introduced in Cyprus, Israel, Morocco, Oman, Syria, Tunisia, Turkey (Schauff *et al.*, 1998), Egypt (Hamed *et al.*, 1999), Greece (Michelakis, 1997) and Spain (Garcia Marì *et al.*, 1997).

 In Italy the parasitoid has been detected on *P. citrella* for the first time in 1998 (Mineo *et al.*, 1998), performing interesting parasitism activity (Caleca *et al.*, 1998). Further observations have shown its spontaneous diffusion in all citrus orchards in Southern Italy (Viggiani, 2001). This record together with Algerian (Schauff *et al.*, 1998) and Jordanian ones

(Mineo, 1999), reveals its spontaneous dispersal capability in Mediterranean citrus areas (Siscaro *et al.*, 2000).

In 1999-2000 the eulophid contribution to the total CLM biological control in Italy was around 90% (Mineo & Mineo, 1999b; Conti *et al.*, 2001).

In summer 2001 *S. petiolatus* was still the most efficient *P. citrella* parasitoid, showing an incidence on the total parasitization activity near 80%; nevertheless during fall of the same year, the main biocontrol activity was also due to *C. phyllocnistoides*.

In 2002 *S. petiolatus* has been recovered in all Sicilian citrus areas with a parasitization activity mainly focused in early summer, as observed in the previous year.

Indigenous parasitoids

During 1995-1998 several Hymenoptera parasitoids belonging to the family Eulophidae have been obtained from samples collected in Sicilian citrus groves infested by *P. citrella*. The main species were *Cirrospilus pictus* (Nees) and *Pnigalio agraules* (Walker) and, as observed in other Mediterranean citrus areas (Garrido Vivas, 1995; Argov *et al.*, 1995), the incidence of the first one on the parasitization has reached 80-90% (Caleca & Lo Verde, 1998; Caleca *et al.*, 1998; Conti *et al.*, 2001). Moreover the following species have been occasionally detected in Sicily: *Apotetrastichus postmarginalis* (Bouček), *A. sericothorax* (Szelényi), *Asecodes delucchii* (Bouček), *A. erxias* (Walker), *Neochrysocharis formosa* (Westwood) and *Ratzeburgiola incompleta* Bouček. Finally, other eulophids have been rarely recovered: *Aprostocetus* spp., *Baryscapus* sp., *Chrysocharis pentheus* (Walker), *Cirrospilus diallus* Walker, *C.* nr. *lyncus* (Nees), *C. vittatus* Walker, *Diglyphus isaea* (Walker) and *Pnigalio soemius* (Walker) (Viggiani & Giorgini, 1995; Benfatto, 1996; Liotta *et al.*, 1996; Caleca *et al.*, 1996; Caleca & Lo Verde, 1998; Caleca *et al.*, 1998; Giorgini *et al.*, 1998; Lo Pinto & Salerno, 1998; Mineo, 1999; Conti *et al.*, 2001).

In 1999-2002 the presence of the exotic eulophids *C. phyllocnistoides* and *S. petiolatus* has progressively substituted almost all the indigenous parasitoids previously detected on *P. citrella*, inducing a decrease of their parasitism to less than one third of what recorded up to 1998.

Conclusive remarks

The higher degree of specifity reached in the last four years by *P. citrella* parasitic complex represents an important element in biological control of the leafminer. The elaboration of the data collected in the main Sicilian citrus areas from 1996 up to now revelead an activity of the parasitoids (parasitization + host feeding) that has grown from 32% (1996-98) up to 58% (1999-2002), mainly thanks to the biocontrol activity of the 2 exotic eulophids *S. petiolatus* and *C. phyllocnistoides* which are permanently established in Sicilian citrus groves showing a contribution of 90% to the total parasitization.

Data collected in 2001-02 have shown that there is a seasonal alternation in the activity of these 2 parasitoids; *S. petiolatus* parasitization is in fact mainly concentrated in the first months of CLM infestation (June – August), while *C. phyllocnistoides* activity is higher in the second part of the season (September – October).

Moreover, the establishment of these eulophids seems to be related to the presence of alternative hosts (Massa *et al.*, 2001; Massa & Rizzo, 2001; Lo Duca *et al.*, 2002), and their seasonal alternation could be partly explained by the different biological and ecological attitudes the two species showed on hosts of native flora (Rizzo, in press). Therefore it is important to maintain a rich biodiversity in citrus groves in order to provide alternative food

and shelter to CLM parasitoids, mainly in winter and spring, when CLM populations are at their minimum levels.

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34

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Lessons from the biological control of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae): the importance of native natural enemies and their conservation

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Abstract: Biological control programs developed against *Phyllocnistis citrella* in the Mediterranean Region have almost exclusively focused on importation of exotic parasitoids. Surveys made in Spain from 1997 through 1999 have demonstrated the important contribution of native general predators (lacewings, spiders, ants, minute pirate bugs, etc.) to mortality experienced by this pest in the field (predation rates being usually as high as twice parasitism rates). This case is used to illustrate the importance of this group of entomophagous arthropods in citrus. Examples on the impact of lacewings and ants are given. The conservation of these native natural enemies should be taken into account more seriously in any citrus IPM strategy.

Tri-trophic interactions involving Eulophid parasitoids (Hymenoptera, Eulophidae) of the citrus leafminer *Phyllocnistis citrella* Stainton[1]

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Abstract: The leafminer community living on about 50 spontaneous plants (herbs, shrubs and trees) has been studied in Sicily (Italy) from 1997 to 2002 to find possible relationships between its parasitoid complex and the one of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillariidae). 49 leafminers (22 Diptera, 26 Lepidoptera and 1 Hymenoptera) were obtained. Most of them can be considered "indifferent" to cultivated plants, being related with a single botanical genus or species. Leafminers are widely present all over the year supplying alternative food and refuge to the natural enemies of pests of cultivated plants. 150 host-parasitoids associations were detected; many of them were unrecorded and 89 (59.3% of the total) regarded 15 native Eulophid parasitoids already known as antagonists of *P. citrella* in Italy or in other Mediterranean countries. 10 of them (66.6%) are among the most polyphagous and frequent species on alternative hosts of spontaneous flora. Two exotic species, *Semielacher petiolatus* (Girault) and *Citrostichus phyllocnistoides* (Narayanan), considered among the most effective in the control of *P. citrella*, were obtained from 9 hosts living on native plants. The comparison of the values of parasitisation found on *P. citrella* and on hosts of spontaneous plants along the year, showed that the latter increases from winter to spring and decreases onwards, while that on *P. citrella* shows its minimum in May and its maximum between August and September. Between the respective values resulted a statistically significant negative correlation (r = -0.60; P = 0.04). Results suggest that spontaneous plants, with their variety of alternative hosts, help in maintaining populations of both native and exotic parasitoid, mainly in the seasons of low availability of *P. citrella* larvae. However, the recovery of the two cited exotic species, previously considered to be specific antagonists of the citrus leafminer, draws attention on the need of improving biological and ecological knowledge of parasitoid species, to better use them in biological control programs.

Key words: spontaneous plants, alternative hosts, native *vs.* exotic parasitoids, enhancing biological control.

Introduction

The accidental introduction of an exotic pest causes, besides other consequences, an adaptative answer by native predators and parasitoids living in the environment colonised by the pest. Indeed, among these organisms we can find the first natural enemies of a new noxious phytophagous. In these cases predator and/or parasitoid species belonging to the predator/parasitoid complexes of phytophagous of native plants move to the exotic pest, producing new prey-predator or host-parasitoid associations and contributing to control the pest (Altieri, 1991; LaSalle, 1993). A great number of researches have been made on this matter confirming that native plant patches are the most important source of useful species in agroecosystems (McMurtry & Johnson, 1965; Powell, 1986; Cerutti *et al.*, 1989; Lo Verde *et*

39

al., 1997; Tsolakis *et al.,* 1997; Barbosa & Benrey, 1998; Barbosa & Wratten, 1998; Bribosia *et al.,* 1998).

The arrival of the citrus leafminer *Phyllocnisits citrella* Stainton (Lepidoptera, Gracillariidae) in Italy in 1994 (Benfatto, 1995) was the occasion to study the relationships that can be established among phytophagous of native plants and those of cultivated ones through their common polyphagous parasitoids, migrating from one host to the other.

This research was carried out on spontaneous flora related to citrus groves and in fragments of natural habitats at the edges of citrus groves in Sicily (Italy) from 1997 to 2002 and its results were partly published (Caleca *et al.* 1997; Caleca 1998; Caleca & Lo Verde 1998; Caleca *et al.* 1998; Mineo *et al.* 1997a, b; Mineo & Sinacori 1998; Rizzo & Mineo 1997; Rizzo *et al.* 1999; Massa & Rizzo, 2000a,b; Massa *et al.,* 2001; Lo Duca *et al.,* in press; Rizzo & Massa, in press a,b). Tri-trophic interactions "native plants > leafminers > parasitoids" were investigated to find out possible host-parasitoid associations involving Eulophid species already known as parasitoid of *P. citrella.* In fact, in the Mediterranean countries more than fifteen parasitoid species belonging to the family Eulophidae (Hymenoptera: Chalcidoidea) were found to be antagonists of this pest (cf. Siscaro *et al.,* in press, and references therein).

Moreover, the relationships between two exotic parasitoid species, *Semielacher petiolatus* (Girault) and *Citrostichus phyllocnistoides* (Narayanan), and the native vegetation has been studied. *S. petiolatus* spontaneously spread in Sicily in 1998 (Mineo *et al.,* 1998), while *C. phyllocnistoides* was actively introduced for *P. citrella* biological control (in western Sicily: Mineo & Mineo, 1999, and in eastern Sicily: Siscaro *et al.,* 2000).

Materials and methods

Samples of about 50 native plants (herbs, shrubs and trees) have been collected from 1997 to 2002 in Sicily (Italy) in the surroundings of citrus cultivated areas. Leaves infested by leafminers were placed in Petri dishes with wet paper at 26° C, 70 % r.h., and L14:D10. All phytophagous species and relative parasitoids that emerged were mounted and identified (for more details cf. Massa & Rizzo 2000b; Massa *et al.* 2001). Moreover, from July to September 2002, additional samples were periodically collected in four citrus groves in the provinces of Palermo and Agrigento (Sicily), where in the same time a survey on the relative abundance of the different parasitoid species of *P. citrella* has been carried out (cf. Siscaro *et al.,* in press).

Results

The leafminer community
The leafminer community comprised 49 species (22 Diptera, 26 Lepidoptera and 1 Hymenoptera) (Tab. 1, from Rizzo & Massa, in press *b*); most of them can be considered "indifferent" to cultivated plants, being related to a single botanical genus or species. All Lepidoptera and the only Hymenoptera resulted to be monophagous or oligophagous species, while Diptera resulted more polyphagous, only few species being strictly monophagous. Just 18.4% of all the obtained species (1 Lepidoptera and 8 Diptera) can also damage cultivated plants, even if the so considered highly polyphagous Diptera species, such as *Liriomyza congesta* (Becker), *L. huidobrensis* (Blanchard), and *L. trifolii* (Burgess), have a smaller host plant range and are less frequent on spontaneous flora than they are on cultivated plants.

The phenology of each leafminer species along the year is described in Tab. 1, where leafminers are divided in three groups: living on herbaceous plants, on shrubs and on trees. The leafminer community is widely present all over the year, even if three kind of trends are

recognizable: 1) species present all over the year with a more or less wide pause in summer; 2) species mainly present in winter; 3) species mainly present in summer.

Table 1. Phenology of the leafminers along the year. Species are divided in three groups living on herbaceous plants, shrubs and trees respectively. Botanical genus are indicated only for oligophagous species

Leafminers – host plants	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Pegomya hyoscyami (Panzer) - Herbaceous plants												
Cosmopterix pulcherimella Chambers -*Parietaria*												
Chromatomyia horticola (Goureau) -Herbaceous plants												
Euleia heraclei (L.) - Umbelliferae												
Liriomyza sp. – *Mercurialis annua*												
Agromyza hiemalis Becker - *Urtica*												
Phytomyza conyzae Hendel - Inuleae												
Ophiomyia pulicaria (Meigen) - Compositae												
Scaptomyza flava (Fallén) - Leguminosae												
Chrysoesthia drurella (Fabricius) - Chenopodiaceae												
Scaptomyza pallida (Zetterstedt) -Herbaceous plants												
Liriomyza strigata (Meigen) - Herbaceous plants												
Ophiomyia cunctata (Hendel) - Herbaceous plants												
Liriomyza huidobrensis (Blanchard) - Herbaceous plants												
Liriomyza bryoniae (Kaltenbach) - Compositae												
Chrysoesthia sexguttella (Thunberg) – Chenopodiaceae												
Liriomyza congesta (Becker) - Herbaceous plants												
Liriomyza trifolii (Burgess) - Herbaceous plants												
Ophiomyia beckeri (Hendel) - Herbaceous plants												
Chromatomyia aprilina (Goureau) - *Lonicera*												
Stigmella aurella (Fabricius) - *Rubus*												
Acalyptris minimella (Rebel) - *Pistacia lentiscus*												
Stigmella sp. - *Rosa*												
Stigmella rhamnophila (Amsel) - *Rhamnus*												
Emmetia angusticolella (Duponchel) - *Rosa*												
Stigmella oxyacanthella (Stainton) - *Crataegus*												
Emmetia marginea (Haworth) - *Rubus*												
Cupedia cupediella (Herrich-Schäffer) - *Pistacia terebinthus*												
Stigmella roborella (Johansson) - *Quercus*												
Phytomyza ilicis Curtis - *Ilex aquifolium*												
Tischeria dodonaea Stainton - *Quercus*												
Phyllonorycter kusdasi (Deschka) -*Quercus*												
Phyllonorycter parisiella (Wocke) -*Quercus*												
Acrocercops brongniardella (Fabricius) -*Quercus*												
Tischeria ekebladella (Bjerkander) -*Quercus*												
Phytomyza phillyreae Hering - *Phillyrea*												
Phyllonorycter suberifoliella (Zeller) -*Quercus*												
Aulagromyza tridentata (Loew) - *Salix alba*												
Phyllonorycter dubitella (Herrich-Schäffer) - *Salix pedicellata*												
Messa hortulana Klug - *Populus*												
Stigmella trimaculella (Haworth) - *Populus*												
Lepidoptera unidentified - *Salix alba*												
Stigmella sp. - *Salix pedicellata*												
Japanagromyza salicifolii (Collin) - *Salix purpurea*												
Phyllonorycter sublautella (Stainton) -*Quercus*												
Phyllonorycter millierella (Staudinger) - *Celtis*												
Leucoptera malifoliella (Costa) - Rosaceae												
Stigmella plagicolella (Stainton) - *Prunus*												
Aulagromyza fraxini (Beiger) - *Fraxinus*												

Fig. 1 shows the contribute that each kind of vegetation gives to the total amount of species present during the year. Herbaceous plants and trees support most of the species, amounting to 39 and 43%, respectively, of the total number of leafminer species recorded. Moreover, even if all the three kinds of vegetation are more or less present all over the year, most of the species living on shrubs resulted absent from the end of April to the start of July.
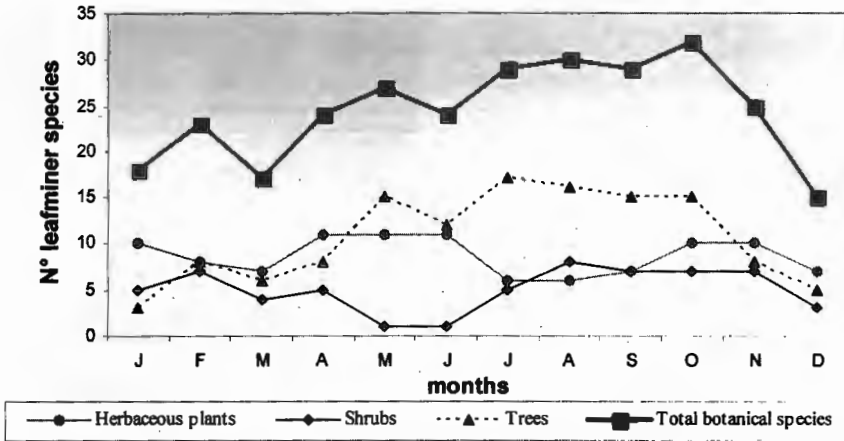
Figure 1. Trend of the number of leafminer species recorded for each kind of vegetation and for the total of botanical species along the year.

Besides, as Fig. 2 shows, the three kind of plants do not sustain similar leafminers communities. In fact, most of Lepidoptera live on shrubs and trees, which, on the whole, host 88.4% of all their species, while Diptera (mainly Agromyzidae) are chiefly present on herbaceous plants, which host 72.7% of them.
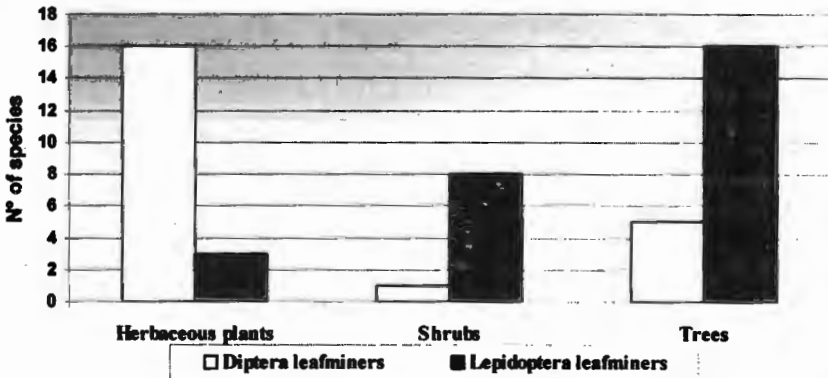
Figure 2. Distribution of Diptera and Lepidoptera leafminers in the three kinds of vegetation.

The different composition of the leafminer communities living on the three kinds of vegetation comes out also observing the trend of the number of species of Diptera and Lepidoptera leafminers along the year (Fig. 3). The formers have their maximum in spring, while the latters in summer, both the peaks corresponding with the maximum development of their respective preferred host plants.
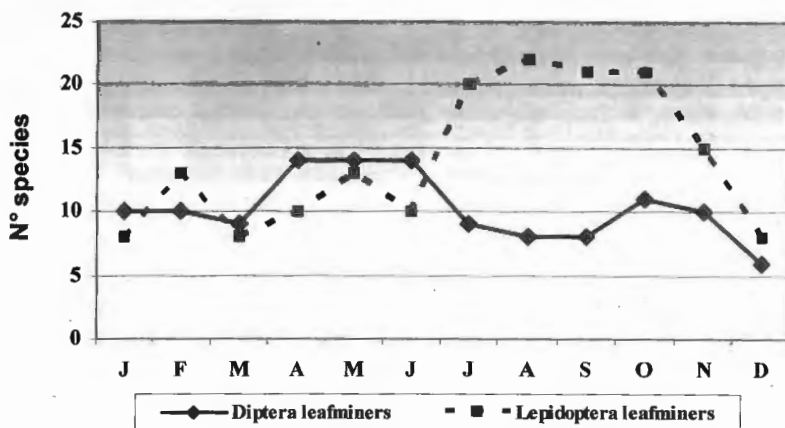
Figure 3. Trend of number of species of Diptera and Lepidoptera leafminers along the year.

The Eulophid parasitoid community

41 Eulophid parasitoids (Hymenoptera, Eulophidae) were obtained from the 49 leafminer species recorded during this research (Tab. 2, modified from Rizzo & Massa, in press *b*). On the whole, 150 host-parasitoids associations were detected; many of them were unrecorded and 89 (59.3% of the total) regarded 15 native Eulophid parasitoids already known as antagonists of *P. citrella* in Italy or in other Mediterranean countries (in bold in Tab. 2; cf. Schauff *et al.*, 1998). Moreover, the two exotic species, *S. petiolatus* and *C. phyllocnistoides*, were obtained, respectively, from 5 and 2 alternative hosts in Sicily, and each one from 1 host more in Jordan (Massa & Rizzo, 2000a; Massa *et al.*, 2001; Lo Duca *et al.*, in press).

The parasitoid phenology described in Tab. 2 shows that three groups of species can be observed: 1) a group of 7 highly polyphagous species (8-14 hosts) widely spread and frequent all over the year; 2) a second group of 8 less polyphagous species (4-7 hosts) with a more restricted presence during the year; 3) a third group, the largest one, of 26 rare or oligophagous species (1-3 hosts), occasionally recorded. The number of parasitized hosts and the number of months in which each parasitoid species was detected resulted significantly correlated (r = 0,83; P<0,001; DF: 39), indicating that the more polyphagous are the species the more they are present all over the year, developing many generations on different hosts.

It is interesting to note that 66.6% of the native parasitoids attacking *P. citrella* (10 species) are included among the first two groups of species illustrated in Tab. 1, which show the most polyphagous behaviour. The remaining 5 native species parasitizing the citrus leafminer are comprised among oligophagous species and are parasitoids which prefer Lepidoptera leafminers.

Table 2. Phenology of Eulophid parasitoid detected on leafminers living on spontaneous flora in Sicily from 1997 to 2002 along the year. (In bold: native species already known as antagonists of *Phyllocnistis citrella*. In grey: exotic species till now present in the island).

Parasitoid species - N° of hosts	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Neochrysocharis formosa (Westwood) – 14												
Pnigalio soemius (Walker) – 8												
Chrysocharis pentheus (Walker) – 10												
Chrysocharis nephereus (Walker) – 12												
Chrysocharis gemma (Walker) – 10												
Pnigalio agraules (Walker) – 9												
Cirrospilus pictus (Nees) – 8												
Semielacher petiolatus (Girault) – 5												
Diglyphus minoeus (Walker) – 4												
Ratzeburgiola incompleta Boucek – 7												
Diglyphus isaea (Walker) – 5												
Cirrospilus variegatus (Masi) – 4												
Cirrospilus viticola (Rondani) – 4												
Ratzeburgiola cristata (Ratzeburg) – 4												
Pediobius saulius (Walker) – 4												
Chrysocharis pubicornis (Zetterstedt) – 2												
Pediobius metallicus (Nees) – 2												
Chrysocharis entedonoides (Walker) – 2												
Elachertus inunctus (Nees) – 1												
Necremnus artynes (Walker) – 1												
Apotetrastichus sericothorax (Szelényi) – 3												
Hemiptarsenus ornatus (Nees) – 3												
Chrysocharis prodice (Walker) – 3												
Cirrospilus diallus Walker – 2												
Diglyphus crassinervis Erdös – 2												
Diglyphus poppoea Walker – 2												
Citrostichus phyllocnistoides (Narayanan) – 2												
Chrysocharis orbicularis (Nees) – 2												
Sympiesis prob. *lucida* Storozheva – 2												
Derostenus prob. *gemmeus* Westwood – 2												
Pnigalio pectinicornis (L.) – 1												
Apotetrastichus postmarginalis (Boucek) – 1												
Achrysocharoides zwoelferi (Delucchi) – 1												
Sympiesis notata (Zetterstedt) – 1												
Baryscapus fossarum Graham – 1												
Hyssopus olivaceus (Thomson) – 1												
Minotetrastichus prob. *platanellus* (Mercet) – 1												
Cirrospilus lyncus Walker – 1												
Cirrospilus pulcher Masi – 1												
Sympiesis sericeicornis (Nees) – 1												
Achrysocharoides butus (Walker) – 1												

Referring to the two exotic species, *S. petiolatus* and *C. phyllocnistoides,* their ecological and biological attitudes seem rather different. In fact, the first appears to be more polyphagous and seasonally widespread, while the second more oligophagous and less frequent till now on alternative hosts living on spontaneous flora. In addition, *S. petiolatus* has been found on leafminers on herbaceous plants (3 Diptera and 3 Lepidoptera), while *C. phyllocnistoides* on species living on shrubs and trees (3 Lepidoptera Nepticulidae) (Tab. 3). Moreover, data collected until now show that phenology of *S. petiolatus* matches quite well with the one of its alternative hosts, while that of *C. phyllocnistoides* appears to be much more restricted (Tab. 3).

Table 3. Phenology on leafminers of spontaneous flora of the two exotic species attacking *P. citrella* in Sicily, compared with that of their respective alternative hosts.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Semielacher petiolatus – 6 hosts												
Agromyza hiemalis Becker – *Urtica* spp.	■	■	■				■		■	■	■	
Chromatomyia horticola (Goureau) - Herbaceous plants	■	■	■	■	■	■		■				
Liriomyza sp. – *Mercurialis annua*					■							
Cosmopterix pulcherimella Chambers –*Parietaria* spp.	■	■	■	■	■							
Stigmella aurella (Fabricius) – *Rubus ulmifolius*	■	■	■		■							
Dialectica scalariella Zeller – *Echium* sp.					■							
Citrostichus phyllocnistoides – 3 hosts												
Acalyptris minimella (Rebel) - *Pistacia lentiscus*	■	■	■		■							
Unidentified Nepticulidae – *Salix alba*					■							
Stigmella sp. – *Rubus ulmifolius*					■							

The total number of parasitoid species recorded along the year (Fig. 4) compared with that of leafminers shows a decrease at the beginning of autumn, even if in the same period the maximum number of leafminer species is present. However, quantities of parasitoid individuals do not decrease. In this period the parasitoid community could mainly consist of few polyphagous species very widespread. Samples collected in summer 2002 seem to confirm this hypothesis (Tab. 4).

In fact, no individual of the two exotic species has been reared from alternative hosts of spontaneous plants during this period, while the parasitoid community, despite the quantity and variety of leafminers species, appeared rather poor in respect to other seasons and mainly consisting of few very polyphagous species (Tab. 4).
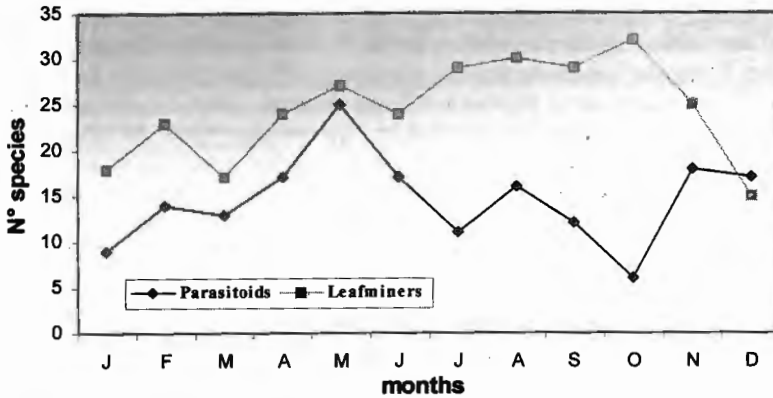
Figure 4. Trend of the number of species of leafminers and parasitoids along rhe year.

Table 4. Summarized results of the samples collected on summer 2002 in four citrus groves.

Botanical species	Leafminers	Parasitoids	
12 herbaceous plants	12 Diptera	*Diglyphus* spp. (22.3%)	Others (28,2%)
2 shrubs	13 Lepidoptera	*Neochrysocharis formosa* (West.) (23.5%)	
4 trees	1 Coleoptera	*Chrysocharis* spp. (23.5%)	

Finally, the comparison of the values of parasitisation on *P. citrella* and on hosts of spontaneous plants along the year, showed that the latter increases from winter to spring and decreases onwards, while that on *P. citrella* shows its minimum in May and its maximum between August and September (Fig. 5). Between the respective values resulted a statistically significant negative correlation (r = - 0.60; P = 0.04).
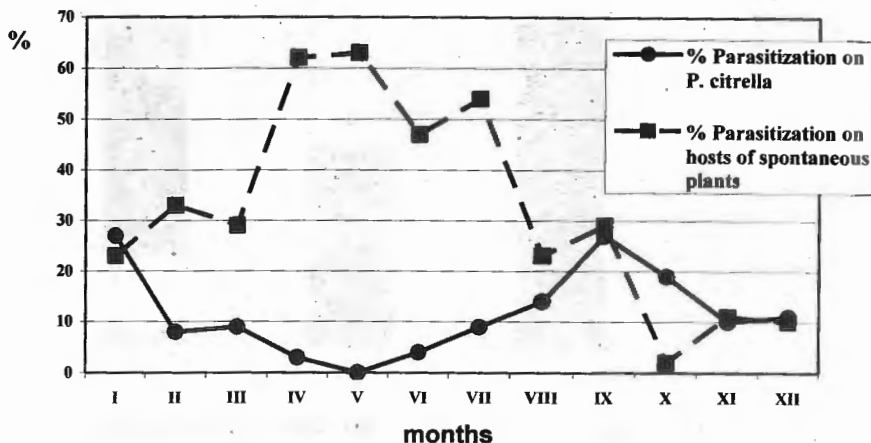
Figure 5. Parasitisation trend on leafminers of spontaneous plants and on *P. citrella* along the year (modified from Massa & Rizzo, 2000b; data of parasitisation on *P. citrella* from Caleca *et al.*,1998).

Discussion

Plant species diversity and plant structural diversity are the main factors in determining parasitoid diversity in agroecosystems (Altieri *et al.*, 1993). The research confirmed that this diversity ensures a potential source of natural enemies as. In fact, the first environmental answer to the arrival of an exotic pest, like *Phyllocnistis citrella*, is provided by the antagonists already present in the agroecosystem. This study showed that native parasitoids till now detected on *P. citrella* come from the parasitoid complex of the leafminers living on spontaneous vegetation and belong to a group of rather polyphagous and very widespread species (Rizzo & Massa, in press *a,b*).

Moreover, the abundance of accessible hosts has been also exploited by exotic polyphagous parasitoids, as *S. petiolatus* and *C. phyllocnistoides*, recorded on 6 and 3 alternative hosts, respectively (Massa & Rizzo, 2000b; Massa *et al.*, 2001; Lo Duca *et al.*, in press). This fact has probably contributed to their establishment and to support them in maintaining their populations in the periods of scarce availability of *P. citrella* larvae.

In western Sicily, like in other Mediterranean citrus cultivated areas, the role of native parasitoids in controlling *P. citrella* was never very effective, not reaching much more then 10% from 1995 to 1997 (Caleca & Lo Verde, 1998; Caleca *et al.*, 1998). The spontaneous spreading of *S. petiolatus* in 1998 (Mineo *et al.*, 1998) and the successive introduction of *C. phyllocnistoides* (Mineo & Mineo, 1999) led to a progressive reduction of native parasitoids relative abundance in the parasitoid complex of *P. citrella* (Caleca & Lo Verde, 1998; Mineo & Mineo, 1999; Conti *et al.*, 2001, Siscaro *et al.*, in press) (Fig. 6). Conversely, total parasitisation on the citrus leafminer gradually grew up due to the two exotic species activity (Siscaro *et al.*, in press).
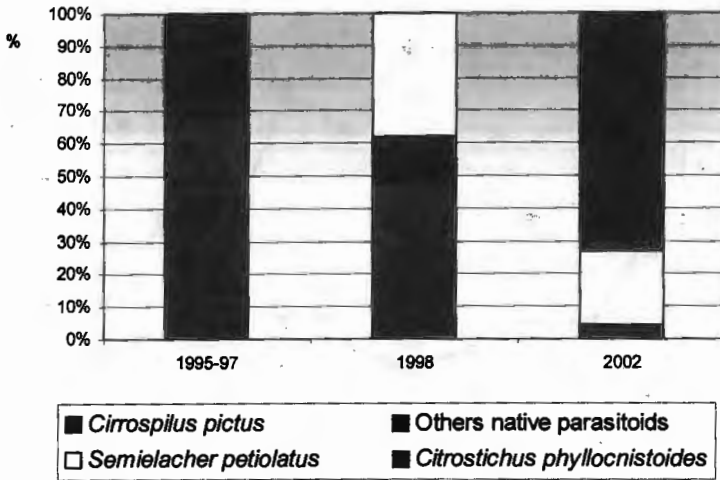
Figure 12. Relative abundance of native and exotic parasitoids of *P. citrella* in citrus groves of western Sicily: a) before the arrival of both the exotic species (1995-97), b) soon after the natural spreading of *S. petiolatus* (1998), and c) three years after the introduction of *C. phyllocnistoides*.

Moreover, Siscaro *et al.* (in press) noted a seasonal alternation in the activity of the two exotic species in Sicily, *S. petiolatus* dominating the first phases of *P. citrella* infestation, while *C. phyllocnistoides* strongly prevailing from mid summer onwards. This alternation could be partly due to the different distribution of the two species till now observed on alternative hosts of spontaneous plants (cf. Tab. 3). In fact, populations of *S. petiolatus* might be quicker and more immediately available to parasitize *P. citrella* thanks to their diffusion on different hosts along the year, while the slow but prevailing increasing of *C. phyllocnistoides* seems the behaviour of a less generalist species, even if more data are needed to confirm this hypothesis.

Finally, referring to the impact of *S. petiolatus* and *C. phyllocnistoides*, their low number of individuals reared from alternative hosts of native plants seems to suggest that the presence of the two exotic species did not affect until now either the structure or the composition of the parasitoid complex of the leafminer community of spontaneous vegetation (Massa *et al.*, 2001; this study). Concluding, it can be pointed out that:

- Tri-trophic interactions (plants > hosts > parasitoids) recorded during this study show that spontaneous plants represent a reservoir of potentially useful species;
- Native vegetation can sustain populations of both native and exotic parasitoids providing them alternative hosts, especially in periods of scarce availability of *P. citrella* larvae;
- More attention should be given to the knowledge of biology and ecology of parasitoid species, both to better use them in biological control programmes and to enhance natural biocontrol.
- Fragments of native vegetation represent the main source of biodiversity in agroecosystems. Agronomical techniques increasing this resource should be preferred.

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Development of a sequential sampling program for *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) in Sicilian citrus nurseries

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Abstract. In Italy the citrus leaf miner (CLM) *Phyllocnistis citrella* Stainton is considered to be an economic problem only on ornamental citrus, young plants in nurseries and re-grafted plants in the field. The aim of the research was to develop a sequential sampling program. In order to define the CLM population density and distribution, samplings on young plants were carried out in Sicilian nurseries from 1998 to 2001, from June to October. The theoretical distribution model better describing the real CLM distribution is Poisson. This result allowed to develop a suitable sequential sampling program for monitoring the CLM populations. The divergence between data obtained from sequential and standard samplings was less than 5%, a level usually considered acceptable. The sequential sampling program may be easily used by inexperienced operators, allowing to treat only when strictly needed. The reduction of treatments to control *P. citrella* in citrus nurseries may result relevant, about 40% in our tests, contributing also to a reduction of insurgence of other pests.

Key words: CLM distribution, economic thresholds, treatments reduction

Introduction

In Italy the citrus leaf miner (CLM) *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) is considered to be an economic problem only on re-grafted plants in the field (Caleca *et al.*, 1996, 1998, 2000; Ortu & Acciaro, 1998), on ornamental citrus (Del Bene & Landi, 1999), and on young plants in nurseries only for aesthetic damage (Caleca, 2000).

In Sicilian citrus nurseries, because of the low economic injury thresholds and the difficulties related to periodic samplings involving laboratory analyses, CLM is usually controlled by weekly treatments with various insecticides, from the end of May to the beginning of November. On older ornamental plants, trunk and root treatments are also used. These control programs imply about 24 foliar sprays, or 2-4 trunk or root treatments per year, thus causing, as we observed, an increase of *Tetranychus urticae* Koch infestations.

Due to the decrease of CLM infestation levels recorded after 1999, some of the cited chemical treatments are unnecessary.

In order to reduce treatments in nurseries, this research was carried out to define the CLM population density and distribution, and to develop a sequential sampling program that could be easily used also by inexperienced operators.

Materials and methods

Standard sampling
In order to define the CLM population density and distribution, weekly samplings on young untreated plants of sour orange (*Citrus aurantium* L.) were carried out from June to November in two Sicilian nurseries of Terme Vigliatore (Messina province) from 1998 to 2001. Each sample, regarding only tender leaves, consisted of 20 leaves 1-3 cm long plus other 20-40 leaves 3-5 cm long; they were randomly collected from about 20 shoots. The number of 1st, 2nd, 3rd, 4th instar larvae, pupae of CLM and preimaginal stages of parasitoids per leaf was recorded. Infestation level was calculated as the mean number of CLM larvae and pupae per leaf of the two different lengths.

Data analysis
To investigate about the theoretical distribution of CLM data collected during standard sampling from 1998 to 2000 were used. Since data regarding only one leaf size in each sample were not enough to test whether they fit with any theoretical distribution, data regarding leaves of both sizes were aggregated, after checking that there were no significant differences between their means.

The aggregated data were confronted with the following theoretical distributions: normal (N), Poisson and negative binomial (n. b.). When all data have not a normal distribution and almost all data better fit to Poisson distribution, the mean is the only measurable parameter required describing it. Applied formulae to construct the sequential chart under the condition of Poisson distribution, as well as for the estimation of the average sample number (ASN) curve, were derived by Boivin & Vincent (1983) and they are relative to Wald's procedure (1947).

Sequential sampling
Only leaves of 3-5 cm were chosen for the sequential sampling because observations on leaves of this size are simpler, as they bear the higher number of larvae longer than 2 mm (most of 3rd instar and 4th instar), visible with the naked eye; furthermore, data from this leaf size resulted strictly correlated to the infestation level calculated from four different leaf sizes (Caleca *et al.* 1996). Therefore, during summer 2000 and 2001 the sequential sampling program was tested analysing the leaves in the field with the naked eye, both on young citrus plants cultivated to be sold from July to the next spring flush, and in the rootstock plants cultivated to be grafted.

According to data collected in a previous work (Caleca, 2000), the adopted tolerance and intervention thresholds were: A) 0.05 and 0.15 larvae longer than 2 mm and/or pupae per leaf, for the plants to be sold in few months; B) 1.5 and 2.5 larvae longer than 2 mm and/or pupae per leaf, for the rootstocks.

The leaves observed in the field were also analysed in the laboratory, to confirm field data and to record other larvae on them. Data collected during the sequential sampling were compared with those of the standard sampling.

During 2000 the reliability of the sequential sampling was tested; in this year treatments were not linked to sequential sampling results.

On the contrary, during 2001 treatments followed the sequential sampling results; these samplings were repeated 7 days after each treatment or 3 days after a sampling not exceeding the intervention threshold.

Results and discussion

Since no significant differences emerged by comparing the mean values of the two considered leaf sizes, the relative data were pooled. The theoretical distribution that better describe the real CLM distribution is Poisson (Tab. 1).

Population density in all samples ranged around values that included the tolerance and intervention thresholds proposed for both kinds of plant (those to be sold in few months and plants to be grafted). The sequential sampling plans were based on the above mentioned tolerance and intervention thresholds:(A) 0.05 and 0.15 larvae longer than 2 mm and/or pupae per leaf, for the plants to be sold in few months (Fig.1); (B) 1.5 and 2.5 larvae longer than 2 mm and/or pupae per leaf, for the rootstocks. The formulae for the parallel lines of the fore mentioned tolerance and intervention thresholds are respectively: A) $y'= -2 + 0.09x$; $y= 2 + 0.09x$; B) $y'= -4.3 + 1.95x$; $y= 4.3 + 1.95x$.

The maximum ASN named (A) corresponding to population means of 0 and 0.09 ($b=$ slope of the stop lines) resulted 30 and 80 respectively. Instead in the B case the maximum ASN corresponding to population means of 0 and 1.95 ($b=$ slope of the stop lines) resulted 3 and 17 respectively.
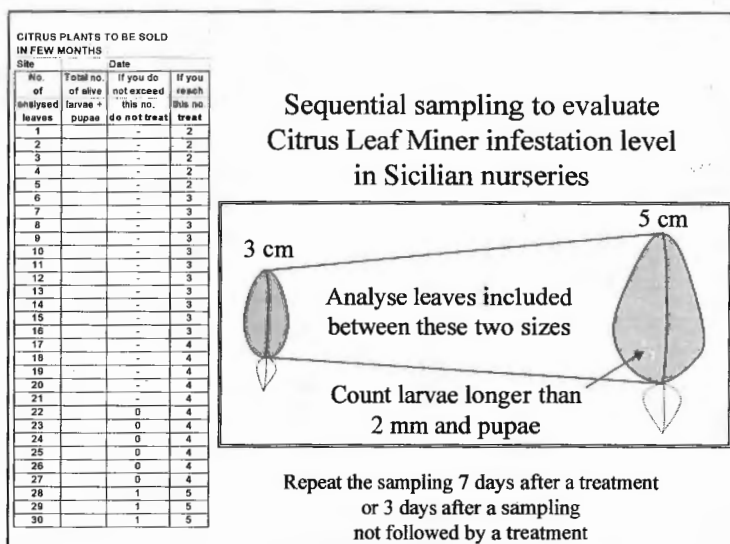

Figure 1. Sampling form for evaluating CLM infestation levels on citrus plants to be sold in few months.

As expected, the results of all 54 tests on sequential sampling on the rootstocks indicated not to treat; these data confirmed results coming from the analysis of 40 leaves in the laboratory; the average number of leaves necessary to obtain a decision in the sequential samplings was 3.3 (3÷7).

The sequential sampling on plants to be sold from July to the next spring flush, suggested 60 times to treat and 18 not to treat; only in three cases these data did not confirm data coming from the analysis of 40 leaves in the laboratory. These errors derived twice from recording

Table1 – Test of goodness of fitness to Poisson and negative binomial distributions of samplings. Data with mean = 0 were excluded from this table.
*= significant differences; n. s.= no significant differences

Sampling Date	Nursery	Mean	s. d.	χ^2 (p-value) Poisson	K (p-value) Negative binomial (n. b.)
12 Jun. '98	Salicà	1.41	1.14	2.44 (>0.05) n. s.	No n. b.
26 Jun. '98	"	3.33	1.80	8.24 (>0.05) n. s.	No n. b.
10 Jul. '98	"	2.15	0.96	7.49 (>0.05) n. s.	No n. b.
27 Jul. '98	"	1.97	1.09	5.53 (>0.05) n. s.	No n. b.
13 Aug. '98	"	1.49	1.02	3.67 (>0.05) n. s.	No n. b.
11 Sep. '98	"	2.23	1.22	6.12 (<0.05)*	No n. b.
24 Sep. '98	"	0.49	0.6	1.36 (>0.05) n. s.	No n. b.
16 Oct. '98	"	1.54	1.50	3.33 (>0.05) n. s.	9.8
18 Nov. '98	"	0.41	0.68	1.36 (>0.05) n. s.	No n. b.
13 Jul. '99	"	1.28	1.11	9.69 (<0.05)*	No n. b.
21 Jul. '99	"	0.85	0.92	1.58 (>0.05) n. s.	No n. b.
27 Jul. '99	"	1.43	1.08	1.7 (>0.05) n. s.	No n. b.
3 Aug. '99	"	1.2	0.88	3.18 (>0.05) n. s.	No n. b.
10 Aug. '99	"	1.03	1.17	1.23 (>0.05) n. s.	No n. b.
24 Aug. '99	"	1.11	1.26	5.4 (>0.05) n. s.	No n. b.
7 Sep. '99	"	1.28	9.99	8.95 (<0.05)*	No n. b.
21 Sep. '99	"	1.58	0.93	2.73 (>0.05) n. s.	No n. b.
7 Oct. '99	"	1.3	0.82	6.88 (<0.05)*	No n. b.
19 Oct. '99	"	1.13	0.94	7.17 (<0.05)*	No n. b.
3 Nov. '99	"	1.85	1.17	2.77 (>0.05) n. s.	No n. b.
2 Jul. '99	Cannotta	1.3	1.42	0.91 (>0.05) n. s.	No n. b.
7 Jul. '99	"	1.53	1.15	4.88 (>0.05) n. s.	No n. b.
13 Jul. '99	"	2.5	1.40	5.46 (>0.05) n. s.	No n. b.
21 Jul. '99	"	1.73	1.47	2.56 (>0.05) n. s.	6.6
27 Jul. '99	"	2.6	1.97	1.86 (>0.05) n. s.	No n. b.
3 Aug. '99	"	1.6	1.04	4.98 (>0.05) n. s.	No n. b.
10 Aug. '99	"	1.6	1.24	2.36 (>0.05) n. s.	No n. b.
24 Aug. '99	"	1.4	0.81	8.47 (<0.05)*	No n. b.
7 Sep. '99	"	1.1	0.67	11.4 (<0.05)*	No n. b.
21 Sep. '99	"	2.3	1.17	4.43 (>0.05) n. s.	No n. b.
7 Oct. '99	"	2.1	0.99	10.6 (<0.05)*	No n. b.
19 Oct. '99	"	1.15	0.96	3.56 (>0.05) n. s.	No n. b.
3 Nov. '99	"	1	0.91	3.49 (>0.05) n. s.	No n. b.
20 Sep. '00	"	0.6	0.99	2.61 (>0.05) n. s.	2.5
11 Oct. '00	"	1.3	1.03	2.81 (>0.05) n. s.	No n. b.
17 Oct. '00	"	1.22	1.15	5.97 (>0.05) n. s.	No n. b.
24 Oct. '00	"	1.45	1.10	9.41 (<0.05)*	10.34
2 Nov. '00	"	1.97	1.47	3.75 (>0.05) n. s.	No n. b.
15 Nov. '00	"	1.15	1.46	10.48 (<0.05)*	1.36

died larvae and once from not recording small larvae. So the total error in this kind of plants was 5%; the average number of leaves necessary to obtain a decision in the sequential samplings was 4.1 (1÷16) when the sampling results indicated to treat, and 23 (22÷28) when the sampling results indicated not to treat.

In the plants managed by the sequential sampling program 4 chemical treatments were necessary instead of 7 weekly treatments done on the plants managed according to the common practice of Sicilian nurseries.

Conclusions

Several studies have revealed an aggregated spatial pattern of populations of many insect pests. On the contrary the theoretical model that better describe the real distribution of CLM on tender leaves 1-5 cm long, is Poisson. It is used to describe a population that is randomly distributed in space. In our case this kind of distribution is probably due to the choice of the only susceptible leaves in the sampling.

The efficiency of the sequential sampling plan results fairly high and compared to the standard one it results with less than 5% to make an error, level usually considered acceptable.

The sequential sampling program may be easily utilised by inexperienced operators, allowing to treat only when strictly needed. The reduction of treatments to control *P. citrella* in citrus nurseries may result relevant, about 40% in our tests, contributing to a reduction of insurgence of other pests.

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58

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MEDFLY
(*Ceratitis capitata*)

Genetic structure of *Ceratitis capitata* species: within and between populations variability

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Abstract: In pest populations, the distribution of genetic variability can reveal not only their history but also the direction and patterns of their evolution. An understanding of the within- and between-population genetic variability is crucial in defining appropriate strategies for eradication or control. Seven Spanish populations of *Ceratitis capitata* (Wiedemann) were studied by gel electrophoresis and RAPD-PCR to determine the degree and pattern of their genetic variability. The results show a relatively low level of variation at enzymatic loci and a high degree of variability with respect to RAPDs. The results are discussed in relation with the polyphagy of this species and the host origin of the flies. The validity of both molecular methods for population studies was evaluated.

Key words: genetic variability, MLEE, RAPD-PCR, *Ceratitis capitata*

Introduction

Over the last 150 years, the tephritid Mediterranean fruit fly, or medfly, *Ceratitis capitata* (Wiedemann), has expanded from its putative source area in Central Africa, probably Kenya (Hagen *et al.*, 1981; Gasperi *et al.*, 1991; Malacrida *et al.*, 1992, 1998) to all regions with temperate or tropical climate (the Mediterranean region, South Africa, Central and South America, and Australia, Fletcher, 1989), and from its original host, *Argaria spinosa* (L.), to more than 250 species and varieties of agriculturally important plants (Fimiani, 1989).

The cost of preventative and eradication resources, as well as fruit losses and imposed quarantine, probably make *C. capitata* the most important fruit pest in the Mediterranean basin. It is particularly important in Spain, especially in the *Comunidad Valenciana*, were there is a large fruit growing industry.

To control this pest, the genetic structure of its populations must be known, and genetic markers found that correctly identify and determine the origin of populations invading new areas. Until recently, the molecular genetic studies performed in this respect have been inconclusive (Milani *et al.*, 1989; Gasperi *et al.*, 1991; Haymer *et al.* 1992, 1997; Malacrida *et al.*, 1992, 1998; Sheppard *et al.* 1992; Haymer & McInnis 1994; McPheron *et al.*, 1994; Reyes & Ochando, 1994, 1998a, b; Baruffi *et al.,* 1995; Gasparich *et al.* 1995, 1997; McPheron & Steck, 1996; Reyes *et al.*, 1996; Roda *et al.*, 1996; Steck *et al.,* 1996; Gomulski *et al.,* 1998; Reyes & Ochando, 1998a, b; Villablanca *et al.*, 1998; Davies *et al.*, 1999; He & Haymer, 1999; Bonizoni *et al.*, 2001).

The study of the between- and within- genetic variation of the populations of a species provides valuable information on its genetic structure and its future evolutionary potential. Several techniques are now available that allow variability to be studied. Protein-based polymorphism is a valuable tool in the study of the structure and dynamics of pest populations (Menken & Ulenberg, 1987; Loxdale & Hollander, 1989; Robinson & Hooper, 1989), and gel electrophoresis is a simple, unbiased method for investigating this. Since its introduction

nearly four decades ago, it has been a mainstay for quantifying inherited variation in population genetic studies (Hubby & Lewontin, 1966; Harris, 1966). The wide use of electrophoresis in population biology has led to many advances, especially in the elucidation of population structures and evolutionary processes (Selander & Yang, 1969; Ayala *et al.*, 1972; Ayala, 1975; Markert, 1975; Powell, 1975, 1994; Nevo *et al.*, 1984; Allard, 1988; Loxdale & Hollander, 1989; Gillespie, 1991; Lewontin, 1991; Murphy *et al.*, 1996). However, DNA and PCR-based techniques now provide a more sensitive array of genomic markers (RFLP, microsatellites, etc.). Of these methodologies, random amplification of polymorphic DNA by the polymerase chain reaction using single primers of arbitrary nucleotide sequence (RAPD-PCR) has considerable appeal. It is generally faster and less expensive than other previous methods of detecting DNA sequence variation, no previous knowledge of the genome is required, the whole genome can be screened, only small quantities of DNA are needed, and no radioactive material is used. Problems concerning reliability can be eliminated by optimising experimental conditions and by following experimental protocols to the letter. Thus, it is not surprising that over recent years RAPD has been used to investigate different biological problems in all manner of organisms (Black *et al.*, 1992; Apostol *et al.*, 1993; Fani *et al.*, 1993; Huff *et al.*, 1993; Lynch & Milligan, 1994; Frey & Frey, 1995; Furman *et al.*, 1997; Dhar *et al.*, 1997; Callejas & Ochando, 1998, etc.).

Our group has used different molecular techniques to investigate the genetics of *Ceratitis capitata* populations. The present paper summarises this work, but it also includes new data. Its aim is to investigate the genetic structure of Spanish medfly populations, i.e., to determine their within- and between-population genetic variability. This was done through the use of two different molecular methodologies: MLEE (multilocus enzyme electrophoresis) and RAPD-PCR (random amplification of polymorphic DNA by PCR). The information obtained on the quantity and patterns of genetic polymorphism in these populations could help determine whether host-dependent races or biotypes exist, and to evaluate the suitability of these two methodologies for the continued study of this pest.

Material and methods

Populations of C. capitata.
Medflies were collected by harvesting infested fruit from different areas of Spain (although mostly from the *Comunidad Valenciana*), and allowing the adults to emerge in the laboratory. Four populations were obtained from peaches and four from figs. A laboratory population, established more than 30 years ago, was also studied. Table 1 shows the population names, their origins and the method assayed. Not all populations underwent analysis with both methodologies.

Table 1.- Populations, geographic origins, host origins and method assayed.

Populations	Geographic origin	Host	Assayed
ALH	El Ejido, Almeria	Figs	MLEE
BAH	Basta, Valencia	Figs	MLEE, RAPD
BAM	Basta, Valencia	Peaches	MLEE, RAPD
CAH	Campo Arcis, Valencia	Figs	MLEE, RAPD
JBM	Jardin Botánico, Madrid	Peaches	RAPD
LAB	Laboratory		MLEE, RAPD
MAH	Atajate, Málaga	Figs	RAPD
RADM	Rincón de Ademuz, Valencia	Peaches	MLEE
REM	Requena, Valencia	Peaches	MLEE

Multilocus Enzyme Analysis.

Single flies were homogenized in 50 μl of 0.1 M Tris-HCl pH 7.1 and 0.1 % Triton X-100 at 4° C. Starch gel eletrophoresis and staining procedures were performed according to Ayala *et al.* (1972) with minor modifications, except for aconitase, for which the procedure of Harris and Hopkinson (1976) was followed, and fructokinase, for which the method of Brewer and Singh (1970) was employed.

Near 100 individuals (but at least 25) from each population were assayed for most of the 28 isozymic loci shown in Table 2.

Table 2.- Systems and loci analysed.

Enzymes	Analyzed loci
Aconitase	*Aco-1*
Aldehyde oxidase	*Ao*
Alcohol dehydrogenase	*Adh-1, Adh-2*
Adenylate kinase	*Ak*
Colinesterase	*Ce-1, Ce-2*
Diaphorase	*Dia-1, Dia-2, Dia-3, Dia-4*
Esterase	*Est-1, Est-2, Est-3*
Fructokinase	*Fk*
Fumarase	*Fum*
α -Glycerol phosphate dehydrogenase	α - *Gpdh*
Hydroxybutyrate dehydrogenase	*Hbdh*
Hexokinase	*Hk-1, Hk-2, Hk-3*
Isocitrate dehydrogenase	*Idh*
Lactate dehydrogenase	*Ldh-1, Ldh-2*
Malate dehydrogenase	*Mdh*
Malic enzyme	*Me*
Mannose phosphate isomerase	*Mpi*
Phosphoglucomutase	*Pgm*

RAPD-PCR Analysis.

Genomic DNA was extracted from single flies according to Reyes (1995) and Reyes *et al* (1997). Six populations (Table 1) and twenty individuals from each population and primer were scored. For amplifications, 8 oligonucleotide primers (see Table 3 for sequences) 10 bp long, from kit C of Operon Technologies (Operon Technologies Alameda, CA), were used.

Table 3.- Sequence and percentage of G+C of the primers used in the amplification of the genomic DNA.

Primer	Sequence 5′ → 3′	% G + C
OPC-01	TTCGAGCCAG	60
OPC-02	GTGAGGCGTC	70
OPC-05	GATGACCGCC	70
OPC-06	GAACGGACTC	60
OPC-07	GTCCCGACGA	70
OPC-08	TGGACCGGTG	70
OPC-09	CTCACCGTCC	70
OPC-10	TGTCTGGGTG	60

Amplification reactions were performed in 25 μl solutions of 10 mM Tris-HCl pH 8.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 100 μM of each dNTP, 5 picomols of primer, 25 ng of template DNA, and 0.4 units Dynazyme (Finnzymes, Espoo, Finland) or TAQ Stoffel (Perkin-Elmer).

A Peltier PTC-100 programmable thermalcycler (M.J. Research, Watertown, MA) was used for PCR reactions. The thermal profile for RAPD-PCR was 94°C for 5 min. for initial denaturation followed by 45 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min, and finally 72°C for 6 min.

Amplification products were resolved in 2% agarose gels with TAE buffer containing EtBr (0.5 μg/ml) (Sambrook *et al.*, 1989). Phage φX174 DNA digested with *Hae* III or 100 bp DNA Ladder Plus (MBI Fermenta) were used as molecular markers.

According to some authors, the main problem with RAPD is reproducibility. In this work, all amplifications were repeated, the protocol was carefully followed, and the same reagents used for all assays.

Data Analysis:

MLEE: The three classical statistics for quantification of electrophoretic variability were used: H, heterozygosity, the average number of heterozygous individuals, P, polymorphism, the proportion of polymorphic loci, and n, the average number of alleles per locus. Two polymorphism criteria were used, the 95% criterion and the 99% criterion. Significance differences between values for flies infesting different hosts were evaluated using the Student *t* test.

RAPD-PCR: RAPD-PCR products were scored as either present or absent for each fly (intensity variations were not taken into account). For each population, the total number of bands and their frequencies were calculated, as well as the proportion of monomorphic and polymorphic markers. Simpson-Gini biodiversity indices were also calculated. Significance differences between values for flies infesting different hosts were evaluated using the Student *t* test.

Results and discussion

Table 4 and Figure 1 show the results of the allozyme variation survey of seven populations of *Ceratitis capitata*. Twenty eight loci of 18 randomly chosen enzymatic system were studied. Although not all systems were studied in all populations, the sample can be considered representative of the true enzymatic variability of this species. Sing and Rhomberg (1987) compiled extensive data on enzymatic variability in different species and conclud·· .d that even doubling the number of loci studied did not affect the mean heterozygosity

proportion of polymorphic loci. Thus, taking into account the large sample size used (about 100 individuals in the majority of cases) and that indicated by the latter authors, the results can be regarded as representative of the true genetic variation of the medfly populations studied.

Table 4.- Measures of genetic variation in the Spanish populations of *Ceratitis capitata*: mean number of alleles per locus, **n**, heterozygosity, **H**, and polymorphism, **P**, at 95 % and 99 %. ALM, Almeria, BAH, Basta, Valencia, CAH, Campo Arcis, Valencia, BAM, Basta, Valencia, REM, Requena, Valencia, RADM, Rincón de Ademuz, Valencia.

Populations	Host	n	H	P 95%	P 99%
ALH	figs	2.0000	0.0574	0.2857	0.3570
BAH	figs	2.0454	0.0969	0.4545	0.6111
CAH	figs	1.7231	0.0732	0.3612	0.5000
MEAN FIGS		1.9218	0.0758	0.3667	0.4894
BAM	peaches	1.9545	0.0954	0.4090	0.5260
REM	peaches	2.1842	0.0527	0.2701	0.6423
RADM	peaches	1.8182	0.0928	0.2727	0.3846
MEAN PEACHES		1.9842	0.0803	0.3172	0.5169
Laboratory		1.7634	0.0914	0.4010	0.5000
Mean Wild		1.9530	0.0781	0.3420	0.5031
TOTAL MEAN		1.8582	0.0847	0.3715	0.5015

The mean number of alleles per locus was 1.8565, mean heterozygosity was 0.0847 and polymorphism 37.15 % or 50.15 %, depending on whether the 95% or 99% criterion was used. If the present data are compared to those of other *C. capitata* populations they generally agree with the rule of decreasing genetic variability from the source area of a species toward the periphery of its range. In other words, the variability detected in the present populations was lower than that observed in Kenyan populations, but greater than that observed in other north Mediterranean countries, and much greater than that observed in American and Australian populations. Huettel *et al.* (1980) found an H value of 0.167 in a South African population and values between 0.034-0.071 in other populations. Similarly, while polymorphism values for the South African population were 52 %, values of 9-17 % were seen for the others. Further, the number of alleles per locus varied between 1.1-1.2 in non-African populations but was 2.3 for the South African population. Morgante *et al.* (1981) studied four Brazilian populations and found a heterozygosity value of 0.030. Other authors have found similar results (Loukas, 1989; Malacrida *et al.*, 1992; Baruffi *et al.*, 1995).

The source area of *Ceratitis capitata* is thought to be in central Africa (Sub-Saharan Eastern Region). It is proposed that the insect first invaded Spain (were it was detected in 1842), then spread from the Iberian peninsula to other northern Mediterranean countries, and

finally to the Middle East and other regions (Hagen *et al*. 1981). The present results, as well as our previously published data on allozyme variability in Spanish populations of this pest (Reyes & Ochando, 1994; Roda *et al*., 1996) seem to support this hypothesis.
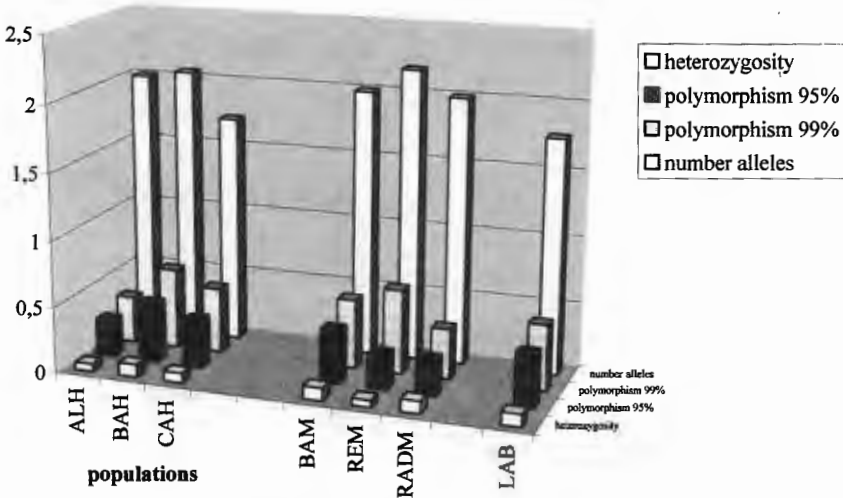

Figure 1.- Electrophoresis variability

Ceratitis capitata is an extremely polyphagous species which, from its original host *Argaria spinosa,* has extended to infest more than 250 different plant species. According to some authors (Powell, 1971; Levinton, 1973; Gillespie & Langley, 1974; Yong, 1992), a positive correlation can be expected between the range of environmental diversity and the amount of genetic variability of a species. Accordingly, the genetic variation in *C. capitata* populations ought to be very high. At first sight this does not seem to be the case for this species, at least as far as enzymatic variability is concerned. Comparisons of the genetic variability level observed in *C. capitata* to that observed in other flies with a more restricted host range do not follow these expectations. Species such as *Rhagoletis pomonella* shows means heterozygosity between 0.095-0.189 and means number of alleles per locus between 1.5-2.8 (McPheron *et al*., 1988). *Bactrocera oleae*, a strictly monophagous species that infest olives, has reported heterozygosity values of 0.1321-0.1077, means number of alleles per locus of 2.6218-2.0 and values of polymorphism of 75-77 % and 83-95% (criterion 95% and criterion 99%, respectively, Ochando *et al*., 1994; Ochando & Reyes, 2000).

With respect to RAPD-PCR, variability was regarded as the presence/absence of a given band, since, owing to the dominance of RAPD markers, homozygous and heterozygous conditions cannot be distinguished.

RAPD patterns revealed between 5-21 bands per fly, ranging from 280 to 2500 bp in size (the majority between 350-1500 bp). Each primer generated a variable number of different bands. However, the total number of markers as well as the mean number of polymorphic bands per primer and population, were relatively similar for all primers and in all populations - except for primer OPC-06 which showed less variability, and the laboratory population that showed a slightly lower number of polymorphic bands (Table 5, Figure 2).

Table 5.- Number of bands per primer in each population. First line: total; second line: polymorphic; third line: monomorphic. Means are in parenthesis.

| Primers	Populations						Total figs	Total peaches	Total wild
	BAH	CAH	MAH	BAM	JBM	LAB			
OPC-01	19	10	12	21	10	7	41	31	72
	14	6	7	16	7	5	27	23	50
	5	4	5	5	3	2	14	8	22
OPC-02	14	10	11	13	10	11	35	23	58
	9	4	7	8	5	7	20	13	33
	5	6	4	5	5	4	15	10	25
OPC-05	14	17	11	17	16	11	42	33	75
	11	16	9	14	16	10	36	30	66
	3	1	2	3	0	1	6	3	9
OPC-06	9	9	9	10	8	7	27	18	45
	5	8	8	6	7	4	21	13	34
	4	1	1	4	1	3	6	5	11
OPC-07	15	12	13	13	14	11	40	27	67
	8	11	11	8	12	10	30	20	50
	7	1	2	5	2	1	10	7	17
OPC-08	12	16	19	12	17	14	47	29	76
	8	15	17	8	15	11	40	23	63
	4	1	2	4	2	3	7	6	13
OPC-09	11	12	14	12	13	6	37	25	62
	6	10	10	8	10	3	26	18	44
	5	2	4	4	3	3	11	7	18
OPC-10	11	13	14	9	15	12	38	24	62
	8	12	12	7	14	11	32	21	53
	3	1	2	2	1	1	6	3	9
Total	105	99	103	107	103	79	307	210	517
	(13.12)	(12.37)	(12.87)	(13.37)	(12.87)	(9.87)	(12.79)	(13.12)	(12.92)
Total polym.	69	82	81	75	86	61	232	161	393
	(8.62)	(10.25)	(10.12)	(9.37)	(10.75)	(7.62)	(9.67)	(10.06)	(9.82)
Total monom.	36	17	22	32	17	18	75	49	124
	(4.50)	(2.12)	(2.75)	(4.00)	(2.12)	(2.25)	(3.12)	(3.06)	(3.10)
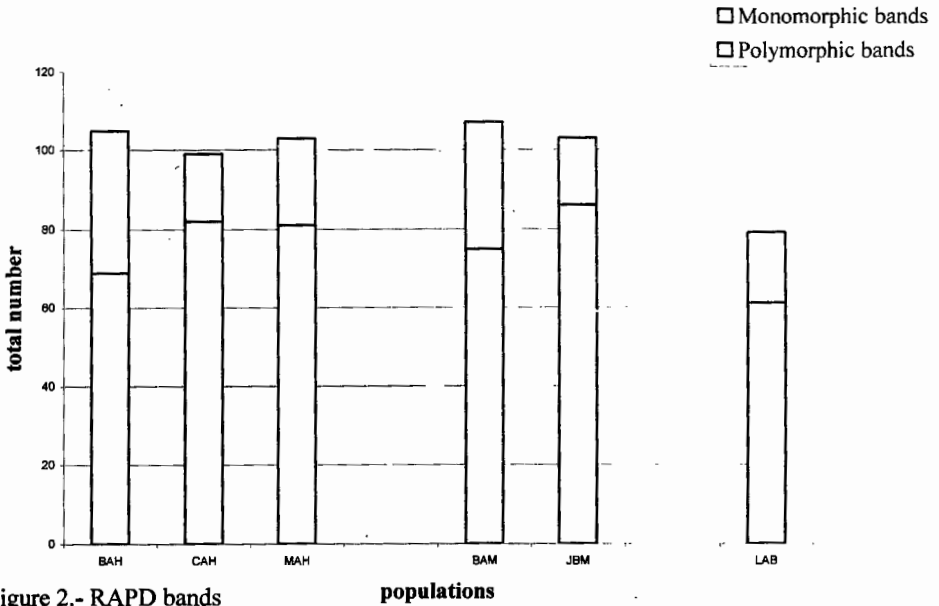
Figure 2.- RAPD bands

RAPD analysis revealed a high degree of polymorphism in the studied populations. The average proportion of polymorphic markers was 67.98 % for the seven studied populations, increasing to 76.31 % if only the six wild populations are taken into account (Table 5, Figure 2). The Simpson-Gini biodiversity index, which provides information on intrapopulational variability, showed values of 0.8733 for the laboratory population and between 0.95 – 0.98 for wild populations (Table 6). The maximum value this index can take is 1, meaning that all the studied flies are different.

Table 6.- Simpson-Gini biodiversity index for each population and primer.

OLIGO	BAH	CAH	MAH	BAM	JBM	LAB	Figs	Peaches	Wild	Total
OPC-01	1	0.9526	1	1	0.9368	0.6526	0.9842	0.9684	0.9779	0.9237
OPC-02	1	0.9298	0.9333	1	0.9474	0.9494	0.9544	0.9737	0.9621	0.9600
OPC-05	1	1	1	1	1	0.9842	1	1	1.0000	0.9974
OPC-06	0.6667	0.9684	0.9890	0.8667	0.9895	0.8421	0.8747	0.9281	0.8961	0.8871
OPC-07	1	0.9883	1	0.9778	0.9947	0.9579	0.9961	0.9862	0.9922	0.9864
OPC-08	0.9778	1	1	0.9556	0.9947	1	0.9926	0.97515	0.9856	0.9880
OPC-09	1	0.9412	0.9778	0.9556	0.9421	0.6474	0.9730	0.94885	0.9633	0.9107
OPC-10	0.9556	1	1	0.8667	0.9804	0.9526	0.9852	0.92355	0.9605	0.9592
Mean	0.9500	0.9725	0.9875	0.9528	0.9732	0.8733	0.9700	0.9630	0.9672	0.9516

Previous work performed by our group revealed a high degree of polymorphism with the RAPD technique in other medfly populations (Reyes, 1995; Reyes *et al.*, 1996; Reyes & Ochando, 1998; Fernández *et al.*, 2001). Only two others investigations have used this methodology with *C. capitata* populations, one for identification rather than quantification purposes (Haymer & McInnis, 1994), and one which revealed high polymorphism (higher for Kenyan populations and lower for other populations, Baruffi *et al.*, 1995). Curiously, with respect to their degree of variability, the present populations were more similar to Kenyan than to Mediterranean populations. In any event, it is clear that a high degree of unknown polymorphism is present in the nuclear DNA of our populations.

There are few RAPD data on tephritid flies that allow a comparison between flies of restricted range of host and *Ceratitis capitata*. We previously published some data on *Bactrocera oleae* which showed Simpson–Gini values of about 0.97 -in the same range as that found for *C. capitata* in the present work. Thus, in contrast to the enzyme data, the RAPD data show by polyphagous and monophagous species appear to be similar (this should be taken with caution since only two species are considered; to generalize, data from other fruit fly species are needed) and show much more genetic variability. This confirms that there is no relationship between degree of polymorphism and host-use diversity.

What could be the reason for the differences between the RAPD and enzymes markers results with respect to the variability detected?. Probably, the ability of *C. capitata* to feed on many types of fruits, and the change from one kind of fruit to another in consecutive generations (depending on the moment of the year and the availability of hosts), requires adaptation to a wide range of hosts. This would tend to restrict variability since the selection of "generalist" alleles conferring the ability to metabolise different kind of nutrient substrates would be required. Variability is therefore ultimately prevented by adaptation to different kinds of fruit host. On the other hand, the majority of RAPD markers represent random sequences of the genome, probably in a high number polymorphisms of non-codifying and repetitive sequences (Williams *et al.*, 1990; Black, 1993; Haymer, 1994; Haymer & McInnis, 1994), but neither kind have the selective restrictions shown by the enzymatic systems. It is possible that these different regions amplified by PCR evolved at a faster rate than did the protein enzymatic loci. This supports the idea that greater variation could exist in RAPD markers.

Given the above, no differences should be expected in variability with respect to host-fruit. When data for populations collected on figs (ALH, BAH, CAH and MAH) were compared with those corresponding to populations collected on peaches (BAM, JBM, RADM and REM), the results were similar. With respect to the electrophoresis data (Table 4 and Figure 2), mean number of alleles per locus, mean heterozygosity, as well as mean polymorphism, are similar for fig- and for peache-collected populations, i.e., 1.9218 versus 1.9842, 0.0758 versus 0.0803, and 0.3667 versus 0.3172 (95% criterion) or 0.4894 versus 0.5169 (99% criterion). No significant differences were found in any statistical comparison (Student *t* values were 0.4260, 0.2486, -0.7386 and 0.2638, respectively, p >0.50). In the same sense, data on RAPD markers were also very similar for fig- and peache-collected populations (Table 5, Figure 3). The total average number of bands was 12.79 in flies from figs and 13.12 in those from peaches, and the number of polymorphic bands was 9.67 and 10.06, respectively (t= -0.9798 and -0.3047, p>0.40). The same is true for Simpson–Gini biodiversity index with values of 0.97 in fig flies and 0.96 in peach flies. Further, no single allele, either enzymatic or RAPD, was detected as characteristic of flies coming from one class of fruit, i.e., no single, host-specific genetic marker was detected. Some rare alleles were present in a very reduced number of flies from one or another kind of fruit, but these markers

cannot be considered host-specific due to their very low frequency. They are therefore of no use as host or population markers. In summary, there is no host-dependent genetic differentiation or host biotypes in *Ceratitis capitata* populations. Results totally coherent with the expectations for a generalist strategy.

The laboratory population maintain a degree of genetic variability only slightly lower than the wild populations and only in some aspects (number of alleles per locus, and percentage of polymorphic RAPD bands). Two factors probably encourage this situation: the length of time that has elapsed since the population was established (more than 30 years ago), and the provision of "fresh" flies. Both facts may have helped the population adapt to laboratory conditions, though some kind of balancing selection could be at work. In fact, the degree of heterozygosity was slightly higher than that of the wild populations. Tsakas and Zouros (1980) indicate that laboratory populations of *Dacus –Bactrocera- oleae* show wider genetic variation than do wild populations, specifically they show increased heterozygosity.

Finally, the results show the different quantity and quality of variation detected by the two methodologies. Depending whether one is investigating adaptive variability or general variation, one or the other method might be better, but the simultaneous use of both techniques gives a more complete and congruent picture of the genetic structure of the populations of this harmful pest. This information, in its turn, may be useful in designing more efficient and environmentally safe methods of control.

Acknowledgements

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Comparison among trap-attractant combinations for the control and monitoring of *Ceratitis capitata* (Wied.)

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Abstract: We carried out an essay for testing the compared efficacy of different trap-attractant combinations. For this, we used two citrus orchards in Guadalhorce Valley (Malaga) with the Salustiana and Navel-Late orange varieties. The trap-attractant combinations compared were: (a) Mc-Phail trap with di-ammonium phosphate + Buminal; (b) Tephri-Trap with three lure compounds: (1) di-ammonium phosphate + Buminal + DDVP, (2) Nu lure + di-sodium tethra-borate + DDVP, and (3) tripack (ammonium acetate + trimethylamine hydrochloride + putrescine) + DDVP; (c) Elkofon trap with Entomela 50SL. These devices were held up in the orchards from early November 1999 to August 2000. We checked the traps at weekly intervals, so that male and female individuals were counted separately. The data obtained along the monitoring period were processed statistically using analysis of variance and the Scheffée and LSD tests for mean comparison. The Thephri-Trap with tripack + DDVP was significantly the most effective for capturing females and total individuals, and the Tephri-Trap with Nu lure + di-sodium tetra-borate + DDVP was the best for capturing males.

Key words: *Ceratitis capitata*, citrus, trap, attractant, monitoring, mass-trapping.

Introduction

Ceratitis capitata (Wieds.), the Mediterranean Fruit Fly, is one of the most serious phytosanitary problems in Spanish citrus crops. The damages derived from it cause the fall and the rotting of fruits (Moner et al., 1988), and also restrict the exportation possibilities of oranges and tangerines, since the detection of Fruit Fly larvae in a consignment implies quarantine in many countries.

Chemical spraying is, at the present time, the most used technique for the control of this pest. However, in the last decades, as the public opinion has become conscious of the problems derived from chemicals, the legislation in many countries has established maximum limits for the chemical residuals in agricultural products (LMR), and has even prohibited the use of many substances (Santaballa et al., 2001). With the aim of diminishing the chemical inputs to the agro-environments, from the beginning of the 20th century, the development of pest monitoring methods and of alternative techniques for their control have driven to the definition of the "integrated control" (Division of Agriculture and Natural Resources, 1991). Undoubtedly, traps are one of the most important tools for this type of management. By using capture devices for insects, not only a precise monitoring of their populations is done, but also efficacious control for many pests can be achieved by mass-trapping.

The search for an optimum trap to capture the Mediterranean Fruit Fly dates from the 1930s. Then, the di-ammonium phosphate (4%) was considered the best lure for attracting females (Moner et all, 1988), and so it has been, for a long time, a reference to express the population levels in the orchards and to define the treatment thresholds. In more recent times, other lures have been developed and, according to some studies, they are more effective than

the di-ammonium phosphate for the Mediterranean Fruit Fly. Among these alternatives, we find the combination of hydrolyzed proteins, combined with di-sodium tethra-borate which, besides having a certain attractant capacity, avoids the quick decomposition of proteins and of the insects captured (Ros et al., 2001; Lloréns & Lloréns, 2002). Other very efficacious lures have been proposed, based on the combination of the synthetic substances ammonium acetate, trimethylamine hydrochloride and putrescine, often distributed on a low emission format commercially called "tripack" (Ros, 1997; Ros et al., 2.001; Heath, 2001). A sexual attractant based on pheromones has been isolated, reproduced in laboratory, and successfully tried in the field (Vila & Rama, 1998; Lloréns & Lloréns, 2.002).

The efficiency of a capture system does not only depend on the lure, but also on the trapping device containing it. Its geometry, colour, access ways for insects, and capacity to give the lure trail off, are decisive to ensure the success of the trap (Vila & Rama, 1998). Many different traps have been used so far, but the Mc-Phail glass trap (Ros et al., 1.997; Lloréns & Lloréns, 2002) has been traditionally the most accepted one, typically lured with di-ammonium phosphate. Other devices are Tephri, Nadel, Funnel, Delta, Jackson, FRUTEC, Bait Stations, Elkofon and other traps (Ros et al., 1.996; 1997; 2001; Lloréns & Lloréns, 2002).

In view of this variety of possibilities, it is necessary to reach agreement about which is the most efficacious device to capture *Ceratitis capitata* individuals, and especially which is the best for trapping females, as they are the direct responsible for damages. This task is necessary for simplifying the application of economic injury thresholds, which should be referred to a standardised way of measuring the population size. The aim of this work is to compare the efficacy of five trap-attractant combinations, based on three types of trap and four different lures. The criteria used to choose the devices tested here were their traditional condition, their trapping efficacy (as suggested in the existing literature), or their novelty in the market.

Material and Methods

This essay was made in two citrus orchards with the Salustiana and Navel-Late orange varieties, located in the Guadalhorce Valley (Malaga, Spain). The study period lasted from early November 1999 to August 2000. The trap-attractant combinations compared were: (1) Mc-Phail trap with di-ammonium phosphate and a hydrolyzed protein (Buminal); (2) Elkofon trap, lured with Entomela 50SL; (3) Tephri-Trap with three lure compounds: (a) di-ammonium phosphate + Buminal + DDVP (toxicant strip as retention method), (b) hydrolyzed proteins (Nu lure) + di-sodium tethra-borate + DDVP, and (c) tripack (ammonium acetate + trimethylamine hydrochloride + putrescine) + DDVP (see figure 1).

In the two study orchards, we held up four traps of every type, at least 20 m apart from each other, to avoid interferences between devices. We checked the traps at weekly intervals, and interchanged their positions after every check to reduce any positional effect. The lures composed by di-ammonium phosphate and hydrolyzed proteins were replaced every week (Ros et al., 2001), whereas the Entomela and the tripacks were replaced monthly, as recommended by the commercial distributors. We collected weekly data referred to the male, female and individual numbers per trap and day, respectively. To compare the trap efficiency, we processed the daily percentage of flies per trap and lure-trap combination type, considering separately the males, the females and all the individuals with analysis of variance. Pair-wise mean comparisons between combination types were made using the Scheffée and LSD (least significant difference) tests (Snedecor & Cochran, 1967).
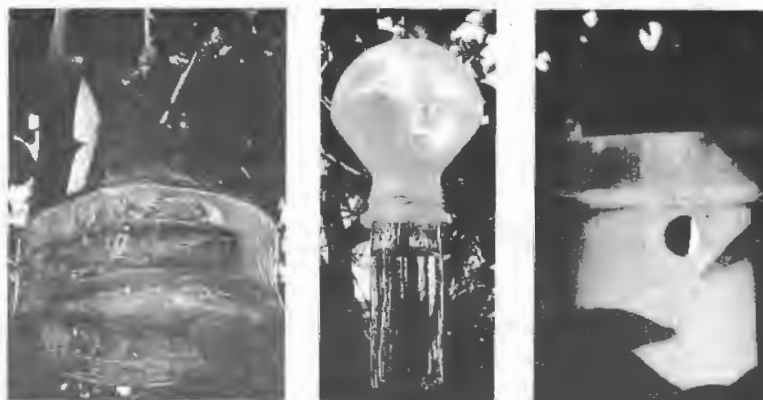
Figure 1. Used traps Mc-Phail , Elkofon, and Tephi-Trap.

Results and discussion

Figure 2 shows the Mediterranean Fruit Fly capture curves recorded by the different types of lure-trap combinations tested. Two periods of high capture volumes are registered: from September to November, and from May to July. In this chart, it is already remarkable a higher number of captures by the Tephri-Traps lured with tripack, and by the same trap with Nu lure and di-sodium tethra-borate. The analyses of variance show significant differences between types of lure-trap combinations for males (F_s=8.00; $p<0.001$), females (F_s=27.72; $p<0.001$) and total individuals (F_s=16.73; $p<0.001$). Tables 1, 2 and 3 represent the results of the mean pair-wise comparison Scheffée and LSD tests. In any case, the lure-trap combinations have been ordered downward from the most to the least efficacious one.
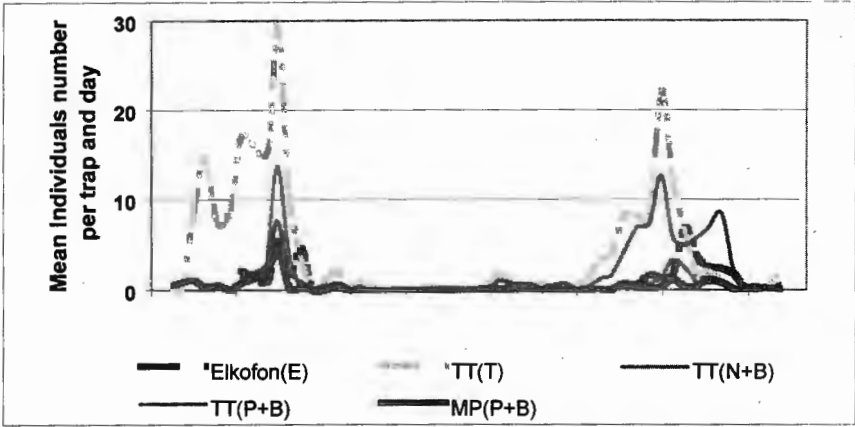
Figure 2. Capture curves of *Ceratitis capitata*. TT: Tephri-Trap; MP: Mc-Phail. Abbreviations in parentheses are the lures used: E: Entomela 50SL; T: tripack; N+B: Nu lure and di-sodium tethra-borate; P+B: di-ammonium phosphate and Buminal.

Table 1. Mean comparison test for males. Abbreviations as in Figure 2.

	T-T (N+B)	T-T (T)	T-T (P+B)	Elc (E)	McP (P+B)		T-T (N+B)	T-T (T)	T-T (P+B)	Elc (E)	McP (P+B)
T-T (N+B)						T-T (N+B)					
T-T (T)						T-T (T)					
T-T (P+B)						T-T (P+B)					
Elc (E)						Elc (E)					
McP (P+B)						McP (P+B)					
LSD test						Scheffée test					

According to the results for male trapping, the most efficacious combination was the Tephri-Trap lured with Nu lure and di-sodium tethra-borate, and the least efficacious was the Mc-Phail trap lured with di-ammonium phosphate and Buminal. The LSD test shows that both the most and the least efficacious devices significantly differ from the remaining ones. The more conservative Scheffée test shows differences only between the most efficacious device and the others.

Table 2. Mean comparison test for females. Abbreviations as in Figure 2.

	T-T (N+B)	T-T (T)	T-T (P+B)	Elc (E)	McP (P+B)			T-T (N+B)	T-T (T)	T-T (P+B)	Elc (E)	McP (P+B)
T-T (N+B)							T-T (N+B)					
T-T (T)							T-T (T)					
T-T (P+B)							T-T (P+B)					
Elc (E)							Elc (E)					
McP (P+B)							McP (P+B)					
		LSD test							Scheffée test			

As shown in table 2, the best lure-trap combination for trapping females is the Tephri-Trap lured with tripack, and the worst one is again the Mc-Phail trap lured with di-ammonium phosphate and Buminal. In general, the results of both the LSD and the Scheffée tests are similar to each other, showing significant differences between the most efficacious device and the other ones, but also between the second most efficacious (the Tephri-Trap lured with Nu lure and di-sodium tethra-borate) and the worst ones.

Table 3. Mean comparison test for individuals. Abbreviations as in Figure 2.

	T-T (N+B)	T-T (T)	T-T (P+B)	Elc (E)	McP (P+B)			T-T (N+B)	T-T (T)	T-T (P+B)	Elc (E)	McP (P+B)
T-T (N+B)							T-T (N+B)					
T-T (T)							T-T (T)					
T-T (P+B)							T-T (P+B)					
Elc (E)							Elc (E)					
McP (P+B)							McP (P+B)					
		LSD test							Scheffée test			

For all the adult individuals captured (table 3), most combinations show significant differences between each other according with the LSD test, but the Scheffée test points out the differences between the best device (the Tephri-Trap lured with tripack) and the other ones left.

So, this essay has provided the following concluding remarks: (1) the Tephri-Trap was always, (for males and for females) a more efficacious trap than both the Elkofon and the Mc-phail ones, whatever the lure contained in it; (2) the lures based on the tripack and on the Nu lure and di-sodium tethra-borate combination were the best attractant for *Ceratitis capitata*, tripack, was the best lure for trapping female flies and it also captured the highest number of individuals, whereas males were significantly more attracted by the Nu lure and di-sodium tethra-borate combination; (4) the Mc-Phail trap was in any case the worst trap.

78

According with the results of this work, and taking into account the trapping volumes of the best devices (figure 1), it is worth considering the Tephri-Trap lured with tripack as a good tool, not only for population monitoring, but also as a device to be used in mass trapping techniques for the control of *Ceratitis capitata*.

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Field evaluation of the attractancy of a synthetic male pheromone blend and of an extract of ripe coffee berries on wild and reared females of Medfly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

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Abstract: In the present work a synthetic pheromone blend based on the five major components of the natural male sex odor, and an aqueous extract of ripe coffee berries (*Coffea arabica* L.), both developed by USDA-ARS PBARC of Hilo, Hawaii, USA, have been tested on reared and wild Medfly females in different locations in Italy. The primary purpose of the current study was to look at the effects of abiotic factors such as temperature, seasonality and climate on response of these putative female attractants in field cages. We also wanted to confirm earlier studies which suggested that biotic factors such as mating status could influence olfactory behavior. In a second phase, the attractants were tested in open field conditions, to verify the results obtained in field cage tests on the wild Italian populations. The results of field cage experiments showed a good attractancy of the synthetic sex pheromone blend to unmated Medfly females, while the coffee extract did not perform as well on mated females. Moreover, field cage tests highlighted marked differences between mated and unmated females circadian rythms of activity, and the absence of differences of the response to the compounds at the various climates. The open field tests on wild populations, showed a low response of the insects to both the attractants tested. As a global result, this work showed a good potential of the synthetic pheromone blend to be used as attractant for early monitoring of Medfly infestation, prior the optimization of the open field formulation.

Key words: Medfly, pheromone, behavior, attractants

Introduction

The Mediterranean fruit fly, (Medfly), (*Ceratitis capitata* Wiedemann) is an economically important pest worldwide. Medfly is known to infest over 250 types of fruits and vegetables and is responsible for millions of dollars in lost crop, pesticide usage and postharvest quarantine treatments. Because of its economic importance, new or improved detection tools that could be used to control this pest or keep it from becoming established are needed.

Semiochemical lures and attractants are currently used in most detection programs for Medfly worldwide (Jang & Light, 1996). Until recently male lures such as Trimedlure have been considered the standard detection and delimitation lure of choice. Protein-based food lures are used for females but are considered less potent and have been less specific in regards

to other insects. Recently, Biolure®, a food-based three component lure which attracts primarily female medflies and some male medflies has been used in some programs (Heath *et al.*, 1995). The development of additional female attractants could further improve tools to cover all bases in a detection program.

Previous research conducted on the identification of the components of the male pheromone of Medfly (Jang *et al.*, 1989) identified over 50 components of which five major components were the basis of follow-up laboratory studies conducted to develop a pheromone-based female attractant. The five major component ensemble was reported to attract at least 50% of virgin laboratory female Medflies released in laboratory windtunnel tests (Jang *et al.*, 1994). When compared to the authentic male odor, only 30% of the released flies responded to the synthetic five component ensemble. Open field tests of the five component ensemble has been highly variable in tests using both released laboratory-reared flies as well as wild flies. The full cause of this variability is not known, although factors such as mating status (Jang, 1995) has been shown to influence female response to volatile semiochemicals.

Extracts from ripe coffee berries were also found to attract female Medflies over short distances on field cages (Prokopy, 1997). 28 volatile compounds were identified from crushed ripe *C. arabica* coffee berries (Warthen *et al.*,1997). Another 28 volatiles were identified from whole ripe *C. arabica* coffee berries (Mathieu *et al*, 1996). As in the case with pheromone, these compounds were not effective alone in the field suggesting blends may be more effective. Both coffee juice and the odor of ripe coffee berries were found to be attractive in the field to female Medflies (Vargas *et al.*, 1997).

The primary purpose of the current study was to look at the effects of abiotic factors such as temperature, seasonality and climate on the response of these putative female attractants (coffee and pheromone components) to females in field cages. We also wanted to confirm earlier studies which suggested that biotic factors such as mating status could influence olfactory behavior.

This paper describes research that was conducted under natural conditions in field cages to test the attraction of mated and unmated female Medfly to the five major pheromone component ensemble and a water extract of fresh ripe coffee berries. Experiments were conducted in different locations in Italy where the fly is known to occur to look at the effects of climate on female attraction.

Materials and methods

Insect rearing
For field cage experiments, laboratory-reared Medfly pupae were obtained from the CRAS-LAIU (Sardinian Regional Agricultural Experimental Center, Useful Insects Rearing Laboratory) rearing facility located on the Island of Sardinia (Italy). Flies were allowed to emerge and held in cubical screened cages 30 cm x 30cm x 30cm and given water, sugar and hydrolyzed protein (yeast hydrolysate enzymatic, Sigma-Aldrich). Emergence dates were recorded on each cage to track the age of the flies. Emergence normally occurred over a 2 day period. All adult flies were kept at 24°C, 70% RH and 12/12 LD cycle.

Unmated females were separated out at the pupal stage as described in Cunningham, 1969. Groups of fifty females were placed in smaller screen covered containers (11.5cm x 6.5cm) and given water, sugar, and protein. Unmated females were kept in a separate room from the males.

After mating occurred, mated females were separated after immobilization of flies in a cold room (4°C) and placed in groups of fifty, 24 hours before testing. Both mated and unmated females were tested.

Attractants

Four treatments were used for both field cage and open field experiments: a 5 component male pheromone ensemble (ethyl -E3- octenoate, geranyl acetate, ethyl acetate, EE alpha farnesene and 1- pyrroline), coffee water extract, Nulure®, a protein based fruit fly attractant used for monitoring and detection, and an aqueous control.

Field cage experiments

Field cage tests were conducted in ENEA Casaccia Research Center (near Rome), and fruit orchards in Cisterna (near Naples) and Catania on the island of Sicily. In each location, three replicates for both mated and unmated females were performed separately each month for the duration of a complete year. 3m x 3m x 3m mesh field cages were placed each over a live host tree (usually citrus), without fruits.

Attractants were distributed into odor chambers made from a 500 ml cylindrical Nalgene® bottles with the bottom third cut off and a mesh cover placed on both ends. The odor chambers were placed in the middle of 15cm x 21cm green sticky panel traps, extending through a 7cm hole in the panel so that natural air movement facilitated the volatilization of chemicals or extracts from the substrates.

The five major pheromone components were presented in 5 and 10 ul glass capillaries as described in Jang et al.(1994), and taped inside the odor chamber. The water extract of fresh ripe coffee berries was prepared as described in Vargas 1997. Nulure® was prepared by mixing 9% Nulure® plus 5% borax and 84% water. 15 ml of coffe water extract, 15 ml of Nulure® and 15 ml of aqueous blank control were presented on a 6.5 cm richmond wick inside the odor chamber.

In each field cage, flies were allowed to respond to all four treatments; the four traps were hung on branches approximately at the four corners of the cage. 150 females, 5-7 days old were released in each cage and allowed to respond to the attractants for six hours, from mid morning until early afternoon (9.30 AM – 3.30 PM).

Every 30 minutes the traps were rotated, shifting their positions clockwise, and the following independent variables were recorded: trap captures, temperature, relative humidity, weather conditions.

Open field experiments

Open field tests were conducted in three locations in Italy. Istituto Sperimentale Frutticoltura in Rome, Sardinia and Sicily. Experiments took place into pesticide-free fruit orchards during the season of activity of Medfly in Italy (June-December). In each location three bucket trap replicates for each treatment were hung on the southern side of trees following a randomized complete block design.

The distribution of the attractants was done using 5l covered bucket traps with sticky inserts. Pheromone compounds were placed in 1ml disposable pipettes with the tip cut off at different lengths according to the release rate of each single component; pipettes were taped to the inside of the bucket traps. 50 ml of Nulure®, coffee water extract and water blank control were adsorbed on a 6.5cm richmond wick and placed in the bucket traps.

A Trimedlure Cromotrap (Isagro SpA, Italy) was placed into the orchards to compare male and female captures. Traps were serviced weekly and temperature, humidity,male and female trap captures were recorded.

Results and discussion

Tests were run over the course of four seasons to encompass the entire range of climatic conditions within which we measured fly responses to the three attractants. When data was analyzed for difference in location in respect to response we found no significant differences attributable to location during the years of the study. Thus data was combined for the three different locations in Italy. For the purpose of data analysis, the mean proportion of flies responding to each of the attractants in each field cage was summed over a three-month period, which was considered a season.

Field cage tests confirmed marked influence of mating status on the response of Medfly females to proposed attractants. Overall unmated female response was greater than mated females. Unmated females significantly preferred pheromone ensemble and Nulure® to coffee and control while mated females showed significant preference for Nulure® followed by coffee extract and pheromone (Tables 1 and 2). Additionally, the mated females also preferred coffee extract and Nulure® to pheromone and control at the higher temperatures.

Table 1. Mated females. Field cage experiment results.
Means in each line followed by the same letter are not significantly different (LSD test significance level $\alpha = 0,05$).

Treatment	N	Mean captures	
Pheromone	216	$17,20 \pm 12,15$	b
Nulure®	216	$25,70 \pm 18,66$	a
Coffee extract	216	$16,41 \pm 13,46$	b
Control	216	$10,94 \pm 8,24$	c

Table 2. Unmated females. Field cage experiment results.
Means in each line followed by the same letter are not significantly different (LSD test significance level $\alpha = 0,05$).

Treatment	N	Mean captures	
Pheromone	232	$33,57 \pm 17,12$	a
Nulure®	232	$29,90 \pm 16,01$	a
Coffee extract	232	$14,10 \pm 8,69$	b
Control	232	$14,03 \pm 10,52$	b

The primary goal of field cage experiments was to highlight the effects of abiotic factors on the response of Medfly females to the proposed attractants. It has been shown that temperatures lower than 16°C suppress field cage responses; as reported by Tremblay, 1982. We also found that at temperatures higher than 33°C the responses to treatments were also suppressed (Figure 1). Within this range of temperature, the activity of flies remained relatively constant; mated females showed lower levels of activity and a preference for higher temperatures when compared to unmated females. These differences were also reflected on

the time of day in which the most activity of flies took place: unmated females captures were concentrated from mid morning to midday, and rapidly decreased during the afternoon; on the other hand, mated females started to be active later in the morning continuing until the end of the experiments. These observations confirm the behavioral data previously reported by other authors (Hendrichs & Hendrichs 1990; Warburg & Yuval, 1997).
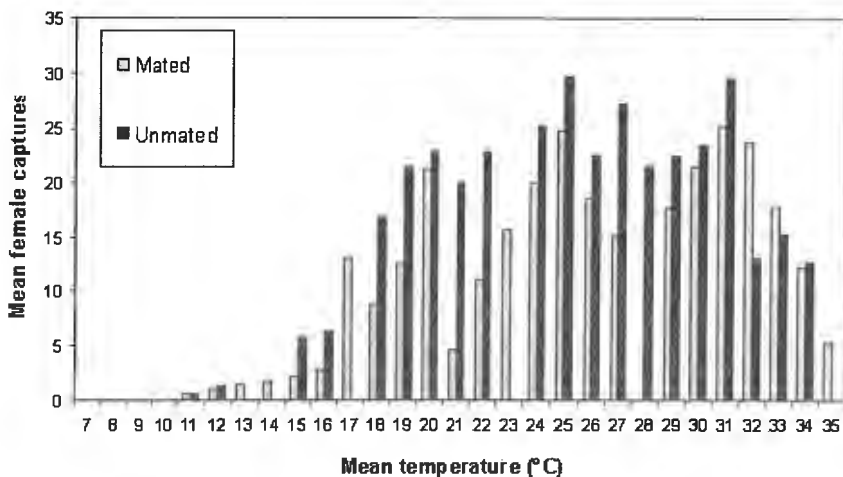

Figure 1. Relation between mean temperature and captured females in field cage tests (all treatments considered)

Concerning the effects of seasonality on insects reception of proposed attractants, although responses were still quite variable, there were definite differences comparing the four seasons. Unmated females showed greatest activity during spring, summer and fall seasons at which time when temperatures were increasing and most optimal. Mated females did not show most activity until the summer and fall seasons despite that spring temperatures were quite high; this fact may be correlated with host availability.

Following the field cage assays, the compounds were tested on the wild Medfly populations in the open field. In Sardinia, Nulure® captured the highest number of wild females compared to pheromone, coffee extract and blank control. In Sicily, there were no significant differences in trap captures among all treatments in bucket traps. In Rome, which was the third field location, pheromone captured the highest number of females but not significantly different from Nulure® and coffee but significantly more than blank control.

Although there was some wild fly response in the field it was not the most optimal response. It is possible that the formulations tested were out of the range of attractancy, which could be improved with some changes in release rates in the right proportions. Determining the right combination of trap design and formulation will be important in improving the range of attractancy which will optimize the effectiveness of the pheromone blend as a strong attractant for female Medflies.

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Evaluation and comparison of mass-trapping methods for the control of *Ceratitis capitata* Wied. in Citrus orchards

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Abstract: Within the framework of the Citrus Integrated Production Agreement between the Consejería de Agricultura y Pesca (Junta de Andalucía) and some citrus cooperatives in Malaga, in 2001 we carried out an essay for the control of *Ceratitis capitata* using the mass trapping technique. We installed trap batteries according with two alternative control strategies, in two crops with Clementina de Nules Mandarins located in Guadalhorce Valley (Malaga). In the first strategy, we used the Agrisense BCS LTD trap, lured with a sexual and food attractant combination; in the second strategy, we used the Teprhi-Trap with a combination of food attractants. We placed one trap by every 64 trees, and kept them in the crops from late August to the harvest time, late November 2001.

To test the effect of the control strategies, we monitored the damages in fruits as well as the adult flight volume using traps, with a weekly periodicity. The same monitoring was carried out in crops run with a traditional chemical management, in order to have blank controls available.

The damage level was insignificant in all crops, and so this parameter could not be used for comparison. However, the adult flight volume was clearly lower in crops with a mass trapping system than in the blank controls. A better control of *Ceratitis capitata* was observed in crops with Tephi-Traps compared with crops with Agrisense BCS LTD traps.

Medfly chemosterilization using an IGR

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Abstract: Current *Ceratitis capitata* (Wiedemann) control methods are mainly based on organophosphate bait sprays. Nevertheless, mass-trapping, the Sterile Insect Technique and biocontrol techniques are eventually employed. Spinosad® is an insecticide of bacterial origin that may replace organophosphates in the coming years. We have developed a medfly control method based on autosterilization in the field with the active ingredient lufenuron®. This Insect Growth Regulator (Chitin Synthesis Inhibitor) is employed in the field in a suitable way to allow medflies become sterilized. Three field trials were done in Valencia (Spain) in order to test the diminishing effect over wild medfly population. The efficacy of this method was compared with malathion treatments, mass trapping and untreated fields. In all cases the sterilising method was at least as efficient as malathion and mass trapping. Large-scale field trials are currently under way.

Keywords: sterilant, insect growth regulator, lufenuron, *Ceratitis capitata*, medfly

Introduction

We have tested chemosterilization as an alternative to insecticide treatments against the medfly *Ceratitis capitata*. This method bases on our previous investigation about an IGR that avoids medfly reproduction (Casaña et al., 1998). The activity of lufenuron prevents medfly egg hatching when females ingest a bait with 0.1% of lufenuron. Moreover, females that mated with lufenuron treated males (0,5% a.i. in diet) lay non hatching eggs, in laboratory tests. In addition, our research group has tested lufenuron in field caged grapefruits infested with medflies also, observing an effective reduction of population density (unpublished data). Small and medium sized field trials have been made in order to check efficacy in real conditions over wild medfly population.

Materials and methods

Chemicals
Corn Steep Liquor from Dadelos (Valencia, Spain), meliose from Roquette Laisa España S.A. (Valencia, Spain), sorbitol 70% from Guinama (Valencia, Spain), lufenuron tech. (99,1%) from Syngenta (Basel, Switzerland), emulsogen and calcium phenyl sulfonate (FSCa) from Agrevo España (Valencia, Spain), agar-agar from Panreac and trimedlure (TML) + DDVP dispenser from AgroAlcoy (Alicante, Spain).

Fields characteristics
The field trials were done in Sagunto (Valencia, Spain), Denia (Alicante, Spain) and Gandía (Valencia, Spain). The characteristics of the field trials were: Sagunto (3 hectares, *Citrus sienensis* Osbeck cv. New-Hall, 350 trees per hectare, reference zone treated with malathion); Denia (5 hectares, *Citrus sienensis* Osbeck cv. Valencia-late, 320 trees per hectare, reference zone untreated); Gandía (1 hectare, *Citrus reticulata* Blanco cv. Marisol, 500 trees per hectare, reference zone treated with mass trapping (Frutect traps from Rhône Poulenc).

Attractants and sterilants

For these trials we have developed a bait gel that contains the lufenuron. The bait had the following composition: corn steep liquor (CSL) 60%; meliose 30%; sorbitol 10% (vol:vol:vol), agar-agar 1% (wt:vol) and lufenuron 1% (wt:vol). The CSL has a protein content of 14.8% (wt:vol) (Hull et al., 1996). Meliose is a glucose-fructose mixture (39% : 21% (wt:wt)) with 76% dry weight. We have observed that meliose increases the ingestion of the bait by the flies (unpublished results). This gel is active for three months, therefore, to maintain sterilant activity in field from June to October we replace the gel once in a year. The gel consists on a protein bait in order to attract the medflies and to cause lufenuron ingestion. The gel is put into a delta trap to protect it from the rain.

There were 40 traps placed per hectare in the field. Inside one out of 3 traps we placed an attractant for males (trimedlure emitter). Besides, all the traps contained an attractant for females (trimethylamine, putrescine and ammonium acetate (1%:0,1%:1%) (wt:wt)) included in the gel. We needed to place three more times female attractants due to the fewer efficacy of them compared with TML ones. In order to attract a big percentage of females to the traps we needed that the attractants for females stayed closer.

Male and female attractants were placed to be effective at large distances. Once near the trap, the bait gel will attract flies to eat it. When flies become satiated with protein bait they will leave the trap. All the females that eat from the gel will become sterilised, and males that ingest the gel will transmit the sterility when copulate with females. Therefore, we use male flies to transmit sterilization to the medfly population.

Sterilant traps were hanged on first of July in Sagunto while malathion treatment began on first of August. Malathion bait spray was repeated every 7 days until fruit harvest.

In Denia sterilant traps were hanged in the first week of March and were replaced in May.

Finally, sterilant traps were placed in field trials on June 26 and mass trapping was hanged on July 18.

Method efficacy evaluation

To monitoring medfly population we have used five Nadel traps (from AgroAlcoy, Alicante, Spain) with a TML+DDVP emitter per hectare. All the traps were recorded once at week. Emitters of TML were changed every 3 weeks.

To check the efficacy of this method against Ceratitis capitata we also recorded the fruit damage one week before harvest in the field of Gandía. There was selected a sample of 20 trees per hectare in the lufenuron treated zone and 20 trees in the mass trapping zone.

The fruit was left overripe in trees until June in Denia. Then, 9 trees were revised in the untreated zone and 9 trees in the lufenuron treated zone.

One hundred fruits per tree were examined in both fields.

Results and discussion

Evolution of medfly population is shown in figure 1 (Sagunto), figure 2 (Denia) and figure 3 (Gandía).

We can observe that medfly populations were very similar in treated and reference fields at the beginning of the trials. However, about 45 days after hang the sterilizing traps, the population in treated fields was significantly reduced in Denia and Gandía. Reference field was treated with malathion in Sagunto and, in this case, there was no significant differences between the two orchards in medfly population.

First positive results obtained in population dynamics appeared two moths after the beginning of the treatment with lufenuron. This could be explained because it is necessary to let one generation to occur for observing a reduction of the population.

Evaluation of fruit damage is shown in table 1. Fruit damage was obviously reduced with lufenuron treatments when compared with the untreated field. However lufenuron treatments were as efficient as malathion for protecting fruit.
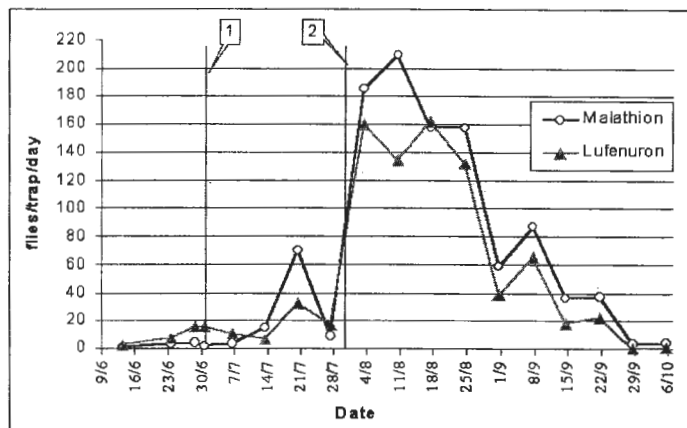

Figure 1: Evolution of captures in the field treated with lufenuron and in the field treated with malathion in Sagunto.
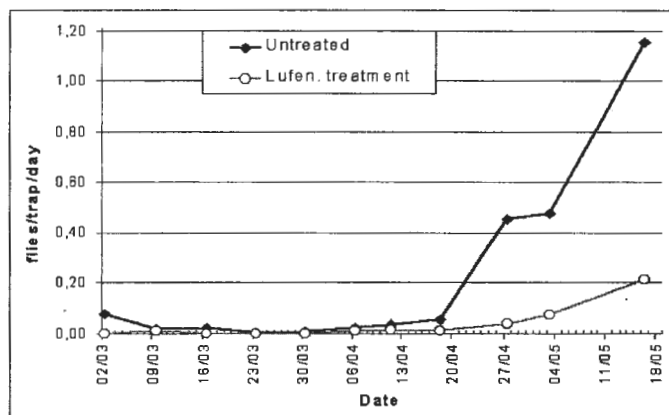

Figure 2: Evolution of captures in the untreated field and in the field treated with lufenuron in Denia.
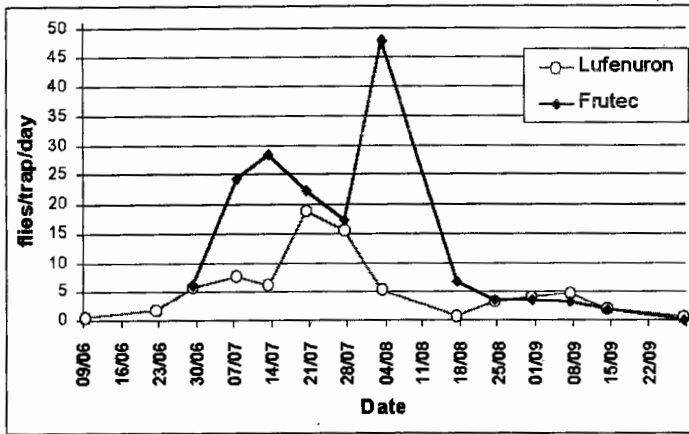
Figure 3: Evolution of captures in the mass trapping field and in the field treated with lufenuron in Gandía.

Table 1. Fruit damage in Gandía and Denia.

	Per cent average of punctured fruits ± E.S.	
	Gandía	Denia
Lufenuron treatment	1,70±0,49 [a]	35,25±1,75 [b]
Reference field	1,71±0,72 [a]	99,67± 0,33 [a]

[a] Values of a period with the same letter within a column are not significantly different in t-student test (P≤0.05).

Reference field in Gandía was treated with Frutec mass trapping. Reference field in Denia was not treated.

In conclusion, first year treatments reduce medfly population, but this treatment is not effective enough to avoid fruit damage completely.

Dimension of trial field is very important in order to obtain the best results. In this case we have searched for three field isolated from other orchards to prevent medfly invasion. It is known that medfly looks for hosts trees and it is able to move searching oviposition hosts (Israely et al, 1997). Therefore this method is no applicable in small orchards or when the frontier with untreated fields is very long.

The sterilizing effect is accumulative (Knipling 1955), so this treatment will be very effective when it will be repeated for two or three years. Currently, a three-year trial is being made in a larger zone in order to verify the efficacy of this method.

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Susceptibility of *Ceratitis capitata* to the control by entomopathogenic fungi

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Abstract: The effectiveness of a new control method of *Ceratitis capitata* using entomopathogenic fungi has been evaluated. Previous works in our laboratory demonstrated the susceptibility of *Ceratitis capitata* to the entomopathogen *Metarhizium anisopliae*.

In this work we describe the design and development of an attractant-contaminant system and its preliminary assessment in field trials. This system is selective due to the use of specific attractants of the medfly. In addition, a long-time attractant-contaminant effect is obtained because of a controlled-release emitter included in the trap, and because the persistence of the conidia is notably increased due to the humidity control in the trap.

The trap showed high effectiveness provoking pathogenicity during more than three months in laboratory conditions. Preliminary results in the field show that the system is able to reduce the medfly population.

Susceptibility of the Mediterranean fruit fly (*Ceratitis capitata*) to entomopathogenic nematode *Steinerma spp* ("Biorend C")

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Abstract : Several laboratory assays were carried out to determine the susceptibility of *Ceratitis capitata* (medfly) immatures to "Biorend C" (a mixture of entomopathogenic nematodes (*Steinernema spp.*) with chitosano, from Idebio/ABF, Spain). Initial laboratory tests showed high susceptibility of medfly larvae to infection by these nematodes, with > 90% mortality. The activity was higher on sandy-loam soil than on clay soil. Further trials were carried out to investigate the response of the medfly to the entomopathogenic nematodes under field conditions. Late third instar larvae and pupae of *C. capitata* were introduced into soil previously infested with 500,000 infective juveniles per square metre of *Steinernema spp.* Different types of soil coverage or insolation were compared. Overall, nematodes produced mortalities of 70% on larvae and no effect on pupae. The highest mortalities were observed in soil covered with mulching, shaded or wet, compared with exposed to the sun, weed covered or bare soil. Nematodes may offer a non-toxic alternative to soil treatments for Mediterranean fruit fly control programs.

Key words: *Ceratitis capitata, Steinernema,* entomopathogenic nematodes, Citrus.

Introduction

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is one of the most damaging pest attacking citrus crops. Usually the adult is the only stage of the medfly targeted when applying pesticides to control de pest, but an important period of the biological cycle of *C. capitata* develops inside the soil, as late third larvae and pupae. Control treatment applied at this stage would reduce the number of adults considerably, avoiding fruit damage and also reducing the population for the next generation. Previous research work demonstrated that parasitic nematodes were able to infest and kill both stages (late third instars and pupae) of *C. capitata* in the soil (Lindegren and Vail, 1986; Lindegren et al, 1990; Gazit et al, 2000).

The objective of this research was to evaluate the mortality caused by enthomopathogenic nematodes in soil stages of *C. capitata*. "Biorend C", a new patented formula including a mixture of chitosano with entomopathogenic nematodes (*Steirnema spp.*), from Idebio/ABF, Spain, was tested.

Material and methods

Laboratory assay
The susceptibility of late third instar larvae of *C. capitata* to "Biorend C" was evaluated. The soil inside a plastic cylinder 1.3 cm high and 14 cm in diameter was infested with nematodes. Two rates, 500,000 nem/m² and 250,000 nem/m², plus an untreated cylinder as control, were used. The experience was carried out in two different soil textures, sandy-loam and clay.

There were four replicates per treatment. The Abbot's formula was applied to calculate the efficacy.

Field trial

The experiences were carried out in three citrus orchards with drip irrigation, with different soil management each, bare soil, with mulching (crushed prunning branches) and weed covered. On each orchard, three soil positions with different moisture levels were compared, wet (soil permanently humid because of its proximity to the drip), shaded (soil under the tree canopy) and sunny (soil out of the tree canopy). On each soil position, a soil plot of 1 m^2 was infested with netamodes and another plot was left unsprayed as control. The plots were irrigated before and after the treatment. The amount of "Biorend" applied was adjusted so that the number of infective juveniles per square metre of *Steinernema spp.* was 500,000. Two plastic cylinders 8 cm high and 3.6 cm in diameter were inserted in the soil, one of them containing 10 late third instar larvae of *C. capitata* (in order to have pupae in this cylinder at the moment of the treatment). Six days later, 10 late third instar larvae were introduced in the second cylinder and the nematode treatment was applied. A plastic vessel 12 cm high and 6 cm in diameter, with a yellow plastic trap of 3x4 cm covered with sticky resin fixed on its bottom, was placed on the plastic cylinders to evaluate the emergency of the adults.

The experimental design included three factors, development stage of medfly at two levels (late third instars and pupae), soil type at three levels (bare, mulching and covered) and soil humidity and three levels (wet, shaded and sunny). All cases included one treated and one untreated cylinder, and four replicates per treatment were done. A factorial analysis of variance (ANOVA) was carried out. The square root transformation was applied to values prior to analysis.

Results and discussion

Laboratory test

Results in laboratory showed susceptibility of third instar larvae of *C. capitata* to infection by nematodes, with similar values for the two rates tested. Efficacy was much higher in sandy-loam soil (88 ± 5% and 99 ± 1% of corrected mortality with 500,000 and 250,000 nematodes/m^2, respectively) compared with clay soil (52 ± 17% and 57 ± 21%).

Field tests

Mortality was rather high in untreated controls and similar in larvae and pupae, ranging from ≈ 30% in sunny and covered soil to ≈ 50% in wet and mulching soil. The treatment with nematodes had apparently no effect on pupae of the medfly as mean mortality observed in treated plots was 41 ± 5%, compared with 45 ± 6% in the controls (F = 0.40; P = 0.5312).

On the contrary, mean mortality observed with larvae was significantly higher (81 ± 4%) than mean mortality observed in untreated plots (36 ± 5%) (F = 46.38; P = 0.0000). This represents that the efficacy or corrected mortality caused by the nematodes to medfly larvae was 70%.

In relation with the other two factors analysed, soil cover and soil humidity, it was observed that mean mortality (considering together data from larvae and pupae, and treated and untreated with nematodes) was significantly higher in soil with mulching (61 ± 5%) compared with weed covered (47 ± 5%) or bare soil (44 ± 4%) (F = 4.29; P = 0.0156). Soil exposed to the sun showed lower mean mortality (42 ± 5%) compared with shaded (54 ± 5%) or wet soil (56 ± 5%) (F = 3.03; P = 0.0518). Both results indicate that, in general, conditions

of shade and humidity in the soil increase natural mortality in stages of medfly developing in the soil.

As a conclusion, the levels of mortality in the laboratory and in the field have been satisfactory for late third instars of *C. capitata*. Nematodes may offer a non-toxic alternative to soil treatments for *C. capitata* to be included in IPM control programmes.

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Rearing methods of two braconid parasitoids used in the biological control of *Ceratitis capitata*

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Abstract: A research work about the possibilities of carrying out the biological control of the Mediterranean fruit fly, *Ceratitis capitata*, with exotic parasitoids has been started in Spain. For that purpose, two hymenopterous braconid species, the larva-pupal parasitoid *Diachasmimorpha tryoni* and the egg-pupal parasitoid *Fopius arisanus*, have been imported from Hawaii, maintained in quarantine facilities, and reared in laboratory conditions. The rearing methodologies for each parasitoid species and the results obtained are discussed.

Key words: Tephritidae, Braconidae, Medfly, *Ceratitis capitata*, biological control, parasitoid rearing, *Diachasmimorpha tryoni*, *Fopius arisanus*

Introduction

The biological control of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera, Tephritidae), has been developed and applied in field in several countries, and in some cases this method has reached a great success in the management of the pest (see Table 1). The first attempt to use biological control methods against the Medfly with exotic parasitoids was performed in Australia in 1902; nowadays classical biological control of Medfly is successfully being used in South America and Central America and in Hawaii (Wong *et al.*, 1991; Headrick & Goeden, 1996; Sivinski, 1996; Bautista *et al.*, 1999; Morales *et al.*, 1999).

In Spain, Medfly biological control had been attempted at the beginning of the XX Century by the importation of exotic parasitoids: in the 1930's two species of braconids (Hymenoptera, Braconidae), *Opius humilis* Silvestri, 1913 (= *Psyttalia incisi* (Silvestri, 1913)) and *Opius tryoni* Cameron, 1911 (= *Diachasmimorpha tryoni* (Cameron, 1911)) from Hawaii were imported into the Valencian Comunity (Spain), but it was not a success due to the failure of the parasitoid rearing process in laboratory (Gómez Clemente, 1932, 1934). Later, the hymenopterous eulophid *Tetrastichus giffardianus* Silvestri, 1915 was introduced into the island of Tenerife (Canary Islands) in 1960, and nowadays this parasitoid can be found in the field parasitizing the Medfly, but no analysis about its beneficial effect has been performed (Moner *et al.*, 1988). Another braconid parasitoid species, *Diachasmimorpha longicaudata* (Ashmead, 1905), was imported by the I.N.I.A. (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain) in 1979 from Greece and it is still being kept on a laboratory rearing, but it has never been tested against the pest in field trials.

Now, once again our research group is considering to study the possibilities of using exotic parasitoid species as biological control agents against the Medfly in Spain, but this work will take into account the new methods to rear insects in laboratory and their later release in field. For that purpose, we have imported two braconid species from Hawaii: *Diachasmimorpha tryoni* (Cameron, 1911) and *Fopius arisanus* (Sonan, 1932) (Hymenoptera, Braconidae, Opiinae).

D. tryoni is one of the candidate species for the control of the Medfly in Hawaii (Wong & Ramadan, 1992), and also it is now being used in biological control programmes in Guatemala (Sivinski, 1996). *F. arisanus* is an important candidate for the augmentative release method against the Medfly in Central America (Harris & Bautista, 1996; Vargas *et al.*, 1999), and its mass rearing has recently been improved (Wong & Ramadan, 1992; Bautista *et al.*, 1999; Calvitti *et al.*, 2002). This will facilitate the use of this parasitoid in control programmes.

In this publication we explain the procedures used for the importation and the laboratory rearing of the insects.

Table 1. Hymenoptera species recorded as parasitoids of *Ceratitis capitata*. Capital letter at right of scientific names indicates the countries where they are extensively used in biological control programmes against the Medfly (H: Hawaii, F: Florida, C: Costa Rica, G: Guatemala, A: Argentina).

Hymenoptera: Braconidae: Opiinae		other Hymenoptera		
		Diapriidae:	*Coptera haywardi* (Oglobin)	
Biosteres fullawayi (Silvestri)	H		*Coptera occidentalis* (Muesebeck)	
Diachasmimorpha longicaudata (Ashmead)	HFCGA		*Coptera silvestri* Kieffer	H
Diachasmimorpha kraussii (Fullaway)	HG	Chalcididae:	*Dirhinus anthracina* Walker	H
Diachasmimorpha tryoni (Cameron)	HG		*Dirhinus giffardii* (Silvestri)	
Doryctobracon crawfordi (Viereck)	G	Eulophidae:	*Aceratoneuromyia indica* (Silvestri)	A
Fopius arisanus (Sonan)	HC		*Tetrastichus giffardianus* Silvestri	H
Fopius vandenboschi (Fullaway)	H	Pteromalidae:	*Muscidifurax raptor* (Girault & Sanders)	
Psyttalia concolor (Szepligeti)	H		*Pachycrepoideus vindemmiae* (Rondani)	A
Psyttalia incisi (Silvestri)		Eucoilidae:	*Ganaspis carvalhoi* (Dettmer)	
			Odontosema anastrephae (Borgmeier)	

Material and methods

Importation and quarantine
Both *D. tryoni* and *F. arisanus* were imported from the U.S. Pacific Basin Agricultural Research Center (USDA-ARS) at Hawaii in August 2002.

The original lots of parasitized pupae of *Ceratitis capitata* were put in the quarantine facility located at our Center. After a few days, adults of both parasitoids began to emerge from parasitized pupae. Some of these wasps were prepared for a voucher collection.

When the *D. tryoni* population reached 3rd generation, and that of *F. arisanus* reached 2nd generation, the parasitoid rearings were transferred to a climatic room.

Parasitoid rearing
The rearing of both parasitoids is being developed on the fruit fly *C. capitata* as host material. The host is being reared on artifical diet in accordance with Albajes & Santiago-Alvarez (1980).

The rearing process is explained in Figure 1. The parasitoids are placed into the Adult Cage. In the Parasitoidism phase, larvae of third instar and eggs of *C. capitata* are exposed to parasitoids in order for them to be attacked by *D. tryoni* and *F. arisanus* respectively in each case: the larvae are put on the mesh of the upper side of the adult cage, and the eggs are placed in an artificial "egg-laying bottle", as described in Calvitti *et al.* (2002) which in turn is introduced into the adult cage. The host, as third instar or egg, continues its development on a plate with the artificial diet up to the pupal stage. The plate with putative parasitized pupae is placed into a new cage in which the adult parasitoids emerge from host pupae.

The general aspects of these rearing methodologies have been provided by colleagues from I.N.I.A. (Spain) for *D. tryoni* (Jiménez & Castillo, 1992) and from E.N.E.A. (Italy) for *F. arisanus* (Calvitti *et al.*, 2002).
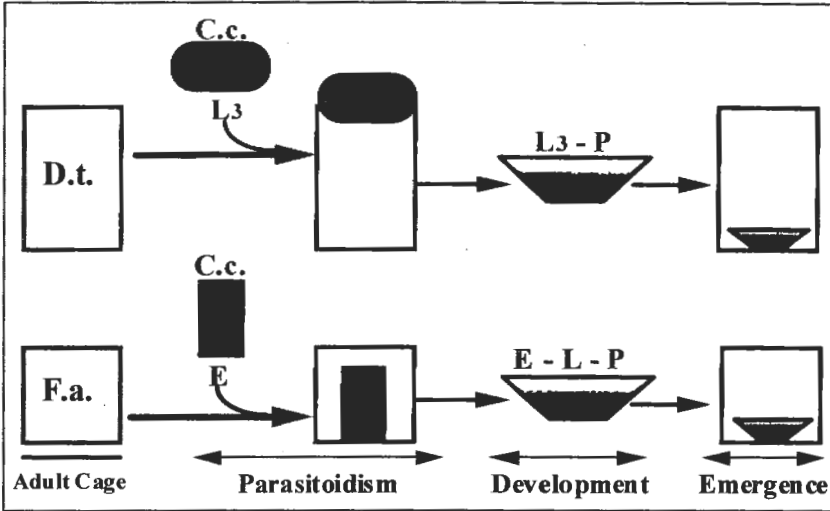

Figure 1. Rearing phases of the parásitoids *Diachasmimorpha tryoni* (D.t.) and *Fopius arisanus* · (F.a.) on the host *Ceratitis capitata* (C.c.). E: egg, L: larval stage, L3: third larval instar, P: pupa.

Results and discussion

In this moment, both parasitoid populations are successfully maintained in laboratory. In the case of *D. tryoni*, it has reached the 5th Spanish generation. *F. arisanus*, because of its longer life cycle, has reached the 3rd Spanish generation.

We are trying to improve the rearing methodology in order to know the best parameters and conditions (i.e. age or stage of the host, time of exposure to parasitoidism, age of parasitoids, ...) as this will facilitate the mass rearing and field releases of these insects.

Acknowledgements

We thank colleagues from I.N.I.A. (Madrid, Spain) and E.N.E.A. (Rome, Italy) for their help and kindness when we visited their laboratories. And Dr. Bautista from USDA-Hawaii for sending the parasitoids to start our rearing.

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COCCID SCALES

Methods of estimating degree-days of scales in citrus

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Abstract: One of the most important factors to achieve a good control of citrus armored scales (Homoptera: Diaspididae) is the timing of application of pesticides, which is related with the percentage of immature stages in the population. Average daily temperature and sampling data from seven citrus orchards were analysed to develop a predictive equation, with accumulative degree days, for the first maximum of immatures of the citrus red scale *Aonidiella aurantii*. The descriptive analysis showed a sigmoidal trend in the graphic result. Relating accumulated degree-days with accumulated percentage of immatures (expresed as probability units or "probits") a generalizable linear model was obtained, with the possibility of comparing significant differences between orchards.

Key words: Degree-day, diaspididae, citrus, *Aonidiella aurantii*.

Introduction

One of thet key issues in the chemical control of citrus armored scales (Homoptera: Diaspididae) is to determine the moment when the phytosanitary treatment must be applied, as different life stages are not equally susceptible to insecticides (Schoones and Giliomee, 1984) and sprays should be timed in relation to the phonological development of the target pest. For example, effectiveness of California Red Scale control was improved by timing insecticide applications to coincide with increases in crawler production in the population (Walker et al, 1990). Estimating experimentally the relative proportion of different development stages in a scale population is a common task for Agriculture Technicians; however, this task requires several worker-hours if representative results want to be obtained.

Phenology models predict timing of events in an organism's development. For many organisms which cannot regulate internally their own temperature, development is dependent on temperatures to which they are exposed in the environment. Insects are heterotermic animals and consequently their development speed is linked to the temperature. The "degree-days" or "effective temperature summation" method can predict population phenology from climatic data and allow to forecast the set-in of a phenological stage (Wilson and Barnett, 1983; Zalom et al, 1983). In diaspidid scales the "degree-days" method is applied to determine the time of the year when immature proportion reaches its maximum. To apply routinely this procedure, previous studies should be made in different orchards and years to correlate population phenology data obtained experimentally with effective temperature sumations above a pre-established threshold.

In this paper we report on the methodology used to adjust these correlations in one of the main species of diaspidid scales in citrus, the citrus red scale *Aonidiella aurantii* (Maskell).

Material and methods

Phenological and climatic data from seven orchards between 1999 and 2000 were used. Climatic data were taken from nearby meteorological stations or from data loggers installed in the same orchards. Orchards were full grown commercial plantations located in an area of 50 km around the town of Valencia.

The low temperature treshold considered in order to determine degree-days with *Aonidiella aurantii* was 11.6°C (Kennett and Hoffman, 1985). Computation of degree-days started on 1st of January. Daily temperatures were obtained as the intermediate between maximum and minimum values for the day.

California Red Scale phenology was monitored by sampling weekly branches and leaves containing scales. In the laboratory scales were checked counting the number of live individuals, separating between immatures (first and second instar larvae) and adults (adult females, and prepupa, pupa and adult males). A minimum of 150 insects were counted on each sampling date.

For each specie, a descriptive analysis was considered in order to obtain lineal regression solutions as adjusted as possible. The model's benefit was obtained by adjusting the correlation coefficient.

Results and discussion

Figure 1 shows the relationship between the phenological development of *Aonidiella aurantii*, expressed as the accumulated percentage of immature stages in the population, and accumulated degree-days since 1st of January, in the seven orchards sampled. The graphic shows a non linear trend with the aspect of a sigmoid line. To obtain a model easier to use for predictive purposes, we proceed to transform the variables to obtain a linear relationship.
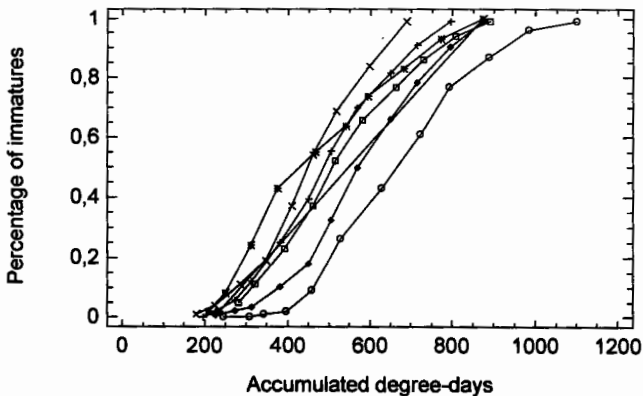

Figure 1. Relationship between percentage of immatures and degree-days for the first generation of *Aonidiella aurantii* in seven citrus orchards.

Some authors have suggested to use of the "Probit" transformation applied to the degree-days accumulated values (Del Tio et al, 2001). Else, the decimal logarithm was applied to the accumulated percentage of immatures of *Aonidiella aurantii*. As the variable that we want to predict is the degree-day accumulated value, this variable is moved to the ordinate axis. Figure 2 shows the results after transforming the variables and changing the axis. The relationship between both variables becomes almost lineal.
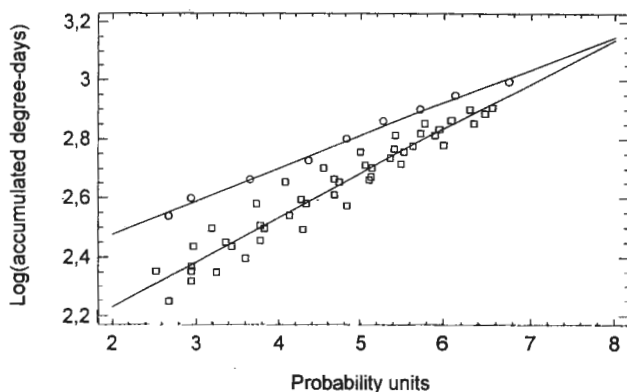

Figure 2. Relationship between percentage of immatures and degree-days for the first generation of *Aonidiella aurantii*, after transforming the axis.

The next step to obtain a generalization of the results was to locate and eliminate from the analysis orchards that differ significantly from the main tendency. In the Fig. 2 one orchard shows apparent differences in the linear regression. This orchard presented coefficients of the model significantly different (student's t-test; for the slope, $t = 3.05$, $P < 0.01$; for the independet term, $t = 5.21$, $P < 0.01$), and consequently these data were eliminated from the general model.

For the six remaining orchards, a unique lineal model was obtained with the equation:

$$logD = 1.928 + 0.1513*P \quad (R^2 = 94\%)$$

Being "D" the accumulated degree-days and "P" the accumulated percentage of immatures, expresed in probits.

The "lack of fit" test was not significant ($F = 1.81$; $P = 0.35$), so the complexity of the model was considered sufficient and adequate for the observed data. With this model, the

predicted values of the accumulated degree-days for several accumulated percentages of immatures are shown in table 1.

Table 1. Predicted values of degree-days and prediction limits as a function of percentage of immatures in the first generation of *Aonidiella aurantii*, with the model described in the text.

Percentage of immatures	Degree-days predicted	Prediction limits (95%)	
0.2	361	287	453
0.4	443	353	556
0.5	484	385	607

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The process of colonization of growing citrus fruits by three diaspidid species

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Abstract: The process of invasion of the fruit surface by three species of armored scale insects (*Lepidosaphes beckii* (Newman), *Parlatoria pergandii* Comstock and *Aonidiella aurantii* (Maskell)), has been studied in Citrus orchards in València, Spain. By means of sticky tape traps located around branches, three moments of intense crawler migration were detected throughout the year. The circulation of crawlers increased along the year and they moved preferentially outwards in the tree canopy.

The three species invaded the fruits continuously during the period of fruit growing, starting at a very early stage of fruit development by crawlers of the first generation. We found differences in settling and fruit invasion behaviour between the three species. First generation crawlers tend to settle under the fruit calyx in *L. beckii* and *P. pergandii*, but not in *A. aurantii*. During the second generation, most scales of *A.aurantii* originate from the reproduction of females already settled on the fruit, whereas in *L. beckii* and *P. pergandii* they come preferentially from other tree substrates or from females settled under the fruit calyx.

Field parasitoids of *Aonidiella aurantii* (Homoptera: Diaspididae) in Valencia (Spain)

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Abstract: California red scale, *Aonidiella aurantii* (Maskell) is considered a citrus key pest in Spain. Nowadays red scale is controlled using non-selective organophosphate pesticides. Fortunately, there is a trend throughout the world to minimise the use of insecticides in citrus and to promote the control of pests by the use of natural enemies. The first step previous to natural enemies releases is to know field parasitoid structure and to study parasitoids interaction. A survey was carried out in a citrus field in Valencia (East coast of Spain). As a result, two parasitoids were found: *Aphytis chrysomphali* and *Aphytis melinus*. *A. chrysomphali* was the predominant species. *A. melinus* was mainly found on third instar female scales and *A. chrysomphali* on male scales. The sex ratio was 1:1 for *A. melinus* and it was dependent on the host size. Only females of *A. chrysomphali* were found.
Key words: *Aonidiella aurantii*, *Aphytis chrysomphali*, *Aphytis melinus*, parasitoid structure, host stage, host size, parasitoid sex ratio.

Introduction

California red scale, *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae), is nowadays one of the most important economic pests in the Spanish citrus areas. The red scale is controlled by organophosphate insecticides and IGR treatments. Fortunately, the present tendency to control pests is focused on reducing chemical treatments and on looking for the most selective products. Furthermore, farmers who wish to develop an integrated pest management program are often interested in natural enemy releases.

The first step to develop or implement an IPM program with natural enemy releases is to study the parasitoid structure present in the field.

The most recent work about California red scale parasitoids in Spanish citrus areas was done between 1989 and 1991 (Rodrigo, 1993). Two species of *Aphytis* were found all through this monitoring: *Aphytis chrysomphali* (Mercet) that represented approximately 98% of red scale parasitoids and *A. melinus* DeBach that was just 2% (Rodrigo, 1993).

A. chrysomphali is an autochthonous parasitoid of the Mediterranean Basin. This parasitoid was first described from Mercet in Spain in 1912. Since that time, its presence in citrus areas has changed from being almost cosmopolitan to be clearly reduced as a consequence of the competitive displacement exercised by other species of the same genus. In California, no *A. chrysomphali* is reported (DeBach & Sundby, 1963), in Southern Italy it does not appear in the parasitoid catalogues of *A. aurantii* (Siscaro et al., 1999), in Cyprus it has been virtually displaced from the interior dry areas, and a similar situation has been observed in South Australia (Furness et al., 1983).

A. melinus was imported from India and Pakistan and introduced in southern California, where it displaced the other species of *Aphytis* (Rosen & DeBach, 1979). In Spain it was first introduced in 1976 from the "Station de Zoologie et de Lutte Biologique" of Antibes (France) to control *Chrysomphalus dictyospermi* (Morgan). Releases were intensified in 1985 when *A.*

aurantii was first detected as a pest. In 1996, more specimens of *A. melinus* were reintroduced from the Insectary of California, Riverside.

The aims of this work are to study red scale parasitoid structure and parasitoid fluctuation after ten years of the last field monitoring. Furthermore, the scale stage attacked by each parasitoid and parasitoid sex ratio are evaluated.

Material and methods

The monitoring was carried out every fifteen days from June 1999 to June 2000 in an orange field located at Alzira (Valencia) close to the first focus of *A. aurantii* as a pest. Red scale in this citrus grove was under biological control.

Ten shoots of 25 cm long, containing leaves, twigs and fruit (from fruit set to harvest) were picked up each time. The three different plant regions were examined individually. For the analysis, twigs and leaves were evaluated together and fruit was studied separately.

The recorded data were:
a) Parasitoid species (*A. melinus*, *A. chrysomphali* or *Aphytis* spp.)
b) Parasitoid stages (egg, larva, pupa, adult or exuvia), parasitoid number and parasitoid sex.
c) Host scale stage (second instar female, third instar female or male scale)

Immature stages were placed inside gelatine capsules until their emergence as adults or their death as immatures.

Adults were identified through microscope identification after preparing them on Hoyer's medium as explained by Rosen & DeBach (1979). Pupae and exuviae were identified using the different colour pattern that they have (Rosen & DeBach, 1979).

When parasitoids died as egg or larva, or pupa and exuvia were unrecognisable, they were added to *Aphytis* spp. column.

Results

Parasitoid species
A total of 3371 identified parasitoids were found throughout one year survey. The two parasitoid species obtained during the monitoring were *Aphytis chrysomphali* and *A. melinus*.
A. chrysomphali was the predominant species with 2619 specimens.
Parasitoid annual distribution
Annual distribution of all parasitoid stages (except exuvia) and parasitoid species was evaluated plotting separately leaves and twigs from fruits. A different tendency in parasitoid distribution was observed between both substrates. Whereas the parasitoid population on leaves and twigs remained constant all through the year, the parasitoid population on fruit showed a maximum in October and stayed with high values until the fruit harvest (Figure 1).

Parasitoid species abundance in the pupa and adult stage was analysed in both substrates. *A. chrysomphali* was the predominant species all through a year. *A. melinus* was also present all along the sampling period but remained at low numbers except for the summer months, when *A. melinus* was the most abundant species (Figure 2 and Figure 3).
Host scale
Host scale stage was different for both parasitoid species. *Aphytis* generally prefer to oviposit on third instar female scales because of their large size. The larger the scale, the larger is the resulting *Aphytis* offspring, or the more offspring can be produced per scale (Forster et al., 1995). Male scales can be considered as scales of medium size and second instar females as scales of small size. However, *A. chrysomphali* clearly preferred male scales, followed by

second instar females and virgin third instar females. On the other hand, *A. melinus* preferred third instar females, followed by male scales and finally second instar females (Figure 4).

The same tendency was observed in both plant substrates.

Parasitoid sex ratio

A. melinus sex ratio was close to 1:1 whereas identified *A. chrysomphali* adults were practically always females (1 male out of 351 females).

A. melinus sex ratio was influenced by the host size and by the number of eggs laid on the host scale, as reported by Luck et al. (1982). *A. melinus* generally laid male eggs when the host scale was of small size and female eggs when the host scale was of large size (Table 1).

When more than one egg is laid in the same scale, several sex ratio combinations for *A. melinus* are possible. However, the unique parasitoid structure recorded in the field was 1:1 when two eggs were laid in the same scale. Host scale was always third instar female. Smaller scales could not hold more than one egg. If more than one egg was found in the scale, only would survive one.

On the contrary, *A. chrysomphali* sex ratio was independent of the host size and of the number of eggs laid on the scale. In all cases, only females were obtained.
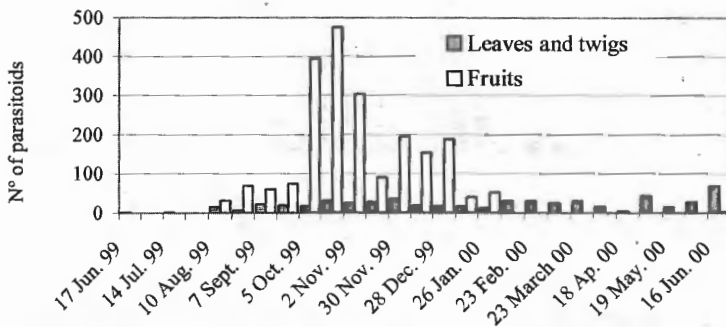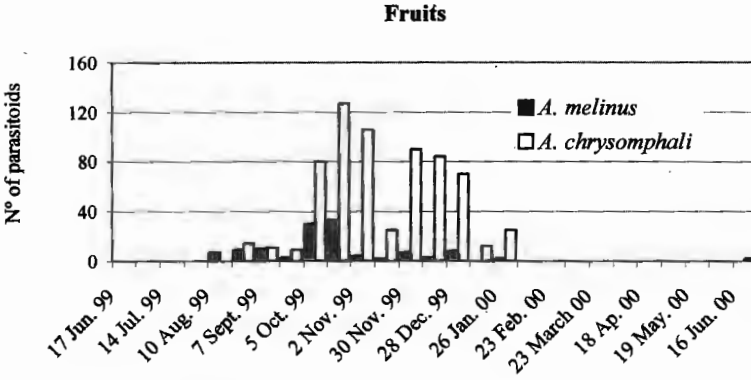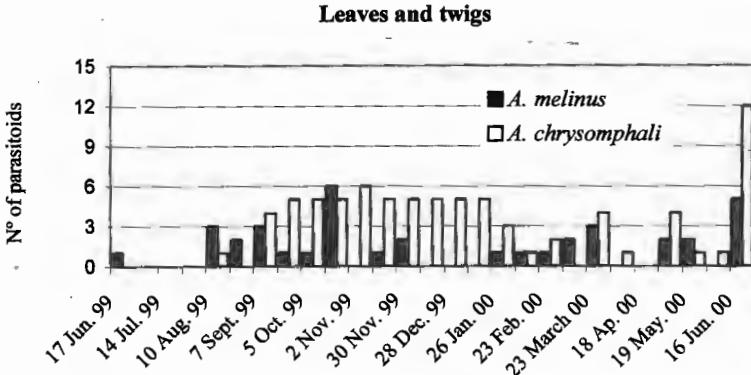

Figure 1. Annual distribution of *A. aurantii* parasitoids on two citrus plant substrates all through a year. All parasitoid species and parasitoid stages except exuvia are shown in this figure.

Figures 2 and 3. Parasitoid species abundance on California red scale, in the pupae and adult stage, in both citrus substrates for one year survey.
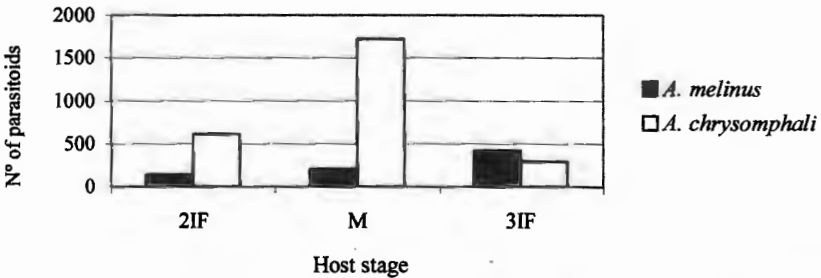

Figure 4. Red scale stage attacked by each parasitoid species. (2IF: Second instar female; M: Male scale; 3IF: Third instar female).

Host scale stage	A. melinus		A. chrysomphali	
	Total adults	*% male presence*	*Total adults*	*% male presence*
2IF	9	89	79	0
M	27	74	240	0.04
3IF	17	24	33	0

Table 1. Percentage of male presence from the total number of adults of each parasitoid species, depending on the host scale stage attacked. In *A. melinus,* as the host scale increases in size, the male parasitoid presence decreases (2IF: Second instar female; M: Male scale; 3IF: Third instar female).

Discussion

After ten years of the last field monitoring, *A. chrysomphali* is still the predominant parasitoid in the East coast citrus fields despite the mass releases of *A. melinus* and the expected competitive displacement exercised by *A. melinus* in other countries. This situation contradicts what happened in other places, where *A. chrysomphali* was complete or partially displaced by *A. melinus* after some years of coexistence (DeBach & Sundby, 1963; Furness et al., 1983; Orphanides, 1984; Siscaro et al., 1999).

It has been speculated that specimens of *A. melinus* introduced from Antibes were a different strain of this species (Troncho et al., 1992). If this hypothesis were accepted, and the strain that really gives good results in terms of parasitism rates was that introduced from California, it would be necessary to wait for a reversal of the situation in some years. However, the actual trend is that *A. chrysomphali* is still the most abundant parasitoid.

Furthermore our results dissent with those obtained by Troncho et al. (1992) and Hafez (1988) as far as *A. chrysomphali* does not show three clear peaks of activity and can be assumed that its presence is almost constant all through the year with a maximum in autumm.

A. melinus also appears throughout the year as *A. chrysomphali* except for the winter months when it disappears, and for the the summer months when it is more abundant than *A. chrysomphali*. Abdelrahman (1974a) described that *A. melinus* presents a better adaptation to extreme hot weather and *A. chrysomphali* to cold weather.

On the other hand, annual fluctuation of parasitoids clearly depends on the substrate. Parasitoid abundance is almost constant on leaves and twigs where a stable scale population can be found. However, scale population on fruit experiments an exponential increase, from the moment they reach the fruit on the first generation (Rodrigo & García-Marí, 1994) and, as a consequence, the parasitism rate also shows a quick rise confirming the density dependence response. McLaren & Buchanan (1973) observed a similar density-dependence trend between scale and parasitoid density.

The preferred host stage by *A. chrysomphali* is the male scale. The same result was obtained by Abderahman (1974b) and Rodrigo (1993). Smith (1978) also pointed that *A. chrysomphali* parasitizes more frequently second instar female, second instar male and prepupa. Third instar scale is the scale stage preferred by *A. melinus* confirming the results obtained in laboratory by Abdelrahman (1974b) and Forster et al. (1995).

All throughout one year of field monitoring, *A. melinus* sex ratio was close to 1:1 and it was clearly variable depending on the host size. Luck et al. (1982) reported that *A. melinus* is

a biparental species that can control the sex of its progeny depending on the host size. However, *A. chrysomphali* is an uniparental species and male presence occurs in a really low percentage in the field (Rosen & DeBach, 1979). During the monitoring, almost all *A. chrysomphali* adults obtained were females and its presence was independent of host size. This fact gives *A. chrysomphali* advantage when the extreme temperatures prevail over the arrhentokous *A. melinus*, because the population of *Aphytis* would be extremely low. *A. chrysomphali* starts reproducing female progeny a few hours after emergence, whereas female *A. melinus* will produce only male progeny until fertilization. This would reduce the possibility of *A. melinus* of leaving female progeny when killed by extreme temperatures (Abdelrahman, 1974a).

The next step is to study the relationship between percentage parasitism and percentage of suitable scales for parasitoid reproduction to determine parasitism rate.

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Effects of the mineral oil Sunspray Ultrafine[R] on California red scale parasitoids *Aphytis chrysomphali, A. lingnanensis, Comperiella bifasciata* and *Encarsia perniciosi*

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Abstracts: California red scale, *Aonidiella aurantii,* is a major pest of citrus worldwide. Parasitoids play a fundamental role in the control of the pest. *Aphytis chrysomphali* is a native species that has not been displaced by the introduced *A. melinus*. The species *A. lingnanensis, Comperiella bifasciata* and *Encarsia perniciosi* have been recently introduced to implement the biological control of red scale.

Laboratory experiments were carried out on larvae and pupae of the natural enemies to determine the effects of mineral oils used against citrus pests.

Oil was applied to parasitoids populations at the recommended dosages (1.5%). Mortality of larvae and pupae were recorded.

Mortality of larvae and pupae of the ectoparasitoids *A. chrysomphali* and *A. lingnanensis* was 98%.

Mortality of pupae of the endoparasitoids *C. bifasciata* and *E. perniciosi* was 85%.

The lack of persistence and the absence of resistance mechanisms on target arthropods, make the use of mineral oils safer to the natural enemies than the conventional insecticides nevertheless the detrimental effects of the application.

Efficacy of the mineral oils Sunspray Ultrafine[R] and Ivenol-G[R] on California red scale *Aonidiella aurantii.*

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Abstract: Mineral oils are considered, mainly, contact insecticides. They penetrate a short distance into the main tracheal trunks and kill the insect by interference with respiration. They have been recommended against scale insects for years, their use only limited by the risk of phytotoxicity.
Sunspray Ultrafine[R] is a paraffinic mineral oil 85% p/v and Ivenol-G[R] is a white oil 72% p/v .
Spray applications were done with a Potter equipment at different volumes, dosage of 1.5% oil concentration and 0.5 bar air pressure.

California red scale is a citrus pest of great economic importance and resistance to organophosphate insecticides has been documented. Oil treatments are recommended when a biological control program is established in the area.

Oils were applied to lemons infested with the following red scale stages: pupa-prepupa, moult II, young female and gravid female.

Efficacy was positively related to volume increase and negatively to red scale size. Sunspray Ultrafine[R] was more effective in all aspects than Ivenol-G[R].

Modelling the mortality of the California Red Scale (*Aonidiella aurantii* Maskell) produced by a mineral oil application in laboratory conditions

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Abstract: The high cost and the negative environmental impact of synthetic pesticide applications increases the use of less aggressive products, such as mineral oils, whose optimal application conditions in Mediterranean citrus orchards need to be established. In this research, trials with a Potter Tower have been conducted to characterise the deposition of a mineral oil by means of the measurement of the coverage, the number of impacts per unit area and the distribution of the size of the impacts. The applications were performed at different volumes and pressures. In parallel, the same applications were sprayed over California red scale (*Aonidiella aurantii, Mask*) populations grown in laboratory in order to model the relationship between the deposition parameters and the mortality of the insect at different growing stages. The models were obtained by fitting the experimental data to predefined functions and allowed to estimate the coverage, number of impacts per square centimetre and impact size distribution required to achieve 95% mortality.

122

Lemon variety preferences by *Aonidiella aurantii* (Homoptera: Diaspididae)

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Abstract: An essential aspect of beneficial insect mass rearing program is to know which is the best host plant that optimise pest growth and consequently improve the natural enemy development.

California red scale, *Aonidiella aurantii* (Maskell) is one of the species whose host plant range has been extensively studied. Lemons, grapefruits, oranges, mandarines and different varieties of squash and potatoes have been used for this purpose. However, there is no work carried out about the preferences of red scale inside the lemon group.

Lemon is the host plant that is often employed in laboratory studies and the second host plant after squash for mass rearing projects. Furthermore, in the field, lemon is the most susceptible citrus species to red scale attack.

The effect of different lemon varieties on the development and survival of California red scale was evaluated. The tested varieties were Eureka, Verna, Fino, Lisbon and Villafranca coming from the germplasm bank of Instituto Valenciano de Investigaciones Agrarias.

Mass rearing of *Aphytis melinus* for biological control of *Aonidiella aurantii* in Sicily

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Abstract: A mass rearing of the Aphelinid *Aphytis melinus*, the main bio-control agent of California red scale (*Aonidiella aurantii*) in Sicily, has been started in 1995 within a Regional Extension Service IPM program. The rearing, maintained in an insectary of the Regional Phytosanitary Service, had the aim to produce parasitoids in order to verify their efficacy in controlling the pest through augmentative releases. The beneficial has been reared, from 2000, on a partenogenetic strain of *Aspidiotus nerii*, originally supplied by the INRA laboratory of Valbonne (France).

Further observations on the main biological features of the wasp have been started in collaboration with the Dipartimento di Scienze e Tecnologie fitosanitarie of the University of Catania (Italy). The biological parameters will be employed to compare commercial and indigenous strains in order to evaluate the possibility of using the indigenous strains in biological control programs, due to the uncertain field results given by the commercial strains.

Key words: *Aphytis melinus, Aspidiotus nerii, Aonidiella aurantii*, mass rearing, biological control

Introduction

Red scale (*Aonidiella aurantii* (Maskell) [Homoptera: Diaspididae]) (RS) was found 35 years ago on lemon groves in Eastern Sicily. In the same period *Aphytis melinus* De Bach and *A. lingnanensis* Compere (Hymenoptera: Aphelinidae) were introduced to Sicily (Inserra, 1966). Currently RS is considered the major pest of the Sicilian citrus industry and it is widespread in all southern Italy citrus areas, being most common in inland citrus orchards (Liotta, 1970, Longo *et al*, 1995, Tumminelli *et al*, 2000). *A. melinus* and *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinellidae) are the main biotic mortality agents (Benfatto & Cucinotta, 1994; Siscaro *et al.* 1999). RS is traditionally controlled with organophosphate insecticides and mineral oil, as carrier or alone. The use of *A. melinus* through augmentative releases in coincidence with peak presence of virgin adult females, could represents a reasonable alternative to the chemical treatment. This approach can suppress armored scale densities below economic injury levels (Forster & Luck, 1997; Forster *et al.*,1995; Luck *et al.*, 1997; Moreno & Luck, 1992; Papacek & Smith, 1992; Smith *et al.* 1997).

A. melinus augmentation for control of RS was included in 1994 in a program for citrus growers, started in Sicily, with the aim to introduce IPM tactics. Commercially strains of *A. melinus* was introduced from abroad. Results of releases of *A. melinus* in May and June, when late second-instar and adult virgin female scales were most abundant relative to the other scale stages (Siscaro *et al.* 1999), did not significantly reduce the proportion of scale-infested

fruit at harvest, when compared with those in non-release orchards. The difficulty we encountered in obtaining statistically convincing results can be attributed to different reasons: uncertain origin of wasps and long storage time required to import parasitoids due to insufficient production in Italy; lack of uniformity of experimental orchards due to the small size area (less than two hectares) of Italian citrus groves; low rates of wasp release due to high cost of *A. melinus* in Italy; late releases due to unavailability of reared wasps in late winter.

The augmentation was not generally an effective tactic for controlling RS in Sicily citrus (Mazzeo *et al.*, 2001), but certainly it helped to dramatically reduce the use of disruptive traditional pesticides by 26% (Raciti *et al.*, 1997) and re-establishing the natural biological balance. On this basis this tactic was included in our IPM guidelines in 1999 (Areddia *et al.*, 2000). Better results could probably be achieved with locally produced *A. melinus*.

To this aim the process of rearing of *A.melinus* and observation about the main biological features was started in Sicily in 2000. The rearing was implemented as proposed in 1960 by P. De Bach and E. B. White (DeBach and White, 1960). Some fitting was necessary to local conditions.

The substrate

Evaluation of suitability of substrate for use in insectary indicated good feature of the squash variety Butternut. Factors evaluated included year round availability, ease of handling, relative suitability to the host scale and the parasite, susceptibility to disease end insect pests, advantageous surface/volume ratio, and length of useful life.

Squash size
Size of butternut squash: a range in weight of between 500 and 1,500 gr and of between 15 and 25 cm in length was found to be satisfactory in the method used. The minimum size of the squash is 10 cm diameter max and 5 cm diameter min and 15 cm length. The maximum is 20 cm diameter max and 10 cm diameter min and length 25 cm. This is the range of squash capable per compartment. Special arrangements for culturing this small variety can be made with some growers.

Squash availability, price, selection and handling
Squash are available in the field from late May or early June through late October. It is possible to obtain tunnel production from March to April, but it is expensive. The price ranged from € 0,40 to € 0,70 /Kg for the field and € 1,40 for the tunnel production.

Storage area was near the field production, in the cooperative farm; a little area for daily supply was in the insectary. In order to maintain a constant production it is desirable to replenish the host plant as early in the spring as possible, in spite of the high market price which prevails at that time.

Some physiological change in the squash appears when it becomes old (about 5 or 6 month) and makes it less suitable for the scale rearing. The crawlers placed on such squash fail to settle properly and many of them leave the squash and are lost, or the scale will remain small and with very low crawler production when settled.

Disease control
In order to reduce the incidence of diseased squash in insectary, the squash must be selected and handled with care to avoid bruises and cut. Mature squash are collected by cutting them to 5 centimetres from the peduncle. Before use, the squash is washed in water. The greater losses for decay in our insectary have been attributed to presence of *Rhizopus* spp., which is considered a pathogen (from hurt) agent of soft rot. We use to dip preventively squash in diclozolinate solution. This treatment has not compromised the development of the oleander scale.

Storage

The Butternut squashes were stored in two different conditions: in climatic room (12° C and 75% of RH) and in an external area. The first condition extended the life of the squash but with an increase of squash creasing. The squash lost to decay started after approximately 80 days in controlled condition and 110 days in the external area. Percentages of losses, after 150 days, are the same in the two conditions (approximately 15%) (Fig. 1).
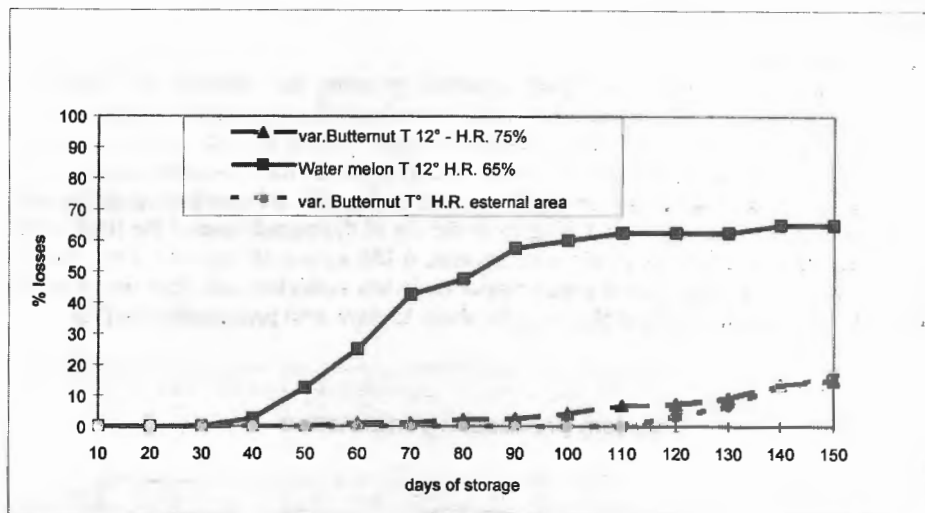

Fig. 1. Squash storage performance.

The host scale

A partenogenetic strain of *Aspidiotus nerii* originally supplied by the INRA laboratory of Valbonne was used; the uniparental oleander strain is superior to the biparental strain in several biological responses like absence of male, absence of prolonged molt stage, reproductive capacity, degree of preference of the parasite for the scale, favorability of the scale in regard to parasite sex-ratio and size. The uniparental form produced 107 crawlers per female at 24°C and RH 50-55 % and it requires approximately 58 days for the beginning of effective progenie production (De Bach and Fisher 1956).

The parasite

Aphytis melinus was first introduced in the insectary on July 20, 2000 from a commercial strain of a private insectary. The originary strain was imported from California. At 26,7°C and 50% RH, the life cycle takes 12-13 days and an average of 2,8 eggs are laid on the oleander scale (Rosen and De Bach 1978). Longevity of the female has been reported to average 29,8 days at 25°C. On the California red scale as host, the fecundity of *A. melinus* is relatively high, averaging 67,4 progeny per female at 25°C, each female destroying an average of 61,6 host scale by oviposition and 50,6 host scale by mutilation and host-feeding.

The rearing method

Scale production

In the routine operation the new squash must be infested with scale insect each day. Squashes are selected from the outside storage area, transported to infesting room, washed, and placed on individually dated squash holders length 15 cm, large 8 cm and high 4 cm. In order to permit to reach room temperature before the infesting process begins, squash are brought into the insectary the day before the infestation. For good crawlers settling the squash are previously moistened with a damp cloth.

Crawler collection

Crawlers are collected from producing squashes by using the "shadow line" technique. Crawlers on the surface of the producing squash move toward a light source and fall from the tip of the squash to a white card below, then move again toward the light until they reach the edge of the shadow boards. The light source is a fluorescent tube mounted vertically. The mother-scale-infested squash are arranged on three racks with the crawlers collection cards placed below to receive crawlers falling from the tip of the squash nearest the light source. The collection crawlers room would accommodate n.180 squash 58 days old. From the card the crawlers are transferred to a paper funnel (crawlers collector) and after into a similar saltshaker. The squash remain in the room for about 15 days until peak production (Fig. 2).
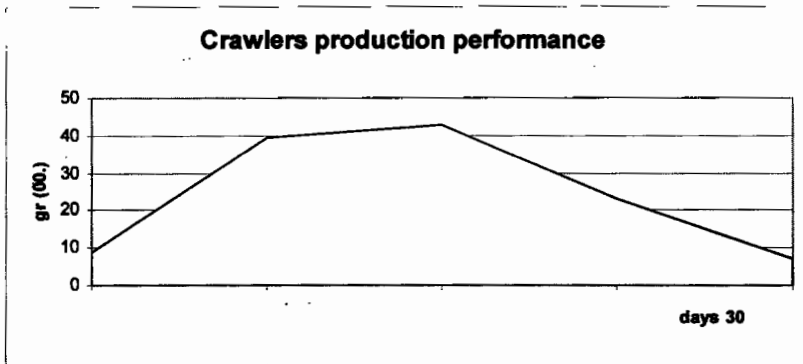

Fig. 2. Crawlers production (one squash of 600 cm square surface)

The infesting process and holding period

Crawlers are distributed over the upper surface of the squash previously moistened. The squash are then rotated and the newly exposed upper surface is infested in turn. This process continues until the squash has been completely rotated and infested. About three gr. of crawlers are necessary daily to prepare 40 squash. An average from 15 to 30 scales per square centimetre are required for the squash infestation. After the surface has been infested completely the squash is removed to a darkened wood box in order to permit all crawlers to settle. After, crawlers settling on the squash are removed to a dark holding room at 25°C and 50- 60 %RH. When scales on squash in the holding room have reached between 45 and 58

days of age they are removed for use in the parasite producing programme and maintenance of mother strain respectively.
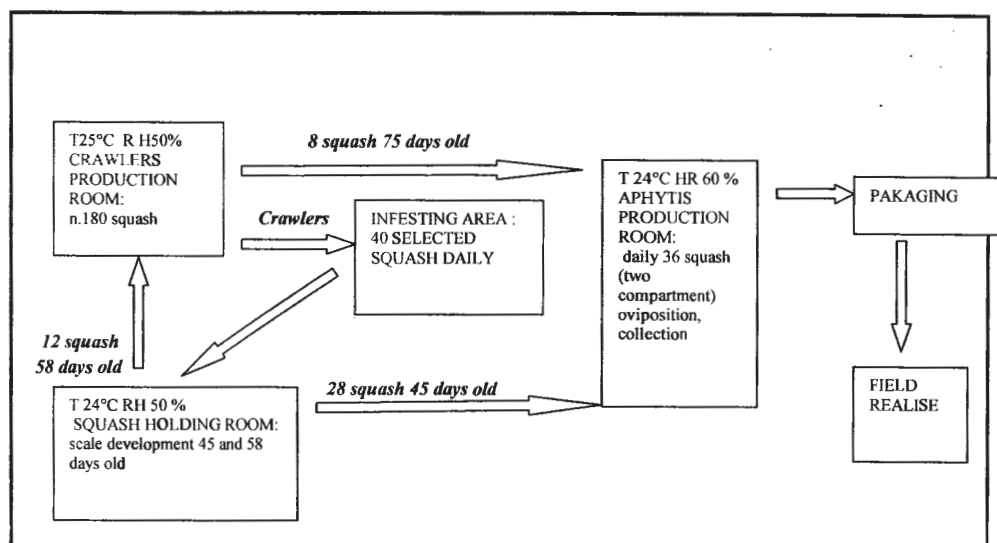

Fig. 3. Handling procedures in the insectary production

Figure 3 shows the host-scale handling procedures in the insectary production. Forty squash per day are infested, all of which go to the holding area for scale maturation. Crawlers production requires not more than 180 squash in the mother scale culture and this need to be completely renewed each 15 days at the rate of 12 squash daily. It follows then that, out of 40 squash, 12 complete the maturation period for the mother culture and twenty-eight 45 days old go directly to parasite production. The best eight of the 12 squash completing the crawlers major production period (75 days) go also to parasite production permitting a total of 36 squash daily.

Parasite production

The oviposition collection room (24°C, RH from 50 to 60%, and 14:10 (L.D.) photoperiod) contains 4 units with 5 wood compartments (20 compartments in total number) each capable of holding about 20 squash. Each compartment is built on drawer- type slides so that it can be drawn from the unit. Two levers at each end of each cage permits that everyone to be lifted approximately 1 cm in order to provide freedom to the base card, which serves as the floor of the drawer assembly. A portion of the top of the compartment is hinged to provide access and a sheet of glass attached to the lower surface of this lid. In order to make the lid and the lower edge of the compartment parasite proof, the sheet of glass forms a wall in order to admit ample light and cloth forms the front external wall to permit adequate ventilation. The front side is fitted with two wood removable cover which makes the compartment gastight (rubber is placed around the wood). The squash in each compartment rest on a wood support.

The oviposition

During the mayor production period, 36 scale-infested squash are transferred daily from the scale-production area of the insectary to the parasite-production area. Eighteen squash are placed in a compartment. An average scale density of from 15 to 30 per square centimetre of squash-surface gives considerable open space between scales. The approximate surface area in square centimetres is calculated like frustum of cone. We used a surface squash averaging from 400 to 900 square centimetres. About 25,000-inoculum parasite per compartment (an average of 1,400 per squash) and food (40% water, 40% honey, 20% sugar and 0.5 gr of agar kept 20 minutes at 100°C in a bain-marie spreading on a half of a plastic dish) are added, and the unit closed.

Parasite recovery

After an oviposition period of about 48 hours the parasite inoculum is recovered by anesthetizing the parasites, then rapidly blowing them from the squash surface to the card below, removing the card and pouring the parasite into a release container. In order to anesthetize the parasites the cloth side of the compartment must be covered. Carbone dioxide is forced to the unit for about 3 minutes (30 litres/min). Recovery of the parasites is accomplished by raising the compartment by depressing the lift levers and removing the base card upon which the anesthetized parasites have fallen. These parasites are poured into the dispensing device, which is used to measure, by volumetric means, the number of parasites placed in a release box.

The holding and collection period

The unit containing the squash is then held for 14 days for parasites emergence to begin. On the thirteenth day from the oviposition date food is put in and the unit is closed. For successive days the newly emerged parasites are collected like in parasite recovery. At each collection, additional food is applied on the dish.

The parasites are poured into a graduate dispenser through a funnel, and the volume recorded. This volume can be transformed into an estimated number of parasite. Every release box contains 5,000 parasites (sex ratio 65♀ : 35♂) and a drop of jam is added to supply food for the parasite.

All parasites are released in the citrus grove as soon as conveniently possible. Storage under the proper condition at 15°C and 75/80 % RH is possible for a period limited to a few days.

Results

Squash storage performance

The storage in external area hasn't energetic cost and under our climatic condition it is suitable from October to beginning of April. The conservation in controlled condition is suitable of substrates to use from April to June when are not available fresh squash (Fig. 1). Watermelons evidenced a good settlement of crawlers but high losses (about 60% in 100 days). Other squash varieties evidenced mealybug infestation and/or low settling of crawlers. Unlike the squash Butternut, other tested squash varieties showed high losses, no good and variable size and difficult handling.

Squash production

The amount of infested squash obtained was 3,113 in 2000, 9,213 in 2001 and 3,093 until June 2002.
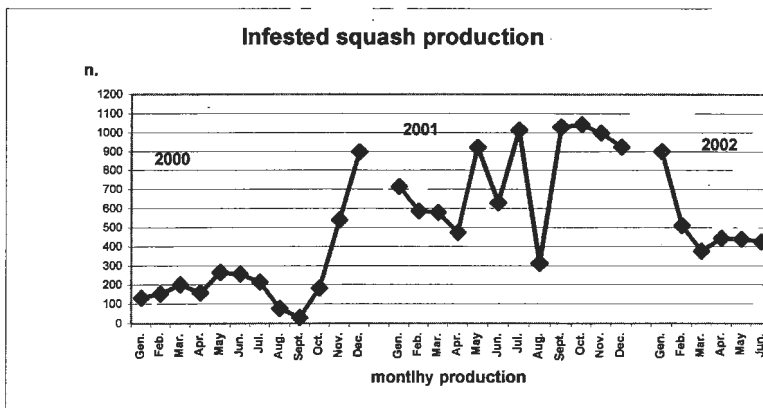
Fig. 4. Infested squash per year

The irregular production of infested squash depends on fresh squash supply because the Butternut variety is difficult to find in the market.

Parasite production

Fig. 5 shows the monthly and total production obtained in the last two years: the parasite production was concentrated in the spring season and at the beginning of summer.
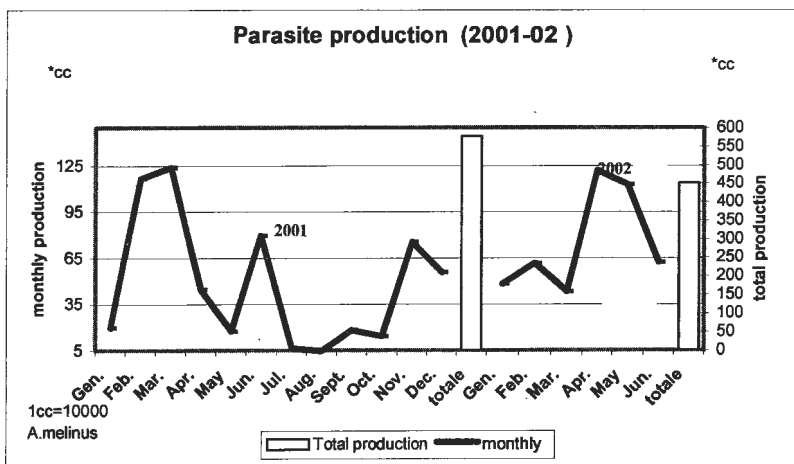

Fig. 5. Parasite production 2000-2001

In 2002, with an inoculum of 25,000 parasites per compartment, an average return of 70,000 parasite progeny per compartment was obtained (about 4,000 per squash). A high increase of yield was reached (Tab.1).

Table 1. *A.melinus* production in 3 years;*until June.

Year	Total (cc) (1 cc about 10000 Aphytis)	Average Inoculum/cage (a)	Average cc) prod/cage (b)	Yield (b/a)
2000	22,35	4,1	0,97	0,24
2001	650,6	3,58	5	1,38
* 2002	450	2,4	7	2,91

Biological features

Recent laboratory observations on some biological features (longevity, host exploitation, fecundity, mobility etc.) of *A. melinus* commercial strain have been carried out, also thanks to a comparison with a "field strain" collected in Citrus groves of Eastern Sicily.

Preliminary results showed that the "field strain" seems to have a higher longevity (over 10dd) than the commercial strain (ca. 4dd). Moreover, investigations on the fecundity showed that the "field strain" parasitizes more than the commercial strain; besides, the commercial strain oviposites during the first 24-48h of life, while the "field strain" reaches higher parasitization levels after 5 days from emergence.

Further observations are needed to verify these data also with field trials.

The insectary

The insectary was installed in a pre-existent basement. Every room is supplied with an air forced system. Light, temperature and humidity are programmable. Protection product system is installed in every room. In the three climatic rooms temperature is about 25°C and relative humidity 60%

- The storage squash area is near the entrance of the insectary. The surface of the walls is shelfful, dimension approximately mt.3x4.The temperature is kept at 15°C and RH 75%.
- The crawlers collection unit is square in shape (dimension approximate 3.2 x 3 x 3 m) composed of five units with seven shelves each.
- The holding room is 5.2 x 3.2 x 3 m; it contains 80 wood supports as shelves.
- The oviposition collection unit is of 2.5 x 3 x 3 m.

Insectary pest control

The production method inhibits the establishment and increase of most insectary pests. In the collection of scale crawlers at a shadow line, possible parasites are automatically separated from the new infesting material. In the parasite-production phase, the relative short life cycle of *Aphytis* is advantageous in holding most pest population in check. The only insectary pests observed were mites and ants.

No control is necessary for the phytophagus mite *Tetranycus urticae* because no measurable damage results from a population build- up by such organism. Since the abundant webbing which covers the peduncle area of the squash interferes with the normal collection of crawlers, it must be removed. Removal is accomplished easily by brushing the squash area whit a soft brush. To keep in check the mite population relative humidity should be lower than 50%. Ants going in from outside of the insectary were controlled by pyretrum inside and clorpiriphos in external nearest area.

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The damage caused by the diaspidid *Parlatoria zyziphi* Lucas on Citrus groves in the Northwest part of Algeria.

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Abstract: The diaspidid *Parlatoria zyziphi* (Homopera: Diaspididae) is one of the major pests for the Algerian citrus cultivation. The present contribution is a part of a large study of the population dynamics of the diaspidid. It was conducted for three years in unsprayed orchards in the vicinity of Mostaganem (N.W Algeria). Heavy infestations occurred in summer season.

Results were expressed as the everage number of insects per cm². A high density of the diaspidid *P. zyziphi* is influenced by cultural practices. Parasitoids are recorded. Their impact reached of 30%.

Management strategies of mealybug pests of citrus in Mediterranean countries

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Abstract: Six mealybug species are reported as citrus pests in the Mediterranean basin: the citrus mealybug *Planococcus citri* (Risso), the citriculus mealybug *Pseudococcus cryptus* Hempel, the long-tailed mealybug *Pseudococcus longispinus* (Targioni–Tozzetti), the citrophilus mealybug *Pseudococcus calceolariae* (Maskell), the obscure mealybug *Pseudococcus viburni* (Signoret) and the spherical mealybug *Nipaecoccus viridis* (Newstead). Some of these species were recently introduced in the region and are still expanding their distribution, e.g., *N. viridis*. Mealybugs are usually occasional or potential pests of citrus. However, some species are considered key-pest in certain situations. Pest status may change with pest management systems and/or other ecological alterations. Management strategies of mealybug populations in citrus orchards have been generally based on biological control (mostly as classical biological control and to a lesser extent as augmentative releases). However, chemical controls are widely used mainly due to low adaptation of the principal natural enemies to the climatic conditions in the Mediterranean. The application of pheromones is still restricted to the monitoring of the citrus mealybug, whose sex pheromone is commercially available. Mass trapping and mating disruption should be consider to be used in IPM programmes as an alternative to the supplementary chemical control. Enhancement of biological control through the management of ant populations is another possible control tactic. The management strategies of mealybug pests of citrus and the possible levels of integration of different tactics based on the pest status will be discussed.

138

Host and parasitoid densities influence on progeny and sex ratio of *Anagyrus pseudococci* (Girault) and *Leptomastix dactylopii* Howard (Hymenoptera:Encyrtidae); two *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) parasitoids

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Abstract: The effects of different *P. citri* densities and *A. pseudococci* and *L. dactylopii* densities on progeny, parasitization, and sex ratio were investigated under controlled laboratory conditions. *L. dactylopii* progeny numbers increased significantly as did host density until the host:parasitoid: ratio was 80:1. Thereafter, progeny numbers began to decline. Numbers of *A. pseudococci* progeny slightly increased as host density increased. Parasitism percentages for the two parasitoids decreased as host density increased. *A. pseudococci* and *L. dactylopii* attained optimum levels for mass production when the host:parasitoid ratios were 10:1 and 80:1 respectively.

Numbers of *A. pseudococci* progeny increased with increasing parasitoid densities (2,4,6,8 and 10 females) when host density was held stable (480 *P.citri*). *L. dactylopii* only showed this trend at lower parasitoid densities. The number of *A. pseudococci* progeny per female was not affected by parasitoid density, though the number of *L. dactylopii* progeny per female fell as parasitoid density increased. Sex ratios of *A. pseudococci* and *L. dactylopii* remained unaffected by modifications to parasitoid densities.

Key words: *Anagyrus pseudococci, Leptomastix dacylopii*, biological control, mass-rearing, *Planococcus citri*, host density, sex ratio, progeny.

Introduction

Anagyrus pseudococci (Girault) and *Leptomastix dactylopii* Howard are two solitary parasitoids of the citrus mealybug *Planococcus citri* Risso. Both parasitoids have been widely used as biological control agents for *P. citri* (Franco *et al.* 1994). *A. pseudococci* is of Mediterranean origin, while *L. dactylopii* is exotic (originating in the West Indies). The latter was first introduced into Spain in 1948 (Gomez, 1950). It was later re-imported from Italy in 1977 (Ripollés, 1983) and has subsequently been reared and released here. It has not been possible to establish it in Spain because, as also occurred in Portugal, it has proved impossible to recover despite numerous releases (Ripollés, 1983), (Tranfaglia *et al.* 1994; Carvalho, 1997).

Numerous laboratory studies have been carried out to determine the biology of the two species (Lloyd, 1966; Avidov *et al.*, 1967; Chandler *et al.*, 1980; De Jong and Van Alphen, 1988; Tingle and Copland, 1988, 1989; Krishnamoorthy, 1989; Islam and Jahan, 1993; Battaglia *et al.*, 1996; Islam and Copland, 1997; Islam *et al.*, 1997). Studies have also investigated the introduction and establishment of populations of the two parasitoids in several different countries: (Krishnamoorthy and Singh, 1987; Mineo and Viggiani, 1976; Spicciarelli *et al.*, 1992; Sudha *et al.* 1992; Carvalho *et al.*, 1994).

One aspect of particular importance for developing mass rearing procedures is to determine the critical host and parasitoid densities that make it possible to obtain optimal production. Many studies of *L. dactylopii* have sought to determine these critical levels (Su and Su, 1993; Smith *et al.*, 1996; Jalali *et al.*, 2000), though rather different results have been obtained. It is therefore necessary to investigate the responses of the two species under mass-rearing conditions, in order to understand the reasons for the differences observed in the different studies referred to above.

Thus, the aim of this comparative study of the two parasitoids under laboratory conditions was to determine the effects of different *P. citri* densities on the responses of the two parasitoids, by studying the resulting progeny production and sex ratio. Studies were also conducted to investigate whether there was any mutual interference between female parasitoids. This was undertaken by analysing responses obtained from several parasitoid densities with a fixed host density.

Materials and methods

All insects used in these experiments were collected from the Estació Experimental de l'Ebre (IRTA) mass-rearing insectary for natural enemies of *P. citri*.

Two independent experiments were conducted in order to determine the respective effects of host density and parasitoid density on progeny production and sex allocation. The host density experiment consisted of two mated female parasitoids being exposed to: 10, 20, 40, 80, 160 and 320 mealybugs. The parasitoid density experiment involved 480 mealybugs being exposed to 2, 4, 6, 8 and 10 female parasitoids. Six replications were carried out for each density level.

P. citri rearing was carried out with sprouted potatoes *Solanum tuberosum* L., though newly hatched crawlers were subsequently transferred onto ripe pumpkins *Cucurbita moschata* Duchesne under conditions of 25°C, 60-70% RH and 12:12 (L:D). This procedure was carried out 25-26 days prior to conducting the experiment in order to allow the host insects to reach the desired age by the date of the experiments. These insects were then settled on the surface of the pumpkin.

The parasitoids used were 2-3 days old, and were obtained from (150 ml) plastic vials with mummies. They were raised on potato sprouts, which came from mass rearing cages for both species of encyrtid parasitoid. In order to obtain numerous parasitoids on the same day, all the newly emerged parasitoids were removed 3 days before the experiment. In this way, the ages of all the insects used in the experiments were < 2-3 days. To ensure mating, 24 hours before the experiment began, female parasitoids were placed in small plastic vials with two males per female. They were provided with honey as food, but no hosts.

The choice of pumpkins as the experimental patch was justified by the need to count the number of *P. citri*. This was easier on the surface of a pumpkin than on potato sprouts. Pieces of pumpkin were cut that carried the exact quantity of *P. citri* required. These different patches were then put into plastic vials (140x60x80 mm) along with female parasitoids. The vials were ventilated by leaving a (80 x 50 mm) cloth mesh window in their lids. Small droplets of honey were left inside the vials as a source of carbohydrates for the parasitoids.

The parasitoids were allowed to oviposit for 24 hours in a climatic chamber: 25°C, 60-70% RH and 12:12 photoperiod (L:D) with light provided throughout the experimental period by 3x37W fluorescent tubes. After this exposure period, the parasitoids were removed and the pumpkin pieces containing *P. citri* were individually placed in larger ventilated cages to avoid damage due to rotting of the pumpkin. The cages containing the parasitized mealybugs were

finally transferred to a rearing chamber maintained under the same temperature and HR conditions until the emergence of offspring, 17-22 days later.

After the emergence of the offspring, the number and sex ratio of the parasitoids were recorded. Where appropriate, data were transformed $\sqrt{x+0.05}$ or $\arcsin\sqrt{x+0.05}$ before means separation. Analysis of variance (ANOVA) was conducted in order to analyse the results (PROC GLM, SAS institute 1998). Significant differences among means were determined by Duncan's Multiple Range Test, with a 95% level of significance.

Results and discussion

Host density

L. dactylopii responded to rising host densities, with its progeny significantly increasing in number until reaching a density of 160 *P. citri*. *A. pseudococci*, on the other hand, did not show such a marked trend. This was evidenced by the absence of significant differences between progeny obtained with 20 and 320 *P. citri* densities. (Tables 1 and 2). The response obtained for *L. dactylopii* confirmed many results obtained in similar experiments under laboratory conditions: Smith (1996), Jalali (2000) with *L. dactylopii*, Su and Su (1997) with *Anagyrus sawadadii* and *L. dactylopii*, and Sagarra (2000) with *Anagyrus sawadadii*, in which increases in the number of progeny were also associated with rising host densities. Reduced production of *L. dactylopii* along with higher densities was also reported by Smith (1996). This could perhaps be attributed to the failure of parasitoids to emerge.

Table 1. Progeny (mean ± SE) obtained for two *A. pseudococci* females with 10, 20, 40, 80, 160 and 320 *P. citri*

No. *P. citri*	A. pseudococci		
	Males	Females	Total
10	2.00 ± 0.45 c	5.67 ± 0.42 ab	7.67 ± 0.79 c
20	3.67 ± 1.45 bc	8.00 ± 1.24 ab	11.67 ± 1.09 ab
40	3.83 ± 0.54 bc	7.33 ± 1.41 a	11.17 ± 1.30 b
80	6.83 ± 1.35 ab	8.17 ± 1.08 a	15.00 ± 1.65 a
160	3.50 ± 1.23 c	7.17 ± 1.14 ab	10.67 ± 1.02 b
320	10.17 ± 1.54 a	4.67 ± 0.76 b	14.83 ± 1.19 a

Progeny data were transformed for variance analysis. Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

Table 2. Progeny (mean ± SE) obtained for two *L. dactylopii* females with 10, 20, 40, 80, 160 and 320 *P. citri*

No. *P. citri*	L. dactylopii		
	Males	Females	Total
10	2.00 ± 0.26 c	4.00 ± 0.58 c	6.00 ± 0.45 e
20	4.67 ± 1.23 bc	11.33 ± 1.08 b	16.00 ± 0.77 d
40	7.33 ± 1.11 b	14.50 ± 2.14 b	21.83 ± 2.36 c
80	12.67 ± 0.71 a	13.83 ± 2.15 b	26.50 ± 2.69 bc
160	12.50 ± 1.41 a	25.50 ± 2.74 a	38.00 ± 2.70 a
320	15.83 ± 2.82 a	14.33 ± 1.48 b	30.17 ± 2.67 b

Progeny data were transformed for variance analysis. Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

When the two parasitoids were compared at low host densities, numbers of progeny of the two species were similar (at 10 *P. citri* density, *A. pseudococci* numbers of progeny were greater than *L. dactylopii*), however, when host:parasitoid ratios were greater than 20:1, the production obtained with *L. dactylopii* was twice that obtained with *A. pseudococci* . (Tables 1 and 2).
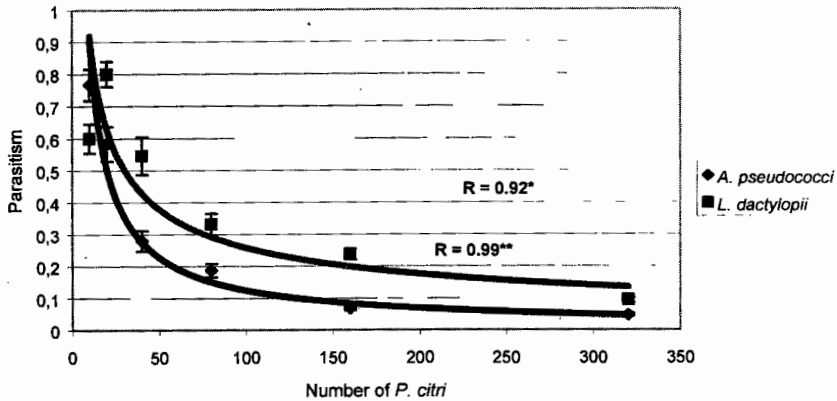

Figure 1. Influence of host density on parasitism by two female parasitoids of *A. pseudococci* and *L. dactylopii*

The percentage of *P. citri* parasitism by *A. pseudococci* varied significantly (F=105.81, df=5, p<<0.001) as host density increased (R=0.99, n=6). This was logical since progeny obtained with different densities were similar (increasing host densities led to lower parasitism percentages). However, *L. dactylopii* showed maximum parasitism at 20 *P. citri*, from which point the rate then declined as host density increased (R=0.92, n=6). (Fig.1)

Table 3. Female percentage (mean ± SE) on progeny for two *A. pseudococci* females with 10, 20, 40, 80, 160 and 320 *P. citri*

No. *P. citri*	% (females/total)	
	A. pseudococci	*L. dactylopii*
10	74.40 ± 5.31 a	65.28 ± 6.03 ab
20	71.95 ± 7.42 a	71.29 ± 7.10 a
40	65.88 ± 6.33 a	65.69 ± 4.93 ab
80	55.29 ± 6.55 a	50.57 ± 3.49 bc
160	68.04 ± 9.24 a	66.49 ± 4.15 ab
320	32.92 ± 6.48 b	48.70 ± 4.88 c

Female percentage data were transformed for variance analysis. Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

A. pseudococci demonstrated the effect of host density upon offspring sex ratio; there were significant differences between the 320 *P. citri*. host density and the other densities assessed. Lower female proportions were obtained at higher *P. citri* densities (R=0.88, n=6). *L. dactylopii* responses were not as clear (R=0.66, n=6), but significant differences were also observed between the highest density tested and the rest (except for the 80 *P. citri*). (Table 3). Jalali (2000) confirmed this pattern with *L. dactylopii*; lower proportions of females were obtained from higher *P. citri* densities. On the other hand, in a comparative experiment with Anagyrus sawadadii and *L. dactylopii* at different *P. citri* densities, Su & Li(1997) confirmed that sex ratio was not affected by host density. In a previous experiment (1993), the same author reported that the percentage of *L. dactylopii* males increased when host density rose from 20 to 300 *P. citri*. Sagarra (2000) proved that *Maconellicoccus hirsutus* host density had no effect on the sex ratio of *Anagyrus kamali* parasitoids.

Several factors must be taken into account when attempting to determine the host:parasitoid ratio that provides optimum efficiency for mass-rearing parasitoids. Progeny production and rate of parasitism are just two of them. Jalali (2000) working with *L. dactylopii* fixed the optimum host:parasitoid ratio at 100:1, with a production rate of 78.3 parasitoids per female. The laboratory conditions, however, were different; 27°C and 14:10 (L:D). However, this author avoided recommending a greater host density because, although it would produce a greater number of parasitoids, he considered it a waste to reduce the percentage of parasitism by more than 50%. Su and Li (1993) considered the optimum host:parasitoid ratio to be 50:1, obtaining 4.1 parasitoids per female. Considering only this aspect, in our case the host:parasitoid proportion of 80:1 for *L. dactylopii* seems to be the most suitable rate for providing greater numbers of progeny (19 per female) and higher percentages of females, though the parasitism percentage was low (23.75±1.68). However a 10:1 host:parasitoid ratio would seem preferable for *A. pseudococci*, as it would provide a high parasitism percentage (58.33±5.43) and also 5.83 progeny per female.

Parasitoid density

Higher parasitoid densities resulted in greater total numbers of progeny on *A. pseudococci* (R=0.97, n=5). However, although *L. dactylopii* production increased when parasitoid density rose from 2 to 6 females, the results were not significantly different from increasing it from 6 to 10 females. (Table 4 and 5). The two parasitoids showed different trends with lower and high densities. *A. pseudococci* similar numbers of progeny than *L. dactylopii* at the highest parasitoid density, but when a density of 2 to 8 females was tested, *L. dactylopii* progeny were greater in number.

Table 4. Progeny (mean ± SE) obtained per 2, 4,6,8 and 10 *A. pseudococci* females with 480 *P. citri*.

No. females	*A. pseudococci*		
	Males	Females	Total
2	9.67 ± 1.50 c	7.33 ± 1.96 c	17.00 ± 1.59 d
4	16.50 ± 3.25 bc	13.50 ± 2.70 b	30.00 ± 4.62 c
6	17.67 ± 3.98 bc	16.00 ± 1.79 b	33.67 ± 4.17 c
8	25.17 ± 2.23 ab	21.50 ± 2.55 ab	46.67 ± 3.26 b
10	36.17 ± 3.64 a	31.50 ± 4.07 a	67.67 ± 4.48 a

Progeny data were transformed for variance analysis. Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

Table 5. Progeny (mean ± SE) obtained per 2, 4,6,8 and 10 *L. dactylopii* females with 480 *P. citri.*

No. females	L. dactylopii		
	Males	Females	Total
2	8.17 ± 1.47 c	15.50 ± 1.02 c	23.67 ± 2.18 c
4	14.67 ± 1.91 b	24.00 ± 2.40 b	38.67 ± 2.48 b
6	28.50 ± 1.56 a	30.33 ± 2.46 ab	58.83 ± 2.48 a
8	29.67 ± 5.87 a	38.33 ± 4.83 a	68.00 ± 3.00 a
10	28.83 ± 2.10 a	35.50 ± 2.76 a	64.33 ± 3.36 a

Progeny data were transformed for variance analysis. Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

Sagarra (2000,1), in a related studio about mutual interference among female parasitoids under mass-rearing conditions, found that increasing *Anagyrus kamali* density at lower levels resulted in a greater increase in numbers and found no significant differences for higher density production.
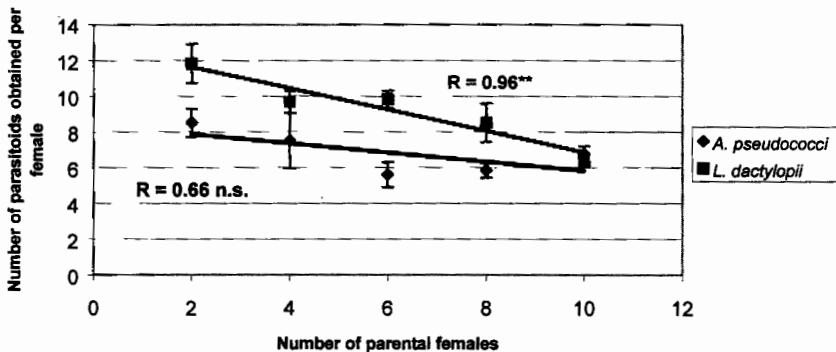

Figure 2. Parasitoids progeny per female.

When comparing progeny obtained per female, no significant relationship was evident between the number of *A. pseudococci* females studied and the number of offspring obtained per female (R=0.66, n=5). However, increasing parasitoid densities of *L. dactylopii* led to a decline in the number of progeny per female (R=0.96, n=5). (Fig 2). This suggests that there is a mutual interference effect among parasitoid females. Sagarra (2000,1) demonstrated this effect by increasing *Anagyrus kamali* density from 1 to 20 parasitoids per 50 host *Maconellicoccus hirsutus* with a resulting decrease in the rate of oviposition.

Results relating to female ratios obtained in progeny proved that they were unaffected by parasitoid density. No significant differences were observed between cases of *A. pseudococci*

(% of female progeny between 41 and 49). In *L. dactylopii* (between 51 and 67%) differences that were observed, failed to suggest any significant trends in this sense. (Table 6). Thus, contrary to predictions based on local mate competition (LMC), the offspring sex ratio for the two parasitoids was apparently not influenced by the number of parental females. In parasitoids that display LMC, competition among offspring for access to mates is affected by the number of females exploiting a common host resource , (Godfray, 1994). Sagarra (2000,1) proved that this effect was not applicable for *A. kamali*, as did Bernal (1999) working with two *Metaphycus* species. Other authors, including Su and Su, (1997) confirmed this response in comparative studies involving *Anagyrus sawadadii* and *L. dactylopii* and also in experiments with *L. dactylopii*, as did Su and Li, (1993) working with different parasitoid densities.

Table 6. Female percentage (mean ± SE), obtained with 2, 4,6,8 and 10 parasitoid females with 480 *P. citri*

No. females	% (females/total)	
	A. pseudococci	*L. dactylopii*
2	41.08 ± 9.86 a	66.76 ± 3.89 a
4	47.00 ± 8.18 a	62.04 ± 4.95 ab
6	49.63 ± 5.57 a	51.33 ± 2.31 b
8	45.77 ± 3.89 a	57.00 ± 4.00 ab
10	46.37 ± 4.74 a	55.05 ± 2.98 ab

Female percentage data were transformed for variance analysis. Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

Other factors, such as the host density that the host plant is able to support without losses of production due to rotting, should also be considered. Along these lines, after taking into account the percentage of the surface area that was rotting, Smith (1996) determined the host density (20 per cm^2) and *L. dactylopii* density (3.3 per cm^2) of the substract required to achieve maximum efficiency mass production. However, in order to determine optimum numbers of hosts and parasitoids for mass rearing of the two species, it is necessary to consider numerous other aspects in addition to the ones described above and also the quality of female parasitoid offspring.

Acknowledgements

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Population dynamics of *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) in citrus groves in Spain.

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Abstract: Nine non-sprayed citrus groves located in the south of Tarragona and north of Castellón (Spain) were periodically monitored from 1992 to 1995 in order to determine the population dynamics of *Planococcus citri* throughout the year. Five male flights were detected, between April and November, and we observed significant differences in abundance between flights and years. The life cycle of *P. citri* is well defined by the thermal parameters of 8.3°C as the lower developmental threshold and 562°DD (C) as the degree-days to complete a generation. Increases in female on-trunk populations coincided with male flights. *P. citri* invaded the calyxes of newly settled fruits between May and July. By the end of this process, more than 70% of the calyxes were occupied by the insect. *P. citri* subsequently developed on the fruit, though in smaller percentages (20%). We observed increasing populations in the absence of natural enemies and decreasing populations of *P. citri* when natural enemies were present. Among the natural enemies, the coccinellidae *Cryptolaemus montrouzieri* Muls. stands out as well as several species of hymenopteran parasitoids.

Key words: *Planococcus citri*, citrus, male flights, lower developmental threshold, degree-days, natural enemies,

Introduction

The citrus Mealybug, *Planococcus citri* (Risso) is a poliphagous and cosmopolitan insect pest. It damages many outdoor crops in the tropics and subtropics and also greenhouse crops in temperate regions.

In citrus, it is an occasional but serious pest, which mainly attacks Navel oranges and lemons. *P. citri* develops several generations in a year and infests early fruits, when they have just settled, and feeds on them. In cases of major infestation, fruit is rendered non commercial as a result of both the sooty mould growing on the honeydew excreted by *P. citri* and the loss of colour and hypertrophy that result from feeding.

P. citri has several natural enemies. These include coccinellidae *Cryptolaemus montrouzieri* Muls. and several hymenopteran parasitoid species.

This paper reports on studies into several biological aspects of *P. citri* in Spain. Aspects investigated include the number of generations per year, the number of degree-days required to complete a generation, the fruit invasion process, development on fruit and the influence of natural enemies.

Material and methods

The study was carried out over a 5-year period (1992-1995 and 1998) in nine unsprayed orange groves (*Citrus sinensis* (L) Osbeck, var Navelina and Washington Navel) located in Tarragona and Castellón (Spain)

Male flights

Pheromone traps were set out in the groves from 1st January in order to monitor male flight activity. Each trap consisted of a yellow wooden frame containing a piece of glass coated with a sticky spray (Soveurode) and baited with a pheromonc capsule (Inagra). Pheromone capsules were changed every six weeks and the pieces of glass were checked on a weekly or monthly basis, according to the season. Checks were more frequent in warmer periods and less frequent in wintertime. The pieces of glass were then taken to the laboratory and examined under a microscope. *P. citri* males captured on 6 x 10-cm glass surfaces were then counted.

Some groves contained more than one trap. In these cases, not all of the pheromone capsules were changed on the same day. Changes were made according to a weekly rotation in order to avoid any potentially distorting influence of recently installed pheromone capsules.

Temperature data were obtained from automatic meteorology stations located from four to fifteen km from the groves.

Lower developmental threshold temperature and thermal constant

The lower developmental threshold temperature for *P. citri* was estimated from the male population over a total of twenty-two one-year periods and following the methodology proposed by Tokeshi (1985). He determined the relationship between development and field temperature by employing a maximum likelihood method to estimate the minimum temperature threshold and minimum number of degree-days required for development in a Lepidoptera species. He studied the variation in the coefficient of determination (r^2) for the regression between body length and cumulative degree-days for different minimum temperature threshold values. This coefficient of determination peaked at a minimum threshold temperature.

In our case, the biological data considered was the degree-day distance between consecutive maximum male captures for a range of temperatures between 5°C and 11°C measured at 0.1°C intervals. That maximum was calculated as the median value of the interval that defined the beginning and end of each flight. The coefficient of variation was obtained for the different temperature values: the development temperature threshold was chosen as the value that minimised this coefficient.

Trunk and main branches observations

Between April and December, *P. citri* populations on the trunk and main branches were studied at different cadencies in the Frudelta (1995,1996), Xalet (1995), Xeminavel (1995, 1996, 1998) and Irida (1995,1996) groves. The total number of samplings made per grove varied from 9 to 12 per year, though 31 samplings were made in the Xeminavel grove in 1998. Each sampling consisted of a random selection of 20 to 30 trees per grove. Total *P. citri* and *Cryptolaemus montrouzieri* present on trunk and main branches in the space of one minute were counted.

Population evolution on the fruit: calyx fruit invasion and cycle on the fruit

P. citri calyx fruit invasion was investigated for the years 1993, 1995, 1996 and 1998. Sixty-six samplings were made during May, June and July, in Frudelta, Xeminavel, Irida, Prat, Marxala and Xalet. From 1 to 8 samplings were made per grove per year. Each sampling consisted of randomly selecting 6-10 fruits per tree from 20-30 trees per grove (120-300 fruits per sample). These fruits were then taken to the laboratory and examined using a stereoscope

microscope. All *P. citri* on the fruit, mainly located under the calyx, were counted. Distinctions were made between first instars, second instars, third instars, young females and females with eggs, and parasitized insects. Fruit diameter and phenology were also recorded in some cases.

The on-fruit cycle was studied from July, when *P. citri* become visible on fruit, until December. A total of twenty-six 6-month periods were studied, and 7 samplings were made per period. Each sampling consisted of 6-12 randomly chosen fruits per tree picked from 25-30 trees per grove. In the field, the number of individuals, belonging to third instar, young female and female with eggs stages of development were counted on these fruits. First and second instars were not counted, but when more than two young larvae were present, this was recorded as a colony.

Results and discussion

Generations per year
Five flights were detected in the different groves in the months of May, June-July, August, September and November, though not all were found in each grove every year. The onset of male captures varied with the grove and the year, with the first males tending to appear on, or around, 9th April (± 2.43 days). The first peak for males was on 11th May (± 1.56 days), the second on 28th June (± 2.07 days), the third on 6th August (± 1.93 days), the fourth on 21st September (± 2.47 days) and the fifth on 10th November (± 2.60 days). Each flight lasted between one and two months. This duration seemed to be related with the temperature in each period. The third flight, in August, was the shortest (40.83 ± 2.39 days) and differed significantly from the rest ($F=7.88$; $df=4, 8$; $p<0.01$). The durations of the other flights were 49.55 ± 2.05 days for the second (June-July), 50.35 ± 2.47 for the fifth (November), 52.51 ± 1.97 for the fourth (September) and 53.18 ± 2.03 for the first (May).

These male flights are believed to be produced just when females reach the mature adult stage and are ready for mating with males. There will then be eggs and new crawlers. The different flights identified therefore imply five generations of *P. citri* per year in the citrus groves in the area studied.

These results partly confirmed those obtained by other authors. Quayle (1941) and Ebeling (1959) concluded that only two to three generations occur each year in California. Harlan (1977) showed three generations per year in Texas: the first in April-May, the second in August and the third in October-November. Bodenheimer (1951) detected eight generations per year in Palestine and Avidov et al. (1969) showed that in Israel *P. citri* was able to produce six generations per year. In the north of the Jordan Valley, where temperatures are higher, *P. citri* was capable of producing seven generations per year. In Greece, Santorini (1977) recorded four to five generations per year. Hibernating stages marked the start of activity at the end of April, with the first generation developing by the end of May. The second generation appeared during June and July, and the third in August. When climatic conditions were favourable, a fourth generation was produced between the end of August and September. A fifth generation was also possible in October-November if there was no rain. In Sardinia (Ortu, 1985), male captures started in May and ended in December, with catches peaking in June-July, August-September and October-November, prompting the conclusion that there were three to four generations per year. Also in Sardinia, Fronteddu et al. (1996) registered two significant male peaks; at the end of June and at the beginning of August. Longo et al. (1985) and Barbagallo et al. (1993) found four to six generations per year in Sicilia. Katsoyannos (1996) reported two to three generations per year in the northern Mediterranean.

In Spain, Gómez Menor (1937) reported a generation that appeared in February-March and whose *P. citri* development depended on temperature. Gómez Clemente (1943) found three to four generations per year, with the first adults being observed in April. Gonzalez-Sicilia (1963) said that *P. citri* developed four to five generations in a year. Ruiz Castro, in Toledo (1965), observing *Vitis vinifera*, found six generations per year, with different durations. Garrido (1991) observed that *P. citri* completed six to seven generations per year, and even more if climatic conditions were favourable for its development.

Male flight abundance

Analysis of the number of males captured per flight in the pheromone traps allowed us to determine certain regular characteristics of *P. citri* development. In general, male captures were more abundant in 1994 and 1995 than in 1992 and 1993. The average number of males captured per trap and flight in 1994 (1470.1±423.6) was significantly higher than values for other years (F=9.43, df=3,88, P<0.01), followed by 1995 (971.2±268.3), and 505.8±114.1 in 1993 and 444.2±93.1 in 1992.

Comparing the abundance of males captured in the five flights of each year, our data showed that abundance was greater in the second and third flights than in the rest (F=27.34, df=4,88, P<0.01). Males were least abundant in the first and fifth flights; the fourth was intermediate and significantly different to the other two (Table 1). The peak number of males captured per trap and day followed the same tendency as abundance. The highest values were observed in the second and third flights (78.5±20.3 and 61.1±8.5 respectively), and were significantly different to values for the other three (F=32.92, df=4,89, P<0.01). The lowest male captures were obtained in the first and fifth flights (11.5±4.8 and 7.8±1.8 respectively). The peak in the fourth showed an intermediate value (17.0±3.0) that was significantly different from the others.

Table 1. Number of captured adult *Planococcus citri* males per trap in each flight.

Year/Flight	1st. Flight	2nd. Flight	3rd. Flight	4th. Flight	5th. Flight	Average per flight and year
1992	257.5±151.2 (7) b B	408.6±129.9 (8) c AB	816.4±362.7 (8) b A	376.3±155.5 (8) a AB	338.8±114.8 (8) ab AB	444.2±93.1 (39) bc
1993	22.0±7.00 (6) c C	691.6±139.4 (6) b AB	1396.7±279.6 (6) ab A	384.7±141.1 (6) a B	33.9±9.6 (6) c C	505.8±114.1 (30) c
1994	311.1±175.3 (4) ab B	3657.3±1395.9 (4) a A	2100.1±616.6 (4) a A	561.0±161.1 (4) a B	412.6±176.8 (4) a B	1470.1±423.6 (20) a
1995	452.3±269.3 (4) a BC	2899.1±533.8 (4) a A	1055.8±417.0 (4) ab B	333.7±179.1 (4) a C	115.3±71.9 (4) bc D	971.2±268.3 (20) b
Average captures per flight	237.6±84.1 (21) C	1529.2±384.2 (22) A	1251.6±213.4 (22) A	404.4±77.5 (22) B	228.4±60.5 (22) C	

The number of captured males has been transformed to ln (males+1) for variance analysis. Means were compared using the General Linear Models Procedure (SAS Institute, 1996). Different small letters in columns indicate different averages. Different capital letters in rows indicate different averages. (Duncan's Multiple Range Test. P< 0.05). Averages are presented at original scale. Numbers of observations appear in parentheses.

None of the groves were treated with pesticide during the study period. Male captures in pheromone traps may, therefore, at least in part, have been a reflection of *P. citri* population abundance in the trees. But it must also be remembered that captures also depend on male activity in each flight period and also on the ratio of males to total population in each

generation. In this species, this ratio can vary according to the season of the year. Bodenheimer (1951) showed that the ratio of males in the *P. citri* population increased in the period from June to September, and decreased in winter. Avidov et al. (1969) also showed that the ratio of males decreased in winter. Thus, male flight abundance, on one hand reflects the evolution of *P. citri* population abundance on the tree during the year, and on the other is related to a series of climatic factors that influence insect behaviour and physiology. The latter are associated with the season of the year and affect all groves in the same way. The seasonal evolution of abundance also exhibits both a common component for all groves and years and a characteristic component for each individual grove. This is closely bound to the different factors that influence mortality.

We can try to estimate to what extent changes in abundance from one flight to the next are due to these common factors and to what extent they are the result of the specific oscillations of each grove by calculating the regression coefficient between the total number of males captured in the five flights (Table 2). When flights are consecutive there is always a significant relationship between the abundance of one and another. Between 25% and 40% of variability in male abundance corresponds to common factors, with the rest being due to specific factors associated with particular groves and years. But, when we try to relate male abundance between non-consecutive flights, the great variability of specific grove and year related factors eliminate any kind of relationship. This is a reflection of the rapidity with which the population abundance of this species can vary over even a short period of time: the male population after two generations bears no relation to that of the initial male population.

Applying this correlation procedure for abundance between two consecutive generations, we were able to estimate the importance of mortality factors that influence *P. citri* populations during winter by correlating the abundance of the last male flight of one year with the abundance of the first male flight of the next. We examined thirteen sets of data and found a total absence of correlation ($R^2=0.03$, df=12, P<0.1). These results suggested the existence of important climatic or biological mortality factors during winter that did not affect all the *P. citri* populations in the same way.

Table 2. Coefficient of regression between male abundance in the different flights

	F2	F3	F4	F5	Flight 1 of the next year
F1	0.29 (22) *	0.03 (22)	0.00 (22)	0.04 (22)	
F2		0.38 (23) *	0.11 (23)	0.00 (23)	
F3			0.24 (23) *	0.02 (23)	
F4				0.31 (23) *	
F5					0.03 (13)

For regression analysis data were transformed to Ln (x+1). Numbers of observations appear in parentheses.
F1, F2, F3, F4 and F5: Number of captured males in the first flight (May), second flight (June-July), third flight (August), fourth flight (September) and fifth flight (November) respectively.
*: Significant model (P<0.01)

Temperature parameters: Lower developmental threshold and degree-days to complete a generation.

The lower developmental threshold temperature was estimated by analysing all the male flights in our study and investigating the temperature value that minimised differences between degree-days obtained for two consecutive male capture peaks. When we studied the

variability between degree-days obtained from field data in all groves (measuring degree-days between male flights) and the lower threshold temperature (Fig.1), we observed that the minimum coefficient of variation corresponded to a temperature value of 8.3°C. Taking this value as the lower developmental threshold, we calculated the degree-days between maximum peaks of consecutive male flights, which averaged 562.4±11.6 degree-days (n=70). We assumed this value to be the heat accumulation required to complete a generation for this species. With this threshold temperature we then calculated the average degree-day distance for the four intervals between the five male flights of a given year. Analysis of these data revealed no significant differences between them (F=1.43, df=3, 62, P=0.2437). This result implied that the number of degree-days did not vary for the five flights produced every year and that, consequently, they can be applied to all of them as a thermal constant.
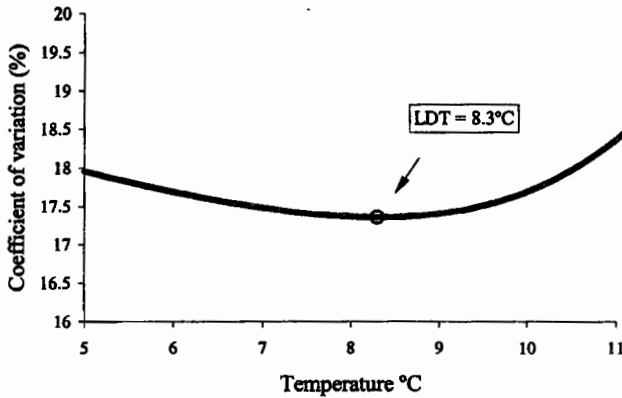
Figure 1. Lower developmental threshold.

We represented the average evolution of male captures in all groves and years, versus the thermal constant in a graph. The result showed that the first male flight occurred at approximately 500 degree-days after 1st January. The second and third flights were the most evident, because of their greater abundance (Fig. 2).

These calculated thermal parameters were similar to those reported by other authors, who generally obtained their values in laboratory assays. Bodenheimer (1951), working in laboratory conditions with insects reared on potato sprouts, calculated the lower developmental threshold and the thermal constant for *P. citri*, obtaining values of 8.4°C and 525 degree-days (from eggs to oviposition) respectively. Avidov et al. (1969) reported that the lower developmental threshold was 8.2°C and that 698 degree-days were required to complete a generation. Arai (1996) obtained values for the lower developmental threshold of female larvae and for the preoviposition period of 7.7°C and 8°C respectively. The respective thermal constants for these periods were 401 degree-days and 378 degree-days.
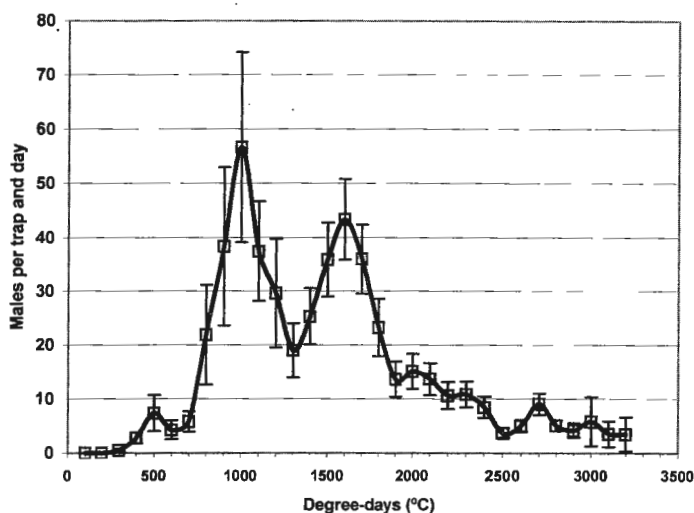
Figure 2. Average evolution of male captures in all groves and years versus degree-days.

Females on the trunk and main branches

At certain periods of the year, increases in *P. citri* populations, mainly young females and females with eggs, were detected on the trunk and main branches. These females were looking for a place to settle for ovipositing, and accounted for an important part of population dispersal. *P. citri* trunk populations showed important fluctuations throughout the year, with increases in May, from the end of June to early July, and in August. These increases in female populations on the trunk coincided with those of previously defined male flights. Peaks for females on the trunk corresponded with the first three male flights. In September and November the number of trunk females also increased, coinciding with the fourth and fifth male flights. But this did not occur in all the groves studied. In 1998, these coincidences between maximums for females on the trunk and male flights were most evident in the Xeminavel grove, as reflected by the five female trunk peaks. These female populations increased from May to July and from July to August (Fig. 3). Trunk populations then decreased in September and almost disappeared in November. Other authors reported similar migrations or increases in numbers of *P. citri* females, with them travelling to trunks and settling for ovipositing, though cases varied with times of the year and climatic conditions.

Santorini (1977) showed that the overwintering individuals started their activity in May, and that this first generation settled on trunks. Franco (1992) also observed a female migration to the trunk in March and April and in summer. Toledo (1965) observed that dry-windy days prompted individuals on grapevines to leave the green organs and move to older wood, crevices, and even buried parts of trunks
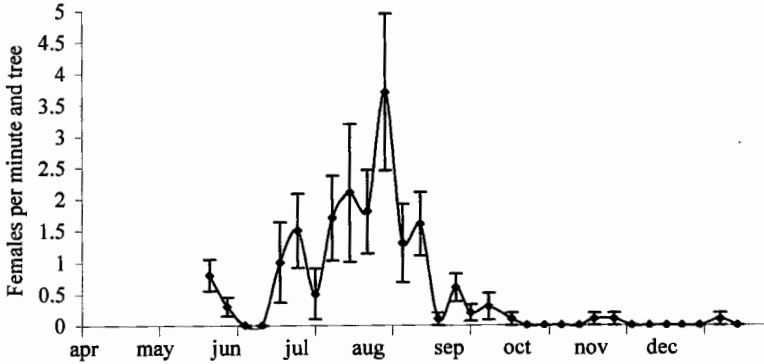
Figure 3. Average number of females per minute on trunk in the Xeminavel grove 1998.

Population dynamics on fruit

P. citri population dynamics on fruit were investigated from May, when the fruit set and started to develop, until the harvest, distinguishing the *P. citri* population under the calyx of the fruit when the insects were not yet visible on the fruit, and the *P. citri* population that was externally visible on the fruit. From petal falls in May to fruit drops in mid July, we noted a major *P. citri* larvae migration from all parts of trees to recently settled fruits. Until July it was difficult to observe *P. citri* in the field, because it sheltered under calyxes. Meyerdirk et al. (1981) concluded that the optimal site for finding *P. citri* when infestation is extremely low is under fruit calyxes. Berlinger et al. (1978) demonstrated that in grapefruit, the calyx is an ideal habitat for *P. citri*, as it protects them from pesticides and natural enemies.

    P. citri started invading fruit in mid May, beginning on recently settled fruit when their petals and pistils had finished dropping. Dispersal stages in this period were first instars from the first generation of the year. At this moment the first instars represented more than 95% of the total number of individuals under calyxes (Table 3). At the end of May and beginning of June more than 80% of the population consisted of first and second instars. Third instars and young females started appearing, but in small quantities. Females with eggs appeared in mid June, and the third instars and young females represented 30% of the total population. In mid July, the proportion of first and second instars increased again, with the arrival of a new generation. Until this point, all the individuals under the calyxes had come from outside the fruit. From May to June the number of insects per fruit experienced a linear increase, and then until mid July the increase was exponential.

    In order to determine the calyx zone where *P. citri* were located, 2140 fruits were investigated and a total of 2043 insects counted. The majority (86.6%) were located on the sepals, on the inner part of the calyxes, and of the rest, 8% were located on the fruit surface under the calyxes and the other 5.4% on the sepals, on the outer part of the calyxes.

    The fruit invasion coincided, therefore, with the month of June, and also included part of the end of May and part of July. During these months the percentage of *P. citri* infested fruits increased rapidly, with values increasing by more than 80%, in many cases, in mid July. In this period, the percentage of infested calyxes averaged 72.0±5.8% (Fig 4) for all the groves

investigated. This invasion period coincided with initial fast fruit growth and with physiological fruit drop, which drastically reduced fruit numbers.

Table 3. Evolution of developmental stages of *P. citri* under calyxes.

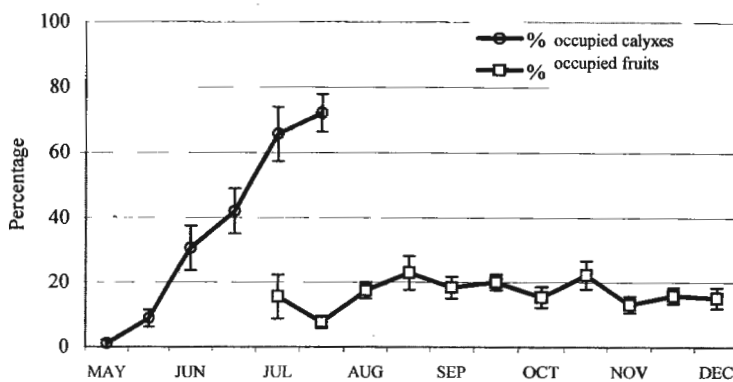| Date	Number of fruit	Insects per fruit	Percentage of insects at different stages of development				
			1st Larva	2nd Larva	3rd Larva	Young Female	Female with eggs
8/5 - 20/5	1000	0.24	96.4±1.4	1.5±1.3	1.1±0.9	1.1±0.9	0.0±0.0
21/5 – 5/6	1400	1.19	57.3±8.3	31.5±8.3	7.5±3.8	3.8±2.4	0.0±0.0
7/6 – 15/6	1240	2.49	32.3±5.6	36.0±2.4	21.3±2.9	10.3±3.2	0.2±0.1
20/6 – 29/6	920	3.19	37.7±6.8	31.2±6.3	18.4±3.9	7.4±2.3	5.3±2.1
13/7 – 31/7	560	9.48	41.2±6.1	35.2±3.6	17.5±3.7	3.8±1.2	2.3±1.1

Figure 4. Population dynamics of *P.citri* under the calyx and on the fruit.

As the percentage of infested fruits increased, so did the number of insects per infested fruit. Initially this was because infested calyxes were invaded by new individuals entering from the outside, but at the end of July, it was due to the females that developed under the calyxes initiating reproduction.

The described process refers to calyx invasion, and in the majority of cases that invasion is hardly appreciable in the field. When we studied the percentage of fruits externally occupied by *P. citri* in the field in mid July, there were initially few fruits in which it was possible to externally detect the incipient *P. citri* population that was developing under the calyxes (Fig. e). At that point in time, *P. citri* could be observed externally on an average of 7.7±1.9 % of occupied fruits, while under calyxes, that percentage was much greater. Although the percentage of invaded calyxes increased rapidly over time and reached values of almost 80%, the external population coming from those calyxes did not follow the same growth pattern. In July and August percentages of occupied fruits increased in some groves,

but generally fluctuated, and in many cases decreased from August onwards. The percentage of occupied fruits that might have been expected if population from invaded calyxes had progressed was never reached. We studied the evolution of the average percentage of fruits occupied in all the monitored groves. In mid August, the percentage of occupied fruits from 26 groves averaged 22.95±5.24%, and in mid September this value slightly decreased to an average of 20.04±2.41%. There must, therefore, have been some important mortality factors that limited *P. citri* population growth on fruit in summer and prevented the full development of the initially very high proportion of invaded fruit calyxes.

We were able to find other indirect proof that important mortality factors were involved in the evolution of *P. citri* on fruits from July onwards, once the invasion of fruit had been completed. We did this by relating the percentage of externally occupied fruits in each grove in mid July and the percentage of invaded calyxes (or number of insects per invaded fruit calyx) during the same period (Fig. 5). During this period, it was observed how, in each grove, the more frequent or abundant was *P. citri* under the calyxes, the more there were the external attacks. There was, however, no observed relationship between the invasion of fruit under the calyx in mid July and the percentage of externally occupied fruits during the months of August and September (Fig. 6). Although the percentage of occupied fruits for the group of groves studied remained relatively constant from July to October (Fig. 4), in some groves this percentage increased and in others it declined. As a result, when harvesting approached, the groves with greatest *P. citri* populations at that particular moment were not necessarily the ones that had had the greatest populations in July.

In conclusion, it seems that the *P. citri* populations that can be found under calyxes or on fruit in July do not serve as a reliable indicator of the potential for attacks in October. Major mortality factors, presumably dominated by natural enemies, are what really determine the future evolution of populations in August and September. Early monitoring in June and July and defining an economic threshold for pesticide applications would not, therefore, seem the most appropriate ways to combat *P. citri*.
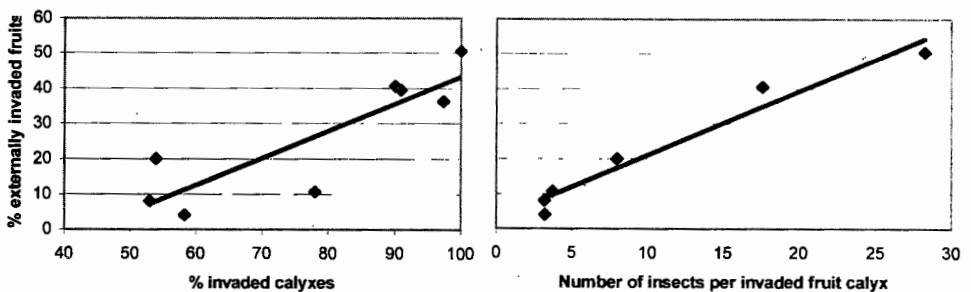

Figure 5. Relationship between percentage of invaded calyxes and number of insects per invaded fruit calyx, and the percentage of externally invaded fruits in July.
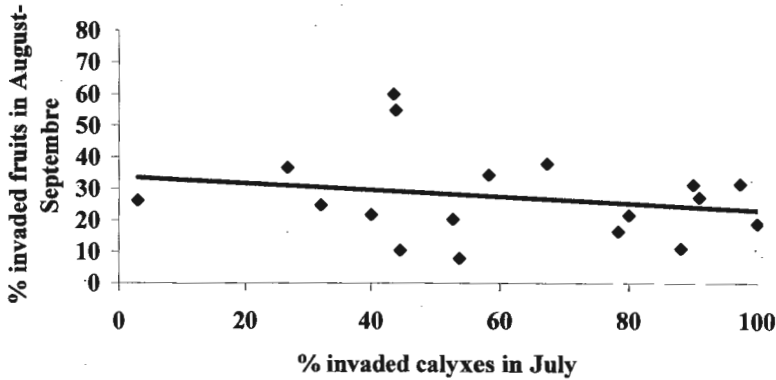
Figure 6. Relationship between the percentage of invaded calyxes in July and the percentage of invaded fruits in August-September.

Natural enemies

Parasitism on *P. citri* located under fruit calyxes was detected as early as mid June. The percentage of calyxes with parasited *P. citri* increased from this time until the end of July, in cases reaching values of over 50%. This parasitism observed under the calyx influenced the subsequent evolution of *P. citri* populations. When the percentage of calyxes with parasited *P. citri* in July was less than 20%, there was a close relationship between the percentage of invaded calyxes in July and the percentage of externally infested fruits in August (R=0.887, n=5). However, when the percentage of calyxes with parasited *P. citri* in July was greater than 20%, this relationship did not apply (R=0.014). In all of these latter cases, the percentage of occupied fruits in August was less than 25% (Fig. 7).
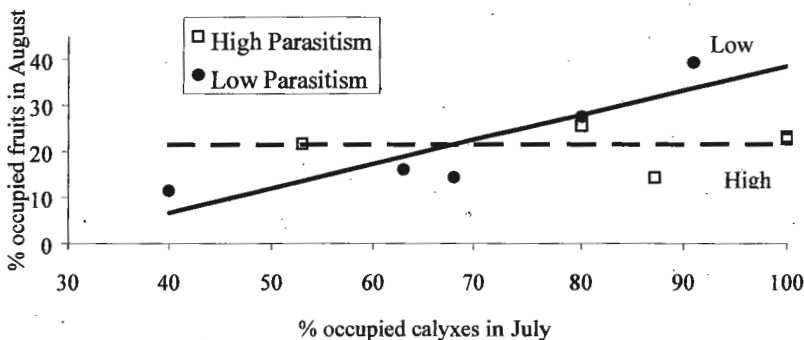

Figure 7. Relationship between the percentage of occupied calyxes in July and the percentage of invaded fruits in August, according to parasitism under the calyx in July.

It seems that in August, the presence on fruit, of the *P. citri* predator *Cryptolaemus montrouzieri* Muls. influenced the evolution of *P. citri* populations from then until November.

In August, in the absence of *C. montrouzieri* a close relationship was observed between *P. citri* populations in August and November (R=0.907, n=6). However, this relationship was not observed when *C. montrouzieri* was present (Fig. 8).
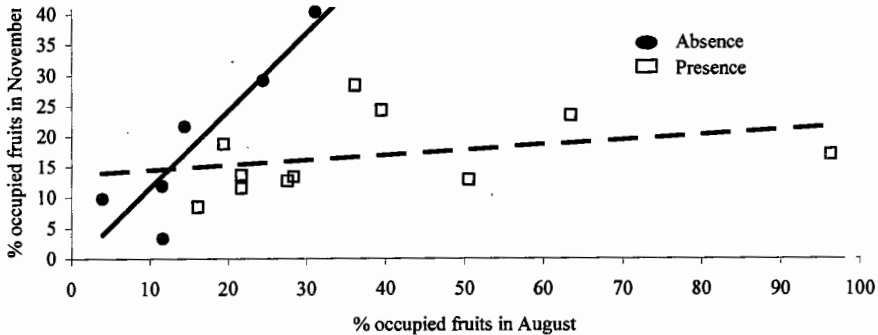

Figure 8. Relationship between the percentage of fruits occupied in August and November related to the presence of *C. montrouzieri*.

In conclusion, *P.citri* developed five generations per year. For this species the thermal constant was 562.4 degree-days, as the heat accumulation required to complete a generation. The lower developmental threshold temperature was 8.3°C. First instars invaded the fruit calyxes from May to July, reaching high values in percentage of invaded calyxes, averaging 72%. *P. citri* invaded the fruits from the calyxes in August, averaging 22% of invaded fruits. Parasitoids reduced *P.citri* population under the calyxes and on the fruit from June to July, so did *C. montrouzieri* on the fruit from August onwards.

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Defining a method to determine the release dose of *Cryptolaemus montrouzieri* Muls. based on the incidence of *Planococcus citri* Risso in Citrus orchards

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Abstract: The use of *Cryptolaemus montrouzieri* as an exotic predator for the biological control of *Planococcus citri* (Citrus Mealybug) dates back to 1892 in California (United States), and from then many countries have adopted this method for controlling the pest. Despite the long history behind its use in citrus crops, there is still no consensus about the suitable doses to be applied for a successful control of the mealybug. In this work we design a mathematical model to estimate the release doses of *Cryptolaemus montrouzieri* needed to ensure an effective control of the pest, based on a statistical procedure. So, along three spring seasons (1998, 1999 and 2002), we noted down the amounts of adult *Cryptolaemus* released per hectare in many citrus orchards. The initial incidence of Citrus Mealybug was known in all these orchards, and we weekly monitored the incidence of the pest in a sample of fruits. Finally, we considered that the biological control had been successful if the fruit percentage with Citrus Mealybug became lower than 5% at least two months after the predator release. With the data collected, we designed a probability model based on the logistic regression method, which allows us to define the release doses suitable for every initial incidence level of *Planococcus citri*. For this, we assume different risk margins about the probability of a control success being reached.

Key words: *Cryptolaemus montrouzieri*, *Planococcus citri*, biological control, citrus, release dosage, logistic model

Introduction

The biological control by the release of exotic beneficial organisms has been often reported as a more effective method than chemical inputs (Llorens, 1990; Vacante, 1992). This is the case of *Cryptolaemus montrouzieri* Muls., a predator which, either alone, or combined with the release of parasites as *Leptomastix dactylopii* How. (Llorens, 1990; Ripolles, 1992a) or *Nephus reunioni* Fürsh. (Magro & Hemptinne, 1999), is the most commonly used insect for controlling *Planococcus citri* Risso, the Citrus Mealybug (Division of Agriculture and Natural Resources, 1991; Magro & Hemptinne, 1999). *Cryptolaemus montrouzieri* was discovered in Australia (Llorens, 1990), and first releases in other countries date back to 1892 in California (United States of America) (DeBach, 1964). Since then, many countries all over the world have reared and released this insect to control Mealybugs in citrus and other crops, and at present its use is widely recommended in regulations and guidelines for the citrus Integrated Production (Consejería de Agricultura y Pesca, 2000; Coscollá, 2000).

The persistence of introduced *Cryptolaemus* in the field is not good, since it does not survive winters well (Division of Agriculture and Natural Resources, 1991; Ripolles, 1992b; 1992c), and so must be supplied to the orchards every year. However, despite the long history behind its use in citrus crops, there is still no consensus about the suitable doses to be applied for a successful control of Mealybug.

Among the doses usually recommended in literature, it is possible to find: 10 adults per infested tree for low to medium-sized pest incidences (DeBach, 1964), 5 to 20 adults per infested tree depending on the pest incidence and the date of the release (Llorens, 1990; Ripolles, 1992c), or 5 to 15 if used combined with *Leptomastix dactylopii* (Llorens, 1990; Ripolles, 1992a); 500 adults per acre (about 1236 per hectare) if the pest was a problem the previous year (Division of Agriculture and Natural Resources, 1991; 1994); 200, 600 or 800 adults per hectare for less than 5%, 5 to 15%, and more than 15% fruits infested, respectively (criteria used in the rearing room of Silla, Valencia, Spain). Other suggestions are usually given to complement the measures above, as the division of the dose chosen into two releases ten days time distant from each other, or to make the releases some weeks after a chemical treatment if pest populations are great (Division of Agriculture and Natural Resources, 1991). One thing everybody seems to agree with is when to make the releases: from April to September, although the optimum season is Spring, from April to June (DeBach, 1964; Alexandrakis, 1983).

The control of Citrus Mealybug run by *Cryptolaemus montrouzieri* is the result of the interactions between this predator and some physical and biotic factors (climate, local auxiliary complex) that are more or less characteristic of each region. So, the doses of *Cryptolaemus* needed to ensure an effective control of Mealybug could differ in different zones. In this work, we develop a mathematical model to estimate the release doses of *Cryptolaemus montrouzieri* in the southern citrus agroecosystems of the Iberian Peninsula. The model is based on an empirical procedure, for which we use the data collected from successful and failed releases made, along three years, using different predator doses. We start from the premise that a good criterion must be based on the initial incidence of Mealybug in the orchard, and the time for the releases must be subordinate to it (DeBach, 1964).

Material and methods

Study area

Along three years (1998, 1999 and 2002), in Spring and Summer, we released adults of *Cryptolaemus montrouzieri* in about 130 to 150 orchards per year, within the three citrus areas in Malaga province: Guadalhorce Valley, Western Coast, and Axarquia. All these orchards showed different infestation levels by Citrus Mealybug, and included plantations of most of the orange, lemon and tangerine varieties grown in the province. All the insects released were self-produced, reared in the official insectary (Consejeria de Agricultura y Pesca, Delegación de Málaga).

Releases and pest monitoring

The criteria used to decide the release doses varied along the study period, and so it included several predator doses for similar incidences of Mealybug. Before releasing the predator, we checked a 300 fruit sample in every parcel, in such a way that three fruits per geographical orientation were checked in each of 25 trees chosen at random. For every fruit, we noted down the presence/absence of Citrus Mealybug individuals. We did not probe under the calyx by raising its edges, to make our sample method resemble that one expected to be followed by a farmer or by a citrus co-operative field technician. In 37 orchards, we repeated this sampling procedure 15, 30, 45 and 60 days after the *Cryptolaemus* releases.

An effective control of Citrus Mealybug populations is usually achieved starting from the second generation of *Cryptolaemus montrouzieri* (DeBach, 1964). At 25-26 °C, this predator completes a cycle in 30-35 days (reported by the Unitat de Lluita Biologica, Servei de Protecció dels Vegetals, Conselleria d'Agricultura i Pesca, Generalitat Valenciana). Because of this, if *Planococcus citri* disappeared from the orchard within the first 30 days after the

release, then we scrapped that orchard from our data set to build the model, since we considered the control of Mealybug to have had other causes than *Cryptolaemus*. Finally, we considered that the biological control had been successful if the Mealybug incidence stood or became lower than 5% fruits at least two months after the predator release.

Mathematic basis

The mathematical model built here is based on the logistic regression (Lawless & Singhal, 1978; 1987). So, the probability of a control success being reached against Citrus Mealybug in an orchard was defined by the following equation:

$$p = \frac{e^y}{1 + e^y}$$

where e is the base of Napierian logarithm, and y is a linear predictor as follows: $y=a+bx$. For the independent variable x, we tried two alternative combinations of the predator release dose (CN: *Cryptolaemus* individual number per hectare) and the incidence of Mealybug (IP: initial proportion of fruits with *Planococcus citri*), to say:

- CN / IP, which assumes a lineal ratio between CN and IP.
- Ln(CN) / IP, which assumes that the predator dose (CN) must increase following an exponential curve as the pest incidence (IP) shows higher values.

We assessed the model goodness of fit by a χ^2 test. Once a statistically significant model was obtained, the resulting equation was used to relate both CN and IP under the assumption of different success probabilities. In order to avoid bias due to the unequal number of successes and failures in the orchards used to build the model (Hosmer and Lemeshow, 1989), a correction was made on the final equation (see Rojas et al., 2001).

Results and discussion

The model

In nine of the 37 orchards where the pest evolution was monitored after the predator release, *Planococcus citri* disappeared in the first month, and so we did not used them to build the model. In 20 orchards the pest disappeared or stood in less than 5% fruits within the second month after the release (successes), and in eight ones, the pest was not controlled at all (failures). Using the logistic regression procedure, we found no significant model for the lineal ratio between CN and IP. On the contrary, a significant model ($\chi^2 = 5.05$; p <0.05) was found for the exponential ratio:

$$p = \frac{e^{1.1590+0.7259 \times [Ln(CN)/IP]}}{1 + e^{1.1590+0.7259 \times [Ln(CN)/IP]}}$$

Solving the equation above for CN:

$$CN = e^{\left[\dfrac{Ln\left(\dfrac{p}{1-p}\right)+1.1590}{0.7259}\times IP\right]}$$

·Figure 1 represents three curves corresponding to the exponential relation between CN and PI for three different success probabilities. In table 1 we show the number of *Cryptolaemus* individuals to be released with different initial incidences of Mealybug, in order to get a given success probability in the control of *Planococcus citri*.
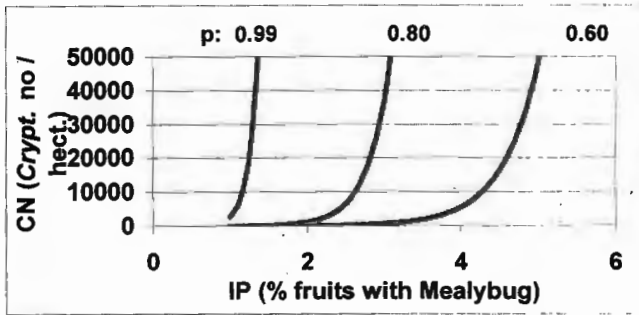

Figure 1. Exponential relation between CN (*Cryptolaemus* number per hectare) and IP (initial proportion of fruits with Mealybug) for three success probabilities, as derived from the equations in the text.

Table 1. Number of *Cryptolaemus* individuals to be released with different initial incidences of Mealybug, in order to get a given success probability in the control of *Planococcus citri*

		IP (initial percentage of fruits with Citrus Mealybug)			
		1%	2%	3%	4%
p (success probability)	0.60	9	74	643	5546
	0.70	16	252	3990	63288
	0.80	33	1111	37018	1000000
	0.90	102	10374	1000000	100000000
	0.95	285	81289	20000000	7000000000
	0.99	2770	8000000	20000000000	60000000000000

The most outstanding thing in table 1 is the great size of predator dose pointed out by the model. For a Mealybug incidence of about 1% of fruits, the number of insects to be released is acceptable even when a high success probability is required. For 2% of fruits infested by Citrus Mealybug, the doses for a 0.8 probability of a control being reached are also acceptable, but if the required probability is 0.9 the predator doses required are over 10000 adults. For incidences about 3% or 4%, the success probability using acceptable doses is not higher than 0.7. More severe attacks by Citrus Mealybug seem to have, according to the model, low possibilities of being controlled with *Cryptolaemus*. The most remarkable conclusion, directly derived from the exponential relation observed between CN and IP, is that the predator must be released when the Citrus Mealybug has a very low incidence in the orchard, if an effective biological control is desired. Thus, a very accurate sample method is needed to be able to detect the presence of *Planococcus citri* at early stages.

The model and dosages in literature

Compared with the doses recommended in literature, our model is usually more conservative. The dosages recommending a given adult number per infested tree (DeBach, 1964; Llorens, 1990; Ripolles, 1992a; 1992c) are to a certain degree equivalent to those derived from our model when the pest incidence is low, but the predator dose / pest incidence ratio in the former is lineal, and this makes them much less predator-demanding than the model as the pest incidence increases. On the other hand, the Division of Agriculture and Natural Resources (1991; 1994) suggests a fixed quantity of *Cryptolaemus* per hectare: about 1236, but also advises to make the releases early in the spring (when the Mealybug incidence is low), and to reduce the pest population with a chemical treatment before the release if the incidence is high. According with our model, 1236 adults per hectare ensure, in fact, a 0.95 probability of a success being reached when the pest affects 1% of fruits, a 0.80 probability if the pest incidence is 2%, and a 0.60 to 0.70 probability if 3% of fruits are infested by Mealybug.

A model for a geographical context

Nevertheless, much care should be taken when applying our model to a geographical context different from the one considered to build the equations. As we said in the Introduction section, an effective control of Citrus Mealybug run by *Cryptolaemus montrouzieri* is the result of the interactions between this predator and many other factors, which are more or less characteristic of each region. Higher summer temperatures, or a wider indigenous auxiliary complex of the Citrus Mealybug, could mean a need for lower doses of *Cryptolaemus* to control the pest. The model presented in this paper was built from data obtained in the field, not in experimentally controlled crops but rather in private orchards in operation. So, all possible factors influencing *Planococcus citri* populations were included in the control, though not detached in the mathematical equation. This means that the validity of our model embraces these geographical areas with similar agro-environmental (climatic, biotic, agronomic) conditions to those in Malaga province. The contribution of this work, thus, from our point of view, is not yet a definite table to relate predator doses with pest incidences. Instead, we propose a methodological approach to be used in order to determine, in any citrus area in the world and using the regional data available, the release doses of *Cryptolaemus montrouzieri* for the biological control of the Citrus Mealybug.

submit the citrus Mealybug to the exclusive control of *Cryptolaemus*, and specially to Carlos Larráyoz, field technician of the S.C.A.A. Malaca, for his valuable collaboration in the sampling works.

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Field trial to determine the effect of pyriproxifen on *Icerya purchasi* Mask. and *Rodolia cardinalis* Muls.

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Abstract: A field trial was carried out in spring, when Icerya purchasi Mask. is present, to determine the effect of the pesticide pyriproxifen on the pest and its predator *Rodolia cardinalis* Muls.

An orchard of clementine cultivar "Marisol" was selected in the municipality of Orihuela and 160 shoots (two per tree from 80 trees) which contained colonies of *I. purchasi*, were previously tagged. Pyriproxifen 10LE at the rate of 0.075% plus a wetting agent was applied with an atomizer (Hardy) consuming 3,472 l/Ha, on March 3, 1997. Population levels of the pest and its natural enemy were monitored immediately before the treatment and 14, 21, 33, 39, 46 59 and 66 days after the treatment. Larvae of *R. cardinalis* appeared at the end of March and its number increased in unsprayed trees until April 22, with an spectacular decrease in the number of the pest. In the plots sprayed with pyriproxifen the number of *R. cardinalis* larvae was low, and neither nymphs nor adults were seen. With time, adults of *R. cardinalis* originating from the untreated parts of the orchard eliminated the *I. purchasi* population from the treated plots.

Key words: *Icerya purchasi, Rodolia cardinalis*, cochinilla acanalada, piriproxifen.

Biological Control in Citrus Groves in the Last 50 Years: Three Successful Cases in Western Sicily

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Abstract: As is well known, the development of trade and the increased speed of transport have facilitated the passive spread of phytophagous insects. Several dangerous new species affecting citrus groves have been reported on in Sicily. In the present paper we will report on the appearance in western Sicily of three dangerous species, *Dialeurodes citri* (Ashm.). *Aleurothrixus floccosus* (Mask.) and *Lepidosaphes gloverii* (Pack.). Chemical insecticides proved unable to control them, while biological means achieved decisive results in keeping these phytophagous species below the economic threshold.

Dialeurodes citri (Ashm.) arrived in Sicily in 1968 and rapidly spread through Palermo citrus groves. The action performed by the non-specific indigenous predator *Clitostethus arcuatus* (Rossi) failed to bring the whitefly down to tolerable levels. In 1977 *Encarsia lahorensis* How. was introduced and after a few years it brought the populations down to levels well below the economic threshold.

Aleurothrixus floccosus (Mask.) was found in the Trapani area in 1980 and it probably spread, over a period of about five years, due to the absence of specific natural enemies all over Sicily and in all other citrus areas in the South of Italy. On the basis of experiences in other European countries, *Cales noacki* How. was introduced from Spain and *Amitus spiniferus* (Brèthes) from France. The aphelinid settled in rapidly, and in the second year, parasitization levels above 70% were achieved in the release area. Similar parasitization levels were attained in the ensuing years in many citrus groves infested by *A. floccosus*.

Lepidosaphes gloverii (Pack.) was discovered in 1947 in Palermo. For several decades it has been the main problem for citrus groves in western Sicily, requiring legislation which made it compulsory to combat it and prohibited transporting citrus plants or parts of them from Palermo province to other provinces of Italy. *Encarsia herndoni* Girault was introduced from Spain and spread rapidly in the areas where it was released, leading to active parasitization levels up to 50%. Despite the high mortality of the parasitoid (70-90%), it has led to the almost total disappearance of *L. gloverii* in all the infested areas of Sicily.

Introduction

As is well known, the development of trade and the increased speed of transport have facilitated the passive spread of phytophagous insects. Several dangerous new species affecting citrus groves have been reported in Sicily. In the present paper we will report on the appearance in western Sicily of three dangerous species, *Dialeurodes citri* (Ashm.), *Aleurothrixus floccosus* (Mask.) and *Lepidosaphes gloverii* (Pack.).

Results

Dialeurodes citri (Ashm), a pest of citrus already known from the USA, France, Spain and parts of mainland Italy, was found for the first time in Sicily in January 1969 on the leaves of orange, mandarin and lemon trees over a small area near Palermo (Genduso 1969).

In a few months it spread rapidly all over the citrus groves of the island, causing heavy damages. The use of white oils against the overwintering stages of *D. citri* had the effect of reducing its population levels, but were ineffective to reduce it under the economic threshold (Liotta and Maniglia, 1975).

Initially, *Clitostethus arcuatus* (Rossi) was the most important predator of *D. citri* observed in Sicily. However, this coccinellid was not able to exert an acceptable control of the pest (Liotta, 1976). In 1977 the aphelinid *Encarsia lahorensis* How., already introduced in Italy (Portici-Naples) in 1973-75 from California to control *D. citri* (Viggiani, 1976), was released in the experimental citrus orchard of S.En.Fi.Mi.Zo. Department, in Palermo, using citrus leaves bearing parasitized host (Liotta, 1978). *E. lahorensis* rapidly became established, its percentage of parasitism increasing progressively, and after three years the biological control of *D. citri* with the aphelinid *E. lahorensis* proved to be a complete success.

Established population of *Aleurothrixus floccosus* (Mask.) were first discovered in July 1980 in a narrow citrus areas near Trapani in western Sicily (Genduso and Liotta, 1980). Since then, and over a period of six years, this pest spread all over Sicily and southern Italy, causing heavy damages to citrus groves (Liotta and Maniglia, 1982).

The results of field observations showed three indigenous species, *Clitostethus arcuatus* (Rossi), *Chilocorus bipustulatus* L. and *Chrysopa* sp. feeding on *A. floccosus*, but they could not reduce the attacks of *A. floccosus* below the economic damage threshold (Liotta, 1982).

On the basis of experiences in other European countries, the parasites of *A. floccosus Cales noacki* How. and *Amitus spiniferus* (Brèthes) were introduced at the end of 1980 in western Sicily. *Cales noacki* How. was obtained from Spain and *Amitus spiniferus* (Brèthes) from France (Liotta and Maniglia, 1982). The first species settled in rapidly and already in the second year, in the release area, parasitization level above 70% were obtained and the population level of the phytophagous decreased progressively from 1980 to 1984 (Liotta and Maniglia, 1984).

Amitus spiniferus (Brèthes) showed initially a poor adaptation capacity, with parasitism levels always below 0,5%. However, five years after its introduction an increasing parasite activity was observed in various areas, and about ten years afterwards *A. spiniferus* attained high levels of parasitism which ranged from 52,9% up to 80,8% in the Palermo Province.

Lepidosaphes gloverii (Pack.), found in Sicily in late 1947, was one of the most harmful armoured scales infesting citrus orchards in Sicily. Observations made in the forty years after its first finding showd that there were no parasitoids of this scale in the island. A few rare reports, as, for example, *Encarsia citrina* (Monastero, 1954), were not subsequently confirmed.

In 1988, in collaboration with the Department of Entomology and Agrarian Zoology of the University of Naples (Portici), through the offices of Dr. Garrido of the Instituto Valenciano de Investigaciones Agrarias, the aphelinid *Encarsia herndoni* (Girault) was introduced in Sicily from Spain (Garrido, 1985; Viggiani and Liotta, 1989). Following its introduction, *E. herndoni* spread rapidly over the citrus orchards around the areas where it was released showing after few months a good ability for settlement and a promising capacity for biological control of *L. gloverii*.

Six years after its introduction in western Sicily, field observations confirmed that the aphelinid showed an active parasitism, ranging from 12,5% to 42,9% on scale males, 45% to 61% on second instar larvae and 56,4% to 76,5% on young females. The high levels of parasitization were accompanied by similar high levels of mortality of parasitoids in the larval stage, in the pupal stage and in the already formed adults which had not yet emerged from the host.

Since its introduction, *E. herndoni* has continually expanded to new areas. A year after being released, in a radius of about 10 km from the point of release, and the following year it was found in citrus orchards 70 km away from the release point. Today the species can be considered present in all citrus areas of Sicily and southern Calabria.

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Petroleum-derived spray oils: current status in the Italian citrus IPM

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Abstract: We introduce a three-level, hierarchical new classification scheme related to degree of refinement of petroleum spray oils (PMOs) used in agriculture for pest control: "mineral oils" (MOs), "agricultural mineral oils" (AMOs) and "horticultural mineral oils" (HMOs). The concept of equivalent *n*-paraffin carbon number and related *nCy* simbology is proposed. We recommend the adoption of median *nCy* values and *nCy* range, as determined by gas chromatograph distillation to replace 50% distillation temperature and 10%-90% distillation temperature range as the standard for describing AMOs and HMOs. In this paper the different PSOs products currently sold in Italy are described. Unfortunately for lack of information, only one of these is included in the list of HMOs. In the period 1995/2001 six commercial PSOs of varying specifications were applied to citrus groves in order to determine the impact to natural enemies, efficacy and influence on fruit production. In 1994/2000 IPM demonstration program, summer applications of PSOs decreased the pressure of *Aonidiella aurantii* on citrus grove. In 1995 experimental fields, the parasitisation of red scale by *Aphytis melinus* was not affected by PSOs, but the parasitisation of woolly whitefly by *Cales noacki* was affected. In 2000/2001spraying seasons, summer application of heavier PSOs reduced crop yield and increased fruit size, compared with lighter oil. Most growers are familiar with the winter application, as they see only negative consequences of summer PSOs (leaf drop, sunburn and yield reduction). But in summer applications the new generation PSOs were safer. These recommendations were included in the IPM programs funded by the European Union.

Key words: classification, selectivity, efficacy, phytotoxicity, narrow range oils

Introduction

Petroleum spray oils (PSOs) have been widely used in Italian citriculture for the past century, owing to their insecticide-miticide characteristics against numerous pests. After the appearance of chlorinated and organophosphate compounds in the 1950s, their use was drastically reduced. These compounds were more effective and had no side effects on plant physiology. But they quickly began to show damage for beneficial arthropods in citrus orchards and to the environment in general (Chapman, 1967; Viggiani *et al.,* 1978; Benfatto 1982 a). At the beginning of the 1980s, certain effects and greater awareness to environmental pollution brought about a rethink in the use of PSOs, whose quality had greatly improved (Riehl, 1981, Benfatto, 1982 b, c; Benfatto & Longo, 1982; Di Martino & Benfatto, 1982; Nucifora, 1984). Nevertheless some doubts remained about possible phytotoxic effects particularly for the plant productivity (Furness & Maelzer, 1981; Di Martino *et al.*, 1986). These concerns were at least partly, overcome by the introduction in Italy of new formulations

of much more refined products commonly known narrow range oils (Luck *et al.*, 1986; Conti, 1987), although consumption data show a continuos decline until the mid 1990s. The slight increase in 1996 was probably due to programs for biological and the integrated control funded by European Union (Raciti *et al.*, 1997) and to better knowledge of the new PSOs. In1997/ 2001 period the total number of organic farms increased from 31,000 to 60,000 units. This increase – from 564,000 to 1,183,000 ha, about 6-7% of the total farms (ISTAT, 2002) – explain the need of the greater definition of PSOs composition. Even though scientific publications indicate that there was a vigorous exchange of information, no common classifications and nomenclature emerged or, it appears, were even seriously attempted. There are almost as many classifications as there are manufactures, administrative regions and authorities. Over the past 20 years, researches for PSOs development culminated in the first international conference on "Spray Oils Beyond 2000- Sustainable Pest & Disease Management", held in Sidney in October 1999 (Eds. Beattie *et al.*, 2002). It covers the various types of PSOs and botanical spray oils, their chemistry and other characteristics, their formulation, modes of action and field use. In particular, it highlights the issue of international standardization of PSOs nomenclature. In this paper we review the specification of PSOs products used in Italian citriculture since the 1940s and a new classification scheme is proposed related to degree of refinement, according to conference recommendations. We also report the impact on natural enemies and phytotoxicity of selected products and their incorporation in integrated pest management (IPM) programs.

A classification scheme for Italian PSOs

Italy PSOs are classified solely on unsulfonated residue (UR). This classification does not consider other important factors, such as the 50% distillation point and the distillation range of 10-90% (Ebeling, 1950; Davidson *et al.*, 1991). Even thought progress has been made in the formulation and labeling of some new PSOs formulations, in general, there is no classification uniformity. A universal system of classification is considered necessary in light of the growing global exchange of information and trade and the expansion of integrated pest management in which PSOs play an increasingly important role. A three-level, hierarchical new classification scheme for Italian product is proposed related to degree of refinement; the three level proposed are, in increasing order of refinement: "mineral oils" (MOs) (Tab.1), "agricultural mineral oils (AMOs) (Tab.2) and horticultural mineral oils (HMOs)" (Tab.3). A simple classification-based approach to nomenclature is proposed with extensions to clarify intended applications as well as essential sub-classifications by molecular size. The concept of equivalent *n*-paraffin carbon number and related *nCy* symbology is introduced. We recommended global adoption of median *nCy* values and *nCy* range, as determined by gas chromatograph distillation to replace 50% distillation temperature and 10%-90% distillation temperature range as the standard for describing AMOs and HMOs (Kuhlmann & Jacques, 2002).

Table1. Proposed standards for mineral oil standards (Kuhlmann & Jacques, 2002)

Source	Virgin petroleum distillate
UV absorbance limit	Meets standard
Initial boiling point	232°C
Colour (ASTM D 1500):	≤ 5.5

Table 2. Proposed standards for agricultural mineral oil (Kuhlmann & Jacques, 2002)

Mineral oil	as table1
source	Highly paraffinic crude
UR (ASTM D 483)	$\geq 92\%$ (minimum)
Paraffin content (% C_p) (ASTM D 2140)	$\geq 60\%$ (minimum)

Table 3. Proposed standards for horticultural mineral oil (Kuhlmann & Jacques, 2002)

Mineral oil	as table 1
Agricultural mineral oil	as table 2
Median-equivalent n-paraffin carbon number (median nCy) (ASTM D 2887)	21, 23, 25
Equivalent n-paraffin carbon number range (nCy range) (ASTM D 2887)	5-6
Colour (ASTM D 1500)	0.5

In the table 4 are described the different most common PSOs products currently sold in Italy with the new classification: only Ultra fine oil (Intrachem, Grassobbio, Italy) can be considered a HMO.

Impact on natural enemies

The effects of PSOs on the population dynamics of pests and beneficial insects are poorly understood. Many variables, such as timing of application, concentrations, and spray coverage probably have a profound effect on the ecology of the system when PSOs was used. In Italy the pressure of red scale (RS) *Aonidiella aurantii* (Maskell) decreased when PSOs were used in IPM citrus demonstration programs because of its greater selectivity than quinalphos (Tumminelli *et al.*, 2002). The effects of different spray timing of Oliocin on parasitisation of red scale by *A. melinus* were not significantly different (Benfatto *et al.*, 2002). Low rates (0.25% - 0.5% v/v product) of Oliocin significantly reduced parasitism of woolly whitefly by *Cales noacki* Howard but the effect of oil alone were significantly less than oil + broad-spectrum pesticides (Benfatto *et al.*, 2002).

Efficacy

During the last 25 years in Italy, PSOs have been widely used as insecticides and miticides (Di Martino & Benfatto, 1973; Benfatto, 1980; Longo & Benfatto, 1982; Benfatto, 1983 a, b; Benfatto, 1994; Benfatto, 1996; Nucifora, 1995; Ortu, 1996; Schilirò *et. al.*, 1996, Maniglia *et al.*, 1996; Mineo *et al.*, 1998; Serges *et al.*, 1998; Conti *et al.*, 1998). The improved characteristics of the standards PSOs and new generation PSOs have extended their use beyond the original application times and range of pests. As general international trend, application rates have been reduced while the number of applications per year has increased, mainly against red scale *Aonidiella aurantii* (Maskell) in the spring and summer and citrus leafminer *Phyllocnistis citrella* Stainton, on young trees or nurseries, in summer and autumn

Table 4. List of PSOs properties used in Italian citrus pest management. (Source: manufactures)

Specification/ Trade names	Oliocin®	Biolid E®/ Agroil®	HMOs Ultra Fine Oil	Newoil®	Ovipron®	Citrole®
Distillation temperature (°C)						
at 101,33 KPa (ASTM D 447)						
50% point				365		321
10-90 %				24		30
at 1,33 Kp (ASTM D 1160)						
50% point	230	226	213	216	213	
10-90 %	≥70	45	36-40	25	36	
Colour (ASTM D 1500)	?	?	0.5	?	?	?
Unsulfonated residue (%) (ASTM D 483)	> 95	> 95	> 95	≥ 98	96	≥ 99
Density at 15°C (Kg/L) (ASTM D 4052)		0.847		0.860	0.8259-0.832	0.82
Viscosity (mm²/s)	270 cps a 20°C					
Saybolt Universal Seconds (SUS) at 37.8°C (ASTM D88)		75		14.1		
Kinematic at 40 °C (ASTM D 445)				13.7	9	6.5
Median equivalent n-paraffin carbon number (median nCy)	24	23	22	21	21	19
Equivalent n-paraffin carbon number range (nCy) (ASTM D 2887)	?	6	5	?	?	?
Carbon-type (%) (ASTM D 2140)						
Ca (aromatics)			2	0.5		0
Cn (naphtenics)			33	43.5		60
Cp (paraffins)			65	56		40
active ingredient (a.i. %) (oil content)	80	80	98	85	98	98
Manifacturer in Italy	Bayer, Milan	Sipcam, Milan Serbios, Rovigo	Intrachem, Grassobbio	SCAM, Modena	Cerexagri, Catania	Total, Puteaux (France)

(Smith *et al.*, 1997). The actual volume applied range from 2000 to 5000 L ha^{-1} depending on foliar densities, tree height, and planting densities. Handgun spraying is the mainly adopted PSOs application system, so that greater precision is achieved in supplying the correct amount of pesticide. Low volume application of PSOs is not recommended because of the insufficient coverage of target pests (Di Martino, 1959; Neubauer, 1981; Beattie & Smith, 1997).

The first results of a 2-years demonstration project (1995-96) concerning the IPM of red scale showed that among those of the new generation PSOs, the lightest PSOs are less effective in summer than the heavier formulations (Davidson *et al.*, 1991; Benfatto *et al.*, 1998). But in cooler periods, the light PSOs guarantee better results, minimizing side-effects (Grafton-Cardwell *et al.*, 1994).

Phytotoxicity

Trials conducted in recent years have shown that the older-type standard products (currently the most used), have a greater risk of leaf toxicity in summer, causing burn and leaf drop, and fruit toxicity in winter, causing insufficient color development of the rind, a low soluble solids content (Brix°), premature ageing, water spot and susceptibility to frost (Furness, 1981 a, b; Riehl, 1981; Di Martino, 1985). New generation of PSOs with intermediate characteristics is considered more suitable for summer temperatures (Grafton-Cardwell *et al.*, 1994; Tumminelli & Conti, 2000). While in cooler periods the light PSOs guarantee better results, minimizing side effects.

The effects of repeated treatments on main physical, chemical and qualitative parameters surveyed during control trials against the citrus leaf miner in Sicily were tested in 1996 (Benfatto *et al.,* 2002). None of the PSOs tested caused any permanent evidential alterations to leaves and twigs or burns to fruit. Absorption spots (soaking) appeared in all oil treatment soon after spray were applied disappeared slowly without leaving marks. In the trees treated with Oliocin, yield per tree was reduced by >50%, and the percentage of green fruit was significantly greater, compared with the control. With Biolid E, the percentage of green fruit was significantly greatly lower than in the control.

Experimental trials carried out during 95-96 on mature Valencia and Navel citrus orchards, demonstrated that the residues level of organophosphates (methidathion, chlorpyrifos, chlorpyrifos methyl, quinalphos) on fruits and leaves are much greater when PSOs are added (Marano *et al.* 1996).

Early ripening "Tarocco" orange was treated in different seasons (winter and summer) with two PSOs (Oliocin, Biolid E) and one HMO (Ultra Fine Oil) to observe their effects on crop yield and fruit quality: Oliocin caused slight reduction in crop yield but increased fruit size (Conti *et al.*, 2002).

PSOs application in the IPM programs funded by European Union

Since 1994, EU has funding a program for growers for implementing IPM in Italy. About 32.000 ha of citrus orchards receive about 600 Euro per ha for integrated production and 1200 Euro per ha for organic production. In these programs PSOs have a strategic role and their utilization is emphasized (table 5).

Table 5. Application of PSOs in the IPM programs funded by European Union

PSOs	Cultivar	Internal area	Coastal area	Pest
213	Lemon		Mar. - Jun. Sept. - Dec.	mites, scales and leaf miner
	Orange/ Mandarin	Aug. - Oct.	Apr. - Jun. Aug.- Nov.	
226	Lemon		Jul. - Sept.	
	Orange/ Mandarin	Jul. - Sept.	Jul. - Sept.	
235 Standard	Lemon		Jul. - Aug.	mites and scales
	Orange/ Mandarin	Jul. - Aug.	Jul. - Aug.	

Source: *Gazzetta Ufficiale Regione Siciliana*, 53, n° 31, Aug. 27, 1999

Most growers are familiar with the winter (post-harvest) application, as they see only negative consequences of summer sprays (leaf drop, sunburn and yield reduction). Table 5 shows that the suggested spray timing is different from the traditional winter application. This is because of evidence that the PSOs applications increase the risk of frost damage (Riehl, 1981; Di Martino, 1985), and detrimental effects on floral induction (Conti, 1987; Beattie *et al.*, 1989; Smith *et al.*, 1997) and are not strictly correlated with pest monitoring (Walker *et al.* 1990; Benfatto & Carroccio, 1996; Tumminelli *et al.*, 1997). As a general tactic, application rates of 0.5-1.2 % of a.i. are suggested for minimizing negative side effects.

Conclusion

Ultra fine oil (Intrachem, Grassobbio, Italy) is a HMOs.

The diverse range of the PSOs used in Italian citriculture is evidence of the interest that growers and pesticide firms have in the products. It is also relevant that, under EU rules, PSOs are often the only broad spectrum active ingredient allowed and available at a reasonable price. With the introduction of these new products it is advisable to implement and adopt more predictive pest and climate monitoring systems to avoid unnecessary treatments and failures in pest control. As a consequence of the application of monitoring system, we predict and suggest a shift of application timing from winter to summer for a closer correlation with the activity of each pest. But unfortunately, we notice difficulties in adopting new compounds and technologies, because Italy have barriers in education. The new IPM programs implemented by EU are working to break down those barriers.

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182

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BENEFICIALS

Identification and abundance of Neuropteran species associated with citrus orchards in Valencia, Spain

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Abstract: Samples were collected fortnightly throughout the year during three years (1999-2001) in 10 citrus orchards from an area extending 50 km around the town of València (at the center of the eastern-Spain citrus belt) in order to identify the species of Neuroptera, quantify their abundance and determine their seasonal fluctuations in abundance. Orchards were sampled with a commercial leaf suction aspirator adapted to collect citrus foliage. Out of 7,038 adults recovered, eight Neuropteran species were identified. The species, in decreasing order of abundance, were *Semidalis aleyrodiformis* (Stephen), *Chrysoperla carnea* (Stephen), *Conwentzia psociformis* (Curtis), *Coniopteryx (Xeroconiopteryx) loipetsederi* Aspöck, *Mallada prasinus* (Burmeister), *Chrysopa septempunctata* Wesmael, *Micromus angulatus* (Stephen) and *Mallada genei* (Rambur). More than 90% of the adults identified belonged to the three most abundant species, *S. aleyrodiformis* (a species not reported previously from citrus in Spain), *C. carnea* and *C. psociformis*. The coniopterygids *S. aleyrodiformis* and *C. psociformis* were usually found together in the same orchards and were associated with diaspidid populations. *C. psociformis* was also associated with populations of *Panonychus citri* McGregor. Population trends along the year were different in the two coniopterygid species.

Key words: Neuroptera, Citrus, *Semidalis aleyrodiformis*, *Conwentzia psociformis*, *Chrysoperla carnea*

Introduction

Many species of arthropod predators live in cultivated citrus trees and contribute to the regulation of populations of phytophagous insects and mites. The order Neuroptera includes three main families, Chrysopidae, Hemerobiidae and Coniopterygidae, which play an important role in biological pest control. These Neuroptera are considered polyphagous predators of microarthropods as mites, scales, whiteflies and aphids, including many important pests of the crop (Killington, 1936; Principi and Canard, 1974;Aspöck et al, 1980;

In the Mediterranean area only Portugal has studied all the fauna of Neuroptera in citrus orchards, describing 10 species (Pantaleao et al, 1993; Pereira and Franco, 1993). Other countries have reported occasionally Neuropteran species acting as biological control agents. Among the Chrysopidae, *Chrysoperla carnea* (Stephens) is abundant and widespread (Katsoyannos, 1996). Among the Coniopterygidae, *Semidalis aleyrodiformis* (Stephens) is the most common species, being reported in citrus from several countries in or near the Mediterranean basin as Georgia (Agekyan, 1979), Turkey (Davarci, 1996) Greece (Katsoyannos, 1996) and Italy (Letardi and Pantaleoni, 1996); *Conwentzia psociformis* (Curtis) is abundant in Spain (Ripollés and Meliá, 1980).

The objective of this paper is to determine the identity and abundance of the Neuropteran species that live in Citrus orchards from Valencia (eastern Spain), and study its seasonal trend and association with different prey species.

Materials and methods

The study has been developed in 10 commercial citrus orchards located in the País Valencià area, all of them lees than 50 km away from the town of Valencia, in the center of the main Spanish citrus belt. All the orchards were under the cultural practices usual in the area, including chemical sprays to control pests. These sprays included one to two applications per year, in spring or summer, usually to control scale insects.

The orchards were sampled fortnightly for three years, 1999 to 2001. On each sampling date, four samples were collected per orchard, each of them from a group of 10 randomly selected citrus trees. The sample consisted of 70 aspirations (approximately 2 minutes) with a custom built suction device made out of a commercial garden two-stroke engine-powered blower (McCulloch, model 320 BV, 32 cc, 1.1 Kw) with an added cylindrical plastic suction mouth 30 cm high and 30 cm in diameter. Each aspiration included the part of the plant canopy contained in the mouth, which represented 20 to 30 leaves plus associated small branches.

The samples were placed in plastic bags and translated to the laboratory for identification. There, samples were frozen for 48 hours to kill the insects, and examined for the presence of Neuropterans using a dissecting scope. All adults were counted and identified to the species level. The Neuropterans found were conserved in 70% ethanol and identified with the keys of Killington (1936 and 1937), Meinander (1972), Aspöck et al (1980). Prey species were estimated by an index of abundance as follows: 0 = no insects; 1 = 1 to 3 insects; 2 = 4 to 10; 3 = 11 to 30; 4 = 31 to 100. The following prey species or groups of species were considered: *Panonychus citri* (McGregor) , *Icerya purchasi* (Maskell), *Lepidosaphes beckii* (Newman), diaspidids with circular scale (*Aonidiella aurantii*(Maskell) and *Parlatoria pergandii* Comstock), *Aleurothrixus floccosus* (Maskell), aphids (*Aphis gossypii* Glover and *Aphis spiraecola* Patch) and *Phyllocnistis citrella* (Stainton) .

Results and discussion

Species identified
The total number of Neuropteran adults found in all the samples taken in this study was 7,038. Eight different species were identified, four included in the family Chrysopidae, three in the Coniopterygidae and one in the Hemerobiidae (Table 1).

Table 1. Species of the order Neuroptera identified in citrus orchards from Valencia (Spain) in 1999-2001

Family	Species	total n°
Coniopterygidae	*Semidalis aleyrodiformis* (Stephens, 1836)	3228
	Coniopteryx (Xeroconiopteryx) loipetsederi Aspöck, 1963	158
	Conwentzia psociformis (Curtis, 1834)	1260
Hemerobiidae	*Micromus angulatus* (Stephens, 1836)	20
Chrysopidae	*Mallada prasinus* (Burmeister, 1839)	128
	Mallada genei (Rambur, 1842)	10
	Chrysopa septempunctata Wesmael, 1841	57
	Chrysoperla carnea (Stephens, 1836)	2177
		7038

Wide differences in abundance were found bewtween these species. Overall the three most abundant, *S. aleyordiformis, C. carnea* and *C. psociformis,* represented more than 90% of all the Neuropteran adults found. The three species were also the most abundant in every individual orchard and in each of the three years of sampling. The remaining species appeared also distributed in the 10 orchards except for *M. angulatus*: most of the 20 adults identified in this species were found in one orchard.

S. aleyrodiformis has been reported in citrus from Georgia, Turkey and Greece (Agekyan, 1979; Davarci, 1996; Katsoyannos, 1996), but this is the first time it is reported in spanish citrus orchards. It is a species of paleartic distribution very common in Europe on trees and bushes, in cultivated as well as in spontaneous plants (Zeleni, 1978; Sziraki, 1994; Vidlicka, 1995; Letardi and Pantaleoni, 1996). It is considered the most common coniopterygid on trees in the Iberian peninsula (Monserrat and Marín, 1991). *C. carnea* is a common and widespread species in citrus of the Mediterranean area, feeding on aphids, scales, mites and other microarthropods (Katsoyannos, 1996; Garrido, 1999; Llorens, 1990; Paulian, 1999). *C. psociformis* is a well known predator of aphids and mites in Spanish citrus orchards (Ripollés and Meliá, 1980; Llorens and Garrido, 1992).

Association between species

Some significant correlations in abundance have been found between the species of Neuroptera identified. Abundance was measured as total number of adults identified in one orchard throughout a year. As populations were sampled for three years and in 10 orchards, a total of 30 abundance indices were considered for each species. Populations of the two common species of coniopterygids, *S. aleyrodiformis* and *C. psociformis*, showed similar abundance indices in the same orchards and years ($r = 0.432$; $P < 0.05$; $n = 30$). Conversely, orchards with higher populations of *C. psociformis* showed usually lower populations of the two chrysopids, *C. carnea* and *C. septempunctata* ($r = - 0.445$; $p < 0.05$; $n = 30$ and $r = - 0.385$; $P > 0.05$; $n = 30$, respectively). *C. loipetsederi* and *M. prasinus* were also found associated in the same orchards and years ($r = 0.503$; $P < 0.01$; $n = 30$). This relation in abundance could be related with similar environmental requirements or with the presence of common prey species.

Abundance indices per orchard and year were also measured for eight possible prey species or groups of species. When estimating the coefficient of correlation between the abundance of prey and the abundance of Neuropteran species, some significant values were found. Populations of the two common coniopterygids, *S. aleyrodiformis* and *C. psociformis*, were higher in orchards where the diaspidids were also higher (for *S. aleyrodiformis and L. beckii*, $r = 0.663$, $P < 0.01$, $n = 30$; for *S. aleyrodiformis* and circular scales, $r = 0.433$, $P < 0.05$, $n = 30$; for *C. psociformis* and *L. beckii*, $r = 0.535$, $P < 0.01$, $n = 30$; for *C. psociformis* and circular scales, $r = 0.558$, $P < 0.01$, $n = 30$). *C. psociformis* was also positively correlated with *P. citri* ($r = 0.407$, $P < 0.05$, $n = 30$). Orchards and years with high populations of *A. floccosus* showed higher populations of *C. carnea* ($r = 0,607$, $P < 0,01$, $n = 30$. Finally, a positive correlation in abundance was found between the prey *P. citrella* and the Neuropterans *M. prasinus* and *C. septempunctata* ($r = 0.573$, $P < 0.01$, $n = 30$ and $r = 0.436$, $P < 0.05$, $n = 30$, respectively). No significant correlation could be established between the species of Neuroptera and *I. purchasi*. These correlations in population density between prey and predators do not necessarily imply the existence of a trophic relationship as they could be produced by environmental conditions acting on the two populations simultaneously, but some general tendencies arise from them. Small sized Neuropterans, such as coniopterygids, feed apparently on diaspidid scales and mites, whereas bigger Neuropterans like Chrisopids prefer prey of higher size as *P. citrella*.

Seasonal trend of abundance

The high number of individuals found and the sampling program which included the collection of samples twice every month all along the year for the three years allowed us to study the seasonal trend throughout the year in the three most common species of Neuroptera (Figure 1). Adults of *S. aleyrodiformis* showed two annual peaks, the first in March and the second, more pronounced, between August and October. The seasonal population trend for *C. psociformis* differed from that found with *S. aleyrodiformis*, in spite of the fact that they are very similar species and appear associated in the same orchards. *C. psociformis* showed the highest adult populations in winter (from January to March), and the lowest in summer (July and August). *C. carnea* showed two periods in which adult populations peak, June-July and November-December. These trends include only adults and they not represent the tendency of the whole population as other developing stages are not included. The overall pattern of two annual peaks per year could reflect two generations that these species reportedly develop annually (Killington, 1936; Vidlicka, 1995; Von Johann Geep, 1986).
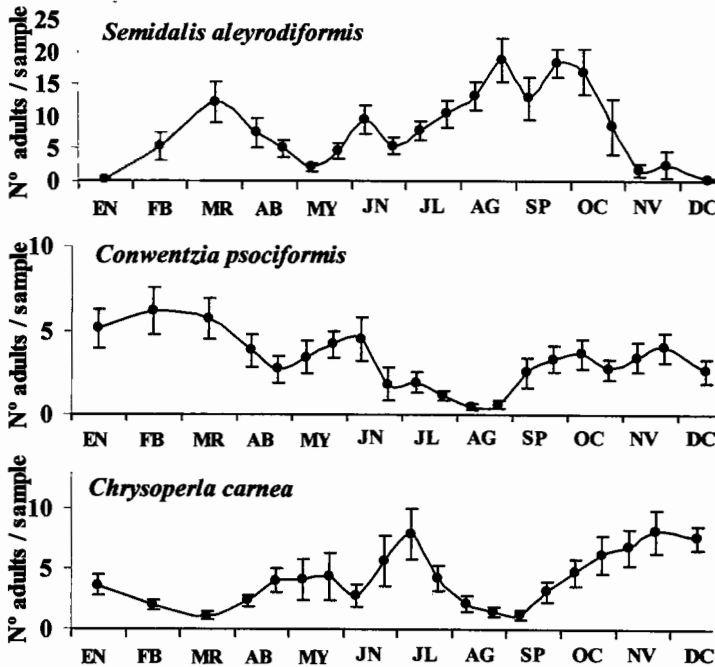
Figure 1. Seasonal trend of Neuropteran species in Citrus orchards in Valencia (Spain). Mean values of 3 years and 10 orchards

In conclusion, eight species of Neuroptera have been identified in spanish citrus orchards and the most abundants are *S. aleyrodiformis*, *C. carnea* and *C. psociformis*. The abundance of *C. psociformis* is positively correlated with the abundance of *S. aleyrodiformis* and negatively correlated with the abundance of the chrysopids. Coniopterygids reach higher populations in

orchards where armored scales and mites are also abundant and the same relationship was found between chrysopids and *P. citrella*. Finally, the seasonal trend in adult abundance differed between species and usually two periods of maximum were found along the year.

Acknowledgements

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Sampling Methods for Faunistic Evaluations in Citrus (Used in Studies for Registration of Plant Protection Products)

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Abstract: For the registration of plant protection products, studies on non-target arthropods are demanded. ESCORT II described a segmental testing scheme. For lower tier testing (laboratory and extended laboratory) study designs are validated and precise guidelines are available. On the contrary for field studies (higher tier) the frame is very flexible. For field trials to assess effects of pesticides on non-target arthropods cereals and orchards (e.g. apples) are used as "model crops". Although there are no detailed recommendations or study designs, basic criteria exist for necessary plot size, possible sampling methods, etc. However, citrus together with olives and hops are not covered by these recommendations, but considered as exceptions that have to be evaluated separately. Actually the field trials in citrus are adapted to the general criteria given for orchards. Nevertheless, test designs should be elaborated taking into account the particularities of citrus crops. With this aim experiences with different sampling methods for non-target arthropods in citrus are presented and discussed.

Key words: citrus, field studies, side effects on non-target arthropods

Introduction

Testing plant protection products for registration purpose on non-target arthropods is based on the sequential testing scheme described by the SETAC/ESCORT guidance document (Barret *et al.*, 1994). The studies start in the laboratory and continue either in extended laboratory or in semi-field. At this lower tier, study designs are conveniently validated and precise guidelines are available.

However, for higher tier studies, i.e. field studies and partly semi-field studies, the frame is very flexible and no concrete regulations exist. Nevertheless basic criteria and good experience exist for test designs, following the guidance document published in 2000 that gives an expert view on the principles, methodological aspects and interpretation of semi-field and field testing (Candolfi *et al.*, 2000). Furthermore, there exists a guidance document for predatory mite field studies on orchards and vineyards, published in 2000 by the IOBC, BART and EPPO Joint Initiative (Blümel *et al.*, 2000).

The crops used as model crops in order to assess effects of pesticides on non-target arthropods in field trials are cereals and orchards. However, citrus together with olives and hops are not covered by any of these guidance documents. Differences between these crops and the ones used as models are considered to be so essential, that results obtained for model crops cannot be extrapolated e.g. to citrus.

Due to the lack of a convenient methodological framework for field studies in citrus, methodologies used commonly in orchard trials have been adapted in several studies concerning side effect testing on non-target arthropods for plant protection product registration. The aim of this paper is to present and discuss different sampling methods for the assessment of side-effects on NTA which could constitute the first step to develop a helpful set of regulations.

Sampling methods

A sampling method for faunistic evaluations has to provide us with quantitative data, which can be used for statistical analysis. It also has to be standardised and reproducible. Furthermore, the method has to prove its efficiency in detecting treatment effects on non-target arthropods. The fauna susceptible to be investigated in orchards in general can be divided into 3 main groups: mites (mainly predatory mites), aerial arthropods and leaf dwelling or foliar arthropods.

Predatory mites

For the assessments of predatory mites, leaf samples are taken and mites are extracted using the washing method (Boller *et al.*, 1984). As the method for side effect testing with predatory mites in vineyards and orchards (Blümel *et al.*, 2000) is validated and standardised for these crops, some aspects have to be taken into consideration, when adapting the method to citrus.

The population level of predatory mites in citrus is clearly lower than in the case of apple and vine. In several trials in France, Italy and Germany up to twenty predatory mites per leaf in vines and about two or three predatory mites per leaf in apple have been observed. In citrus, populations decreased clearly to less than one mite per leaf.

The differences in predatory mite species composition are also an important aspect to consider, due to the fact that their habits and behaviour might vary from one crop to another. For example, the population development differs substantially between the vine, apple and citrus crops. A typical population development for predatory mites in apple in northern Europe reaches its maximum in summer (July and August), while in the case of citrus the minimum is reached at this same time (Figure 1).
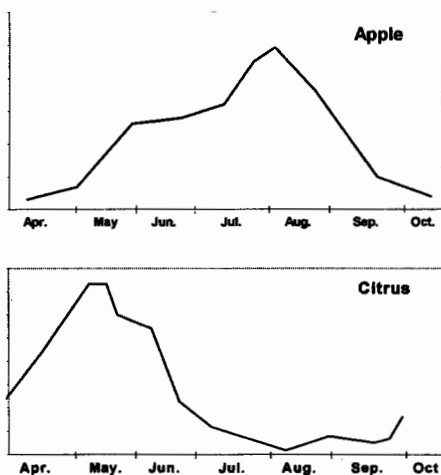
Figure 1. Predatory mite population development in apple and citrus orchards.

The different population development curves demand a completely different trial planning. Particularly when recovery has to be recorded, special care has to be taken while doing the planning for citrus, taking into consideration the breakdown during the hot summer months.

Aerial arthropods

There are several ways to assess aerial arthropods with attracting traps, like water and sticky traps, or with flight interception traps, like window traps. **Sticky traps** have not been used at GAB yet, due to serious difficulties in identifying the arthropods.

When working with **yellow water traps**, the procedure is the following: one trap is placed in the centre of each plot. After an exposure of three to five days, the number of insects caught per trap is collected. This number is about 30-100, and the composition show that the orders Diptera (34%), Homoptera (25%), Thysanoptera (18%) and Hymenoptera (14%) are the most abundant in the samples (Figure 2). Although there are non-target arthropods within these groups, it might be difficult to use the data for evaluation, because the total numbers in the different taxa are often too low for statistical analysis. Treatment effects can be appreciated mainly in the overall amount of insects caught.
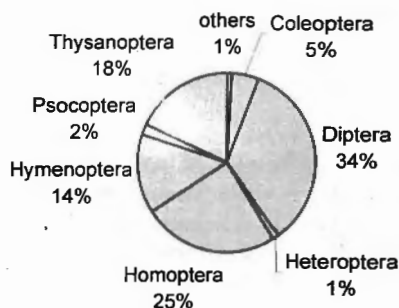
Figure 2. Composition of a yellow water trap sample in citrus

Leaf dwelling or foliar arthropods

The most extended method for the assessment of foliar arthropods is **visual control**, which is used mainly to evaluate the infestation level of the trees with phytophagous insects. In addition, this method is very useful to assess the population development in an orchard. An advantage of the visual control assessment is that results are immediately available and no further determination work has to be done. A clear disadvantage is that results are simply qualitative information, in most cases not suitable for statistical analysis.

The main methods to assess foliar arthropods quantitatively are beating samples and inventory sampling. The **inventory sampling** starts with the erection of collection sheets beneath the selected trees. An insecticide (e.g. dichlorvos) is then applied with a knapsack sprayer. All specimens that fall onto the sheets are collected for a fixed period, e.g. 2 hours. Although it varies during the season, the mean sample size obtained with inventory sampling is approximately 200 arthropods per sampled tree. The composition of the set of insects caught with the inventory sampling illustrates that the predominant groups in citrus are usually Diptera (32%), Hymenoptera (23%) and Homoptera (21%) (Figure 3). Statistically significant treatment effects have been verified in the spiders, beneficial Hymenoptera, Diptera and Coleoptera.
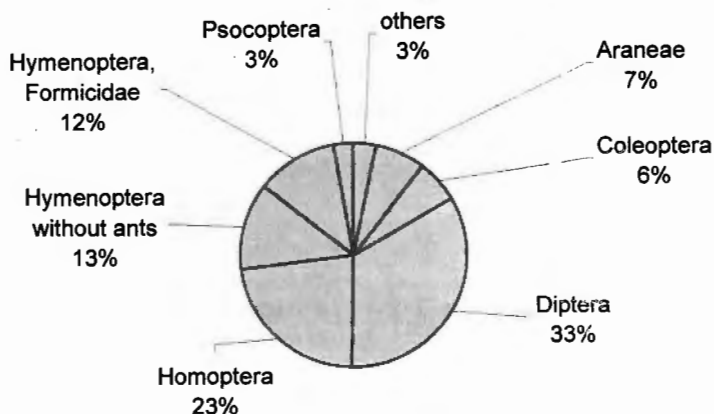
Figure 3 . Composition of an inventory sample in citrus

At the **beating sampling** a fixed number of branches are beaten and arthropods are collected in a funnel. Beating sampling in citrus has not yet been applied, because the amount of arthropods caught with this method is generally lower than when using the inventory sampling method. It might be possible to assess approximately the same spectrum of species, but numbers might be too low to fulfil a reasonable evaluation of the results.

Conclusion

As a conclusion the "standard" methods used in the model crops like apple can be adapted to citrus. Nevertheless, some problems appear in comparison to the trials conducted in apple, most of them because the studies that helped to elaborate the guidelines were located in regions where climatic conditions differ highly from those usually present in areas typical for citrus production.

This results in a completely different population development of arthropods and consequently in a completely different timing of the trials. Especially when recovery has to be observed, it has to be kept in mind, that populations break down during the hot summer months.

The daily temperatures in citrus regions have a strong influence on the amount of arthropods and their location, and sampling methods must adapt to these circumstances. One example is leaf sampling for the assessment of predatory mites. Whereas according to the guidance document the leaves have to be taken randomly from the tree canopy in apple, i.e. from the inner part as well as from the outer part of the tree canopy, it might be more adequate, in the case of citrus, to take the leaves mainly from the inner part of the canopy, as predatory mites concentrate there to avoid direct sunlight and heat.

Finally, sample sizes have to be adapted, because there are generally lower amounts of arthropods in citrus than in apple, and plot sizes have to be adequate.

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Use of "Vortis" arthropod suction sampler for monitoring natural enemies in citrus orchards

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Abstract: Captures of predators (Anthocoridae, Coccinellidae, Coniopterygidae, Chrysopidae, Hemerobiidae, Araneae) and parasitoids (Hymenoptera Parasitica) were compared using the "Vortis" suction sampler and the beating method in lemon orchards from the Oeste region in Portugal. Both methods collected the same *taxa*. Anthocoridae, Conyopterygidae and Hemerobiidae were not sampled by both methods. Although the mean captures were higher in the "Vortis" suction sampler, except for coccinellids, only the Hymenoptera showed significant differences ($p < 0.05$) between the two sampling methods, suggesting that the obtained results using the suction method are similar to those produced by the beating method. Captures were dominated by Araneae and Hymenoptera in both methods, representing 61-67% and 24-35% of the identified specimens, respectively, suggesting a similar structure.

Key words: sampling, predators, parasitoids, IPM

Introduction

The monitoring of citrus pest's natural enemies is recommended in IPM and Integrated Production systems in order to help decision making and to evaluate ecological impact of the pest management strategies. It is usually based on sampling with yellow sticky traps and/or with the beating method. Although these techniques are relatively simple and useful for sampling certain groups of pests and beneficial arthropods, they present important limitations: besides being fastidious, both approaches produce biased estimates in the activities of certain *taxa*. While the beating method favours certain arthropod groups of low mobility which drop readily from branches (Suckling et al. 1996), yellow traps are mainly directed to flying insects that are attracted to yellow colour. Furthermore, the results obtained using the beating methods are influenced by the operator.

Suction sampling may be an alternative method for monitoring natural enemies in fruit orchards, including citrus. It has been used extensively in field crops and grasslands (Suckling et al. 1996). Few examples of application of suction samplers are reported on tree fruits, e.g., apple (Lord 1965, Suckling et al. 1996). "D-Vac" is one of the most widely used suction samplers (Dietrick et al. 1959). It is an effective system, but the apparatus is rather large and heavy. Recently, a number of lightweight blower/vacuums has become available, as the "Vortis" suction sampler. Contrarily to other suction devices, "Vortis" does not require the fitting of a net, bag, or gauze panels. The presence of these restriction systems directly in the air stream, together with a minimal amount of trash, can quickly reduce the airflow to values bellow the accepted critical level of 27 m/s (Southwood 1978, Arnold 1994).

This paper reports on preliminary results of a study comparing the "Vortis" suction sampler and the beating method for sampling predators and parasitoids in citrus orchards.

Material and methods

Sampling methods

In both methods, samples were collected from 20 trees in each of five lemon orchards from Mafra, in the Oeste region in Portugal. The trees sampled in each method belonged to alternate rows in order to prevent interference between methods.

Arthropods were sampled with a wood beating tray in the shape of a four-side funnel (2 x 45 cm x 76 cm x 64 cm). The funnel tapered to a 8 cm threaded opening into which a hollow metal handle was screwed. A vial was inserted in the handle to receive the dislodged arthropods. In each tree, the tray was held under a randomly selected branch from the SW side that was struck three times with a rubber-coated rod.

"Vortis" (see Arnold (1994) for a complete description) samples were collected by suctioning the foliage with a 8 cm diameter flexible tube (estimated airflow = 34,8 m/s) in three different positions in one orientation per tree, during four seconds in each position, i.e., a 12 seconds sampling period per tree. The time duration of sampling was selected based on the results of a previous experiment (Fig. 1), where different periods of time were considered , i.e., 2,

All groups of natural enemies

Hymenoptera Parasitica
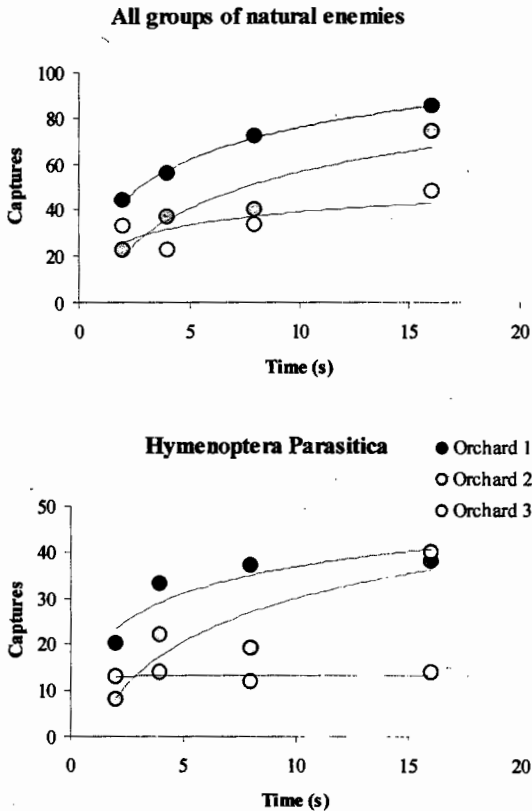
Figure 1. Effect of suction time duration on captures of natural enemies in lemon orchards (September 2002).

4, 8 and 16 seconds. It was suggested that captures of natural enemies (Anthocoridae, Chrysopidae, Coccinellidae, Araneae and Hymenoptera Parasitica) tend to almost saturate after a sampling period of 8 to16 seconds. Saturation may occur after a shorter period, when the abundance of natural enemies is low. The pattern of time response seems to depend on the group of natural enemies. For example, the Hymenoptera Parasitica apparently saturates more rapidly than other *taxa* (Fig. 1) and Araneae did not show a saturation response in some orchards.

Sample processing

In both methods, samples were taken to the laboratory and required the removal of extraneous plant material, in order to allow the identification of the arthropods. A considerable amount of work is needed to sort and identify the specimens.

Taxa

For the preliminary analysis, only the following *taxa* were considered for identification: Anthocoridae, Coccinellidae, Coniopterygidae, Chrysopidae, Hemerobiidae, Hymenoptera Parasitica and Araneae.

Results and discussion

Both methods collected the same *taxa* (Fig. 2). Anthocoridae, Conyopterygidae and Hemerobiidae seem not to be present or just in undetectable levels during the sampling period, because they were not sampled by both methods.
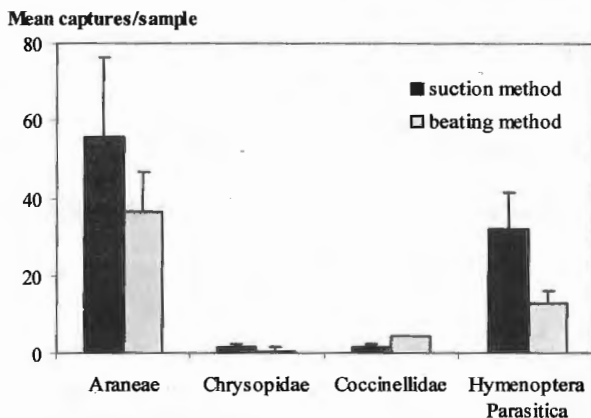
Figure 2. Comparison of Vortis suction sampler and beating method for sampling predators and parasitoids: samples collected from five lemon orchards in October 2002 (mean ± SE).

Although the mean captures were higher in the "Vortis" suction sampler, except for coccinellids (Fig. 2), only the Hymenoptera showed significant differences ($p < 0.05$) between the two sampling methods, suggesting that the obtained results using the suction method are similar to those produced by the beating method. Further studies are needed to confirm this hypothesis.

Captures were dominated by Araneae and Hymenoptera in both methods, representing 61-67% and 24-35% of the identified specimens, respectively (Fig. 3), suggesting a similar structure.

Natural enemies are at the same time important components of the sustainability of agroecosystems, because of their role as regulators of pest populations, and potential environmental bioindicators, because of their sensitivity to ecological factors and to secondary effects of phytosanitary sprays. Several natural enemies groups are being considered with this aim, e.g., Syrphidae (Sommaggio 1999), Neuroptera (Stelzl & Devetak 1999), Heteroptera (Fauvel 1999), Araneae (Marc et al. 1999). "Vortis" suction sampler may be used as a monitoring tool to collect information about the diversity and activity of natural enemies in different components of the orchard, namely, the crop, the hedgerows and the ground cover.
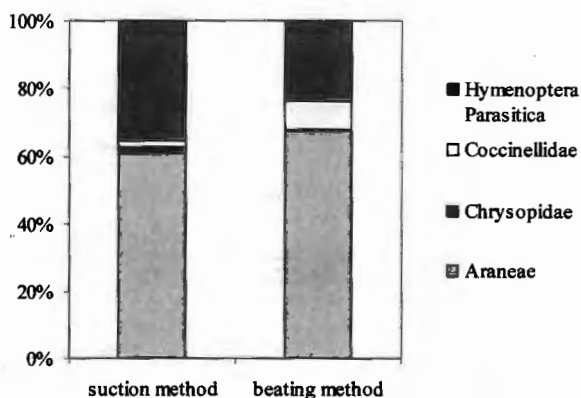
Figure 3. Proportion of the different *taxa* of predators and parasitoids collected by the two sampling methods

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THRIPS

An IPM system for new citrus thrips in Italy

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Abstract: Citrus thrips *Pezothrips kellyanus* (Bagnall) was first recorded in Italy in 1998. Since its first appearance monitoring on different citrus varieties has been conducted in Southern Italy. *P. kellyanus* was the main thrips species in all samples, followed by *Thrips flavus* Schrank and *Thrips tabaci* Lindemann, which are considered secondary pests. In 2002, *Frankliniella occidentalis* Pergande was also detected. In all monitoring sites, the presence of the predatory mite *Amblyseius degenerans* Berlese was observed, but the role of phytoseiids is unclear. Lemon variety was the most commonly attacked, followed by orange and bergamot. The most prevalent alternative host plants were *Lonicera* spp (Caprifoliaceae), *Pittosporum tobira* (Pittosporaceae) and *Jasminum frutificans* (Oleaceae). In the period 1999-2002, citrus thrips activity was monitored on lemon orchards to compare sampling methodology and level of citrus thrips fruit scarring at harvest. The ability to predict season-end scarring from May-July counts, of wingless stages, on fruits was encouraging. According to this data, action thresholds are being implemented. In several field trials white sticky traps were the most attractive for adult thrips, followed by blue traps. The captures on sticky traps demonstrated poor correlation to year-end fruit damage, but showed potential to determine when to time insecticide applications. Pesticide-screening field trials on lemon and orange identified abamectin and spinosad as potential candidates for integrated pest management programs. Organic-compatible compounds as rotenone, pyrethrum, NR oils and azadirachtin did not achieve a sufficient thrips control.

Key words: chemical control, Citrus IPM, *Frankliniella occidentalis*, *Pezothrips kellyanus*

Introduction

Since 1996 the presence of a new species of thrips attacking citrus in Italy has been suspected because of the dramatic increase of characteristic scarring on lemon and orange fruits in coastal areas. In 1998, *Pezothrips kellyanus* (Bagnall) (Kelly's citrus thrips – KCT) was first detected in Sicily, associated with the flower thrips *Thrips flavus* Schrank and *Aelothrips ericae* Bagnall, a predatory thrips (Frittitta *et al.*, 1998; Marullo, 1998). This new pest is suspected to compromise the production of export quality citrus in the coastal area of Italy, as evidenced by the damage caused in Australia and New Zealand (Blank & Gill, 1996; Smith *et al.*, 1997; Baker *et al.*, 2002). Until recently KCT was thought to be an Australian flower thrips species. However, KCT was recently redesignated from *Megalurothrips,* which is an Old World tropical genus, mainly from South East Asia, to *Pezothrips* which are all Mediterranean and Southern European species (Froud *et al.*, 2001). KCT is now believed to have originated from the Mediterranean (Mound, zur Strassen, *pers. comm.*)

The most severe damage occurred on lemon and Navelina orange (18% culled). Lemon fruits were predominantly damaged around the calyx (halo damage, 17%). Tarocco and Valencia orange received similar damage as well (Conti *et al.*, 2001a). Bergamot, whose rind is utilized in the cosmetic industry, can be severely damaged (Marullo, 2000). The causal agent for orange fruit scarring is questioned, because the damage is usually between touching fruit and only occasionally at the stem end of the fruit (halo damage).

A major problem facing the pest control advisor is adequate monitoring of thrips populations in the period shortly after petal-fall when the fruit are most susceptible (Morse, 1996; Baker *et al.*, 2002). Monitoring methods include sticky traps (Childers & Brecht, 1996; Grout & Richards, 1992), counts of fruit infested (Grout *et al.*, 1986) and number of predatory mites on leaves (Pehrson *et al.*, 1991). But little information is available concerning how many adults of *P. kellyanus* per traps are needed or what constitutes an accurate sample. In recent years in Italy several studies are being conducted with the aim to develop an Integrated Pest Management system (IPM) for thrips in citrus. The abundance of thrips species was surveyed. The relationship between the number of fruit infested, of thrips captured on sticky traps, of predaceous mites on leaves and the fruit scarring at season-end was investigated. The potential of several active ingredients to be included in IPM programs was tested. Preliminary results are reported in this paper.

Survey

In 1998-2002 surveys, *P. kellyanus* adults were found on the flowers of most citrus varieties examined, usually in mixed populations with *Thrips tabaci* Lindemann and *T. flavus* (Conti *et al.*, 2001a). *Heliothrips haemorrhoidalis* (Bouché), was found only in six sites, four of which were nurseries. Starting from 2001, *Frankliniella occidentalis* Pergande was found in citrus flowers. *P. kellyanus* was the predominant species in all samples (Table 1).

Thrips larvae were commonly found causing feeding damage on immature fruits of lemons and oranges. They were also found on touching fruits several weeks after petal fall.

In several lemon orchards the phytoseiids predaceous mite *Amblyseius degenerans* Berlese was found. This mite plays a significant regulatory role on *F. occidentalis*, on protected vegetable crops (Ramakers, 1995; van Houte & van Stratum, 1995), but it is unclear whether this species is responsible for natural control of citrus thrips.

Table 1. The identity and relative abundance of thrips species collected on flowers and fruit of a range of citrus varieties, Eastern Sicily, 1998-2002.

Citrus variety	Sites	*Pezothrips kellyanus*	*Thrips tabaci*	*Thrips flavus*	*Heliothrips haemorrhoidalis*	*Frankliniella occidentalis*
Navelina orange	2	XX	X	X		
Tarocco orange	11	XX	X	X	X (1 site)	
Valencia late o.	2	XX	X	X		
Washington navel o.	1	XX	X	X	X	
Femminello lemon	19	XX	X	X		X
Star ruby grapefruit	3	XX	X	X		
Nurseries (lemon, orange, ornamental citrus plants	14	XX	X	X	X (4 sites)	

(XX indicates the predominant species)

Alternative host plant inside or outside citrus orchards may be important in the population dynamics of KCT. Hence, the range of alternative host plants was investigated in the Mediterranean area. The host plants are defined as "breeding host" if the adults and both larval instars are present, and all other records are only "incidental plants" (Froud *et al.*, 2001). The most prevalent alternative "breeding" host plants were *Lonicera* spp. [Caprifoliaceae], *Pittosporum tobira* [Pittosporaceae] and *Jasminum frutificans* [Oleaceae] (Marullo, 2002).

Monitoring techniques

Fruit and leaf counts
In the period 1999-2001, citrus thrips were monitored on lemon, orange and bergamot orchards to compare sampling methodology (fruit counts of wingless stages) and level of citrus thrips fruit scarring. On lemon, the ability to predict season-end scarring from May-July fruit counts was encouraging and a close correlation between all severe scar data and all fruit count data was established (Conti *et al*, 2001b). The following action thresholds are under evaluation: bergamot, 14% of fruitlets infested; lemon, 10 - 20 % of fruitlets infested; orange, 13 % of fruitlets infested (Marullo *et al*, 2002).

The ratio of predatory mites: thrips was tested as an "indicator" for predicting fruit damage at season-end. The number of predatory mites per leaf as well as the ratio mites: thrips-infested fruit did not provide a good prediction of scarring (Conti *et al*, 2001b), confirming that the relationship between canopy phytoseiids and citrus thrips is difficult to demonstrate. (Morse, 1996; Baker *et al.*, 2002). However KCT can be expected to have species-specific natural enemies in the citrus canopy, presumably because this thrips is native to Mediterranean area.

Colored sticky traps
Colored sticky traps are proposed as a means for monitoring *Scirtothrips aurantii* Faure in South Africa (Grout & Richards, 1992) and *F. bispinosa* (Morgan) in Florida (Childers & Brecht, 1996), in order to avoid unnecessary treatments.

In 1999, in lemon groves, the captures of adults on white "type A" ($L=95,23$, $a=-1,28$, $b=+1,85$ according to "Commission Internationale de l'Eclairage"), blue ($L=48,85$, $a=-2,39$, $b=-42,90$), yellow ($L=82,53$, $a=-5,49$ e $b=+85,91$) and transparent traps have been compared, after 15 days of field exposure during February-July. In 2001, the captures of white "type B" ($L=96,22$, $a=-0,86$ e $b=+5,32$) and blue traps have been compared, after 1, 2, 3 and 7 days of exposures during March-June. The best results have been obtained with the white "type A" traps in 1999 and with the blue ones in 2001; blue traps catches of citrus thrips increased from 3 to 7 days of exposures, whereas white "type B" traps did not (Conti *et al.*, 2002).

Unfortunately the captures on sticky traps demonstrated poor correlation to year-end fruit damage, but showed potential to determine when to time insecticide applications. In many sites the peak of adults captures was recorded 1 week before the most abundant proportion of infested fruits (Fig. 1). Use of the sticky traps in citrus groves can provide an early warning method for detecting increases in aerial number of thrips adults. Thus the traps can be incorporated as a valuable tool in the management of citrus thrips populations.
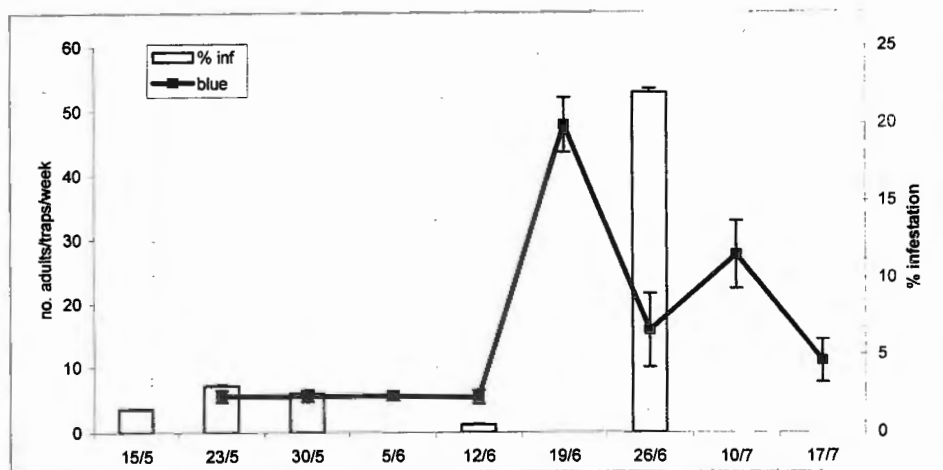
Figure 1. Blue trap catches of adult thrips (KCT predominantly) after 7-days intervals compared with the proportion of fruit infested in lemon groves during 2001.

Chemical control

In the periods 1999-2002, experimental field screenings were conducted against citrus thrips in South Italy on lemon and orange groves (Benfatto *et al.*, 2000; Conti *et al.*, 2001a). Good efficacy of abamectin, reported against *P. kellyanus* in other countries (Blank & Gill, 1997; Baker *et al.*, 2002), was confirmed in lemon orchards. Chlorpyrifos, lufenuron, methomyl and dimethoate achieved good control of the pest. The efficacy and selectivity of spinosad (Morse, 1996) were partially confirmed.

Abamectin and lufenuron were the least disruptive to the phytoseiids populations. Acrinatrin gave the best thrips control but had the most deleterious effect on beneficial phytoseiids mites. Where chemicals with strong miticidal effect have been sprayed (sulphur, NR oil) the numbers of scarred fruits were the highest, suggesting that the predaceous mites were largely responsible for the citrus thrips control (Grout & Stephen, 1996). Abamectin and spinosad are suitable for an IPM program (Luck *et al.*, 1996; Morse, 1996) while insect growth regulators are considered harmful for predacious coccinellids (Hatting & Tate, 1996).

Recent field trials on organic-compatible pesticides as rotenone, pyrethrum, NR oils and azadirachtin did not achieved a sufficient thrips control and they need further investigation on timing and application methodology (unpublished). The design of trials (randomized blocks with unsprayed buffer trees) exposed the treatment to maximum KCT pressure because they permitted adult thrips re-entry from unsprayed trees.

Discussion

In recent years *P. kellyanus* has emerged as the key thrips species of citrus in Southern Italy. This pest jeopardizes the possibility of expanding the production of high quality fruits. Research is needed to resolve the role of phytoseiids in biological control of citrus thrips. A major problem is adequate monitoring of citrus thrips population in the period shortly after petal-fall when the fruit are most susceptible to scarring by citrus thrips. White traps were shown to be very attractive to some thrips species; research is needed to further resolve the

relationship of trap captures with season-end fruit scarring. Direct fruit scouting appear the best method for treatment decision and threshold level for fruit scarring is now under evaluation in Italy. In order to assist pest control decision, current research should also be aimed at improving the KCT phenology model (based on a linear degree-day model) proposed by Baker *et al.* (2002) and at improving the ability to forecast when citrus thrips population will lead to economic levels of fruit scarring. Similar research have been successfully conducted for *S. citri* (Moulton) in California (Schweizer & Morse, 1999). Pesticide screening field trials on lemon and orange identified abamectin and spinosad as potential candidates for integrated pest management programs, but they are not yet permitted for citrus thrips in Italy.

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208

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Thrips flavus Schrank incidence on Primofiori lemon in Málaga province (Spain)

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Abstract: In 1996 a new thrips species, *Trips flavus*, appeared in Malaga province causing important damages in lemon orchards. Because of this, in 1999 the Citrus Integrate Production Agreement between the Consejería de Agricultura y Pesca (Junta de Andalucía) and some citrus cooperatives of Malaga planned to study the biology of this species and its influence on the citrus crops.

We randomly chose nine orchards within the three citrus areas in Malaga province: Axarquia, Western Coast and Guadalhorce Valley. The varieties prospected were mostly Primofiori lemon, but also Berna lemon and Navel-Late orange. We installed three blue sticky card traps in every parcel for a weekly monitoring of flying adults, and with the same periodicity we checked damages in blossoms and fruits. The monitoring lasted from early February to October 1999.

We concluded that *Trips flavus* is a principal pest only in the Axarquia citrus area, where a chemical control is needed. In this zone, damages by *Trips flavus* caused about 60% fruits being considered without commercial value in some orchards. In Guadalhorce Valley we saw similar thrips populations as in Axarquia, but damages scarce, probably due to the customary chemical treatments in the zone. In the Western Coast the *Trips flavus* population was low and irregularly distributed.

The thrips abundance was maximum in April. This time is the best for a chemical control, which could be linked to the first treatment against *Prays citri* usually done in the area.

MITES AND OTHERS

Influence of climatic conditions on population dinamics of *Tetranychus urticae* and *Euseius stipulatus* (Acari: Tetranychidae, Phytoseiidae) on Clementines (*Citrus reticulata*)

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Abstract: A study was carried out during 2000 in citrus orchards (*Citrus reticulata*) from Alicante (Spain) in order to ascertain the influence of climatic conditions on population dynamics of two-spotted spider mite *Tetranychus urticae* and the main phytoseiid mite species *Euseius stipulatus*. Populations of *T. urticae* showed three peaks during winter, late spring and autumn. Apparently population increases in the trees and green cover are related with drops of humidity, whereas fluctuations in temperature have less influence on the seasonal trend. In the same way *E. stipulatus* showed three populational peaks in winter, spring and autumn, with a sudden strong drop at the end of July due to the temperature increase. The influence of temperature, humidity and rainfall are related as well with the stages composition of populations of *T. urticae* and *E. stipulatus* during 2000.

Management of *Tetranychus urticae* in citrus in Spain: acarofauna associated to weeds

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Abstract: *Tetranychus urticae* Koch. (Acari: Tetranychidae) is a serious problem in clementines in the Eastern coast of Spain. Infestations result in a decrease of fruit commercial value. Reasons leading to this problem are poorly understood, but factors such as cover crop management are probably crucial. In this study, the results of sampling weeds at different commercial groves during one year (July 2001-June 2002) are presented. Mites were extracted using Berlese funnels, counted and prepared for microscope observation and determination. In total, 369 samples were processed, corresponding to 45 different weeds. 14,967 mites were extracted from these samples. The genus *Tetranychus* was represented by *T. evansi*, *T. urticae* and *T. turkestani*. Eight different phytoseiidae species were identified: *Euseius stipulatus*, *Typhlodromus phialatus*, *Phytoseiulus persimilis*, *Neoseiulus californicus*, *Typhloseiella isotricha*, *N. cucumeris*, *N. barkeri* and *Anthoseius* sp. Although no relationship could be established between Phytoseiidae and Tetranychidae mite densities, weeds colonized mainly by *T. evansi* could serve as reservoir for phytoseiidae attacking citrus-feeding *T. urticae*.

Keywords: Clementines, weeds, *Tetranychus evansi*, *Tetranychus urticae*, *Anthoseius* sp., *Euseius stipulatus*, *Neoseiulus barkeri*, *Neoseiulus californicus*, *Neoseiulus cucumeris*, *Phytoseiulus persimilis*, *Typhlodromus phialatus*, *Typhloseiella isotricha*,

Introduction

The twospotted mite (TSM henceforth), *Tetranychus urticae* Koch (Acari: Tetranychidae) is a polyphagous and cosmopolitan pest which can occasionally become a serious problem in citrus (Talhouk, 1973). In Spain, this is an almost endemic pest of clementines in La Plana region, the main clementine-growing area in Spain (60,000 ha, ~ 1.5 10^6 Tm). Clementines are especially sensitive to TSM and infestations result in yellowish spots on leaves, and more important, on fruit scarring. Infested fruits become dull-colored on ripening and loose their commercial value. Heavy infestations combined with water stress can result in leaf drop. Reasons leading to TSM problems in clementines are poorly understood, but plant stress is likely to play in important role. On the one hand, water stress increases damage (Wrensh, 1985), but other factors such as climate, citrus variety, or weed management practices could be determinant (Ripollés et al., 1995; UC; 1991).

Control of TSM is complex. Its natural enemies, including species such as *Neoseiulus californicus* McGregor, *Typhlodromus phialatus* Athias-Henriot (Acari: Phytoseiidae) and *Stethorus punctillum* (Weise) (Coleoptera: Coccinellidae) are not considered very effective (García Marí *et al.*, 1991; Garrido & Ventura, 1993; Ripollés *et al.*, 1995). Nevertheless, there

are not specific studies dealing with their effectiveness. Because no reliable sampling method for TSM has yet been developed, growers usually apply preventative chemical treatments, but the results obtained are not always satisfactory. Indeed, resistance of TSM to some miticides - Dicofol, Fenbutatin oxide and Tetradifon- is suspected (Viñuela, 1998).

Polyphagous TSM can also be found feeding on weeds in citrus orchards. These mites, as well as their natural enemies, can move up to the trees and back down to weeds. Therefore, any perturbation of the green cover (either by mowing, plowing, or herbicide applications) may dramatically affect TSM dynamics and as a consequence citrus damage. Green cover management is an important piece of any conservation biological control strategy (Barbosa, 1998), especially when mites on perennial systems, such as citrus, are involved (Nyrop et al., 1998).

As a first step aimed at increasing our knowledge on the relationship existing between ground cover and citrus trees mite populations, we undertook the characterization of the acarofauna present on weeds in citrus orchards.

Materials and methods

Experimental orchards
Orchards sampled in this study were located along the Mediterranean eastern coast of Spain where citrus are grown, between 39°30'N and 41°00'N latitude. From June 2001 to July 2002, different orchards were visited and sampled fortnightly. At each visit, 12 different weed species were collected, put individually in plastic bags and transported to the laboratory for further processing.

Sample processing
About 100 g of each sample was further processed in the laboratory. Microarthropods were extracted by the Berlese method and preserved in ethanol (70% vol.). Afterwards, tetranychid and phytoseiid mites were separated under binocular microscope from the rest of the fauna and preserved in Oudeman's medium (70 % ethanol, glycerin and glacial acetic acid, 87 : 5 : 8 vol.). Prior to species determination, mites were digested in lactic acid (65 % vol.) at 45°C. Then, they were mounted on a slide on Hoyer's medium (distilled water, arabic gum, chloral hydrate and glycerin, 5 : 5 : 20 : 2 weight) for microscope observation.

Mite determination
Based on the morphology of the edeagus, male tetranychid mites were determined. Female tretranychids were distinguished by observation of the chetotaxy of the tarsus of the I pair of legs (Ferragut & Santonja, 1989). Female phytoseiid mites were determined according to their chetotaxy and the morphology of their spermathecae (Garcia-Marí et al. 1990). When representing percentages of abundance (Fig 1-4), undeterminable mites (young stages) were distributed according to percentages obtained from those actually determined. Only when no determinable mites were found on the sample, specimens were classed as undeterminable.

Results and discussion

A total of 369 samples corresponding to 45 different weeds were processed (Table 1). 14,967 mites were extracted from these samples and a minimum of 30 mites per sample further prepared for microscope observation. 7,088 mites were identified: 831 Phytoseiidae, 3,060 Tetranychidae, and 3,197 individuals from other taxa (Oribatida, Acaridae, Tydeidae, Tarsonemidae, Stigmaeidae, Tenuipalpidae and Erythraeidae). The genus *Tetranychus* was represented by: *T. evansi* Baker & Pritchel, *T. urticae* and *T. turkestani* Ugarov & Nikolski. Some specimens of *Bryobia* sp. and *Panonychus* sp. were also found. Eight different Phytoseiidae species were identified: *Anthoseius sp.*, *Euseius stipulatus* Athias-Henriot,

Neoseiulus barkeri Hughes, *N. californicus, N. cucumeris* (Oudemans), *Phytoseiulus persimilis* Athias-Henriot, *Typhlodromus phialatus* and *Typhloseiella isotricha* (Athias-Henriot).

Table 1. Weed species collected.

Family	Weed species (No. of samples)
Amaranthaceae	*Amaranthus* sp. (10).
Apiaceae	*Foeniculum vulgare* L.(2).
Asteraceae*	*Calendula* sp. (9), *Cirsium arvense* (L.) Scop. (1), *Conyza canadensis* L. (5), *Erigeron canadensis* L. (6), *Sonchus* sp. (28), *Senecio vulgaris* L. (13), *Taraxacum dens-leonis* Desf. (2), *Urospermum dalechampii* Desf. (2).
Brassicaceae*	*Capsella bursa-pastoris* (L.) Medicus (4), *Diplotaxis erucoides* (L.) D.C. (21),
Caryophyllaceae	*Silene inflata* Sm.(2).
Convolvulaceae*	*Convolvulus* sp. (24).
Cyperaceae*	*Cyperus esculentus* L. (2).
Equisetaceae	*Equisetum palustre* L. (7).
Euphorbiaceae*	Euphorbia *sp.* (23), *Mercurialis annua* L. (2).
Fumariaceae*	*Fumaria officinalis* L. (12).
Geraniaceae	*Erodium malacoides* Willd. (6), *Geranium rotundifolium* L. (5).
Liliaceae*	*Allium* sp. (4), *Asparagus* sp. (2).
Malvaceae*	*Lavatera trimestris* L. (19), *Malva sylvestris* L. (7).
Papaveraceae	*Papaver rhoeas* L. (2).
Fabaceae*	*Medicago* sp.(6), *Trifolium filiforme* L. (3).
Poaceae*	*Avena fatua* L. (3), *Bromus* sp. (2), *Cynodon dactylon* (L.) Pers.(4), *Echinochloa crus-galli* (L.) P. Beauv. (3), *Lolium rigidum* Gaudin (2), *Poa annua* L. (7), *Setaria glauca* (L.) P. Beauv.(7), *Sorgum halepense* (L.) Pers. (3).
Portulacaceae*	*Portulaca oleracea* L. (10).
Rubiaceae*	*Gallium aparine* (16).
Salsolaceae*	*Beta vulgaris* L. (3), *Chenopodium* sp.(12).
Scrophulariaceae*	*Veronica* sp. (13)
Solanaceae*	*Solanum nigrum* L. (21).
Urticaceae*	*Parietaria officinalis* D.C. (20), *Urtica dioica* L.(9).
Zygophyllaceae*	*Tribulus terrestris* L. (5).

* families including weeds where *T. evansi* was collected.

Table 2 and Figures 1 to 4 present mite abundance per weed. Among Tetranychidae, *Tetranychus evansi* clearly predominated (63.2 %), followed by *T. urticae* and *T. turkestani*. Nevertheless, the most frequent tetranychid mite was *T. urticae*, appearing on 75.5 % of all weeds sampled, followed by *T. evansi* and *T. turkestani*. Differences among species also appeared when considering average mite densities: *T. evansi* presented the highest average densities, followed by *T. urticae* and *T. turkestani*.

Tetranychus evansi, an exotic species from South America, had not been previously found in the study area. This is a polyphagous mite first reported in Spain a few years ago and considered an important pest of annual crops, especially Solanaceae (Ferragut and Escudero, 1999). In our study, this species was very abundant on *Solanum nigrum*, but it also appeared

on weeds belonging to 16 different botanical families (Table1). In addition to presenting the highest average mite densities, *T. evansi* exhibited explosive densities as high as 1,300 individuals in one sample (*Parietaria officinalis*: 16/08/2001). This mite is apparently outcompeting indigenous *T. urticae* and *T. turkestani*. Because *T. evansi* does not feed on citrus, this displacement might be positive for the management of *T. urticae* in clementines.

Table 2. Mite abundance (percentage on total number of mites collected), frequency (percentage on number of weeds where the mite was found) and average mite density (number of mites per sample).

	Abundance	Frequency	Average density
T e t r a n y c h i d a e			
Bryobia sp.	3.6	20.0	0.703
Panonychus sp.	0.4	20.0	0.068
Tetranychus evansi	63.2	55.6	12.267
T. turkestani	10.5	11.1	2.048
T. urticae	22.3	75.6	4.339
P h y t o s e i i d a e			
Anthoseius sp.	0.1	2.9	0.002
Euseius stipulatus	45.9	62.5	2.262
Neoseiulus barkeri	16.7	22.9	0.823
N. californicus	3.8	28.6	0.188
N. cucumeris	0.3	5.7	0.014
Phytoseiulus persimilis	4.7	22.9	0.234
Typhlodromus phialatus	9.9	40.0	0.490
Thyphloseiella isotricha	18.6	14.3	0.916

Euseius stipulatus was, by far, the most abundant phytoseiid mite, representing 45.9 % of all determined Phytoseiidae. It was also the most frequent species, with an average density of 2.26 mites per sample. It was followed by *T. isotricha* (very abundant on pubescent Asteraceae such as *Calendula* sp. or *Conyza canadensis*) and *N. barkeri*, presenting abundances about half that of *E. stipulatus*. Nevertheless, these were not as frequent as *T. phialatus and P. persimilis*, which followed in abundance. According to García Marí et al. (1986), *E. stipulatus* and *T. phialatus* are the most frequent phytoseiid mites in citrus. Therefore, a movement of these mites between the tree canopy and the ground cover is likely to take place.

Although most of the Phytoseiidae found in this study are known to feed on Tetranychidae (García Marí et al. 1991), it was not possible to establish any significant relationship between relative mite densities of either Phytoseiidae and Tetranychidae as a whole or specifically for each possible predator-prey couple. This lack of density-dependent response does not preclude Phytoseiidae are not playing an important role. Actually, no efficient predators have been identified for *T. evansi* yet (Ferragut & Escudero, 1999), and we should not forget that this was the most abundant tetranychid species found. Therefore, weeds, mainly colonized by *T. evansi*, could serve as reservoirs for Phytoseiidae attacking citrus-feeding *T. urticae*. It is for this reason that weeds collected in this study have been ranked according to the difference between Phytoseiidae and *T. urticae* mite densities (Table 3). From this ranking, some interesting weeds can be identified. On the one hand, weeds sheltering the highest *T. urticae* populations should be avoided as much as possible. Weeds

such as *Equisetum palustre*, *Convolvulus arvensis*, *Tribulus terrestris* or *Parietaria officinalis* have a long reputation as mite-harboring weeds. Besides, *Equisetum* sp. and *P. officinalis* have increased their abundance in recent years in orchards were glyphosate has been intensively used (Gómez de Barreda, 1994). Because in most citrus orchards, soil is maintained fairly weed free with herbicides, attention should be paid not to select this kind of weeds. On the other hand, weeds harboring relatively high Phytoseiidae numbers in relation to *T. urticae* could be considered as candidates for establishment of ground covers. Should these weeds exhibit other desirable characteristics, they could be used as recommended by IOBC guidelines on Integrated Production (El Titi et al. 1995) to increase diversity and stability of the orchard. In a recent study (Fibla et al. 2000), one of these weeds, *Poa annua*, was identified as a good candidate for such a use. Another good candidate from that list is *Medicago* sp., because of its belonging to the N_2-fixing family Papilionaceae. Further research on this topic is needed, but the more we know about the citrus ecosystem, the closer we will be to a really sustainable citrus industry.

Table 3. Weeds classified according to the difference (d) between Phytoseiidae density and *Tetranychus urticae* density (d value).

| Difference (d)	Weed (I)
d ≥ 1	*Calendula* sp. (24.6), *Cynodon dactylon* (23.0), *Cirsium arvense* (22.0), *Capsella bursa-pastoris* (16.0), *Portulaca oleracea* (15.8), *Medicago* sp. (7.0), *Asparagus* sp. (5.3), *Lolium rigidum* (4.6), *Bromus* sp. (4.5), *Poa annua* (4.3), *Sonchus* sp. (2.7), *Fumaria officinalis* (2.3), *Foeniculum vulgare* (1.9), *Geranium rotundifoilum* (1.7), *Amaranthus* sp. (1.3), *Taraxacum dens-leonis* (1.0).
1 > d ≥ 0	*Chenopodium* sp. (0.7), *Erodium malacoides* (0.3), *Senecio vulgaris* (0.3), *Solanum nigrum* (0.2), *Conyza canadensis* (0.2), *Sorghum halepense* (0.0), *Mercurialis annua* (0.0), *Echinochloa crus-galli* (0.0).
0 > d ≥ -10	*Avena fatua* (-0.3), *Cyperus esculentus* (-0.5), *Allium* sp. (-1.5), *Urospermum dalechampii* (-2), *Beta vulgaris* (-2.3), *Setaria glauca* (-2.4), *Veronica* sp. (-2.5), *Urtica dioica* (-3.0), *Silene inflata* (-3.0), *Euphorbia* sp. (-3.5), *Gallium aparine* (-3.5), *Trifolium filiforme* (-5.8), *Erigeron canadensis* (-7.0), *Diplotaxis erucoides* (-7.2), *Malva sylvestris* (-7.5).
-10 > d	*Convolvulus* sp. (-10.2), *Equisetum palustre* (-11.9), *Lavatera trimestris* (-12.0), *Tribulus terrestris* (-14.2), *Parietaria officinalis* (-19.5), *Papaver rhoeas* (-23.5).

Acknowledgments
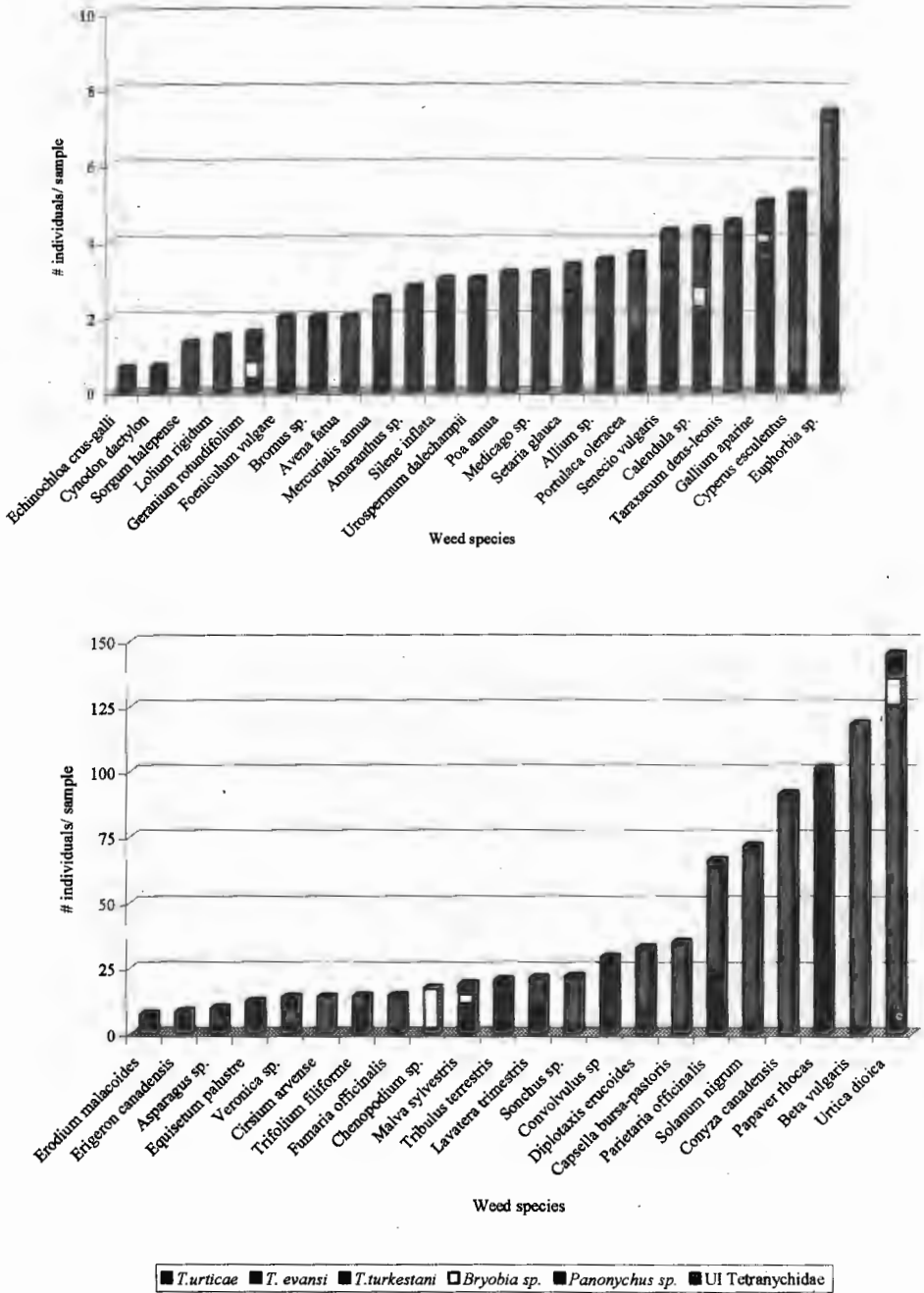
Figure 1. Tetranychidae mite densities (UI: undeterminable specimens).
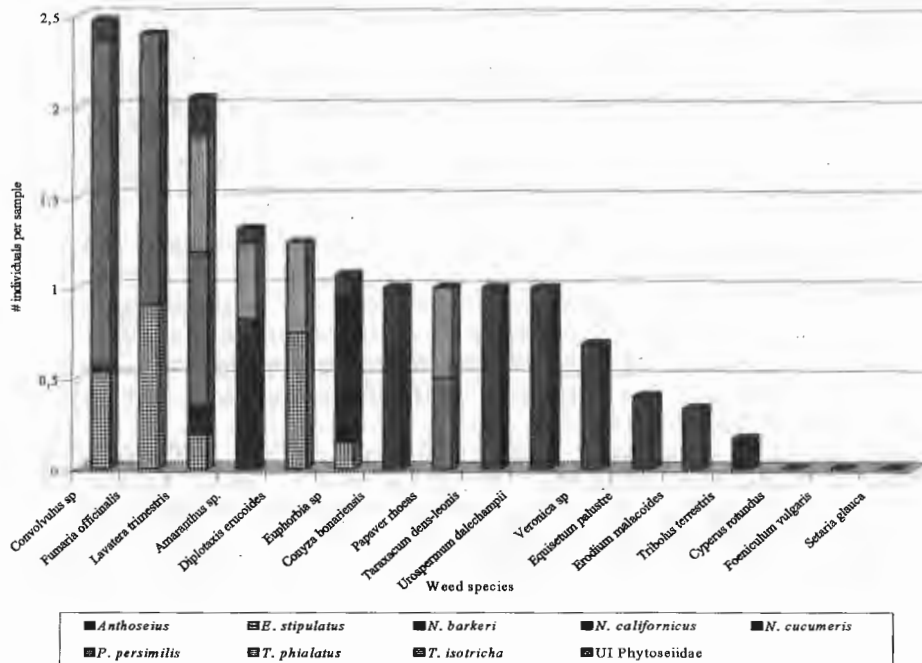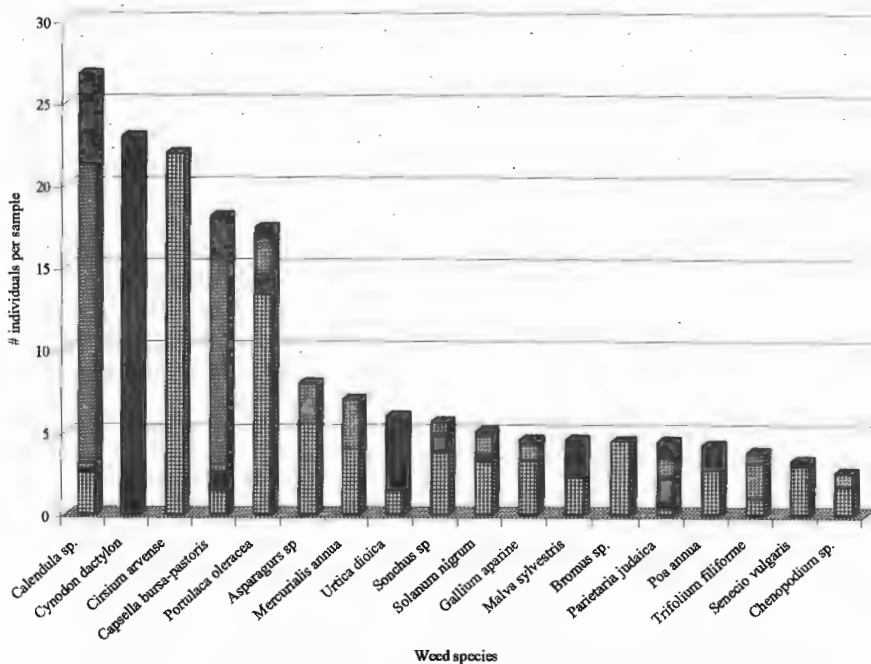
Figure 2. Phytoseiidae mite densities (UI: undeterminable specimens).

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Flora of lemon orchards from the "Oeste" region of Portugal

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Abstract: Fifty four flora surveys were carried out in 50 lemon orchards from Mafra, in the "Oeste" region of Portugal, in February/March 2002. A total of 136 species were identified belonging to 42 families. Compositae, Gramineae and Leguminosae were the predominant families. An abundance index was attributed to each species and the respective mean abundance and infestation degree were estimated. The ecological profiles and preferences were determined, aiming to evaluate the influence of the edaphic factors, pH (H_2O), assimilable phosphorus, assimilable potassium, Mg, and organic matter, on weed distribution.

Key words: flora surveys, lemon orchards

Introduction

Ground cover is a recommended practice in integrated production of citrus. It is a modality of vegetation manipulation in the agroecosystem that attempts to preserve or increase the plant diversity in the surrounding habitats of the orchard. Ground cover may enhance beneficial arthropods in the orchards, constituting therefore a tactic of biological control. With this purpose, knowledge on the diversity and species composition of the flora associated with citrus orchard is very relevant.

The flora of sweet orange (*Citrus sinensis* (L.) Osbeck) orchards are relatively well studied in the major citrus producing regions in Portugal. Several flora surveys were carried out in the Center and South of Portugal since the 70's by Silva (1988), Sá & Fontes (1976), Carvalho *et al.* (1994) and Guerreiro & Martins (1994). However, almost no information is available on citrus orchards from the "Oeste" region, where the major lemon production area of Portugal is located.

This paper is concerned with flora surveys carried out on lemon orchards from Mafra, in the "Oeste" region, during autumn-winter season. Some ecological factors associated with flora composition are also presented. This is part of a research project on the "Management of ground cover and hedgerows in citrus orchards for biological control of pests" that is being conducted on the frame of AGRO Program.

Material and methods

A total of 54 flora surveys were carried out on 50 lemon orchards from Mafra, between February and March 2002.

An abundance index was attributed to the surveyed species, corresponding to the number of plants per square meter, using Barralis (1975) scale. The median abundance of each species and the degree of infestation were determined based on Barralis (1976) and Michez & Guillerm (1984), respectively. Soil samples were collected at 20 cm depth for physical and chemical analysis.

The method of ecological profiles (Daget & Godron, 1982) and the following parameters were calculated: the comprehensive profile for each factor; entropy of factor, maximum entropy and sampling quality; corrected frequency profile; mutual information between species and factors; the mean mutual information; ecological preference and groups.

The edaphic factors, texture, pH (H_2O), assimilable phosphorus, assimilable potassium, magnesium and organic matter were studied. Weed species were grouped in scale imbricated groups (the species are grouped with respect to amplitude of ecological profile, e.g. 301 present in class 1, 2 and 3; 302 present in class 2 and 3) and their ecological preference determined by calculation of the center of gravity of corrected frequency profile (Daget et al., 1971; Daget, 1976).

Results and discussion

A total of 136 vascular plant species were identified, representing 42 botanical families (Table 1). *Asteraceae*, *Poaceae* and *Fabaceae* were the predominant families and therophytes and geophytes the predominant life forms (62.5 % and 19.9 %, respectively).

Table 1. Number of plant species per family.

Family	number	%
Asteraceae	25	18.4
Poaceae	11	8.1
Fabaceae	10	7.4
Apiaceae, Brassicaceae, Geraniaceae, Liliaceae, Polygonaceae	6	4.4
Caryophyllaceae, Euphorbiaceae, Rubiaceae, Scrophulariaceae	4	2.9
Chenopodiaceae, Lamiaceae, Papaveraceae, Urticaceae	3	2.2
Araceae, Cyperaceae, Lythraceae, Plantaginaceae, Ranunculaceae, Rosaceae, Solanaceae	2	1.5
Amaranthaceae, Apocynaceae, Araliaceae, Boraginaceae, Convolvulaceae, Cupressaceae, Curcubitaceae, Equisetaceae, Fagaceae, Hypericaceae, Juncaceae, Malvaceae, Oleaceae, Onagraceae, Oxalidaceae, Pittosporaceae, Primulaceae, Resedaceae	1	0.7

The mean abundance (number of plants per square meter), relative frequency, infestation degree and ecological preferences are shown just for the species with relative frequency equal or higher than 6% (Table 2). *Erodium moschatum, Oxalis pes-caprae, Poa annua* and *Urtica urens* presented the highest infestation degree. These species, as well as *Conyza albida, Lavatera cretica, Solanum nigrum, Sonchus asper* and *Sonchus oleraceus*, were the most frequent species (RF>50%).

The factors with a high equitable sampled were, by decreasing order, magnesium (0.99), assimilable potassium (0.93), organic matter (0.93), texture (0.90), pH (H_2O) (0.84), assimilable phosphorus (0.65). The low equitable sampled of phosphorus is due to the fact that soil samples showed generally high phosphorus content.

The results showed that most of the major weed species in lemon orchards are among those that were able to colonise in all classes of factors studied (species marked with a * in Table 2).

Comprehensive profiles for the factors are: texture (1) sandy and loamy sand = 18, (2) sandy loam and loam = 28, (3) sandy clay loam and clay loam = 8; pH (H₂O) (1) acid (4.6-5.5) = 7, (2) moderate acid (5.6-6.5) = 30, (3) neutral (6.6-7.5) = 8, (4) alkaline (>7.6) = 9; assimilable phosphorus (1) 51-200 ppm P₂O₅ = 9, (2) > 200 ppm P₂O₅ = 45; assimilable potassium (1) 51-100 ppm K₂O = 9, (2) 101-200 ppm K₂O = 23, (3) > 200 ppm K₂O = 22; magnesium (1) 31-60 = 12, (2) 61-90 = 15, (3) 91-125 = 11, (4) > 125 = 16; organic matter (1) ≤ 1.5 % = 35, (2) > 1.5 % = 19.

Table 2. Mean abundance (MA), relative frequency (RF), infestation degree (ID) and ecological preference of the species with a frequency higher than 6 % for texture (T), pH (H2O), assimilable phosphorus (P), assimilable potassium (K), magnesium (Mg) and organic matter (OM).

Species	MA	RF	ID	Ecological preference T	pH	P	K	Mg	OM
Ammi majus L.	0.5	11	0	2.2	2.6	1.5	1.5	3.1	1.5
Anagallis arvensis L.	4.7	44	++	2.5	2.2	1.6	2.1	2.9	1.6
Andryala integrifolia L.	1.0	7	+	2.3	1.9	1.3	1.7	2.8	1.3
Arctotheca calendula (L.) Levyns	5.0	15	++	1.6	2.3	1.7	1.7	2.0	1.0
Arisarum vulgare O.Targ.Tozz.	3.0	28	++	2.6	3.2	1.5	2.4	3.2	1.4
Arum italicum Mill.	2.1	30	++	2.4	3.5	1.5	2.5	3.3	1.3
Asparagus aphyllus L.	0.5	24	0	1.5	1.6	1.8	2.0	2.6	1.7
Avena barbata Link	0.8	7	0	2.0	2.5	1.1	1.8	1.6	1.6
Beta vulgaris L.	0.8	11	0	2.3	4.0	1.5	2.3	3.0	1.4
Bromus diandrus Roth	0.5	13	0	1.6	2.3	1.3	1.8	1.7	1.2
Calendula arvensis L.	7.2	30	++	2.2	2.8	1.4	2.0	2.2	1.3
Capsella rubella Reut.	1.0	7	+	1.4	3.0	1.6	2.5	2.9	1.6
Cardamine hirsuta L.	2.0	15	++	1.9	3.6	1.5	2.0	1.9	1.2
Cerastum glomeratum Thuill.	2.1	15	++	2.0	2.3	1.4	1.8	2.9	1.5
Chamaemelum fuscatum (Brot.) Vasc.	4.7	17	++	1.9	2.6	2.0	1.5	2.6	1.5
Chenopodium album L.	0.5	7	0	1.7	2.2	2.0	1.3	2.2	1.4
Chenopodium murale L.	0.7	17	0	1.7	2.7	1.5	1.7	2.7	1.8
Cirsium arvense (L.) Scop.	1.7	20	+	1.7	3.0	1.4	1.9	2.2	1.4
Coleostephus myconis (L.) Rchb. f.	0.7	24	+	1.9	2.8	1.6	1.5	2.3	1.2
Convolvulus arvensis L.	3.3	15	++	1.8	2.6	2.0	2.1	2.9	1.3
Conyza albida Spreng.	1.8	54	++	2.1	2.5	1.6	2.2	2.5	1.5
Coronopus didymus (L.) Sm.	0.5	11	0	1.6	1.2	2.0	1.9	3.4	1.5
Diplotaxis catholica (L.) DC.	0.7	11	0	1.9	2.7	2.0	2.6	3.1	1.8
Epilobium tetragonum L.	0.8	22	0	2.1	2.2	1.5	1.9	2.4	1.4
Equisetum telmateia Ehrh.	7.5	9	++	2.0	3.3	1.7	2.1	2.2	1.6
Erodium malacoides (L.) L'Hér.	0.8	7	0	2.5	2.9	1.4	2.4	3.1	1.3
Erodium moschatum (L.) L'Hér.	5.0	65	+++	2.1	2.4	1.6	1.8	2.7	1.4
Euphorbia pterococca Brot.	0.5	11	0	2.5	1.9	1.6	2.3	3.0	1.5
Fumaria officinalis L.	0.5	19	0	1.9	2.8	1.5	2.1	3.0	1.6
Galactites tomentosa Moench	0.5	39	+	2.4	2.8	1.5	2.0	2.9	1.4
Galium aparine L.	1.6	43	++	2.2	2.4	1.7	2.2	2.8	1.5
Geranium dissectum L.	4.1	19	++	2.8	3.7	1.3	2.8	3.5	1.2
Geranium molle L.	1.6	20	+	1.6	2.6	1.5	1.4	2.2	1.3
Geranium purpureum Vill.	1.8	19	+	1.8	2.4	1.5	1.5	2.1	1.2
Geranium rotundifolium L.	0.7	9	0	1.8	1.2	2.0	1.8	1.8	1.9

Infestation degree: +++ high, ++ mean, + low, 0 very low.

Ecologycal preference for factors class 1{1-1.5}; class 2{1.5-2.5}; class 3{2.5-3.5}; class 4{3.5-4}.

Table 2. (cont.) Mean abundance (MA), relative frequency (RF), infestation degree (ID) and ecological preference of the species with a frequency higher than 6% for texture (T), pH (H2O), assimilable phosphorus (P), assimilable potassium (K), magnesium (Mg) and organic matter (OM).

Species	MA	RF	ID	Ecological preference					
				T	pH	P	K	Mg	OM
*Hedera helix L.	0.6	31	+	2.0	2.5	1.7	2.3	3.0	1.3
Holcus lanatus L.	6.0	7	++	2.2	1.9	2.0	2.2	1.9	1.3
*Lavatera cretica L.	0.6	69	++	2.1	2.5	1.7	2.1	2.8	1.6
*Leontodon taraxacoides (Vill.) Mérat	0.5	13	0	2.2	2.4	1.6	2.1	1.8	1.6
Lythrum hysopifolia L.	0.5	9	0	1.7	2.0	2.0	1.9	2.3	1.7
*Medicago nigra (L.) Krock.	0.5	19	0	1.9	2.5	1.7	1.9	2.7	1.4
Mercurialis annua L.	0.8	15	0	2.2	2.8	2.0	1.7	2.9	1.4
Oxalis pes-caprae L.	8.3	70	+++	2.2	3.0	1.5	1.9	2.6	1.4
Parietaria punctata Willd.	2.9	9	++	1.4	3.0	1.3	1.2	1.6	1.0
*Picris echioides L.	0.7	48	+	1.9	3.0	1.5	1.8	2.6	1.4
Piptatherum miliaceum (L.) Coss.	0.6	13	0	2.2	2.5	1.4	2.4	3.8	1.4
Pittosporum undulatum Vent.	0.8	15	0	1.3	2.6	1.4	1.9	1.9	1.3
*Poa annua L.	7.3	69	+++	2.0	2.6	1.6	1.9	2.5	1.4
*Polycarpon tetraphyllum (L.) L.	4.1	19	++	2.1	1.5	1.5	1.9	2.1	1.6
Polygonum aviculare L.	3.4	15	++	2.0	2.2	1.7	1.7	3.0	1.7
Pseudognaphalium luteo-album (L.) Hillard & B. L. Burtt	1.8	17	+	2.2	1.9	1.4	1.8	3.2	1.5
Ranunculus trilobus Desf.	4.9	9	++	2.5	3.3	1.6	2.5	3.9	1.8
*Raphanus raphanistrum L.	1.8	19	+	2.1	2.6	1.4	1.8	1.7	1.2
Rosa sempervirens L.	0.8	7	0	2.9	3.7	1.1	2.2	3.9	1.0
Rubia peregrina L.	2.9	9	++	2.5	3.3	1.7	2.1	3.3	1.0
*Rubus ulmifolius Schott	0.6	30	0	2.3	2.7	1.3	2.2	2.4	1.3
*Rumex crispus L.	1.7	24	+	2.1	2.8	1.6	1.5	1.9	1.3
Rumex pulcher L.	2.4	13	++	1.6	2.8	1.4	1.4	3.1	1.3
Senecio jacobaea L.	0.5	13	0	1.8	2.5	1.6	2.1	1.9	1.4
*Senecio vulgaris L.	1.6	43	++	2.3	2.5	1.5	2.1	2.3	1.4
Sinapis arvensis L.	0.5	11	0	2.3	3.3	1.3	2.2	3.2	1.5
*Solanum nigrum L.	0.9	57	++	1.9	2.4	1.5	1.9	2.5	1.6
*Sonchus asper (L.) Hill.	1.0	65	++	2.0	2.3	1.5	1.9	2.6	1.5
*Sonchus oleraceus L.	1.0	69	++	1.9	2.3	1.5	1.8	2.3	1.5
*Stellaria media (L.) Vill.	5.3	37	++	1.8	2.6	1.4	1.7	2.2	1.5
Urospermum picroides (L.) F.W. Schmidt	0.5	13	0	2.2	3.1	1.3	1.8	1.8	1.4
*Urtica membranacea Poir.	17.4	30	++	1.6	2.4	1.5	1.7	2.9	1.2
*Urtica urens L.	11.5	57	+++	2.0	2.6	1.7	1.9	2.3	1.6
*Veronica persica Poir.	6.7	33	++	2.4	3.1	1.4	2.2	3.4	1.1

Infestation degree: +++ high, ++ mean, + low, 0 very low.
Ecologycal preference for factors class 1{1-1.5}; class 2{1.5-2.5}; class 3{2.5-3.5}; class 4{3.5-4}.

Acknowledgements

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