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Organizing Entities

of the

International Conference on

Integrated Control in Citrus Fruit Crops

Instituto Superior de Agronomia,
Universidade Técnica de Lisboa

Estação Agronómica Nacional,
Instituto Nacional de Investigação Agrária e das Pescas

Direcção Geral de Protecção das Culturas

Direcção Regional de Agricultura do Algarve
Preface

These are the proceedings of the meeting of the IOBC/WPRS Citrus Working Group held in Lisbon, in September 2005. This is the second meeting after the new period of activity of the group started in 2002 with the first meeting in Valencia. This second meeting experienced a considerable increase in the number of assistants and presentations. In all, 27 oral papers were presented, together with 38 posters. The number of assistants to the meeting was 106. Portugal (with 45) and Spain (with 34) were the countries with more assistants, although 13 more countries were also represented. However, several Mediterranean countries with relevant citrus production are still largely missing.

One of the topics treated was the introduction of new pests in the Mediterranean area. *Toxoptera citricida*, the main vector of citrus tristeza virus, has been detected recently in mainland Portugal, where it is spreading through the citrus growing area, and Spain, where it is only found for the moment at the north, in non citrus producing areas. Several presentations dealt with this new threat and the possible consequences to the mediterranean citriculture. As in the previous meeting, the medfly *Ceratitis capitata* was the single pest most represented in number of presentations. New techniques of monitoring and control are being developed for this and other pests of the crop. Other problems of pests, diseases and weeds were also presented. The final contribution included of this volume is a survey of the Citrus phytosanitary situation in different Mediterranean countries obtained from a questioner fulfilled by local members. The Lisbon meeting was followed by a scientific trip to Madeira Island, where the assistants had the opportunity to visit the rearing facility of *Ceratitis capitata* sterile males, and to observe pests not yet present in Mediterranean citrus crops as *Toxoptera citricida* or *Trioza erythraeae*.

The exchange of information and experiences among research teams was fully positive and should be encouraged considering that problems of pest and disease management were in many cases similar in different countries. Trying to stimulate contacts and exchange of information among researchers in the period between congresses, a future web page of the group is in project, and a mailing list has already been established as a first step.

The local organizer of the meeting was José Carlos Franco, of the Instituto Superior de Agronomia, Universidade Técnica de Lisboa, the place where the meeting was held. He and his/her collaborators did a very good job in the previous arrangements and during the meeting. On behalf of all the participants I would like to express our gratitude to all of them for the very pleasant stage and for the attentions we received in Lisbon and during the scientific trip to Madeira. Special thanks also to the liaison officer of the group, Mohamed Besri, for his effort and contribution to the success of the meeting.

I look forward to see you in the next meeting.


F. Garcia-Marí, Convenor
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Discovery in Continental Portugal and Spain of the aphid *Toxoptera citricidus*, a potential threat to citrus trees in the Mediterranean Basin

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The recent discovery in Continental Portugal and Spain of *Toxoptera citricidus*, the aphid vector of the most severe strains of citrus tristeza virus, is reported. The areas where the aphid has been collected in Continental Portugal up to the end of April/2005 are indicated. Attention is drawn to the interest of knowing very well the distribution of *Toxoptera citricidus* in Portugal and its biology, as well as the identification of citrus trees infested with tristeza virus, for possible elimination.
Citrus tristeza epidemiology in the Mediterranean basin: a changing scenario?

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Decline of citrus plants grafted on sour orange rootstock is the commonest aspect that people associate to *citrus tristeza virus* (CTV). Manifestation of decline may range from being barely noticeable, extending over a period of years, to quick decline in which the tree dies in a matter of months. Slow decline is very frequent on the Mediterranean Basin. Quick decline (QD) episodes have been occasional (e.g. Spain, Israel, Italy). In citrus producing areas outside the Mediterranean region, where sour orange is not extensively used as a rootstock, it is common to find plants exhibiting stem-pitting (SP) symptoms on the branches; these symptoms which do not lead to death of the tree are associated to a reduction in the size and quality of the fruits. Although less spectacular than QD, the SP syndrome is economically damaging and can not be avoided by changing to tolerant rootstocks. The existence of virus strains is one cause for this wide range of symptoms. It has been generally believed that in the Mediterranean basin only mild CTV strains which produce slow decline exist and that severe ones, when found (e.g., Spain), have been immediately eradicated.

CTV has one of the biggest monopartite RNA genome harbouring 12 genes. In recent years we conducted a worldwide molecular characterization of diverse of these genes, which lead us to recognize the existence of 7 CTV strains. No geographic speciation could be found. Examples of all these strains were found scattered in the Mediterranean basin, frequently in mixed infections. In mainland Portugal, 6 out of the 7 strains have already been detected. Biological characterization of isolates which were determined as "pure" strains enabled to establish a relationship between molecular factors and symptoms. Existence of strains considered as severe (QD or SP) was recognized hiding in mixed infections in diverse parts of the Mediterranean basin. Nevertheless, in some places, the severe syndromes were showing in the field (e.g. SP in Croatia; QD in Italy).

In most citrus producing areas of the world where *Toxoptera citricidus* appeared the CTV incidence has increased. A well documented example refers to Florida (Halbert et al. 2004); the existence of SP strains passed unnoticed until recent years, resembling the Mediterranean situation. According to those authors, two years were sufficient after the establishment of *T. citricidus* for a marked increase in the appearance of severe forms of tristeza (SP and QD). In view of the proved existence of all the CTV strains in the Mediterranean basin (and almost all in Portugal), the introduction of *T. citricidus* in the Iberia peninsula appears to provide the missing ingredient to change the epidemics scenario in the area unless very efficient measures are taken.

References

Halbert et al. 2004: Distribution and characterization of *Citrus tristeza virus* in south Florida following the establishment of *Toxoptera citricida*. Plant disease 88: 935-941.
New Developments in the San Joaquin Valley California Citrus IPM Program

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Abstract: The majority of citrus, primarily navels and Valencia's, for fresh market, is now grown in the central San Joaquin Valley of California. Beginning in the late 1990s a number of new insecticides (pyrethroids, insect growth regulators, neonicotinoids, and fermentation products) were registered for control of citrus pests. These insecticides have improved worker safety, reduced environmental effects, and improved the survival of some natural enemies by greatly reducing organophosphate and carbamate usage. There are, however, some problems with integrating these new insecticides into the California citrus pest management program. First, the two most commonly used insecticides, pyriproxyfen for California red scale Aonidiella aurantii control and spinosad for citrus thrips Scirtothrips citri control are highly selective for these two pests. Their use and the lack of broad spectrum pesticides, releases from control secondary pests such as forktailed bush katydid Scuddaria furcata and citricola scale Coccus pseudomagnoliarum. This necessitates additional insecticide treatments for the secondary pests. Secondly, many of the new insecticides are toxic to the predatory vedalia beetle Rodolia cardinalis. Thus, cottony cushion scale Icerya purchasi problems have increased. Finally, exotic pests continue to invade the region, for example, glassy-winged sharpshooter Homalodisca coagulata, citrus peelminer Marmara gulosa and citrus leafminer Phyllocnistis citrella. These pests require the development of management tactics that must be integrated into the existing IPM program. The use of more selective insecticides, the toxicity of new insecticides to coccinellids, and the invasion of new pests has increased the complexity of the integrated pest management program for San Joaquin Valley citrus.

Key words: integrated pest management, insecticide, insect growth regulator

Introduction

For more than 40 years, the majority of San Joaquin Valley California citrus growers relied upon organophosphate (OP) and carbamate insecticides for pest control because these insecticides were inexpensive and effective. During this period of time, the most damaging pests in this region were California red scale Aonidiella aurantii (Maskell), citrus thrips Scirtothrips citri (Moulton), and to a lesser extent, various Lepidoptera and the citrus red mite Panonychus citri (McGregor) (Table 1, conventional pesticide program). During the 1980s, a number of growers began to use a selective pesticide approach to pest management that utilized the botanical sabadilla for citrus thrips control and Aphytis melinus DeBach releases (Grafton-Cardwell and Stewart-Leslie 1998) for managing California red scale (Table 1, biologically-based IPM program). These biologically-based IPM growers found that pests with a good compliment of natural enemies, such as citrus red mite and Lepidoptera, declined in significance in their orchards (Table 1). They also observed that, when OP and carbamate insecticide use was reduced in frequency, pests with poor biological control, such as katydids (Scuddaria furcata Brunner von Wattenwyl), citricola scale (Coccus pseudomagnoliarum (Kuwana)) and citrus thrips populations were more difficult to control. Katydid nymphs feed...
on young fruit, severely scarring it. Citricola scale can severely reduce the yield of citrus when populations become heavy. Biological control of this single generation pest is ineffective in the San Joaquin Valley, because the extremes of temperature in that region synchronize scale stages so that there are long periods of time when the parasitoids (*Metaphycus* spp.) do not have hosts of sufficient size for oviposition (Bernal et al. 2001). In addition, that region lacks alternative hosts such as brown soft scale *Coccus hesperidum* Linnaeus and black scale *Sassetia oleae* (Olivier). Citrus thrips were difficult to control during this period because the selective botanical insecticide sabadilla had a very short residual life and inconsistent efficacy. Thus, growers practicing biologically-based IPM during the early 1990s experienced the key pests California red scale, citrus thrips, katydid, and citricola scale and treated as often with insecticides as the conventional program growers (Table 1).

<table>
<thead>
<tr>
<th>Target Pest</th>
<th>Level of Biological Control</th>
<th>Conventional Program</th>
<th>Biologically-based IPM program</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus Red Mite</td>
<td>Excellent</td>
<td>0.2 (.2 propargite)</td>
<td>0</td>
</tr>
<tr>
<td><em>Panonychus citri</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrus curtworm</td>
<td>Good</td>
<td>0.5 (.2 OP/carbamate)</td>
<td>0.2 (.2 <em>B. thuringiensis</em>)</td>
</tr>
<tr>
<td><em>Egira curialis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>California red scale</td>
<td>Good</td>
<td>0.7 (.7 OP/carbamate)</td>
<td>0.8 + <em>A. melinus</em></td>
</tr>
<tr>
<td><em>Aonidiella aurantii</em></td>
<td></td>
<td></td>
<td>(.3 OP/carbamate .5 oil)</td>
</tr>
<tr>
<td>Citrus thrips</td>
<td>Poor</td>
<td>2.7 (2.3 OP/carbamate)</td>
<td>2.4 (.2 carbamate 2.2 sabadilla)</td>
</tr>
<tr>
<td><em>Scirtothrips citri</em></td>
<td></td>
<td>(2 sabadilla .2 pyrethroid)</td>
<td></td>
</tr>
<tr>
<td>Katydid</td>
<td>Poor</td>
<td>0</td>
<td>0.2 (.2 OP)</td>
</tr>
<tr>
<td><em>Scuddaria furcata</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citricola scale</td>
<td>Poor</td>
<td>0</td>
<td>0.2 (.2 OP)</td>
</tr>
<tr>
<td><em>Coccus pseudomagnoliarum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average # treatments per orchard</td>
<td></td>
<td>4.1</td>
<td>3.8 + <em>A. melinus</em></td>
</tr>
<tr>
<td>No. of tank-mixes</td>
<td></td>
<td>0.9</td>
<td>0</td>
</tr>
</tbody>
</table>

During the 1980s, numerous populations of citrus thrips, developed resistance to OP and carbamate insecticides (Morse and Brawner 1986, Immaraju et al. 1989). During the 1990s widespread OP and carbamate insecticide resistance developed in California red scale populations (Grafton-Cardwell and Vehrs 1995, Grafton-Cardwell et al. 2001). From 1991-
2002, eight new insecticides were registered for California citrus (Table 2) including four products used primarily for citrus thrips control (abamectin, spinosad, and the pyrethroids cyfluthrin and fenpropathrin), two insect growth regulators for California red scale control (pyriproxyfen and buprofezin), and two neonicotinoids (imidacloprid and acetamiprid) for control of the invasive pest glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* (Say). Growers struggling with OP-resistant citrus thrips and California red scale quickly adopted use of these new insecticides. In particular, use of spinosad for citrus thrips and pyriproxyfen for California red scale reduced organophosphate and carbamate use by more than 70% from 1997 to 2000 (CDPR).

Table 2. First registration of insecticides for citrus pests in California USA during 1991-2002.

<table>
<thead>
<tr>
<th>Insecticides (target pests)</th>
<th>Years of emergency registration</th>
<th>Year of full registration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrethroids (citrus thrips, katydid, glassy-winged sharpshooter)</td>
<td>cyfluthrin 1991-96</td>
<td>1997</td>
</tr>
<tr>
<td></td>
<td>fenpropathrin</td>
<td>2001</td>
</tr>
<tr>
<td>Insect Growth Regulators (California red scale)</td>
<td>pyriproxyfen 1998-99</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>buprofezin 1998-99</td>
<td>2002</td>
</tr>
<tr>
<td>Neonicotinoids (glassy-winged sharpshooter, citricola scale)</td>
<td>imidacloprid</td>
<td>2001</td>
</tr>
<tr>
<td></td>
<td>acetamiprid</td>
<td>2002</td>
</tr>
<tr>
<td>Miscellaneous classes</td>
<td>abamectin</td>
<td>1994</td>
</tr>
<tr>
<td></td>
<td>spinosad</td>
<td>1998</td>
</tr>
</tbody>
</table>

The new insecticides greatly improved worker safety and improved control of key pests of citrus, especially citrus thrips and California red scale. However, the new insecticides made integrated pest management of San Joaquin Valley citrus more complicated. First, it was found that vedalia beetle, *Rodolia cardinalis* (Mulsant) is quite sensitive to pyrethroids, neonicotinoids, and insect growth regulators (Loia and Vigiani. 1992, Grafton-Cardwell and Gu 2003). Thus, the cottony cushion scale *Icerya purchasi* Maskell that had been well-controlled by this predator for 100 years was released from control and outbreaks occurred (Grafton-Cardwell 1999). Secondly, OPs and carbamates applied for key pests such as citrus thrips and California red scale, had incidentally controlled secondary pests such as katydids and citricola scale. When OP and carbamate use was reduced throughout the valley, katydids and citricola scale became primary pests in citrus orchards, because the replacement insecticides spinosad and pyriproxyfen are largely ineffective against these pests. To further complicate the situation, exotic pests continually invade the San Joaquin Valley region. For example, GWSS arrived in the mid 1990s, and citrus growers are required to treat for GWSS with neonicotinoids in order to reduce the overall vector population and so protect vineyards from the bacterium *Xylella fastidiosa* that causes Pierce’s disease. In addition, a new more
damaging strain of the citrus peelminer *Marmara gulosa* Gullen and Davis arrived in 1999, and the citrus leafminer *Phyllocnistis citrella* Stainton is infesting southern California and gradually advancing toward the San Joaquin Valley. These pests require the development of management tactics that must be integrated into the existing IPM program. The following citrus orchard monitoring programs document the effects of changes in insecticide use patterns, the resulting shifts in pest importance, and the impact of the arrival of the exotic pest GWSS on the San Joaquin Valley citrus IPM program.

**Materials and methods**

During 1992, 12 commercial citrus orchards (navel and Valencia oranges) in Kern and Tulare Counties of California, USA were sampled for pests and natural enemies. During 2000-2002, 10 navel orange orchards in Kern County and during 2001-2004 10 navel orange orchards in Tulare County were sampled for pests and natural enemies. Male California red scale were sampled monthly using one pheromone trap per 2.5 acres (4-6 per orchard) with synthetic pheromone lures changed monthly. The female California red scale population was estimated by sampling 100 fruit per orchard monthly from August through October and determining the percentage of fruit that were infested with scale. Adult parasitoids of California red scale, *A. melinus* and *Comperiella bifasciata* Howard, were sampled using four 7.6 x 12.7 cm yellow sticky cards per orchard evaluated monthly. Citricola scale was sampled during July through October by collecting one leaf from the northeast corner of 100 trees and calculating the percentage of infested leaves. Cottony cushion scale populations were sampled monthly from March-October by examining 20 trees per orchard for scale and calculating the percentage of trees that were infested. Citrus thrips were sampled by examining 100 fruit for immature citrus thrips every 3-4 days during May through mid June. The percentage of thrips-infested fruit was calculated. Citrus red mites and the predatory mites *Euseius tularensis* (Congdon) were sampled weekly during spring and fall by counting the number of adult red mites and motile stages of predatory mites on 100 leaves. Katydid densities were estimated once a week during April-June by conducting a 2 minute visual search for immature stages on each of 20 trees per orchard. Citrus cutworm *Egira curialis* (Grote) densities were monitored using a single pheromone trap per orchard sampled weekly during January-April and by vigorously shaking foliage on each of 20 trees over a beating sheet placed on the ground below the tree each week during March-May. The pesticide treatment formulations, rates, dates and water volumes applied by the growers were recorded. Average densities of insects were calculated for each orchard.

**Results and discussion**

Figure 1 shows the number of hectares treated with various insecticides used for citrus thrips and katydid control during 1991-2003 in the San Joaquin Valley California region. Initially, the majority of the acreage was treated with OPs (dimethoate) and carbamates (formetanate). When the pyrethroid cyfluthrin became available in 1991, growers gradually adopted that insecticide as their primary citrus thrips control agent. Spinosad became the dominant insecticide within two years of its introduction in 1998 both because of its effectiveness and because citrus thrips populations had begun to develop resistance to pyrethroids (Khan and Morse 1998). While spinosad provided some control of young katydids (Reagan et al. 1999), it did not have enough residuality to fully control this highly damaging pest and after about 3 years, pyrethroid and OP use began to increase again in response to increased katydid densities. During the 1992 sampling, katydids were not detected.
in the 12 Tulare and Kern County orchards. Table 3 shows that the maximum number of katydids observed on 20 trees per orchard averaged over all orchards in each County increased in Kern County from 0.8 to 1.6 during 2000-2002 and was > 4 per 20 trees in three of four years in the Tulare County region during 2001-2004, coincident with the increase in OP and pyrethroid use shown in Fig. 1.

Table 3 shows that the maximum number of katydids observed on 20 trees per orchard averaged over all orchards in each County increased in Kern County form 0.8 to 1.6 during 2000-2002 and was > 4 per 20 trees in three of four years in the Tulare County region during 2001-2004, coincident with the increase in OP and pyrethroid use shown in Fig. 1.

Figure 1. The number of hectares of San Joaquin Valley, CA citrus treated with insecticides for citrus thrips and katydid.

Table 3. The maximum number of katydids observed per 20 trees in Kern and Tulare Counties during 1992 and 2000-2004 averaged over all orchards in a region.

<table>
<thead>
<tr>
<th>Region</th>
<th>1992</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kern</td>
<td>0</td>
<td>0.8</td>
<td>1.3</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tulare</td>
<td>0</td>
<td>8.1</td>
<td>4.1</td>
<td>0.8</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 contrasts the number of pesticides applied to 12 Tulare and Kern orchards in 1992 with 10 orchards in Kern County during 2000-2002. In 1992, the number of insecticides applied for citrus thrips was high (2.5/orchard) both because of resistance to OPs and carbamates and because of the lack of efficacy of sabadilla. Tank mix combinations of OPs and pyrethroids were common during this period as growers combined insecticides in an effort to reduce pesticide-resistant citrus thrips populations. Spinosad was the primary treatment for citrus thrips during the period of 2000-02 (.5-.9 treatments/orchard). The OP and carbamate insecticides used for control of spring pests initially declined to 0.3/orchard in 2000, but then increased to 0.8 treatments/orchard during 2002, applied primarily as tank-mixes with spinosad. The spinosad was applied for citrus thrips and the pyrethroids and OPs applied for katydid control. Generally, a low rate of OP or pyrethroid (1/2 the labeled citrus thrips rate) was used by the growers to reduce the effect on natural enemies, yet control the highly sensitive katydid. Table 4 shows that initially tank mixes were few in number in 2000 and increased with time as growers experienced chronic problems with katydids. With the
reduction in OP and carbamate use, citrus red mite and Lepidopteran pests declined and were left untreated in the Kern region during 2000-02. Table 5 shows that citrus red mite and Lepidopteran pests continued to persist at treatable levels in the Tulare County region, which is more humid and cooler in climate compared to Kern County. Similar to Kern Co., Tulare County growers depended on spinosad for citrus thrips control (.4-.7 treatments/orchard) and OP, carbamate, and pyrethroid insecticides (.6-.9/orchard) for katydid control during 2001-2004. Tank-mixes of these insecticides increased from 0.1 to 0.6/orchard during that period in the Tulare region.

Table 4. Mean number of pesticide treatments applied to orchards in 1992 in 12 Tulare and Kern County orchards compared with 10 Kern Co. orchards in 2000-2002.

<table>
<thead>
<tr>
<th>Target Pest</th>
<th>Tulare and Kern 1992</th>
<th>Kern Orchards Treatments per orchard (pesticide groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus red mite</td>
<td>.1</td>
<td>0            0  0  0</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>.4</td>
<td>0            0  0  0</td>
</tr>
<tr>
<td>Citrus thrips &amp; katydid</td>
<td>2.5</td>
<td>1.2          1.7</td>
</tr>
<tr>
<td>(1.2 sabadilla 1.2 OP .1 pyrethroid)</td>
<td></td>
<td>(.5 spinosad .8 spinosad .9 spinosad)</td>
</tr>
<tr>
<td>(1.2 OP .1 pyrethroid)</td>
<td></td>
<td>(.2 sabadilla .2 sabadilla .2 sabadilla)</td>
</tr>
<tr>
<td>(1.2 sabadilla .2 abamectin .2 OP .1 pyrethroid)</td>
<td></td>
<td>(.1 abamectin .1 abamectin .1 kryocide)</td>
</tr>
<tr>
<td>Citrus thrips &amp; katydid</td>
<td>1.2</td>
<td>1.7          1.9</td>
</tr>
<tr>
<td>(1.2 sabadilla 1.2 OP .1 pyrethroid)</td>
<td></td>
<td>(.5 spinosad .9 spinosad)</td>
</tr>
<tr>
<td>(1.2 OP .1 pyrethroid)</td>
<td></td>
<td>(.2 sabadilla .2 sabadilla)</td>
</tr>
<tr>
<td>(1.2 OP .1 pyrethroid)</td>
<td></td>
<td>(.6 OP/carbamate .6 OP/carbamate)</td>
</tr>
<tr>
<td>Scales:</td>
<td>.9</td>
<td>.4           .5</td>
</tr>
<tr>
<td>California red scale, citricola, &amp; cottony cushion scale</td>
<td></td>
<td>(.3 Esteem .4 IGR .5 IGR)</td>
</tr>
<tr>
<td>(.3 oil .6 OP/carbamate)</td>
<td></td>
<td>(.1 OP .1 IGR)</td>
</tr>
<tr>
<td>(.3 Esteem .1 OP)</td>
<td></td>
<td>(.4 IGR .3 IGR)</td>
</tr>
<tr>
<td>(.3 Esteem .1 OP)</td>
<td></td>
<td>(.1 neonicotinoid .3 IGR)</td>
</tr>
<tr>
<td>(.1 OP .4 pyrethroid)</td>
<td></td>
<td>(.1 neonicotinoid)</td>
</tr>
<tr>
<td>Glassy-winged sharpshooter</td>
<td>0</td>
<td>.7           .9</td>
</tr>
<tr>
<td>(.3 carbamate .4 neonicotinoid)</td>
<td></td>
<td>(.4 carbamate .2 carbamate)</td>
</tr>
<tr>
<td>(.3 carbamate .4 neonicotinoid)</td>
<td></td>
<td>(.4 carbamate .2 carbamate)</td>
</tr>
<tr>
<td>(.1 pyrethrum .1 pyrethrum)</td>
<td></td>
<td>(.1 pyrethrum .1 pyrethrum)</td>
</tr>
<tr>
<td>Avg. # insecticides per orchard</td>
<td>3.9</td>
<td>2.3          3.1</td>
</tr>
<tr>
<td>No. tank mixes</td>
<td>.5</td>
<td>.1           .5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.6           .6</td>
</tr>
</tbody>
</table>

Pyriproxyfen was adopted for California red scale control in 1998 and within 2 years reduced OP and carbamate use by more than 70% (CDPR). Citricola scale is relatively unaffected by insect growth regulators, especially pyriproxyfen and growers generally use chlorpyrifos to control it. In 1992, citricola scale was undetectable in the 12 Kern and Tulare orchards. Table 6 shows that citricola scale populations appeared in all orchards sampled in Tulare County during the period of 2001-04. Initially, in 2001, there were only 5 orchards with detectable populations of citricola scale. However, the infestations of citricola scale increased to 8-9 orchards in 2002-04. Citricola scale maintained detectable population levels in spite of OP treatments applied to control this pest in orchards 2, 4, 5, 6, 9, and 10, indicating the severity of this pest. Table 5 shows that in the Tulare County region, .3-.6 treatments of IGRS, oils or OPs were applied for scale control per year. Fig. 2 shows the use of chlorpyrifos at various
rates during 1991-2003. Citricola scale is treated with rates of 0.6-3.4 kg/ha and katydid is treated with < 0.6 kg/ha. Chlorpyrifos use at these rates increased during 2001-2003, approximately 3 years after the introduction of spinosad for citrus thrips and pyriproxyfen for California red scale control (1998). Increased use of low rates of chlorpyrifos is a good indicator of the increased problem with katydid and citricola scale.

Table 5. Mean number of pesticide treatments applied to orchards in 1992 in 12 Tulare and Kern County orchards compared with 10 Tulare Co. orchards sampled during 2001-2004.

<table>
<thead>
<tr>
<th>Target Pest</th>
<th>Tulare and Kern Co.</th>
<th>Tulare Co. Treatments per orchard (pesticide groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus red mite</td>
<td>.1</td>
<td>0.2 0 .2 0 .3</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>.4</td>
<td>0 .3 0 .1 0</td>
</tr>
<tr>
<td>Citrus thrips &amp; katydid</td>
<td>2.5 (1.2 sabadilla 1.2 OP .1 pyrethroid)</td>
<td>1.0 (.4 spinosad .3 OP .3 pyrethroid)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6 (.7 spinosad .1 abamectin .3 OP .5 pyrethroid)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1 (.4 spinosad .1 pyrethrum .3 OP .3 pyrethroid)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4 (.4 spinosad .1 pyrethrum .3 OP .6 pyrethroid)</td>
</tr>
<tr>
<td>Scales: California red scale, citricola, &amp; cottony cushion scale</td>
<td>.9 (.3 oil .6 OP/carbamate)</td>
<td>.6 (.2 oils .4 OP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.6 (.5 IGR .1 OP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.3 (.1 IGR .2 OP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.6 (.2 IGR .4 OP)</td>
</tr>
<tr>
<td>Avg. # insecticides per orchard</td>
<td>3.9</td>
<td>1.8 2.5 1.7 2.5</td>
</tr>
<tr>
<td>No. tank mix combinations</td>
<td>.5</td>
<td>.1 .3 .5 .6</td>
</tr>
</tbody>
</table>

Table 6. Percentage of leaves infested with citricola scale in 10 Tulare County citrus orchards.

<table>
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<td>2</td>
<td>20</td>
<td>33</td>
<td>27</td>
<td>42</td>
<td>56</td>
<td>79</td>
<td>29</td>
<td>26</td>
<td>*4</td>
<td>3</td>
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<td>0</td>
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<td>1</td>
<td>8</td>
<td>4</td>
<td>15</td>
<td>30</td>
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<td>0</td>
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<td>1</td>
<td>*0</td>
<td>0</td>
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<tr>
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<td>0</td>
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<td>+0</td>
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<td>7</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
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<td>0</td>
<td>+0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>3*</td>
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<td>8</td>
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<td>0</td>
<td>0</td>
<td>+0</td>
<td>0</td>
<td>0</td>
<td>*+0</td>
<td>0</td>
<td>1</td>
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<td>1</td>
<td>2</td>
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<td>4</td>
<td>16</td>
<td>15</td>
<td>*3</td>
<td>4</td>
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<td>10</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>*20</td>
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<td>0</td>
<td>0</td>
<td>*2</td>
<td>2</td>
</tr>
</tbody>
</table>

+ IGR treatment for California red scale
* OP treatment for scale insect pests
Figure 2. The number of applications of chlorpyrifos applied at various rates to San Joaquin Valley citrus from 1991-2003.

During 2000, populations of GWSS in Kern County began to expand and a control program consisting of preharvest carbamate treatments and in-season systemic imidacloprid treatments was instituted. Fig. 3 shows that these treatments, as they expanded over a three-year period to include all citrus orchards in the region, greatly reduced GWSS egg masses in the orchards. Table 4 shows that these treatments had the effect of reducing scale treatments in 2002, but increased the total number of insecticides applied to citrus by an average of 1 per orchard. Sampling of citricola scale in this region (Table 7) showed that imidacloprid suppressed citricola scale populations and research has shown that it also suppresses California red scale (Grafton-Cardwell and Reagan 2003). Thus, in this region, treatments of imidacloprid for GWSS reduced the need for treatments for both scale pests. However, Table 7 shows that imidacloprid suppressed citricola populations only slightly below the economic threshold (approximately 20% infested leaves) in blocks 2 and 3. Thus, this neonicotinoid only provides short-term control of citricola scale.
Table 7. Percentage of leaves infested with citricola scale and groups of insecticides used for scale and GWSS control in 10 Kern County citrus orchards.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>^1 +0</td>
<td>0</td>
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<td>^0</td>
<td>0</td>
<td>^0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>18</td>
<td>17</td>
<td>^24</td>
<td>14</td>
<td>^+17</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>^4</td>
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<td>0</td>
<td>83</td>
<td>75</td>
<td>^5</td>
<td>2</td>
<td>11</td>
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<td>^+0</td>
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<td>0</td>
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</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>^0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
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<td>0</td>
<td>0</td>
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<td>^0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>^0</td>
<td>0</td>
<td>0</td>
<td>^0</td>
<td>0</td>
<td>^1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>^2</td>
<td>*0</td>
<td>0</td>
<td>+0</td>
<td>0</td>
<td>^3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>+0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>+0</td>
<td>^+3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>+0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>^+1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+ IGR treatment for California red scale
* OP treatment for scale insect pests
^ Imidacloprid for GWSS

In the late 1990s, San Joaquin Valley citrus growers shifted from an organophosphate and carbamate-based control program to one that includes IGRs for California red scale control and spinosad for citrus thrips control. This shift has reduced the number of insecticides applied per orchard from 3.9 insecticides per orchard in 1992 to 1.7-2.5 in Tulare County where GWSS is not present. This change is due to the fact that spinosad is more effective than sabadilla, OPs or carbamates for citrus thrips control and pyriproxyfen is more effective than OPs and carbamates for California red scale control. There is no longer a dichotomy in grower pesticide practices (conventional versus biologically-based), because the insecticides that are most effective are selective and allow many species of natural enemies, especially Hymenoptera, to survive (Ishaaya et al. 1992, Mendel et al. 1994). However, this shift to more selective insecticides resulted in increased use of low rates of pyrethroids and organophosphate insecticides for katydids and chlorpyrifos for citricola scale control. Neonicotinoid treatments applied for the exotic pest GWSS, provided some reduction in sprays for scale pests, however, they increased the total number of treatments per orchard. Research to study the long-term effects of neonicotinoids on the citrus IPM program is currently in progress.

Acknowledgements

I thank cooperators Craig Kallsen (Kern County University of California Cooperative Extension) and Neil O’Connell (Tulare County University of California Cooperative Extension) for research assistance and San Joaquin Valley citrus growers for use of their orchards. This project was funded by the California Citrus Research Board and the California Department of Pesticide Regulation Pest Management Alliance Fund.
References


Evolution of integrated pest management and integrated production in citrus, in Portugal

M. Cavaco, H. Gomes and F. Mendes
Direcção-Geral de Protecção das Culturas, Edifício 1, Tapada da Ajuda, 1300 Lisboa, Portugal (http://www.dgpc.min-agricultura.pt)

Since the end of the 1950s integrated pest management (IPM) has evolved slowly in Europe. However, since the beginning of the 1980s significant development was achieved in countries like Germany, Switzerland, and certain regions of Italy. In Portugal, although the initiatives to develop the practice of IPM have been initiated in the 1980s and continued in the following decade the practise of this technology was almost incipient until the 1990s. In Portugal, since the implementation of the agro-environmental measures in 1994, an intensification of the IPM practice was observed.

Citrus is one of the crops addressed in the agro-environmental measures, together with pomefruits, vineyard, cereals, vegetable crops, olives, stone fruits and nut crops. Since 1997, DGPC has carried out the recognition of farmers’ associations in the scope of IPM and integrated production. The present paper describes some data, namely in relation with recognised farmers’ associations, involved areas, farmers and technicians.
The Phytosanitary problems that affect orange groves on Terceira Island, Azores

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Abstract: In orange orchards several pests (insects and mites) trips) damage the trees, reducing production and contributing to low quality fruits. The INTERFRUTA project has as one of its goals a better understanding of the phytosanitary problems (pests and diseases) that appear in the orange orchards in the three most productive areas of Terceira Island (Biscoitos, Angra do Heroísmo and São Sebastião). Mites, thrips, aphids, whiteflies and the citrus leaf miner are the most important pests in orange orchards and they mainly concentrate their action in the upper part of the citrus trees. The methodology used to estimate damage was direct observation with a biweekly registration of all data collected from the orange orchards (such as occupation percentage, for almost all the pests found, and the number of mobile forms and eggs for the mites), and for thrips monitoring captures on yellow traps coated with glue. Two collecting methods were used to identify both harmful and beneficial insects: the Battage and the Mailaise trap, in a seasonal strategy with three periods of sampling (Spring, Summer and Autumn). In this study we have analyzed the seasonal evolution and occupation percentage for each pest registered in the orange orchards, at each of the three most important production areas of Terceira Island. The disease and virus survey was made in six orange orchards using direct sampling by observation of symptoms, at different times according to the development of the orange trees, searching for symptoms in 10% of all the trees present in each orchard. Eight species of fungi were found, but tests showed that the CTV virus was not present in all the orange orchards studied.

Key words: pests, disease, virus, orange, damage, Azores

Introduction

The Azores Islands are an archipelago situated in the Atlantic Ocean, between America and Europe, comprising nine islands covering 2.352 km², distributed in three groups: the Oriental
group (Santa Maria, São Miguel), the Central group (Terceira, Graciosa, São Jorge, Pico, Faial) and the Ocidental (Flores, Corvo).

The INTERFRUTA Project, a project for the promotion and development of fruit production and research of bioactive plants to support Integrated Pest Management and Integrated Production in the Atlantic Island groups, has as a major goal stimulating interest in fruit production. This project was an interregional cooperation project between the three European Atlantic regions (Azores, Madeira and Canary Islands) and involved around 30 investigators from several Institutions (University of Azores - Agricultural Sciences Department, University of La Laguna, University of Trás-os-Montes e Alto Douro, a Producers Association - FRUTER, Imperial College London, and the Government Agricultural Services of Azores and Madeira). It was financed by FEDER in the EC program INTERRREG–III-B (Lopes, 2004a; 2004b).

In this project we looked in an integrated way at the fruit ecosystem as a whole, and we analyzed climactic and other important parameters that affected productivity (fertility, soil conditions, and the different varieties of fruit trees). We also wanted to know which pests, fungi and viruses appeared and caused problems in the orchards. Seasonal sampling was done to catalogue the beneficial insects present in orchards.

The INTERFRUTA project had, among others goals, one to understand the phytosanitary problems and beneficial insects that are present in fruit orchards, especially in orange groves. All the field work was carried out between January 2004 and June 2005, working with 28 fruit producers on Terceira Island.

Materials and methods

Starting phase

Working closely with the fruit producers association (FRUTER), it was possible to interview 160 fruit producers registered with the agricultural services, from seven different areas of the island (Biscoitos, Porto Judeu, S. Brás, Lajes, Vila Nova, S. Sebastião and Angra). That also permitted a GIS application, in an Arc View environment and using digital photographs, determining the exact location of the orange groves, their limits, and the first definition of the boundaries of the fruit production areas on the island.

Part of the field work of this project was conducted in orange groves in three different areas on Terceira Island (Angra do Heroísmo, Porto Judeu/São Sebastião e Biscoitos). These experimental orchards were identified and maps were made of the distribution of the fruit trees in each orchard, identifying the variety and its distribution. In each experimental orchard an Automatic Meteorological Station (EMA) was placed to register important climate parameters. These data were important to the field observations.

Pest survey

To make a quantitative risk assessment, the method used was visual observation (occupation percentages, mite mobile forms and egg counts) once every two weeks in the field, with subsequent laboratory identification. At each sampling time three observations were made from three of five trees in each orchard, over a total of nine orange orchards, from each of the three zones studied.

To determine the occupation percentage of citrus leaf miner, whitefly, scale insects, mealy bugs and aphids, a circle of wire (fifty-six centimeters in diameter) was randomly placed over a branch and then the number of organs (new leaves, old leaves, flowers and fruits) inside that circle were counted and the occupied percentage was noted for each pest. In addition, one leaf was collected and taken to the laboratory from each sample point (fifteen leaves from each orchard) to count all mite mobile forms and eggs present.
To evaluate the Thysanoptera species present, we used the visual observation, and chromatic traps were set out in each orchard. These traps were collected and replaced every two weeks. In the laboratory using a magnifier, the adults in the leaves and on the traps were identified, counted and registered.

The Mediterranean fruit fly was monitored using Jackson traps with sexual lures (for the males) and Tephri (3Clure) trap with three different kinds of food attractants (for the females) in all the orange groves. In each orchard one trap of each kind was examined every fifteen days, the adults were counted and separated to determine the number of males and females from each trap. From these data it was possible to know the population fluctuations during the observation period.

**Beneficial insects and pest survey**

In the survey of all the insects and mites present in the orange groves three methods were used: Visual observation of pre-defined parts of the fruit trees; the Battage technique (Santos *et al.*, 2004a); and the Malaise trap (Santos *et al.*, 2004a). With the visual observation of predetermined parts of the plant it was possible to identify the species present but also the occupation percentage of each organism, which contributed to a better knowledge of the different organisms present and their action in the orange groves. The Battage technique was carried out in the orange groves monthly and the Malaise trap was also placed in the experimental orange orchards monthly to collect the pests, predators and parasitoids (Santos *et al.*, 2004a; 2004b; 2004c).

**Fungi and virus survey**

The methodology for pathogens was defined in collaboration with investigators from the University of La Laguna, Canary Islands. Some aphids species transmit viruses, and for that purpose it’s the incidence of aphids in the fruit trees was studied by collecting samples in which the aphids species present in the groves were identified using the “Battage” technique and serological tests (DAS-ELISA) on 44 leaf samples collected in the experimental orchards studied. (Melo *et al.*, 2004).

**Results and discussion**

**Pest survey**

The analysis is based on 990 samples taken from orange trees and 141 observations from the chromatic traps. As a result of all the work done in the orange groves, the following pest groups were detected as pests in Terceira Island: aphids, whiteflies, mites, the Citrus Leaf Miner, scale insects and mealybugs, thrips and the Mediterranean fruit fly.

**Aphids**

From a total of fifteen species of aphids identified in four fruit hosts (banana, orange, apple and peach) studied in the INTERFRUTA project, seven species were found in orange and two of them are for the first time mentioned as existing on Terceira Island (Table 1).

**Whiteflies**

The Aleiroididae family or whiteflies were represented by two species in the orange groves: the citrus white fly (*Aleurothrixus floccosus* (Maskell)) and *Paraleyrodes minei* (Laccarino). On the Azores Islands, *A. floccosus* was observed for the first time in Pico Island in 1988 and from there it spread rapidly to the other Islands (Mota, 1993). *P. minei* was first observed in 1997 in mainland Portugal (Carvalho, 1994), there are no references of its existence in Madeira Island or the Azores and this species is now recorded for the first time in the Azores as a result of the INTERFRUTA project investigation work. This species cohabits normally with *A. floccosus*, but the colonies can be distinguished by the waxy secretions that enclose the adults with a powdery aspect and that are disposed like an
alveolus. It is a polyphagous species that attacks both new and mature leaves. *P. minei* adults have a unique appearance, they have little mobility and they have feint grey stains at the apex of the wings (Carvalho et al., 2000).

Table 1. List of the aphids species found and identified in orange groves in the INTERFRUTA Project on Terceira Island (* indicates species previously unreported on the island). Citrus (C). Areas are Angra (AH), Biscoitos (Bc) and São Sebastião (SS), sampling is by Malaise Trap (AM) and visual observation (RD).

<table>
<thead>
<tr>
<th>Family</th>
<th>Specie</th>
<th>Culture</th>
<th>Area</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphididae</td>
<td><em>Aphis fabae</em> Scopoli, 1763</td>
<td>C</td>
<td>Bc, SS</td>
<td>RD, AM</td>
</tr>
<tr>
<td></td>
<td><em>Aphis gossypii</em> Glover, 1877</td>
<td>C</td>
<td>Bc, SS</td>
<td>RD</td>
</tr>
<tr>
<td></td>
<td><em>Aphis hederae</em> Kaltenbach, 1843 (*)</td>
<td>C</td>
<td>AH, Bc</td>
<td>AM</td>
</tr>
<tr>
<td></td>
<td><em>Aphis solanella</em> Theobald, 1914</td>
<td>C</td>
<td>AH, Bc</td>
<td>AM</td>
</tr>
<tr>
<td></td>
<td><em>Aphis spiraecola</em> Patch, 1914</td>
<td>C</td>
<td>Bc</td>
<td>RD</td>
</tr>
<tr>
<td></td>
<td><em>Toxoptera aurantii</em> (Boyer, 1841)</td>
<td>C</td>
<td>SS</td>
<td>RD</td>
</tr>
<tr>
<td></td>
<td><em>Anoecia haupti</em> Börner, 1950 (*)</td>
<td>C</td>
<td>AH</td>
<td>AM</td>
</tr>
</tbody>
</table>

The average occupation percentage values on leaves changes over the studied period, sometimes exceeding the Economic Threshold (ET= 25% occupied leaves) (Fig.1). Along with these changes, these values are different when comparing new leaves with old leaves. In all the three areas studied in February, May, June, July and August, the ET (25% leaves occupied) of the aphid populations was achieved and in some areas was exceeded. Biscoitos and Angra (Fig.1), were the areas where the greatest densities of aphids were recorded.

Fig 1. Aphid’s population distribution in orange groves from S. Sebastião, Angra, and Biscoitos on Terceira Island.
Among the three areas studied (Fig. 2), S. Sebastião registered the highest population values and the most propitious conditions to their development. This could be explained by the peaks of whitefly presence that coincided with the budding period in the orange groves. In Angra, there were no records of substantial attacks, and they never exceeded the ET (ET = 20% occupied leaves). In Biscoitos, there were two peaks in two different periods, one in July and other in February (when the first budding was observed). The field work demonstrated that there are four important months in which whitefly can cause great damage (January, May, June and November) in the orange groves.

Fig 2. Whitefly population distribution in orange groves from S. Sebastião, Angra, and Biscoitos on Terceira Island.
Mites
Of the seven species of Acari found during the project field work, only three are related to citrus culture (Table 2).

Table 2. Species of mites (Acari) identified in citrus from the INTERFRUTA project field work. (* indicates species previously unreported on the island). Areas are Sao Sebastiao (SS), Angra (AH).

<table>
<thead>
<tr>
<th>Mite Family</th>
<th>Specie</th>
<th>Area</th>
<th>Orchard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetranychidae</td>
<td><em>Panonychus citri (McGregor)</em></td>
<td>SS; AH</td>
<td>S6L; T6L</td>
</tr>
<tr>
<td>Tarsonemidae</td>
<td><em>Fungitarsonemus peregrinus (Bee)</em></td>
<td>SS</td>
<td>S6L</td>
</tr>
<tr>
<td>Phytoseiidae</td>
<td><em>Amblyseius herbicolus (Chant)</em></td>
<td>AH</td>
<td>T6L</td>
</tr>
</tbody>
</table>

The number of adults present in each leaf varied between the months studied and from place to place. The most affected areas were registered in the first half of the year: Biscoitos (almost 15 adults per leaf) in January and S. Sebastião (with almost 25 adults per leaf) in July (Fig. 3b).

Fig 3. Distribution of mites eggs (3a) and mobile forms (3b) in orange groves from S. Sebastião, Angra, and Biscoitos on Terceira Island.
For the first time the results from the field observations show that mites eggs are present in the orchards during all the year (Fig. 3a) and no only during winter as known until now.

**Citrus leaf miner**
The citrus leaf miner (*Phyllocnistis citrella* Stainton, 1856) is, without doubt, the most important Lepidoptera in the citrus ecosystem. In Madeira Island, *P. citrella* was first detected in 1995 and then in the Azores in 1996. *P. citrella* has restricted food host species ranging mostly from the Rutaceae family, specifically the citrus cultures (Vitorino, 1999). *P. citrella* was present throughout the year and in close relation with the appearance of the new leaves in all the seven studied citrus experimental orchards over the three zones.

However, S. Sebastião was the study area that had the most severe levels of attack (90% in January and 40% in July) (Fig. 4). The two orchards in area were at different altitudes, but registered two peaks of attack matching the two to three citrus budding periods observed. There is one peak matching the budding period in the summer (June and July) and another one in autumn (November and December). This last budding in autumn was the most affected by this pest. Biscoitos (Fig. 4) had a peak in April (almost 40% presence on new leaves) and a second peak in June (40% presence). In Angra the populations of this pest started to grow from the middle of May (40% infestation) (Fig. 4) and registered an enormous peak in August that continued until the end of September, reaching values of 60%, which is above the ET of 20% of occupied leaves. The leaf occupation percentage values in all areas exceeded the ET and the peaks for percentage of leaf occupation were different in all the areas studied.

![Fig. 4. Leaf occupation percentage by Citrus leaf miner in S. Sebastião, Angra and Biscoitos.](image)

**Scale insects and Mealy bugs**
The most representative families of Scale insects and Mealybugs in oranges were the Coccideae, with six species, and Diaspididae, with three species. On the basis of frequency and importance, there were two species from the Coccid family that stand out: *Ceroplastes sinensis* (Del Guercio) and *Protopulvinaria pyriformis* (Cockerell). Occasionally others come up without causing damage: *Parasaissetia nigra* (Nietner); *Coccus hesperidum* (Linnaeus); *Saissetia coffeae* (Walker) and *Saissetia oleae* (Olivier).

From the Diaspididae family, *Chrysomphalus pinnulifer* (Maskell) was the most frequent species with the greatest importance. Sometimes others Diaspidideae stand out but not with
the same importance: *Lepidosaphes becki* (Newman) and *Chrysomphalus dyctiospermi* (Morgan).

Pseudococcideae was another family with fewer species found, but no less important, including *Pseudococcus longispinus* (Targioni-Tozetti) most frequently. *Planococcus citri* (Risso) was less important and less frequent. This situation is similar to that in Madeira, and is contrary to what happens in mainland Portugal (Carvalho & Aguiar, 1997). *Icerya purchasi*, from the Margaroididae family, was the other scale insect that occurred frequently in the citrus groves.

**Thrips**

The symptoms induced by the feeding of Thysanoptera can indicate the species present, along with the nature of the attacked organ and even of the phenological stage of the fruit tree. Table 3 shows the species found in the 141 traps in the orange orchards.

Table 3. Thrips found in banana, peach and orange groves on Terceira Island in the INTERFRUTA project work. Sampling is by visual observation (RD) and Battage (TB).

<table>
<thead>
<tr>
<th>Species</th>
<th>Culture</th>
<th>Orchard</th>
<th>Sampling method</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anisopilothrips venustulus</em> Priesner</td>
<td>Banana</td>
<td>B2B; S3B; B5L</td>
<td>RD</td>
</tr>
<tr>
<td><em>Heliothrips haemorrhoidalis</em> (Bouché)</td>
<td>Banana</td>
<td>B2B; B5L</td>
<td>RD</td>
</tr>
<tr>
<td><em>Hercinothrips bicinctus</em> (Bagnall)</td>
<td>Peach</td>
<td>S12P</td>
<td>TB</td>
</tr>
<tr>
<td><em>Apterygothrips longiceps</em> zur Strassen¹</td>
<td>Peach</td>
<td>B10P</td>
<td>TB</td>
</tr>
<tr>
<td><em>Haplothrips</em> sp.</td>
<td>Orange</td>
<td>B5L</td>
<td>RD</td>
</tr>
<tr>
<td><em>Haplothrips</em> sp.</td>
<td>Peach</td>
<td>B10P</td>
<td>TB</td>
</tr>
</tbody>
</table>

On orange, it was possible to identify three species: *Anisopilothrips venustulus* (Priesner), *Heliothrips haemorrhoidalis* (Bouché) and *Haplothrips* sp. From January to November the thrips were captured in all the orchards. Angra registered four peaks (Fig.5) and there this pest was present for a longer period of time and with more constant numbers. In S. Sebastião (Fig.5), July and August were the months with the highest population density of all the orchards studied. Biscoitos registered the lowest population level and had a pattern similar to S. Sebastião, however in Angra thrips appeared in the orchards earlier (on February) than in the other two areas (Fig.5).

**Mediterranean fruit fly**

The Mediterranean fruit fly (*Ceratitis capitata* Wied.) was most abundant from the middle of August to the end of November (Fig. 6). From the Medfly data it was possible to build a digital GIS map to show the Mediterranean fruit fly adults density distribution in all the areas studied (Fig. 7) (Lopes et al., 2005a; 2005b).

**Beneficial insects and pests survey**

From all the work done in the INTERFRUTA project in the four cultures studied (banana, peach, orange and apple) a total of 990 samples from orange orchards were analysed.
Fig. 5. Thrips population distribution in orange groves from S. Sebastião, Angra, and Biscoitos on Terceira Island.

Fig. 6. Mediterranean fruit fly adult captures in Terceira Island orange groves.

Fig. 7. An example GIS map from Biscoitos adult trap captures data showing the Mediterranean fruit fly distribution density at the different altitude levels.
Among the organisms collected in the Battage and Malaise traps 15 aphids species were found, three of which were new (referred for the first time) to Terceira Island. Two of the three new species were found in orange (Aphis hederae Kaltenbach and Anoeclia haupti Börner) as were five other species (Aphis fabae Scopoli, Aphis gossypii Glover, Aphis solanella Theobald Aphis spiraecola Patch and Toxoptera aurantii Boyer) that were already known (Santos et al., 2004b). It is now possible to start a reference collection from the INTERFRUTA project for Terceira.

**Fungi and virus survey**

Surveys collected and analysed 548 samples of fungi and 838 of viruses. From those, 148 fungal samples and 123 virus samples were analysed from orange groves. It was also possible to analyse the distribution of the fungi distribution from early sprout, blossom and throughout the season until fruit appearance. Table 4 shows the fungi species found.

<table>
<thead>
<tr>
<th>Pathogen agent</th>
<th>Colletotrichum gloesporioides</th>
<th>Phoma glomerata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata</td>
<td>Colletotrichum musae</td>
<td>Phoma musaecola</td>
</tr>
<tr>
<td>Alternaria arborescens</td>
<td>Fusarium lateritium</td>
<td>Phytophthora sp</td>
</tr>
<tr>
<td>Alternaria mali</td>
<td>Fusarium oxysporum f. sp. cubense</td>
<td>Rizoctonia solani</td>
</tr>
<tr>
<td>Aspergillus wentii</td>
<td>Goseporium gloesporioides</td>
<td>Verticillum teobromae</td>
</tr>
<tr>
<td>Botryosphaeria lutea</td>
<td>Pestalotia gracilis</td>
<td>Verticillum sp 1</td>
</tr>
<tr>
<td>Botryosphaeria ribis</td>
<td>Pestalotia leprogena</td>
<td>Verticillum sp 2</td>
</tr>
<tr>
<td>Cephalosporium sp</td>
<td>Pestalotia sp</td>
<td></td>
</tr>
</tbody>
</table>

In the virus survey three DAS – ELISA with polyclonal scores LOEWE Phytodiagnostics from Germany were made to find CTV virus, two in March and one in July. From the 118 samples tested, and also from 12 aphids analysed, the results obtained from these were all negatives. In July 38 samples of aphids and 5 from roots were tested and also these were all negative, concluding that the virus tested was not present in the orange groves survey in Terceira Island.

At the end of the INTERFRUTA project it was possible with all this information to prepare and distribute several extension leaflets for distribution to the technicians and fruit producers involved in the INTERFRUTA project.

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To the Azorean Regional Government and to the INTERREG III-B European Community Programme that financed all the investigation work done in the INTERFRUTA project; to all the partners involved, and their investigators that worked in this INTERFRUTA project; to all the fruit producers that allowed us to work in their orchards; to Eng.º Passos de Carvalho, Prof. Doutor António Mexia, Prof. Doutor John Mumford, Prof. Doutora Ana Pereira, Eng.º Fernando Ilharco and Eng.ª Maria do Anjos Ferreira, who helped us create the project goals and its implementation. This work was financially supported by the EC programme INTEREEG III-B and resulted from the INTERFRUTA project (MAC/3.1/A1).
References


Ecological infrastructures and conservation biological control in citrus orchards

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The IOBC/WPRS edited recently a book (Boller et al. 2004b) on ecological infrastructures, i.e., any infrastructure at the farm or within a radius of about 150 m that has an ecological value to the farm, such as hedges, grasslands, wildflower strips, ruderal areas, conservation headlands, stone heaps, whose judicious use increases the functional diversity of the farm. One important aspect of functional biodiversity for pest management is the preventive and sustainable regulation of pests by their natural enemies. In this way ecological infrastructures can contribute for conservation biological control by increasing the density of the natural enemies and enhancing their impact on the pest. A minimum surface of 5% of farmland is required by IOBCwprs to be designated as ecological infrastructures (Boller et al. 2004a, b). A synthesis on the results of a project (nº 29 PO AGRO-Medida 8.1-DE&D 2002-2005) aiming at study the influence of ground cover management and hedges in the diversity and abundance of natural enemies in citrus orchards will be presented and discussed.

References

Abundance and population dynamics of ground-dwelling predators in Spanish citrus orchards

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There is little information on the abundance-activity of predaceous ground arthropods in citrus crops in Spain. In this work a large number of ground predatory species have been identified in citrus groves in Valencia (Spain) for the first time. We monitored ground-dwelling predators in four citrus orchards, during one year (April 2004 to April 2005), by pitfall trapping across the diagonal in each orchard. Ants (Hymenoptera) were the most abundant-active group, followed by spiders (Araneae), rove beetles (Col.: Staphylinidae), ground beetles (Col.: Carabidae), earwigs (Dermaptera) and tiger beetles (Col.: Cicindelidae). Ants were captured in very low numbers during the winter months but they were very active during the rest of the year. Spiders, rove beetles and earwigs were active throughout the year, fluctuating without a clear pattern. Carabids were especially active in spring and at the end of the summer and tiger beetles were only collected during the spring period.
Parasitism disruption by ants of *Anagyrus pseudococci* (Girault) and *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), two parasitoids of the citrus mealybug *Planococcus citri* (Risso) (Homoptera: Pseudococcidae)

Campos, J. M., Martínez-Ferrer, M. T., Forés, V.

Abstract: Numerous ant species are known to disrupt parasitism of citrus pests by natural enemies. *Lasius niger* (Latreille) is the most abundant specie in citrus groves in this area, so the effect of this ant on *P. citri* parasitization by *A. pseudococci* and *L. dactylopii* was studied. *L. niger* significantly reduced parasitism by about 35%, while sex ratio of the progeny remained unaffected in both parasitoid species. Nevertheless, when both parasitoids were tested altogether, ants reduced parasitism by about 50%. Field observation showed that ants rarely left the calyx area where mealybugs were located, and both parasitoids were able to attack *P. citri* in presence of attending ants. Host inspection by *L. dactylopii* was detected by ants in 59% of the events, compared to *A. pseudococci*, which was only detected in 32%. Nevertheless, disturbance by ants led *A. pseudococci* to leave the host, or the fruit itself, more frequently than *L. dactylopii*.

Keywords: Ants, disturbance, disruption, *A. pseudococci*, *L. dactylopii*, *L. niger*, mealybug, *P. citri*.

Introduction

The disruption of biological control of pests by ants has been reported by numerous authors (De Bach *et al.*, (1951), Phillips y Sherk, (1991), Itioka and Inoue, (1996), Buckley and Gullan, (1991), Garcia-Marí *et al.*, (2003), Martínez-Ferrer *et al.*, (2003)). Numerous ant species feed on products with a high sugar content, like honeydew. Thus ants tend to protect insects that produce honeydew from their predators and parasitoids, hence ensuring their source of food for as long as possible. The presence of attending ants in citrus, has been demonstrated as highly effective in preventing biological control of Homopterae (aphids, whiteflies, scale insects, etc.) (De Bach, 1951; Itioka and Inoue, 1996(1); Moreno *et al.*, 1987). Moreover, attending ants disturb natural enemies, for in addition to those which protect honeydew-producing insects there are also ants which protect diaspididae or mites as well (Flanders, 1945; Haney et al., 1987; Martínez-Ferrer *et al.*, 2003) Ants protect such insects by maintaining close contact with them, at times even inside the colony itself (Phillips y Sherk, 1991).

Ant species show different levels of aggressiveness, and depending on that, protection offered to homoptera against parasitization can be more or less effective (the more aggressive the more effective) (Buckley y Gullan, 1991). Aggressiveness depends on factors such as the parasitoid specie, or honeydew abundance (Gross, 1993), ants behaving more aggressively when honeydew is scarce (Way, 1963). As the natural enemy approaches, ants try to disturb it by walking towards it and making it jump off and leave the colony, or by preventing contact between host and parasitoid, depending on their aggressiveness. (Buckley and Gullan, 1991). In the case of predators, larger than parasitoids, ants bite their legs and wings until they leave...
their prey (Iitioka e Inoue, 1996), since usually predators show a greater firmness towards ants than do parasitoids, and ignore them more often.

Another kind of indirect protection provided by ants involves shelters made of grains of earth and wood chips to cover the homopteran colonies on the fruits, as _L. niger_ does with _Pseudococcus comstockii_ (Morimoto, 1976), and _L. humile_ with _P. citri_ (Franco et al., 2000).

With regard to natural enemies of _P. citri_, Garcia-Mari _et al._ (2003) confirmed in field trials the need to exclude ants from citrus trees as a strategy for pest control, in spite of the in-field release of _L. dactylopii_ and _C. montrouzieri_.

Apart from the role of ants as disturbers of the biological control of numerous pests, they have also been reported in citrus trees as pests themselves. _Solenopsis invicta_ Buren for instance, causes damage to fruits, flowers, leaves and flushes on young trees (Smittle _et al._, 1988; Banks _et al._, 1991), and damage to polyethylene irrigation tubes by _Solenopsis geminata_ (Fabricius) has also been noted (Chang and Ota, 1976). Sometimes species like _Solenopsis xyloni_ McCook are bothersome for workers because of their stings and bites (Martínez-Ferrer, 2003).

According to Martínez-Ferrer _et al._ (2003), ant populations in California increase as a consequence of the declining utilization of broad-spectrum pesticides, leading to a disruption of biological control of several pests. With regard to citrus trees in Spain, authors like Palacios _et al._ (1995) achieved satisfactory results in ant control by using products normally used by farmers, such as diazinon and clorpirimfos, which prevented ants from accessing the canopies, and which persisted for three months in some cases.

There are 5 species of foraging ants in the citrus areas in southern Tarragona (Spain). According to Palacios _et al._ (1999), surveys in the Baix Ebre and Montsià areas (Tarragona) showed that the most abundant species is _Lasius niger_ (Latreille) representing a 89.5% of the individuals captured, _Pheidole pallidula_ (Nylander) representing 7.3%, _Linepithema humile_ (Mayr) 1.4%, _Plagiolepis schmitzii_ Forel 1.2%, and finally _Tetramorium caespitum_ (Latreille) 0.3%. According to these authors, _L. niger_ is much more abundant in spring than during the summer. Alvis _et al._ (2003) found that _L. niger_ was the most abundant specie in citrus tree canopies in Valencia, followed by _Pheidole pallidula_. _Lasius niger_ is considered by several authors (Hubner and Volkl, 1996; Iitioka and Inoue, 1996; Kaneko, 2003) as highly aggressive in its defence of the host insect against parasitoids and predators.

Disturbance of natural enemies of _Pseudococcidae_ by ants has been studied by numerous authors (Buckley and Gullan, 1991; Phillips and Sherk, 1991; Iitioka and Inoue, 1996(1); Tumminelli _et al._, 1997; Garcia-Mari _et al._, 2003). However, it is necessary to determine how the parasitoids’ activity is altered by the presence of ants. Apart from the effect on the parasitoid, the ant species is also important. _Lasius niger_, the most abundant species in this area, may behave differently to other species. The direct observation of ant and parasitoid behaviour on _P. citri_ infested fruits in the field should determine how the parasitization sequence is altered.

Therefore the objectives of this study were:

1) To assess the disturbance effect of ants on _A. pseudococci_ and _L. dactylopii_, and to measure their capacity for reduce parasitization.

2) To define the behaviour rules of ants and parasitoids, and their interactions.

3) To describe the behaviour of ants and parasitoids in the field during interaction with each other.
Material and methods

The study was conducted in the field in order to assess ant activity under the same conditions as in the orchard, and to therefore prevent alteration of parasitoid behaviour. Thus two different experiments were conducted. The first (Trial 1) was carried out in order to examine the reduction of parasitization on *P. citri* by both species. The second (Trial 2) was a study of how the ants disturbed and attacked the parasitoids, thus interfering in their relationship with the host. This second trial was carried out by observing and noting ant and parasitoid activity. In both cases the ant species identified and used was *L. niger*.

Insects

Both *P. citri* and parasitoids came from the experimental mass rearing carried out in the insectaries of the Estació Experimental de l'Ebre (IRTA). Mealybugs were reared on pumpkins and sprouted potatoes in nylon mesh cages supported on wooden frames. The cultures were maintained in the dark at 25 °C and 60-70% RH. *A. pseudococci* and *L. dactylopii* were obtained 19-22 days after releasing adult insects in cages containing young female instar larvae of *P. citri*.

The parasitoids were collected, ensuring that they were of equal size. To ensure mating, when emerged, females were exposed to male parasitoids for 24 hours in small plastic vials (Ø= 60 mm., h=32 mm.), in a 1:2 proportion (females:males). Later, female parasitoids were allowed to oviposit on *P. citri* for 24 hours, thus ensuring that they had previous experience. Then females were removed and placed in small plastic vials (Ø=10mm, h=50mm) for 24 hours so that they would have mature eggs when used in the experiments (Cadée and van Alphen, 1997).

This process, lasting three days, was conducted in a climatic chamber under the above-described conditions (25°C, 60-70 % HR and 12:12 L:D photoperiod).

Experimental procedure

Experiment 1.- Effect on parasitism: Small pumpkins were used which would fit inside the small evolutionaries where the experiments were going to be conducted. Twenty-five days prior to the experiments the pumpkins were contaminated with first instar *P. citri* larvae, to ensure the settling of the mealybugs. Plastic boxes with a lid of fine mesh nylon fabric were used (L=20 cm., Ø=25 cm) to allow aeration of the pumpkin and the insects during the field trials (Picture 1). A plastic tube was also placed in some evolutionaries to permit the access of ants to the pumpkins infested with *P. citri* (Picture 2).

Parasitoid activity was allowed for 24 hours. Then the evolutionaries were carried to the laboratory, where the parasitoids and the ants were removed. To prevent the pumpkins from rotting and producing severe damage inside the evolutionaries, the *P. citri* were removed from the pumpkins and placed in evolutionaries along with potato sprouts until the parasitoids’ emergence.

Six treatments were established: each one of the parasitoids together, or alone with ants, or ant-excluded. In the ant-excluded treatments the number of progeny per female alone or with both species was also studied.

Six replications were carried out of each experiment, thus 36 evolutionaries were used. Six female parasitoids were placed in each evolutionary, so overall 108 females of each species were used.
Experiment 2.- Field observations of parasitoid and ant behaviour: The observations were conducted in July (2003), around noon so as to avoid the possible influence of the time of day on the ants’ and parasitoids’ behaviour. It being an observation study, fruits infested with P. citri under the calyx (and around the peduncular area) as well as ants were chosen. Once the fruit was chosen, proximal leaves that prevented visibility were carefully removed, with care taken not to disturb the ants. A parasitoid was released on the fruit and the activities of the parasitoid, P. citri and the ants were recorded. Each female was only tested once. It was necessary keep sight of the parasitoid at all times during its movements to ascertain when it headed for the P. citri colony. A small area surrounding the calyx where the P. citri were hidden was visually defined as the P. citri colony. Consequently, it was considered that when the parasitoid entered that area it was to inspect the hosts located there. The observation ended when the parasitoid abandoned the fruit. Only two A. pseudococci and 15 L. dactylopii females were manually removed, after having remained motionless on the fruit for more than 6 minutes. Overall, 56 A. pseudococci and 57 L. dactylopii were observed, with alternation of species.

Assessment
In the first experiment, the number and sex of the parasitoids emerged per evolutionary was recorded, and corresponded to the activity of 6 females during 24 h in field conditions.

For the second experiment, after the audio recordings, the duration of the different activities observed during the observation period was measured and counted. The frequency of each activity following another was calculated for both parasitoids and ants, and represented graphically through an ethogram.

Results were analysed using analysis of variance (PROC GLM, SAS Institute 1998) and mean separation by means of Duncan’s multiple range test with a 95% significance level. If necessary, data were arcsin-transformed prior to mean separation before the analysis to meet the assumption of normality and homogeneity of variance. Means are showed at original scale.

Results

Experiment 1
The presence of ants significatively reduced parasitism by both parasitoid species. In those evolutionaries that ants entered, a lower progeny per 6 parasitoids was obtained compared to those which were ant-excluded. (Table 1). A greater effect was shown when both parasitoids were tested together, where parasitism was reduced by 50% when ants were attending P. citri.

When parasitoids were tested separately, reduction of progeny was about 35% for A. pseudococci as well as for L. dactylopii (Table 1).

The number of L. dactylopii progeny was unexpectedly low. In previous experiments under laboratory conditions, the number of L. dactylopii progeny was higher than that of A. pseudococci, results which contrast with these of the present study. When P. citri was attended by ants, the number of progeny per female when both species were together and when they were alone was not significantly different. Thus, when A. pseudococci was alone, its progeny per female was (4.08±0.65), similar to that obtained from L. dactylopii (3.33±0.23) ( n=6; t=1.08; P=0.32). This also happened to L. dactylopii, whose progeny per female was 2.36±0.17 and 2.78±0.35 respectively (n=6; t=1.06; P=0.34).
Table 1. Effect of *L. niger* on parasitism: Progeny of 6 female parasitoids after 24 hours along with pumpkins containing *P. citri*.

<table>
<thead>
<tr>
<th></th>
<th><em>A. pseudococci</em></th>
<th><em>L. dactylopii</em></th>
<th><em>A. pseudococci</em> + <em>L. dactylopii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ants absence</td>
<td>38.17 ± 4.35 b</td>
<td>21.83 ± 2.91 b</td>
<td>33.00 ± 3.51 b</td>
</tr>
<tr>
<td>Ants presence</td>
<td>24.50 ± 3.89 a</td>
<td>14.17 ± 1.05 a</td>
<td>17.33 ± 2.27 a</td>
</tr>
<tr>
<td>Parasitism reduction (%)</td>
<td>35.8</td>
<td>35.1</td>
<td>47.5</td>
</tr>
</tbody>
</table>

Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

The sex ratio was never affected by the ants’ presence. In all cases the sex ratio was around 50%, both when ants were present as well as when they were excluded. (Table 2).

Table 2. Effect of ants on parasitoids progeny sex ratio: Male percentage.

<table>
<thead>
<tr>
<th></th>
<th>Ants presence</th>
<th>Ants absence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. pseudococci</em></td>
<td>56.0 ± 5.5 a</td>
<td>49.5 ± 4.9 a</td>
</tr>
<tr>
<td><em>L. dactylopii</em></td>
<td>51.5 ± 8.4 a</td>
<td>49.4 ± 4.3 a</td>
</tr>
<tr>
<td><em>A. pseudococci</em> + <em>L. dactylopii</em></td>
<td>49.9 ± 7.2 a</td>
<td>47.5 ± 7.7 a</td>
</tr>
</tbody>
</table>

Means within a given row followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

**Experiment 2**

The number of ants per fruit ranged from 3 to 7 individuals. The ant used was the dominant specie in citrus canopies in this area, *Lasius niger*. Generally, during the observations ants were located in the area close to the calyx, where the *P. citri* were settled partially beneath the sepals. Only rarely did the ants abandon this area to search for the parasitoids as they approached.

Normally ants remained close to the *P. citri*, touching them with their antennae. They seemed to be unaware of the parasitoids’ presence, which sometimes moved between two ants and managed to successfully parasitise *P. citri*. Physical interactions between parasitoids and ants commonly occurred in the vicinity of the host, particularly during drilling.

Both parasitoids exhibited similar behaviour patterns to that shown in the laboratory, although the inspection and oviposition sequence were more rapid. (Table 3). Only on a few occasions did *A. pseudococcci* have to be removed from the fruit due to inactivity, unlike *L. dactylopii* which had to be removed one out of three times. When the ants headed for the parasitoid, *A. pseudococcci* was disturbed and never reincided in its attempt to parasitise the host, whereas *L. dactylopii* sometimes made a second attempt and walked towards the ant. The parasitoid was caught by the ants only once, at other times when the ants encountered a parasitoid, it abruptly switched directions or jumped off the fruit.

Both parasitoids were able to parasitize *P. citri* successfully while being surrounded by ants, which seemed unaware of their presence. Although only fruits with *P. citri* under the
calyx were selected, parasitoids sometimes went to the navel and oviposited on young *P. citri* located there.

Both parasitoids were also able to parasitize the hosts in difficult areas, such as beneath the calyx or the navel. Curiously, *L. dactylopii* twice landed on the same fruit after jumping off it, something which never happened with *A. pseudococci*.

Generally, the behaviour of both parasitoid species on the fruit was similar: no significant differences were found for any of the variables analyzed (Table 3). Although *L. dactylopii* showed a higher *P. citri* encounter rate, it did not appear to be significantly higher than *A. pseudococci*. The time spent on the fruit, approximately 3 minutes, was similar in both species.

The duration of the inspection prior to oviposition was very similar as well, at just over 4 seconds (Table 3), in contrast to what was obtained in the oviposition behaviour chapter, where the duration of inspection was 4 times longer.

Table 3. Activities observed in both parasitoids in *L. niger* presence. Mean ± s.e. of number of activities, and duration of some behavioral events (seconds).

<table>
<thead>
<tr>
<th>Activities</th>
<th><em>A. pseudococci</em></th>
<th><em>L. dactylopii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Encounters with <em>P. citri</em></td>
<td>0.64 ± 0.17 a</td>
<td>1.02 ± 0.21 a</td>
</tr>
<tr>
<td>Ovipositor insertions</td>
<td>0.27 ± 0.10 a</td>
<td>0.36 ± 0.11 a</td>
</tr>
<tr>
<td>Time of permanence besides the <em>P. citri</em> colony (s)</td>
<td>64.91 ± 10.14 a</td>
<td>50.31 ± 7.51 a</td>
</tr>
<tr>
<td>Time of permanence on the fruit (s)</td>
<td>169.27 ± 40.69 a</td>
<td>160.11 ± 27.28 a</td>
</tr>
<tr>
<td>Duration of inspection of <em>P. citri</em> (s)</td>
<td>4.67 ± 0.76 a</td>
<td>4.24 ± 0.75 a</td>
</tr>
</tbody>
</table>

Means within a given row followed by the same letters did not differ significantly. Duncan's Multiple Range Test P<0.05

After being released on the fruit, 70% of the times the parasitoid headed for the *P. citri* colony area, whereas 30% of the times it left the fruit immediately. After entering the *P. citri* colony area *A. pseudococci*, tried to inspect a *P. citri* one out of five times, whereas 73% of the times it left the area (Fig.1). On the other hand, *L. dactylopii* inspected a *P. citri* 40% of the times, leaving the area 43% of the times (Fig.2). Both species left the area after entering a similar proportion of the times (12% and 15% for *A. pseudococci* and *L. dactylopii*, respectively). After the *P. citri* examination, both species continued and oviposited half the times, and the rest of the times abandoned the colony or the fruit in the same proportion.

After oviposition, whereas *L. dactylopii* left the area close to *P. citri* 66% of the times (Fig.2), *A. pseudococci* only did so half the times (47%, Fig.1). Following that pattern, *A. pseudococci* examined another *P. citri* 38% of the times, whereas *L. dactylopii* only did so 17% of the times. Both insects abandoned the fruit in a similar proportion (about 15%). Another difference is the percentage of times that *A. pseudococci* approached the *P. citri* colony area again (85%), higher than in *L. dactylopii* (65%). This high rate of entries and exits of *A. pseudococci* in the peduncle area means a higher rate of movements across the fruit. In contrast, *L. dactylopii* movements were less constant and more rapid.

*L. dactylopii* frequently remained motionless in the fruit in areas far from the calyx (29%), for several minutes until it was manually removed. This only happened to *A. pseudococci* 2% of the times.

After staying in an area distant from the *P. citri* colony, the parasitoid abandoned the
fruit at a low proportion of the times in both species (4% and 10%, in *L. dactylopii* and *A. pseudococci* respectively).

Figure 1. Behavioural sequence exhibited by *A. pseudococci* during oviposition in the presence of *L. niger*.
( * ) The parasitoid was removed when remained motionless for several minutes
Figure 2. Behavioural sequence exhibited by *L. dactylopii* during oviposition in the presence of *L. niger*.

( * ) The parasitoid was removed when remained motionless for several minutes.

The response of the ants towards the parasitoids offered only two possibilities: actively defending the *P. citri* from the parasitoids’ attack, or ignoring the parasitoids. Generally, after the parasitoid entered the *P. citri* colony and ant area, the ants responded in a similar way towards both parasitoid species: one out of three times ants exhibited an active defense, repelling the parasitoid, whereas approximately two out of three times ants did not appear to be aware of their presence (Fig.3). However, when the parasitoid was inspecting the host (thus closer to the ants), in *L. dactylopii* the trend was the inverse: two out of three times the ants showed an active defense. This only happened to *A. pseudococci* 41% of the times. These results show the different way in which the ants perceived host inspection by the parasitoids, behaving more aggressively with *L. dactylopii* than with *A. pseudococci*. 
Four different reactions of the parasitoids to the ants’ attacks were observed. The first consisted of the parasitoid continuing with the host inspection, meaning that the disturbing effect of the ant was null. This pattern was followed 43% of the times that ants attacked *L. dactylopii*, whereas this never happened with *A. pseudococci* (Fig.4), as every time that this parasitoid was attacked by ants it stopped examining the host. Another response consisted of the parasitoid ceasing the inspection of *P. citri* but staying in an area close to the calyx, where the colony was located. Both parasitoids rarely showed this pattern, only 2% of the times. Another type of response was shifting to another part of the fruit. *A. pseudococci* showed this response 35% of the times and *L. dactylopii* 20%. Finally, it was also observed that after the ant attack the parasitoid abandoned the fruit definitively by jumping off. This response was considered as the most disturbing among all of them to the parasitoid, and represented 63% of the times for *A. pseudococci* and 35% for *L. dactylopii*, showing clearly that the former was more easily disturbed by the ants’ attack.
**Discussion**

**Effects on parasitism**
The reduction of parasitism on *P. citri* due to the ants' activity was similar in both species. This reduction reaches about 35% in both cases. However, a higher reduction could be expected according to previous references (DeBach et al., 1951; Phillips and Sherk, 1991, Martínez-Ferrer et al., 2003). In this study, the low interference caused by ants could be attributed to the excessive density of *P. citri* on the pumpkins. It should be considered that the effect of disturbance by ants declines as homopterae density increases (Gross, 1993) as, according to some authors, there are density thresholds beyond which the protection offered by ants is not enough (Itioka and Inoue, 1996). The *P. citri* density on the pumpkins, about 2,000 young female per pumpkin, was chosen according to experimental design factors. However, in field conditions *P. citri* densities are lower, and thus easier for the ants to protect. Authors like Franco et al.(2000), consider that the reduction of some homoptera populations observed after the exclusion of ants, must be partially due to the favourable effect of ants on scale insects (physiological stimulation, protection through shelter and nests, etc.).

Parasitoid progeny sex ratio was not altered by the presence of ants. This constrasts with the results obtained in many studies, where variations in the hosts and parasitoid density, or the female age, did affect this variable in the case of *A. pseudococci*. Then again, it was also observed that in the presence of ants both parasitoids produced similiar progeny when ovipositing together in the same evolutionary, or alone.

Under the field conditions of this experiment, the number of *L. dactylopii* progeny was abnormally low, even in the absence of ants. The low temperatures during the 24-hour experimental period could be the reason.. Although the experiment was conducted on the 14th and 15th May, the average temperature was 17.5º C (max: 19.2º C, min: 15.8º C), abnormally low for this season of the year. According to Tingle and Copland (1989), the number of

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**Figure 4: Response of parasitoids against ants disturbance.**

![Diagram showing the response of parasitoids against ants disturbance.](image-url)
progeny per female of *L. dactylopii* declined to one sixth when the temperature dropped from 24º C to 18º C, whereas in *A. pseudococci* there was no significant variation. Authors like Su and Su (1997) also observed a reduction of half the progeny of *L. dactylopii* when passing from 25º C to 20º C. This sensitivity of *L. dactylopii* to low temperatures, also reported by other authors (Krishnamoorthy, 1989; Battaglia *et al.*, 1996), could explain this reduction.

Field observations on the behavior of ants and parasitoids.

Except in a few cases, *Lasius niger* stayed close to the *P. citri* colony, in contact with it and touching (inspecting) it through its antennae. This behaviour appears to be related to the increase in the quantity of honeydew produced by *P. citri* when being physiologically stimulated by ants, as confirmed by Panis (1981). The ants were apparently motionless when the parasitoids inspected the fruit or approached the area surrounding the calyx, where they were protecting the *P. citri*. Sometimes, when the parasitoids were located between two ants and ready to oviposit on a *P. citri* that they were protecting, the ants seemed to be unaware of the presence of the parasitoid. This is very similar to the behaviour observed in *Formica aerata* (Francoeur) by Martínez-Ferrer *et al.* (2003), in contrast with other ant species like *Solenopsis xyloni* and *Linepithema humile*, which were constantly moving on the fruit.

The behaviour of *L. niger* towards the parasitoids was very similar during all the observations, and it may contrast with what other authors have observed, as they consider this specie to be a highly aggressive ant (Hubner and Volkl, 1996; Itioka and Inoue, 1996; Kaneko, 2003). Although the aim of this study was not a comparative experience between different ant species, no severe aggressive behaviour of *L. niger* was observed. Proof of this can be seen in the refusal of the ants to go towards the parasitoid to disturb it, and in the fact that the parasitoids managed to oviposit on some *P. citri* located between two ants without their intervention. Moreover, the parasitoids stayed on the fruit for approximately 3 minutes before they were expelled by the ants, or else abandoned the fruit. However, there should be some caution in the interpretation of these results, as according to Martínez-Ferrer *et al.* (2003) observing three different ant species, the ants appeared to be unaware of the presence of parasitoids and remained motionless during parasitoid oviposition, but the parasitism rate of two *Aonidiella aurantii* was severely affected.

The ants may behave differently according to the parasitoid species or if it is a predator. Panis (1981) confirmed that *L. dactylopii* was not affected as a *P. citri* natural enemy by *L. humile*. Itioka and Inoue (1976) observed bites of *L. niger* and formic acid on coccinellidae predators of *Pseudococcus citriculus*. Furthermore, the way the ants behave depends on whether the predator is at the larval or adult stage (Way, 1963). In this experiment a differential response of the ants towards the parasitoid inspection was confirmed, there being a more intensive response towards *L. dactylopii*, probably due to the higher boldness of that species which often locates itself very close to the ants. The observations have shown the bolder behaviour of *L. dactylopii* in face of the ants, regardless of the fact that it was attacked more often when inspecting *P. citri*. On the other hand, *A. pseudococci* was more fearful, always abandoning the host after the ant attack, and often abandoning the fruit definitively. This disadvantage was compensated by its higher ability of remaining undetected by ants when parasitising *P. citri*, as can be seen by observing the constant entries and exits to the *P. citri* colony area, as shown in the ethograms.

The greatest contrast between the two parasitoids was observed when they reacted to the ants’ attack. *Anagyrus pseudococci* ceased its activity or left the immediate area in almost all the cases, while *L. dactylopii* exhibited much more insistent behaviour, continuing with the same activity half the times. Such a difference is not due to the different duration of some activities such as host inspection, as indicated by Gross (1993) or Martínez-Ferrer *et al.* (2003), because these are similar in both species. However, it should be borne in mind that the
duration of host inspection was shorter in the field and with the presence of ants (slightly more than 4 seconds), than in the laboratory and without ants (about 20 seconds).

Hubner and Volkl (1996) reported that the different behaviour of aphid hyperparasitoids to ant attack is responsible for its success in the field, some of them suffering from mortalities of about 26% by *L. niger*, and consider that more aggressive than *Myrmica laevinodis*. Comparatively, the parasitoids in this experiment, *A. pseudococci* and *L. dactylopii*, can be considered as capable in their attempts to prevent the ants’ attack.

Both species showed the capacity to reach the host with their ovipositors, even though the host was young and was completely hidden under the sepals or the navel. These observations, as too those by Martinez-Ferrer (2003), contrast with Berlinger and Gol’berg (1978) who, when after observing lower parasitism of *A. pseudococci* on *P. citri* in grapefruit than in orange, reported that it was due to the difficulty of the species to reach the host under the sepals, which are larger in grapefruit. Another important aspect is that the duration of host inspection prior to oviposition was highly reduced compared to the episode of host inspection and oviposition behaviour, which appears to be indicative of the effect of the field conditions or the ants activity on the parasitoid, which make it act more rapidly. According to Barzman (1992) this is an important aspect, along with others like host acceptance or ant interference, which will eventually determine the parasitism activity of the parasitoids.

Finally, the similar results obtained in the reduction of parasitism, encounters with *P. citri* and ovipositions per insect in both species, show that despite the differences observed in the behaviour of the parasitoids in front of the ants, both species are similarly affected by *L. niger*.

**Conclusions**

The presence of ants reduced the parasitism activity of *A. pseudococci* and of *L. dactylopii* by a similar proportion, about 35%.

Ants tried to disturb the approach of the parasitoids to the host on the fruit by attacking them or by making rapid movements along the fruit.

Direct ant attack was greater on *L. dactylopii* than on *A. pseudococci*, although *A. pseudococci* showed a more fearful response to the attack.

**References**


Natural enemies of the black scale *Saissetia oleae* (Homoptera: Coccidae) in Valencia (Spain)

**Alejandro Tena-Barreda, Ferran Garcia-Marí**

**Abstract:** A survey of the black scale [*Saissetia oleae* (Olivier)] present in citrus orchards in Valencia (Spain) was conducted between March 2003 and March 2004 in order to determine the main species of natural enemies and to study their seasonal abundance, distribution and incidence on *S. oleae* populations. Four orchards were sampled fortnightly, and occasional samples were collected in 13 more orchards. Each sample consisted of 16 branches (15 cm long) and 64 leaves. Adult natural enemies were also sampled with an engine-powered vacuum-machine. Six species of parasitoids of *S. oleae* were identified, being the most abundant *Metaphycus flavus* (Howard), *Metaphycus lounsburyi* (Howard) (Hymenoptera: Encyrtidae) and *Coccophagus lycimnia* (Walker) (Hymenoptera: Aphelinidae). Among three species of egg predators, *Scutellista caerulea* (Fonscolombe) (Hymenoptera: Pteromalidae) was the most abundant. *S. caerulea* was often parasitized by the mite *Pyemotes herfsi* (Oudemans) (Prostigmata: Pyemotidae). The entomopathogenic fungus *Verticillium lecanii* (Zimmermann) reached high incidence in one sampling point in autumn. According to their abundance, distribution and impact on *S. oleae* populations, *M. flavus* and *S. caerulea* appeared as the main natural enemies of *S. oleae* in citrus orchards in Valencia (Spain).

**Key words:** *Saissetia oleae*, citrus, natural enemies, parasitoid, predator, entomopathogen

**Introduction**

The black scale, *Saissetia oleae* (Olivier) (Homoptera: Coccidae) is a cosmopolitan and polifagous insect which has been found in more than 60 different plants (Avidov and Harpaz, 1969; Morillo, 1977). This soft scale is considered a pest of citrus, olives and ornamentals in Spain (Limón et al, 1976; Morillo, 1977; Llorens, 1984; Ripollès, 1990; Costa et al, 2001). *S. oleae* causes economic damage in Mediterranean citrus crops since long time ago. However, the damage caused by *S. oleae* is variable due to the action of the natural enemies and the climate (Panis, 1977).

Different programs of biological control of *S. oleae* have been carried out in several countries on citrus and olives with variable success (Mendel et al, 1984; Barbagallo et al, 1992; Lamson and Morse, 1992; Argov and Rössler, 1993; Waterhouse and Sands, 2001). Among the most important natural enemies of soft scale insects imported into Europe or exported from Europe is the family Encyrtidae (Hymenoptera: Chalcidoidea) and mainly the genus *Metaphycus* (Guerrieri and Noyes, 2000). The egg predator *Scutellista caerulea* (Hymenoptera: Pteromalidae) (Fonscolombe) is also considered an important agent of control of the populations of *S. oleae* in Australia and Israel (Mendel et al, 1984; Waterhouse and Sands, 2001).

Several reports about the natural enemies of *S. oleae* have been carried out in the citrus crops from Valencia during the last 30 years. In the area of Castellón, Limón et al. (1976) found the parasitoids *Metaphycus flavus* (Howard), *M. zebratus* Mercet and *Coccophagus scutellaris* Dalman (Hymenoptera: Aphelinidae), and the predators *Scutellista cyanea*
(synonymy of S. caerulea and name used until the middle of the nineties) and Eublemma scitula Rambur (Lepidoptera: Noctuidae). In 1977, Metaphycus helvolus (Compere) and M. bartletti were introduced from France in order to improve the biological control of the larvae of S. oleae (Ripollés, 1986). Panis (1977) found M. helvolus, Coccophagus lycimnia (Walker) (Hymenoptera: Aphelinidae) and C. scutellaris as the main parasitoids of larvae, and M. lounsburyi (Howard) and S. cyanea as the main parasitoids of adults. Llorens (1984) cited the native parasitoids C. lycimnia, C. scutellaris, M. flavus and M. lounsburyi as well as the introduced parasitoid M. helvolus, and the predators S. cyanea, Chilocorus bipustulatus L., Exochomus quadrimaculatus L., Rhizobius lophantae Blais (Coleoptera: Coccinellidae), E. scitula (Lepidoptera: Noctuidae) and the mite Pyemotes ventriculosus New (Prostigmata: Pyemotidae).

In this paper we present the results of a survey of the black scale present in citrus orchards in Valencia (Spain) to determine the main species of natural enemies and to study their seasonal abundance, distribution and incidence on S. oleae populations.

Materials and methods

The survey was conducted between March 2003 and March 2004 in 17 citrus orchards from Valencia (Spain). Four of the orchards were sampled fortnightly. At each site four trees were selected for their high infestation. Occasional samples were also collected in 13 orchards.

On each sampling date, two samples were collected:

**Sample of branches and leaves**

Sixteen twigs (chosen for their high infestation level) with their leaves were collected. The twigs were between one and three years old and 15 cm long. The twigs and four leaves per twig, chosen randomly, were examined under a binocular in order to count the number of the different stages of S. oleae alive, dead or parasitized.

Afterwards, all the twigs and leaves were placed in an emergence plastic box and held at 25 ± 5°C and 65 ± 5% RH (Argov and Rössler, 1993). After 4 weeks the branches and leaves were removed and the insects from the emergence plastic boxes were collected and identified.

**Sample collected with vacuum-machine**

For each sample, 70 blows in the four trees were given with an engine-powered vacuum-machine. In each blow between 20 and 30 leaves were sampled.

The incidence level of natural enemies was assessed as the number of S. oleae alive with presence of natural enemies (parasitoids, predators or entomopathogens) divided by the total number of S. oleae alive.

Results and discussion

**Biological cycle**

The populations of S. oleae showed a similar evolution along the year in the four orchards studied. For this reason, they have been represented together (Fig. 1). The highest population peak was observed at the end of June, due to the emerging of larvae of a new generation. This peak was preceded by a peak of females in May and June. S. oleae population density decreased during the summer, when the first-instar larvae predominated in the population. Second and third-instar larvae developed along autumn and winter.

The number of S. oleae generations per year reported on citrus varies from one to four depending mainly on the country, cultivation area and weather (Bodenheimer 1951; Panis, 1977). According to Ripollés (1990) in citrus from Valencia S. oleae may present only one
generation, two generations or a partial second generation. Panis (1977) found only one
generation in Castellón. Llorens (1984) reported two generations, with larvae emerging in
February-March and August-September. The fact that we had observed almost identical cycle
in the four orchards studied indicate that the number of generations may vary among years,
but not among the orchards in the same year.

Identification of natural enemies
The natural enemies of *S. oleae* collected in this survey are shown in Table 1. Six species of
parasitoids were identified, three of them belonging to the genus *Metaphycus* and two of them
to the genus *Coccophagus*. The most abundant parasitoid was *Metaphycus flavus*, followed by
*Metaphycus lounsburyi* and the facultative autoparasitoid *Coccophagus lycimnia*.

We also found *Metaphycus helvolus*, which is an exotic species introduced in 1976 and
1979 (Ripollés, 1990). *M. helvolus* is thus present and established, but its population level
appear to be very low for unknown reasons, since *M. helvolus* is considered one of the best
larvae parasitoids of *S. oleae* in other countries.

Three egg predators were collected. The most abundant was *Scutellista caerulea*. The
other two predators of eggs found were the Lepidopteran *Eublemma scitula* and a Diptera
Cecidomidae which possibly belongs to genus *Lestodiplosis*. The larvae of this cecidomidae
appeared frequently, though it has not been reported earlier as a natural enemy of *S. oleae*.

The mite *Pyemotes hersfi* (Oudemans) (Prostigmata: Pyemotidae) was found as a
secondary parasite of the egg predators *S. caerulea* and *E. scitula* in this survey.

Abundance of the natural enemies
*M. flavus* was the most abundant parasitoid in the four orchards sampled fortnightly (table 2).
It was also found in twelve of the thirteen orchards sampled occasionally (table 3). The
distribution of *M. lounsburyi* does not seem so uniform, since its population level was low in
Xilxes, whereas the population levels were very high in other orchards as Vinaroz and
Museros II. *C. lycimnia* appeared in most of orchards at low populations levels.
Table 1. Abundance of natural enemies of *S. oleae* observed in different types of samples carried out in 17 citrus orchards of Valencia between March 2003 March 2004 (nd = no determined; * nº hosts with presence of natural enemies).

<table>
<thead>
<tr>
<th>Parasitoids</th>
<th>On host (nº of cases)*</th>
<th>Emergence box (nº insects)</th>
<th>Vacuum-machine (nº insects)</th>
<th>Total (nº insects)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Metaphycus flavus</em></td>
<td>1449</td>
<td>334</td>
<td>1777</td>
<td>3560</td>
</tr>
<tr>
<td><em>Metaphycus lounsburyi</em></td>
<td>142</td>
<td>511</td>
<td>264</td>
<td>917</td>
</tr>
<tr>
<td><em>Coccophagus lycimnia</em></td>
<td>21</td>
<td>34</td>
<td>100</td>
<td>155</td>
</tr>
<tr>
<td><em>Metaphycus helvolus</em></td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td><em>Coccophagus semicircularis</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Microterys nietneri</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Egg predictors</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scutellista caerulea</em></td>
<td>1006</td>
<td>959</td>
<td>403</td>
<td>2368</td>
</tr>
<tr>
<td><em>Lestodiplosis sp.</em></td>
<td>18</td>
<td>48</td>
<td>53</td>
<td>119</td>
</tr>
<tr>
<td><em>Eublemma scitula</em></td>
<td>15</td>
<td>39</td>
<td>50</td>
<td>104</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2*ary parasites</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pyemotes herfsi</em> (on <em>S. caerulea</em>)</td>
<td>279</td>
<td>Nd</td>
<td>4</td>
<td>283</td>
</tr>
<tr>
<td><em>Pyemotes herfsi</em> (on <em>E. scitula</em>)</td>
<td>3</td>
<td>Nd</td>
<td>nd</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathogens</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Verticillium lecanii</em></td>
<td>849</td>
<td>Nd</td>
<td>nd</td>
<td>849</td>
</tr>
</tbody>
</table>

Table 2. Abundance of the main natural enemies of *S. oleae* (summing the three types of observations) in four orchards sampled fortnightly in the area of Valencia between March 2003 and March 2004.

<table>
<thead>
<tr>
<th>Parasitoids</th>
<th>Albal</th>
<th>Moncófar</th>
<th>Museros</th>
<th>Xilxes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Metaphycus flavus</em></td>
<td>1223</td>
<td>790</td>
<td>612</td>
<td>804</td>
</tr>
<tr>
<td><em>Metaphycus lounsburyi</em></td>
<td>125</td>
<td>270</td>
<td>82</td>
<td>6</td>
</tr>
<tr>
<td><em>Coccophagus lycimnia</em></td>
<td>15</td>
<td>13</td>
<td>1</td>
<td>106</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Egg predators</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scutellista caerulea</em></td>
<td>304</td>
<td>294</td>
<td>284</td>
<td>1064</td>
</tr>
<tr>
<td><em>Lestodiplosis sp.</em></td>
<td>57</td>
<td>24</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td><em>Eublemma scitula</em></td>
<td>42</td>
<td>15</td>
<td>21</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2*ary parasites</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pyemotes herfsi</em> (on <em>S. caerulea</em>)</td>
<td>9</td>
<td>10</td>
<td>36</td>
<td>204</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathogens</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Verticillium lecanii</em></td>
<td>78</td>
<td>0</td>
<td>0</td>
<td>231</td>
</tr>
</tbody>
</table>

*S. caerulea* was the most abundant egg predator in all the orchards and the second natural enemy in abundance (except in Xilxes, where it was the most abundant). Moreover it was found in ten of the thirteen orchards sampled occasionally. Overall, *M. flavus* and *S. caerulea* appeared in this study as the most abundant and widely distributed natural enemies of *S. oleae* in citrus from Valencia.
Table 3. Abundance of the main natural enemies of *S. oleae* (summing the three types of observations) in 13 orchards sampled occasionally in the area of Valencia between March 2003 and March 2004.

<table>
<thead>
<tr>
<th>Natural enemies</th>
<th>Stage of <em>S. oleae</em></th>
<th>Incidence level m ± se (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitoids</td>
<td><strong>Metaphycus flavus</strong></td>
<td>L1 0.35 ± 0.15 (38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L2 + L3 9.12 ± 1.63 (78)</td>
</tr>
<tr>
<td></td>
<td><strong>Metaphycus lounsburyi</strong></td>
<td>L2 + L3 0.07 ± 0.02 (75)</td>
</tr>
<tr>
<td></td>
<td><strong>Coccophagus lycimnia</strong></td>
<td>Female 5.52 ± 2.35 (33)</td>
</tr>
<tr>
<td>Egg predators</td>
<td><strong>Scutellista caerulea</strong></td>
<td>Female 21.8 ± 5.29 (37)</td>
</tr>
<tr>
<td></td>
<td>Lestodiplosis sp.</td>
<td>Female 1.1 ± 0.80 (40)</td>
</tr>
<tr>
<td></td>
<td><strong>Eublemma scitula</strong></td>
<td>Female 0.06 ± 0.04 (30)</td>
</tr>
<tr>
<td>2*ary parasites</td>
<td><strong>Pyemotes herfsi</strong> (on <em>S. caerulea</em>)</td>
<td>24 1</td>
</tr>
<tr>
<td>Pathogens</td>
<td><strong>Verticillium lecanii</strong></td>
<td>540 1</td>
</tr>
</tbody>
</table>

**Incidence levels of the main natural enemies of *S. oleae***

*M. flavus* parasitized all the larvae stages of *S. oleae*, although L1 were rarely parasitized (0.35%) (table 4). About 9% of average of the L2 and L3 were parasitized by *M. flavus*, which represents the highest percentage of parasitism found in this study. The percentage reached the maximum at the end of the spring, coinciding with the last larval stages. In that moment 30% of larvae were parasitized by *M. flavus*. In summer, when females and L1 predominated, the percentage of parasitism was very low (1-2%). In autumn, coinciding with L2 and L3, the percentage increased again.

Table 4. Mean incidence level of the main natural enemies of *S. oleae* in four citrus orchards from Valencia sampled between March 2003 and March 2004.

<table>
<thead>
<tr>
<th>Natural enemies</th>
<th>Stage of <em>S. oleae</em></th>
<th>Incidence level m ± se (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitoids</td>
<td><strong>Metaphycus flavus</strong></td>
<td>L1 0.35 ± 0.15 (38)</td>
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<tr>
<td></td>
<td></td>
<td>L2 + L3 9.12 ± 1.63 (78)</td>
</tr>
<tr>
<td></td>
<td><strong>Metaphycus lounsburyi</strong></td>
<td>L2 + L3 0.07 ± 0.02 (75)</td>
</tr>
<tr>
<td></td>
<td><strong>Coccophagus lycimnia</strong></td>
<td>Female 5.52 ± 2.35 (33)</td>
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<td>Lestodiplosis sp.</td>
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</tr>
<tr>
<td></td>
<td><strong>Eublemma scitula</strong></td>
<td>Female 0.06 ± 0.04 (30)</td>
</tr>
<tr>
<td>2*ary parasites</td>
<td><strong>Pyemotes herfsi</strong> (on <em>S. caerulea</em>)</td>
<td>22.91 ± 7.23 (28)</td>
</tr>
</tbody>
</table>

*C. lycimnia* was also found parasitizing the larval stages L2 and L3, but the parasitism was quite low (0.07%). *C. lycimnia* is a facultative autoparasitoid (Walter, 1983); the females are primary endoparasitoids of soft scales, whereas the males are hyperparasites on heterospecific or conspecific female parasitoids, including species of *Metaphycus*. Thus, an abundance of *C.*
**lycimnia** may suggest that hyperparasitism of scales previously parasitized by *M. flavus* reduces the potential of *M. flavus* to suppress the soft scales (Bernal et al., 2001).

The parasitism of *M. lounsburyi* was 5.5%, although this percentage varied according to the areas. In Albal and Museros II, *M. lounsburyi* parasitized almost 10% of the females.

*S. caerulea* was always observed feeding on the eggs of *S. oleae*. The pteromalid was present in almost 22% of the soft scale females, which represented the highest incidence level of this report. But, *S. caerulea* does not eat all the eggs laid by the *S. oleae* female, the percentage of eggs eaten depending on the scale size (Ehler, 1989).

The incidence levels of the egg predator *S. caerulea* and the female parasitoid *M. lounsburyi* were important in July after the peak of the *S. oleae* females, having no incidence the rest of the year. The percentages reached the maximum in the middle of July, when most females contained already the new born larvae (fig 2). However, these levels were very low in June when the number of females still without eggs or larvae was higher.

![Figure 2](image-url)

Figure 2. Comparison of the seasonal evolution of the *S. oleae* females (average number in four orchards) and the incidence level of its natural enemies *Metaphycus lounsburyi* and *Scutellista caerulea*, and the secondary parasite *Pyemotes herfsi*. Standard errors are indicated by vertical bars.

The secondary parasite *P. herfsi* was found feeding on almost 23% of a high *S. caerulea*. This mite reached high levels of incidence mainly at the end of the development of *S. caerulea*. *P. herfsi* may be an important agent in the regulation of *S. caerulea* populations and consequently a negative factor in relation with the biological control of *S. oleae*.

Finally, the presence of the entomopathogen fungus *Verticillium lecanii* caused important decreases of *S. oleae* populations in autumn when the humidity was higher, as it had been observed earlier on our citrus (Tuset, 1992).
It is difficult to assess the real influence of the natural enemies on the population levels of *S. oleae*, since they are affected not only by biotic but also abiotic factors, mainly the climate. During spring and autumn, when most *S. oleae* populations are in the second and third larvae stages, the widespread general presence of *M. flavus* may be responsible for a high part of the observed mortality. The females of *S. oleae*, which appear in summer-time, are heavily attacked by several natural enemies, but normally too late. The effectiveness of the parasitoid *M. lounsburyi* as a regulation factor of *S. oleae* populations seems to be limited because it parasitizes the females of *S. oleae* when they have already started the oviposition. The effectiveness of the egg predator *S. caerulea* could be more important due to the high incidence levels it reaches.

**Conclusion**

Our analysis between March 2003 and March 2004 shows that the life cycle of *S. oleae* in citrus was very similar in the four orchards studied, larvae emerging between June-July. Six species of parasitoids of *S. oleae* were identified, being the most abundants the parasitoid of larvae *M. flavus* (9.12% of parasitism) and the parasitoid of females *M. lounsburyi* (5.5%). Three predators of eggs of *S. oleae* have been identified, *S. caerulea* (22% of incidence), *E. scitula* and a larva of Cecidomidae. The mite *P. herfsi* was found in 23% of *S. caerulea*. Overall, the most abundant and widely distributed natural enemies of *S. oleae* were *M. flavus* and *S. caerulea*.

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Side-effects of insecticides on natural enemies of citrus scale pests in Italy∗§

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Abstract: Laboratory trials were conducted to test the side-effects of 5 insecticides, Etifos® M (chlorpyrifos-methyl), Applaud® (buprofezin), Admiral® (pyriproxyfen), Laser® (spinosad) and Biolid® E. (narrow range mineral oil) on 4 parasitoids of Citrus scales: Aphytis melinus DeBach, Coccophagus semicircularis (Förster), Coccophagus lycimnia Walker (Hymenoptera: Aphelinidae) and Leptomastix dactylopii Howard (Hymenoptera: Encyrtidae). The tests were conducted using a spray Potter Tower following the standard principles accepted by the IOBC/wprs Working group “Pesticides and Beneficial Organisms”. Contact toxicity on adults after 24, 48 and 72h, the effects on their fertility as well as the sex-ratio and the fecundity of the progeny were observed. A test was carried out also spraying Citrus mealybug mummies parasitized by L. dactylopii. Total mortality (100%) of all tested parasitoids due to contact toxicity was observed 24h after the treatment with chlorpyrifos-methyl and spinosad. The mean levels of mortality obtained after 72h on C. semicircularis and L. dactylopii were 76% and 58% respectively after treatments with mineral oil. Buprofezin after 72h caused 95% mortality on C. semicircularis, 100% on C. lycimnia, 42% on L. dactylopii and 76% on A. melinus. The other IGR (pyriproxyfen) caused lower mortality rates (88% on C. lycimnia, 48% on L. dactylopii and 62% on A. melinus). The average number of progeny per single L. dactylopii surviving female was 13.45±4.10 (buprofezin), 13.67±9.61 (mineral oil), 10.83±5.67 (pyriproxyfen) and 15.09±10.35 (untreated control) with no statistically significant differences. The sex ratio of the progeny (M:F) was 0.8:1 (buprofezin), 2.6:1 (mineral oil), 1:1 (pyriproxyfen) and 0:8:1 (untreated control). The surviving A. melinus females produced a mean number of progeny of 3.75±0.35 (buprofezin), 36.04±4.20 (pyriproxyfen) and 36.44±2.42 (untreated control) with the first value significantly different from the others. The sex ratio of the progeny (M:F) was 0.8:1 (buprofezin), 0.6:1 (pyriproxyfen) and 0.6:1 (untreated control). The surviving C. lycimnia females (pyriproxyfen) didn't produce any progeny while 39.98±7.59 mean progeny were produced by the untreated control. Semi-field and field trials are needed to better define the compatibility of the tested pesticides with IPM strategies in Citrus groves in Italy.

Key words: insecticides, side-effects, Hymenoptera parasitoids, integrated pest control, Citrus.

Introduction

The correct evaluation of the side-effects of agrochemicals on beneficials is necessary for the application of modern Integrated Pest Management (IPM) strategies. In Italian citrus production area (130,000 hectares) Integrated Pest Management is widely adopted. Most of the 90 species of animal organisms reported on Citrus in Italy are Homopterous insects (Barbagallo, 2000; Barbagallo et al., 1994; Longo, 1994). Among these only 15 are considered of primary economic interest and less than 10 are "Key pests", such as the scales Planococcus citri

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§ The Authors equally contributed to the work.
(Risso), *Aonidiella aurantii* (Maskell) and *Aspidiotus nerii* Bouché. Soft scales are secondary pests but they can be classified as potential pests because, although they are widely under natural control by a complex of beneficials, the irrational use of insecticides could cause a disruption in the ecological balance making some of them become harmful.

On the complex IPM on Citrus gives satisfactory results (Barbagallo *et al.*, 1994) and it is largely applied also with the support of Official Extension Services (Siscaro & Mazzeo, 2003). Up to now in Italy 41 insecticides are registered on citrus: 11 Phosphorates, 9 Pyrethroids, 5 IGR (JHA-MAC included), 3 Carbamates, 3 Botanical insecticides, 3 Microbiological insecticides, 2 Neonicotinoids, 2 Inorganic insecticides (mineral oil – polysulfur), 1 Organochlorine, 2 Others (anti-feeding, Abamectin). Fourteen of these compounds are acaricides as well (WinBDF, 2005). Laboratory trials were conducted to test the effects of 5 insecticides on 4 parasitoids of Citrus scales: *Aphytis melinus* DeBach, *Coccophagus lycimnia* Walker, *C. semicircularis* (Förster) (Hymenoptera: Aphelinidae) and *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae).

The pesticides have been selected with the following criteria: one insecticide frequently used in Citrus pest protection in organic and IPM (narrow range mineral oil); two insecticides generally used in case of high infestation (chlorpyrifos-methyl in conventional management and buprofezin in IPM); one IGR recently registered in Italy (pyriproxyfen) and the last one registered on Citrus in other Countries and expected to be registered in Italy as well (spinosad).

The parasitoids tested were selected on the basis of their importance in biological control in Citrus both from a commercial and ecological point of view. *A. melinus* is considered the main biotic mortality factor of *A. aurantii* in Italy (Lizzio *et al.*, 1998) and is currently used in biological control programs by means of augmentative releases (Mazzeo *et al.*, 2004; Tumminelli *et al.*, 2000); *C. semicircularis* and *C. lycimnia* are the most common parasitoids of soft scales of economic importance all over the Mediterranean basin; *L. dactylopii* is the most widely used parasitoid of the Citrus mealybug by means of inoculative releases.

The aim of the work is to acquire preliminary laboratory data on the side-effects of some agrochemicals as starting point for a sequential multi-tier testing that is considered the most helpful tool to select suitable pesticides for IPM (Hoy, 2000; Vogt, 2000a; Ware & Hattingh, 2000).

### Material and methods

The tests were conducted following the standard principles accepted by the IOBC/wprs Working group “Pesticides and Beneficial Organisms”. Contact toxicity on adult parasitoids after 24, 48 and 72h, as well as the effects on their parasitization activity and the sex-ratio of the progeny were observed. A test was carried out also spraying mummies of *P. citri* parasitized by *L. dactylopii* in order to evaluate the effects on the emergence rate and the potential fecundity of the progeny.

#### Insect rearing

The Citrus mealybug was reared in the laboratory from specimens collected in the field. It was maintained on sprouted potatoes of the variety ‘Spunta’ in plastic boxes (37 W × 50 L × 25 H cm) with openings covered with net to ensure proper ventilation. They were kept in a rearing room at 26±1°C, 65-80% RH and 14L:10D. The parasitoid *L. dactylopii* was reared on *P. citri*, in plastic boxes identical to the ones used for the host but placed in a rearing room under continuous light. For the experiments, mummified mealybugs were collected from the mass cultures and put into glass vials (length 5.0 cm, diameter 2.5 cm), which were then kept in an incubator at 26±1°C under continuous light. They were checked twice a day at the same
time, for parasitoid emergence. Newly emerged *L. dactylopii* females were individually isolated with a male 1–3 days old and then observed until mating.

Adults of *A. melinus* were obtained from the Regional Phytosanitary Service’s insectary. The parasitoid is maintained on *A. nerii* fed on Butternut squash following a well known technique, with subsequent minor modifications (Raciti et al., 2003).

*C. semicircularis* was reared on *Pulvinariella mesembryanthemi* (Vallot) fed on its natural host *Carpobrotus edulis* (L.) N.E. Br. (iceplant) which was propagated planting cuttings 30 cm long in 1 l pots in all purpose planting mix. Iceplants were grown until they rooted and produced new shoots in a greenhouse under natural photoperiod. They were then infested with the soft scale by putting healthy plants in contact with previously infested ones, in order to allow crawlers to spread out. The plants were used in the parasitoid rearing when most of the scales were third instar nymphs. The Aphelinid was reared in cubic cages (40 W × 40 L × 40 H cm) constructed of 1.6-cm outer diameter PVC tubing and fine polyester mesh covers. Temperatures were maintained at 26±2.5°C, relative humidity was kept around 60% inside the cages and a light regimen of 16L:8D was provided, using fluorescent lamps. The iceplant leaves were collected after 15 days and were placed inside emergence boxes until the adult parasitoids emerged (starting on average on day 21).

*C. lycimnia* was reared on *Coccus hesperidum* L. fed on excised leaves of *Yucca elephantipes* Regel maintained bouquet-style in vials. The rearing of the phytophagous host was carried out inside clear plastic containers (37 W × 50 L × 25 H cm). Ventilation was provided by holes (20 × 20 cm) covered with fine polyester mesh present on each side of the cage. Cages were held at 25±3°C in a dark room to allow the crawlers to settle on new leaves. Part of the infested leaves were then transferred to the parasitoid rearing facilities where temperatures were maintained at 26±2.5°C, relative humidity was kept around 60% and a light regimen of 16L:8D was provided. The same kind of cages used to rear *C. semicircularis* was adopted for *C. lycimnia*. Two weeks after the release of the adult parasitoids, the *Yucca* leaves were placed inside emergence boxes where the adults of the new generation start emerging on average after 7 days.

**Chemicals**

Five pesticides were tested for their side-effects on *L. dactylopii*, *A. melinus*, *C. lycimnia* and *C. semicircularis* (Table 1). The control was sprayed with tap water.

**Toxicity assays**

The tests were conducted in the laboratory under constant temperature conditions (24±2°C), using a Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd.) with constant pressure, operated by compressed air (Hassan et al., 1994).

*C. semicircularis* was tested only for contact toxicity while on the other species the effects on fertility (number of emerged adults) were also evaluated. Biolid® E. was tested only on *L. dactylopii* and *C. semicircularis*; Admiral® was not tested on *C. semicircularis* while the effects of the other insecticides were evaluated on the 4 species (Table 1).

The exposure cage consisted of 6 glass plates, the 4 lateral ones of 9 × 8 cm, the top and bottom of 9.5 × 9.5 cm, 0.5 cm thick (Viggiani & Tranfaglia, 1978). The plates were joined externally with transparent adhesive tape to form a cube and three of the 4 lateral glass plates had a central hole (diameter 0.5 cm) to allow air-flow and to provide the specimens with food by means of a cotton dispenser imbibed with a sugar and protein solution (Protonecctar™, Lega Italy).

The direct toxicity was tested by exposing the adult parasitoids to a fresh dry pesticide film applied on glass plates at the highest recommended field dose in order to ensure a uniform deposit ranging between 1.5 and 1.8 mg cm⁻². The treated glass plates were kept at room temperature for 2 hours to complete drying and the cages were then assembled. Ten
adults (5 males and 5 females) of each tested species were used in the experiments and 5 replicates per test product were performed. The cages were held at 26±2°C and around 60% RH; a light regimen of 16L:8D was provided using fluorescent lamps. Mortality of adults after 24, 48 and 72h was recorded.

The surviving females were then offered 100 hosts each for *A. melinus* and *C. lycimnia* and 50 hosts for *L. dactylopii* to verify the effects on their fertility and the sex-ratio of the progeny. *A. melinus* was provided young *A. nerii* females cultured on lemon fruits, *C. lycimnia* second and third *C. hesperidum* nymphs while *L. dactylopii* was provided *P. citri* third instar nymphs. The parasitoids were left with the host until death, except for *L. dactylopii* that was left in contact with the host only for 24h. The number of emerged adults was then counted and the sexes were separated.

A preliminary test was carried out also spraying *L. dactylopii* at the pupal stage inside its hosts with Applaud®. Two hundred *P. citri* mummies were selected from the rearing cages 4-5 days before the adults emergence and then sprayed under the Potter Tower using the same concentration adopted for the toxicity test on adults. The number of emerged adults was recorded and then the females were dissected in order to evaluate the potential fecundity, counting the number of mature ovarian eggs. Five replicates were performed.

Table 1. Pesticides and parasitoids tested.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Formulation</th>
<th>Active ingredient (a.i.)</th>
<th>Company</th>
<th>% a.i. (g/l)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admiral®</td>
<td>10EC</td>
<td>pyriproxyfen</td>
<td>Siapa</td>
<td>10.86 (100)</td>
<td><em>A. melinus</em>&lt;br&gt;<em>C. lycimnia</em>&lt;br&gt;<em>L. dactylopii</em></td>
</tr>
<tr>
<td>Applaud®</td>
<td>40SC</td>
<td>buprofezin</td>
<td>Sipcam</td>
<td>40.50 (430)</td>
<td><em>A. melinus</em>&lt;br&gt;<em>C. lycimnia</em>&lt;br&gt;<em>C. semicircularis</em>&lt;br&gt;<em>L. dactylopii</em></td>
</tr>
<tr>
<td>Biolid® E.</td>
<td>EC</td>
<td>mineral oil</td>
<td>Sipcam</td>
<td>80.00</td>
<td><em>C. semicircularis</em>&lt;br&gt;<em>L. dactylopii</em></td>
</tr>
<tr>
<td>Etifos® M</td>
<td>EC</td>
<td>chlorpyriphos-methyl</td>
<td>Du Pont</td>
<td>22.10 (225)</td>
<td><em>A. melinus</em>&lt;br&gt;<em>C. lycimnia</em>&lt;br&gt;<em>C. semicircularis</em>&lt;br&gt;<em>L. dactylopii</em></td>
</tr>
<tr>
<td>Laser®</td>
<td>SC</td>
<td>spinosad</td>
<td>Dow Agrosciences</td>
<td>44.20 (280)</td>
<td><em>A. melinus</em>&lt;br&gt;<em>C. lycimnia</em>&lt;br&gt;<em>C. semicircularis</em>&lt;br&gt;<em>L. dactylopii</em></td>
</tr>
</tbody>
</table>

Data analysis

The data related to the adult mortality as well as to the emergence rate from treated *P. citri* mummies were subjected to a one-way ANOVA and the means were separated by applying the Least Significant Difference (LSD) test. All the percentages were arcsin square root transformed before being analyzed. The levels of mortality were adjusted for control mortality using Abbott’s formula (Abbott, 1925).

One-way ANOVA was also performed to analyze the mean progeny produced per single
survived female. If significance in the analysis was detected, the means were compared using the LSD test.

The capacity of parasitism per single parasitoid female (number of emerged adults/female) and the reduction in this capacity compared with the control (RP%) was used to evaluate the effect of the pesticides on the parasitic activity of the treated females. Pesticides were then classified into four categories, according to the reduction in parasitization: 1, harmless (< 30%); 2, slightly harmful (30-79%); 3, moderately harmful (80-99%); 4, harmful (>99%) (Hassan et al., 1994).

Results and discussion

Contact toxicity tests
The data on mortality of the adults showed that the chemicals used variously influenced the survival of the parasitoids. Laser® and Etifos® M caused 100% mortality of the four species tested (Figs. 1, 2). A. melinus adults exposed to Admiral® showed a mean level of mortality of 16% after 24 h, 40% after 48 h and 62% after 72 h. Applaud® caused higher rates of mortality after 48 and 72 h, respectively 54 and 76% (Fig. 1a). The one-way ANOVA performed on these data revealed statistically significant differences between the treatments (F= 101.99; d.f.= 4; P= 0.00 after 24 h; F= 10.2131; d.f.= 2; P= 0.002 after 48 h; F= 11.64; d.f.= 2; P= 0.001 after 72 h). The results of the LSD test are reported in Figure 1a.

L. dactylopii adults exposed to Admiral® showed a mean level of mortality of 24% after 24 h, 30% after 48 h and 48% after 72 h. When the parasitoids were treated with Applaud® the mean level of mortality was 18% after 24 h, 24% after 48 h and 42% after 72 h. The data recorded after the treatment with the mineral oil, showed that the highest level of mortality was reached after 72 h (58%), while after 24 and 48 h the mean level of mortality was of 44% and 54% respectively (Fig. 1b). The one-way ANOVA performed on these data revealed statistically significant differences between the treatments (F= 50.08; d.f.= 5; P= 0.00 after 24h; F= 6.81; d.f.= 3; P= 0.003 after 48 h; F= 15.26; d.f.= 3; P= 0.00 after 72 h). The results of the LSD test are reported in Figure 1b.

The preliminary test carried out spraying parasitized mummies parasitized by L. dactylopii with Applaud® didn't show any significant influence of the treatment on the emergence rate (F= 1.93; d.f.= 1; P= 0.17), which was 63% in the treated groups and 68% in the untreated control; the mean number of eggs per female was 18.8 and 20.7 respectively.

C. lycimnia adults exposed to the same chemicals showed overall higher rates of mortality. In particular after having been in contact for 24 h with the surfaces treated with Admiral® 74% of the specimens died. After 48 h the dead adults were 78% and the mortality rate increased to 88% after 72 h. Applaud® after having caused a lower initial mortality (20% after 24 h and 30% after 48 h) took to death all the remaining parasitoids (Fig. 2a). The one-way ANOVA performed on these data revealed a statistically significant effect of the treatment (F= 92.21; d.f.= 4; P= 0.00 after 24h; F= 29.55; d.f.= 2; P= 0.00023 after 48 h; F= 28.35; d.f.= 2; P= 0.00028 after 72 h). The results of the LSD test are reported in Figure 2a.

The congeneric C. semicircularis responded similarly to the same insecticides. Total mortality of the specimens (100%) was recorded keeping them in contact for 24 h with Laser® and Etifos® M. Applaud® caused a mortality rate of 68.33% after 24 and 48 h and 95% after 72 h. The effect of the mineral oil (Biolid® E.) was slightly weaker than the other pesticides: 61.90% of the adult parasitoids died after 24 h, this rate didn’t increase after 48 h and it finally reached 76.19% after 72 h (Fig. 2b). The statistical analysis showed significant differences between the treatments (F= 24.14; d.f.= 4; P= 0.0018 after 24h; F= 7.71; d.f.= 2;
Effects on fertility

The surviving \textit{A. melinus} females treated with Admiral\textsuperscript{®} showed a parasitic activity almost identical to the one performed by the untreated females. The mean number of emerged adults per single female was 36.44 in the control and 36.04 in the Admiral\textsuperscript{®} treatment. The negative effect on the parasitoid fertility was instead very strong after placing the adults in contact with surfaces treated with Applaud\textsuperscript{®}. This insect growth regulator (chitin inhibitor) caused a significant reduction in the parasitic activity with 3.75 mean progeny per single female. The results of the one-way ANOVA revealed a significant effect of the treatment on the parasitoid fertility \((F= 90.476; \text{d.f.}= 2; P= 0.000003)\) and the following LSD test showed that the untreated control and the adults treated with Admiral\textsuperscript{®} were statistically different from the other treatments. None of the tested insecticides showed a significant effect on the sex-ratio of the progeny (Table 2).

The effects of all tested insecticides on \textit{C. lycimnia} were much more intense, considering that the only product which didn’t take all the specimens to death was Admiral\textsuperscript{®}. Nevertheless, it caused a total disruption of the Aphelinid parasitic activity with no progeny produced by the few surviving females. Therefore all the tested insecticides, on \textit{C. lycimnia}, could be classified as “harmful” due to a reduction of more than 99\% in the parasitic activity as compared to the untreated control which produced 39.98 progeny per female (Table 2).

On \textit{L. dactylopii} none of the tested compounds had a significant effect on the mean progeny produced per female \((F= 0.292; \text{d.f.}= 3; P= 0.83)\). Nevertheless the insecticides significantly affected the sex-ratio of the adults emerging from parasitized Citrus mealybugs \((F= 3.66; \text{d.f.}= 3; P= 0.03)\); in particular the LSD test revealed that in the Biolid\textsuperscript{®} E. treatment the sex-ratio significantly deviated (Table 2).

Conclusive remarks

The overall high mortality (>50\%) caused by all the insecticides tested on \textit{A. melinus} and \textit{Coccophagus} spp., with Etifos\textsuperscript{®} M and Laser\textsuperscript{®} which took all the specimens to death, needs to be highlighted. No data were up to know available on pesticide toxicity on parasitoids belonging to the genus \textit{Coccophagus}, important natural biocontrol agents of several scale pests. The mortality rates as well as the RP values recorded in these trials underline the particular harmfulness of all the insecticides tested both on \textit{C. semicircularis} and \textit{C. lycimnia}. The disruption of their natural control activity could lead to outbreaks of pests normally considered secondary in citrus groves.

The results obtained exposing \textit{A. melinus} adults to the tested compounds partly confirmed literature data (Michaud, 2003; Morse & Bellows, 1986) but gave useful further information on the side-effects of pesticides commonly used in citrus IPM. In particular, Admiral\textsuperscript{®} was classified as “harmless” while Applaud\textsuperscript{®} had a strong negative effect on the fertility of the parasitoid and was therefore classified as “harmful”.

\textit{L. dactylopii} instead showed a lower sensitivity to the same pesticides, confirming the results obtained in other similar tests conducted in Europe (Jacas Miret & Garcia-Mari, 2001; Vogt, 2000b).
Table 2. The side-effects of pesticides on adult parasitoids, % mortality, reduction in parasitism compared to the control (RP%) and sex-ratio of the progeny produced by the surviving females.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (ml/hl)</th>
<th>Mortality 72h after treatment (% ± SD)</th>
<th>Progeny/female (mean ± SD)</th>
<th>RP%</th>
<th>Progeny sex-ratio (M:F)</th>
<th>Class*</th>
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</thead>
<tbody>
<tr>
<td><strong>Aphytis melinus</strong></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Applaud®</td>
<td>80</td>
<td>71.81 ± 33.60</td>
<td>3.75 ± 0.35 a</td>
<td>89.71</td>
<td>0.8:1 a</td>
<td>3</td>
</tr>
<tr>
<td>Admiral®</td>
<td>50</td>
<td>54.38 ± 16.83</td>
<td>36.04 ± 4.20 a</td>
<td>1.10</td>
<td>0.6:1 a</td>
<td>1</td>
</tr>
<tr>
<td>Etifos® M</td>
<td>250</td>
<td>100.00</td>
<td>–</td>
<td>100.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Laser®</td>
<td>30</td>
<td>100.00</td>
<td>–</td>
<td>100.00</td>
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<td>–</td>
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<tr>
<td><strong>Leptomastix dactylopii</strong></td>
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<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>15.09 ± 10.35 a</td>
<td>–</td>
<td>0.8:1 a</td>
<td>–</td>
</tr>
<tr>
<td>Applaud®</td>
<td>80</td>
<td>35.56 ± 16.43</td>
<td>13.45 ± 4.10 a</td>
<td>10.90</td>
<td>0.8:1 a</td>
<td>1</td>
</tr>
<tr>
<td>Biolid® E.</td>
<td>1000</td>
<td>53.33 ± 4.47</td>
<td>13.67 ± 9.61 a</td>
<td>9.46</td>
<td>2.6:1 b</td>
<td>1</td>
</tr>
<tr>
<td>Admiral®</td>
<td>80</td>
<td>42.22 ± 26.83</td>
<td>10.83 ± 5.67 a</td>
<td>28.23</td>
<td>1:1 a</td>
<td>1</td>
</tr>
<tr>
<td>Etifos® M</td>
<td>250</td>
<td>100.00</td>
<td>–</td>
<td>100.00</td>
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<td>–</td>
</tr>
<tr>
<td>Laser®</td>
<td>30</td>
<td>100.00</td>
<td>–</td>
<td>100.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Coccophagus lycimnia</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>39.98 ± 7.59 a</td>
<td>–</td>
<td>0.3:1</td>
<td>–</td>
</tr>
<tr>
<td>Applaud®</td>
<td>80</td>
<td>100.00</td>
<td>–</td>
<td>100.00</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Admiral®</td>
<td>50</td>
<td>82.83 ± 15.66</td>
<td>0.00 b</td>
<td>100.00</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Etifos® M</td>
<td>250</td>
<td>100.00</td>
<td>–</td>
<td>100.00</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Laser®</td>
<td>30</td>
<td>100.00</td>
<td>–</td>
<td>100.00</td>
<td>–</td>
<td>4</td>
</tr>
</tbody>
</table>

% Mortality is corrected using Abbott’s formula.
RP is the reduction in the parasitism rate compared with the control.
* Evaluation categories of initial toxicity, IOBC classification: 1, harmless (<30%); 2, slightly harmful (30-79%); 3, moderately harmful (80-99%); 4, harmful (>99%).
Means followed by the same letter were not significantly different at P< 0.05 (LSD test).

In spite of the fact that, in our tested conditions, Laser® and Etifos® M were consistently the most toxic insecticides to all the parasitoids tested, several authors report how the methods used in evaluating the mortality caused by the pesticides on beneficial arthropods can considerably affect the results, with subsequent variable indication on how those pesticides would fit into IPM programs. The data obtained in our laboratory tests, which represent the "worst case scenario", need to be confirmed by semi-field and field trials, considering that multiple testing methods should be used in evaluating pesticide effects on beneficial arthropods (Banken & Stark, 1998; Hoy, 2000; Vogt, 2000a; Ware & Hattingh, 2000; Studebaker & Kring, 2003).

Therefore, at the moment no definitive elements are available to define the compatibility of the tested pesticides with IPM strategies in Citrus groves in Italy.


Figure 1. Mean levels of mortality (% ± SD) of *A. melinus* (a) and *L. dactylopii* (b) adults caused by the different chemicals 24, 48 and 72 hours after the treatment. Columns bearing the same letter in the same time interval were not significantly different in a one-way ANOVA followed by the LSD test (P<0.05).
Figure 2. Mean levels of mortality (% ± SD) of *C. lycimnia* (a) and *C. semicircularis* (b) adults caused by the different chemicals 24, 48 and 72 hours after the treatment. Columns bearing the same letter in the same time interval were not significantly different in a one-way ANOVA followed by the LSD test (P<0.05).
The recruitment of native parasitoid species by an invading herbivore: the case of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Eastern Spain

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**Abstract:** The parasitoid assemblage associated with the invading herbivore *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) was studied in Eastern Spain over a seven years period just after its introduction (1995-2001). The recruitment of native parasitoids by the invading leafminer followed the expected patterns of hosts as invaders: lower species richness, generalized habits, idiobiont strategy and low to moderate rates of parasitism. Ten primary parasitoid species were reared from the citrus leafminer, indicating a rapid accumulation of native parasitoids on the invading host. They belonged to the families Eulophidae (nine species) and Pteromalidae (one species). From 1997 onwards, two main species, *Pnigalio* sp. and *Cirrospilus brevis* Zhu, LaSalle and Huang, represented more than 90% of the parasitoids identified. The spatial and temporal dynamics of native parasitoids, some host-parasitoid interactions and other life aspects of parasitoids are also reported. The study of native parasitoids of the leaf-mining niche showed that parasitoids considered minor or secondary on native hosts, such as *Pnigalio* sp., can play a major role as parasitoids of introduced herbivores.

**Key words.** *Phyllocnistis citrella*, *Pnigalio* sp., *Cirrospilus brevis*, parasitism, native parasitoids, species recruitment.

**Introduction**

The citrus leafminer is a good example of alien herbivore that becomes an important pest in invading areas. This microlepidoptera is a pest native of Eastern and Southern Asia that invaded most citrus-growing regions in America and the Mediterranean basin in the early nineties. In the Mediterranean basin, it reached very high population levels, and produced great damage in new flushes, causing problems particularly in young trees, nurseries, and overgraftings (Garcia-Marí *et al.*, 2002).

In Spain, the citrus leafminer was first detected in 1993 and was fully established in all the citrus areas by 1995. In 1996, a classical biological control targeting the citrus leafminer was initiated. Five species of exotic parasitoids were released in the following years (Vercher *et al.*, 2000), but until 1999 only native species were found parasitizing the new host. From 2000 onwards the exotic parasitoid *Citrostichus phyllocnistoides* (Narayanan) became established and dispersed, reaching high parasitism rates and lowering citrus leafminer population (Garcia-Marí *et al.*, 2003). Simultaneously, a long term study was developed in the Spanish Mediterranean area to determine the structure of the community of native parasitoids associated with the new pest. In this paper, we present and analyze the development of the parasitoid assemblage associated with the invading pest over a 7-yr period. To better understand parasitoid recruitment into the orchards, the study included sampling of the parasitoid assemblages of the leafmining guild outside of the orchards.
Materials and methods

Sampling Sites
Samples were made in full production commercial citrus orchards selected within an area of 60 km around the city of Valencia. Samples came from a total of 105 different citrus orchards, each orchard being sampled between one and six times a year. In total, 974 samples were collected between 1995 and 1999. In 2000 and 2001, 10 orchards were sampled every two weeks throughout the citrus growing season, representing a total of 389 samples.

Level of Parasitism and Parasitoid Assemblage Associated with P. citrella
Samples from citrus orchards consisted of 100-150 developed new shoots containing different development stages of the citrus leafminer. One part of each sample was used to estimate the level of parasitism. 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 were isolated in glass vials, placed in climatic chamber (25 ± 2 °C, 50-60 % RH and LD 16: 8 h) and allowed to develop to adult stage for identification. The rest of the sample was enclosed in 3-litter glass jars and kept into the climatic chamber. After 30 days the emerged adult parasitoids were collected and identified. In the samples collected in 2000 and 2001, the parasitism was not estimated, all the shoots were enclosed in the 3-litter glass jar and emerged parasitoids were counted and identified.

Diversity Index
Two measurements of species diversity widely used in ecological studies were calculated to describe the species recruitment all over the 7-year study period, the Simpson Index and the Shannon-Wiener Diversity Index (Krebs, 1999).

Abundance of Parasitoids in Other Leafminers
Herbaceous and woody plants attacked by leafminers and located in the proximities of the target citrus orchards were sampled through the years 1996, 1997 and 1998. Altogether, 145 samples were made. Leaves attacked by leafminers were collected and emerged parasitoids were identified.

Identification of Parasitoids
P. citrella parasitoids were identified using the key published by Schaufl et al. (1998). Other leafminer parasitoids were identified using the keys of Peck et al. (1964), Askew (1968) and Goulet and Huber (1993). Identifications were 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 and discussion

Parasitoid Assemblage Associated with P. citrella
In all the study period (1995-2001), 11,587 adult native parasitoids were reared and identified. They belonged to two families, Eulophidae (nine species) and Pteromalidae (one species). According to these results, the parasitoid assemblage associated with the citrus leafminer can be divided into three functional groups (Fig. 1): major species, that are the more abundant and have high effect on the parasitism rates, secondary species, responsible for lower rates of parasitism and maybe using P. citrella facultatively when it is very abundant or other hosts are rare, and minor species which attack P. citrella seldom and incidentally. The two major species, Pnigalio sp. and Cirrospilus brevis Zhu, LaSalle & Huang, represented more than 90% of the parasitoids identified. Secondary species, representing in all 7% of the parasitoids, were Sympiesis gregori Boucek, Cirrospilus pictus (Nees), C. vittatus Walker and
Ratzeburgiola cristata (Ratzeburg). Finally, minor species included Neochrysocharis formosa (Westwood), Chrysocharis pentheus (Walker), Baryscapus sp. and Pteromalus sp.

![Graph showing percent abundance of parasitoids](image)

Figure 1. Relative abundance of native parasitoids of *P. citrella*. Results of samplings made in citrus orchards in the Eastern-Spain region from 1995 to 2001.

The relative abundance of parasitoids changed between 1995 and 1999. *Pnigalio* sp. was the first species to adapt to the new host, establishing quickly as the main parasitoid in all the orchards in 1995. In contrast, *C. brevis* required 3 yr to become one of the two major parasitoids of the citrus leafminer. In 1995 *Pnigalio* sp. was the prevalent species and *Cirrospilus brevis* represented only 3% of the parasitoids. *C. brevis* increased considerably in 1996 and from 1997 to 1999 both species, *Pnigalio* sp. and *C. brevis*, coexisted in the same orchards and were the predominant parasitoids in most samples.

Eight of the ten species found in our study represented less than 10% of the total native parasitoid species reared from citrus leafminer (Fig 1). Cornell and Hawkins (1993) indicated that when invading hosts reach high densities in the region of introduction, minor species of parasitoids may represent incidental attacks by non-adapted native parasitoids simply because the new host is abundant. Such incidental attacks would not occur at lower, more normal population densities of the new host. This is what apparently occurred in Spain when the exotic parasitoid *C. phyllocnistoides* was introduced, becoming widespread and lowering citrus leafminer populations from the year 2000 onwards (Garcia-Marí et al. 2003). In these circumstances only the two major species of native parasitoids (*Pnigalio* sp. and *C. brevis*) and a few secondary species remained attacking the citrus leafminer.

The results show as process of parasitoid recruitment in Spain was very fast. In 1995, one year after the arrival of the citrus leafminer to Eastern Spain, five species of parasitoids were already found on the invading host. From 1996 onwards 10 species were identified. Though no statistical analysis was conducted, the results show that the Diversity and Dominance Index rose from 1995 to 1999 and declined sharply after *C. phyllocnistoides* establishment (2000 and 2001) due to the great reduction in the presence and number of native parasitoids (Table 1).
Table 1. Number of species and diversity indices for parasitoid assemblage associated with *P. citrella* from 1995 to 2001.

<table>
<thead>
<tr>
<th>Year</th>
<th>Species richness</th>
<th>Simpson dominance</th>
<th>Shannon diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>5</td>
<td>1.24</td>
<td>0.47</td>
</tr>
<tr>
<td>1996</td>
<td>10</td>
<td>1.93</td>
<td>0.97</td>
</tr>
<tr>
<td>1997</td>
<td>10</td>
<td>1.91</td>
<td>0.83</td>
</tr>
<tr>
<td>1998</td>
<td>9</td>
<td>2.28</td>
<td>1.03</td>
</tr>
<tr>
<td>1999</td>
<td>8</td>
<td>3.41</td>
<td>1.35</td>
</tr>
<tr>
<td>2000</td>
<td>8</td>
<td>1.27</td>
<td>0.47</td>
</tr>
<tr>
<td>2001</td>
<td>5</td>
<td>1.02</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Abundance of Parasitoids in Other Leafminers**

The native parasitoid species that act on invading herbivores are likely to be those that attack similar hosts with similar ecology (Askew and Shaw 1986, Godfray 1994). The results of our analysis seem to follow this trend (Table 2). Two of the four main parasitoids of Lepidopteran leaf-mining insects on trees (*Cirrospilus brevis* and *C. vittatus*) were common on the citrus leafminer, whereas the most abundant parasitoids attacking Dipteran leafminers of herbaceous plants (*Diglyphus minoes* (Walker) and *Chrysocharis pubicornis* (Zetterstedt)) never attacked the citrus leafminer or did it only in very low numbers (*Chrysocharis pentheus*). The fact that *Pnigalio* sp., considered as secondary parasitoids on native tree leafminers, was the first to attack the invading host and became its major parasitoid is also remarkable. These results document the benefits of preserving biodiversity (Lasalle 1993), stressing how native fauna has potential value as initial suppliers of biological control agents for introduced herbivores, and can also serve as a seasonal reservoir of natural enemies (Massa et al. 2001, Rizzo 2003).

Table 2. Relative abundance of hymenopterous parasitoids of leafminers collected in herbaceous and woody plants in the vicinity of citrus orchards. Result of 145 samplings made in the Eastern-Spain citrus region between 1996 and 1998.

<table>
<thead>
<tr>
<th>Parasitoid species</th>
<th>Percent parasitoids</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Diglyphus minoes</em></td>
<td>44</td>
<td>-</td>
</tr>
<tr>
<td><em>Chrysocharis pentheus</em></td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td><em>Chrysocharis pubicornis</em></td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td><em>Cirrospilus brevis</em></td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td><em>Cirrospilus variegatus</em> (Massi)</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td><em>Cirrospilus vittatus</em></td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td><em>Neochrysocharis formosa</em></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><em>Pnigalio</em> sp.</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Other hymenoptera</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>365</td>
<td>185</td>
</tr>
</tbody>
</table>
**Host Stage Preference of Parasitoids**

Parasitoid incidence in all susceptible stages of *P. citrella* was studied from a total of 5,508 parasitoids reared individually to adult stage. All species developed mostly from third and fourth instars of *P. citrella*, with apparently two groups, three species which preferred fourth instars (*Pnigalio* sp., *Sympiesis gregori* and *Ratzeburgiola cristata*) and three *Cirrospilus* species (*C. brevis*, *C. vittatus* and *C. pictus*) which developed mainly from third instars.

**Level of Parasitism**

A total of 93,846 live immature stages of the citrus leafminer were observed between 1995 and 1999, of which 21,460 (22.9%) were found to be parasitized. The annual mean parasitism rate, considering all susceptible development stages of *P. citrella*, was similar from 1995 to 1999, with no significant differences between years (*F* = 0.56; df = 4, 715; *P* = 0.689), and with a mean value for the 5 yr of 22.6 ± 0.6 (n = 720). During these years the leafminer population was high and stable (Garcia-Marí et al. 2003).

Important differences in parasitism levels were found between the two main native parasitoids, *C. brevis* and *Pnigalio* sp. The mean percentage of parasitism for third and fourth instars of the citrus leafminer in samples with *C. brevis* as predominant parasitoid was 59%, compared with only 36% in samples where *Pnigalio* sp. predominated. These differences are due to the much higher parasitism produced on third instars by *C. brevis*, whereas fourth instars of the citrus leafminer were similarly attacked by the two parasitoids.

**Host-feeding**

Host feeding incidence varied with the development stage of *P. citrella*. The percentage of feeding punctures was higher in second and third instars (12% and 13% respectively) compared with fourth instars (2%). This is not related with differences in host feeding activity between parasitoid species, as host feeding rates were similar for the two major parasitoids (14.0 ± 3.0% (n=18) for samples where *Pnigalio* sp. was the predominant species, 12.9 ± 1.7% (n=45) for samples where *C. brevis* was the predominant species).

In summary, after the invasion of citrus leafminer in Spain, native parasitoids moved into the new host, following the expected patterns of hosts as invaders such as generalized habits, idiobiont strategy and low to moderate rates of parasitism (Cornell and Hawkins, 1993). The high population level reached by the new pest, associated with a negative density dependent response of the parasitoids at these high population levels (Vercher et al, 2005) suggests that the parasitoid assemblage played only a limited role in regulating the population of the new host. The study of native parasitoids of the leaf-mining niche showed that parasitoids considered minor or secondary on native hosts, such as *Pnigalio* sp., can play a major role as parasitoids of introduced herbivores.

**Acknowledgments**

We thank C. Alfaro, A. Alonso, V. Bueno, D. Castrillón, M. Villalba, C. Granda, C. Moreno, J.M. Rodríguez, and E. Sanz for their technical assistance in many aspects of the project. Financial support was provided by the Consellería d’Agricultura (project GV-CAPA00-18) and de Conselleria de Cultura Educació i Ciència (project GV-D-AG-01-128-96) of the Generalitat Valenciana, and the Ministerio de Agricultura, Pesca y Alimentación (project INIA SC95-108-C5-4) and Ministerio de Educación y Cultura (project CICYT AGF97-0899-C02-02).
References


Two native pupal parasitoids of *Ceratitis capitata* (Diptera, Tephritidae) found in Spain

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**Abstract**: Searching native parasitoids of *Ceratitis capitata* is one of the activities carried out in the Valencian Community in plots of citrus and other fruit trees. Adults of two different species of hymenopterous insects have been obtained from medfly puparia reared under laboratory conditions. The pteromalids *Spalangia cameroni* Perkins and *Pachycrepoideus vindemmiae* (Rondani) have been identified as idiobiont pupal parasitoids of the Medfly.

**Key words**: Tephritidae, Pteromalidae, Medfly, *Ceratitis capitata*, native parasitoid, *Spalangia cameroni*, *Pachycrepoideus vindemmiae*, Valencian Community.

**Introduction**

*Ceratitis capitata* (Wiedemann, 1824) is considered a key pest of stone and citrus fruits in several parts of the world. The medfly has become a cosmopolitan species with a wide range of host plants as a result of its great capacity of dispersion, adaptability and high rate of reproduction.

The life cycle includes four phases that begin with the egg oviposited under the fruit skin. It continues with the larvae developed inside the pulp of the fruit. The larva of third instar falls down to the soil where it buries and becomes pupa. Adults emerge from the pupae in a few days.

In the Valencian Community (East coast of Spain) this insect has become an endemic pest since the 30s, what making necessary to take measures of control to avoid the economic losses caused in the citrus sector. Nowadays, the citrus areas affected by the medfly are under a program of control based in a monitoring system (pheromone & bait traps), terrestrial and aerial treatments with chemicals (malathion+protein), sterilizing traps and the use of sterile insect techniques (sterile males) (Castañera, 2003).

As a result of the importance and interest of this pest in Spain, a multidisciplinary project with different research centres began in 2003. This project is based on an integrated pest management applied before and after the harvest. Biological control is included in the pre-harvest measures. The Valencian Institut for Agricultural Research (IVIA) takes part in a sub-project which aims at studying the possibilities of use of parasitoids against the medfly in the Mediterranean areas of Spain. For this purpose, the IVIA imported two parasitoids from Hawaii that were being used satisfactorily in other parts of the world: the Hymenoptera Braconidae *Fopius arisanus* (Sonan, 1932) and *Diachasmimorpha tryoni* (Cameron, 1911), egg and larva parasitoids respectively (Falcó *et al.*, 2003a,b,c). Other parasitoids of *Ceratitis capitata* such as *Aganaspis daci* (Weld, 1951), *Habrobracon hebetor* (Say, 1836), *Bracon laetus* Wesmuel, 1838 and *Psyttalia concolor* (Silvestri, 1914) have been found in the Mediterranean Basin (Dean, 2003; Papadopoulos & Katsoyanos, 2003) but there are no records of native parasitoids detected in the Iberian Peninsula. Our work group has initiated a
deep search of native parasitoids of the Medfly in the Valencian citrus area in order to find insects that could carry out biological control of *C. capitata*.

**Materials and methods**


The methodology used was based on the collection of fruits attacked by the medfly. These fruits were put on trays with inorganic substrate and placed under the trees to get the developed larvae which could fall down and pupate in that substrate. Eggs, larvae, and pupae were left on the trays in order to allow the possible parasitic action of any parasitoid in the area that could work over the different phases of the medfly. Five days old pupae were collected and transferred to the Entomology laboratory so that they could develop under regulated climate conditions, at 21-26°C, 65-90 % RH and 16:8 (L:D), until the emergence of medfly as well as parasitoid adults. If there was a lack of attacked fruits, young pupae reared in laboratory were placed in the orchards, following the same steps until the adults emerged.

**Results and discussion**

Two native parasitoids were detected. The first native parasitoid found was located in the plot of Bétera (4) where sampling of attacked fruits and reared lab pupae was carried out weekly during the period from September until November, 2003. In a sample of september-2003 several parasitoids emerged from medfly pupae in the laboratory. After a precise taxonomic identification they were recognized as the species *Spalangia cameroni* Perkins, 1910, an hymenopteran pupal ectoparasitoid which belongs to the family Pteromalidae (Fig. 1). This species attacks basically diptera Muscidae and Sarcophagidae, but also the tephritid genus *Dacus* has been recorded as its host in Hawaii and Fidji islands; its geographic distribution is Pacific Islands, Central and South America (Antilles, Brazil), Asia, Africa and Europe (including Azores) (Boucek,1963). It may be emphasized that 5 species of the genus *Spalangia* are recorded as parasitoid of 11 tephritid fruit-flies (Boucek, 1963). However, our finding represents the first record in the world of *Spalangia cameroni* as parasitoid of *Ceratitis capitata*.

This parasitoid is being reared under regulated climate conditions. At the moment the life cycle and several biological parameters are being studied. 11 generations with a maximum rate of 60 % of parasitism were obtained in the first year.

The second native parasitoid was found in the sampling areas number 4 and 6 in the year 2004; it was again found in the field at the same searching sites in the year 2005. It was identified as the species *Pachycrepoideus vindemmiae* (Rondani, 1875), also a hymenopteran parasitoid that belongs to the family Pteromalidae (Fig. 2). Although *P. vindemmiae* is a cosmopolitan species, it has been recently recorded from Spain (Askew et al., 2001). The biological features are Idiobiont Pupal Ectoparasitoid of Diptera (Muscidae, Drosophilidae, Tephritidae (including *C. capitata*) but it is also recorded as hyperparasitoid of beneficial hymenopteran (Rueda & Axtell, 1985; van Alphen & Thunissen, 1983). The species is being
used as a biological control agent of *Ceratitis capitata* in Argentina, Colombia, Hawaii and Costa Rica (Ovruski *et al.*, 2000).

Nowadays the parasitoid is being reared in the laboratory under regulated climate conditions and we obtain regularly a sufficient number of individuals to maintain the rearing. *Spalangia cameroni* represents a new record to the Spanish fauna and a new record in the world as a parasitoid of *Ceratitis capitata*. It will be studied to know its potential as a biological agent against *C. capitata*.

Fig. 1. *Spalangia cameroni*: habitus and oviposition on *C. capitata* pupa.

Fig. 2. *Pachycropoideus vindemmiae*: habitus and oviposition on *C. capitata* pupa.

**Acknowledgements**

This work is included in the RTA Project 2003-05 “Integrated Control of the Mediterranean Fruit-fly, *Ceratitis capitata*, in Citrus Fruits” which is financed by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA).

**References**


Influence of ground predators on the survival of the Mediterranean fruit fly pupae, *Ceratitis capitata* (Diptera:Tephritidae), in Spanish citrus orchards

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A survey of predaceous ground arthropods was conducted in two citrus orchards in Valencia, Spain, and their role as predators of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) pupae was evaluated under field and laboratory conditions. A total of 2,959 predaceous arthropods were collected by pitfall trapping in the two orchards from July 2003 to September 2004. Ants (Hymenoptera) were the most abundant group (83%), followed by Staphylinidae (≈8%), Araneae (≈5%), Carabidae (<1%), Cicindelidae (<1%) and Dermaptera (≈1%). Pupae disappearance rates were higher during the warmer months of the year, from May to October, and in the orchard with the largest ant populations. In the warm season, average survival (mean ± SE) of *C. capitata* pupae was 35.7 ± 6.2% and 14.3 ± 6.7%, respectively, in both orchards. Symptoms of predation, inferred from broken or abnormal pupae, were more frequently observed in the colder months, from November to April, when spiders, Staphylinidae and other predators were present. In the cold season, the combined effect of predation and low temperature during the period, lasting between one and four months, when the pupae stayed in the soil led to an adult emergence rate of only 10.0 ± 6.0% and 4.7 ± 3.4% in both orchards. In no-choice laboratory trials, all predator species tested fed on the *C. capitata* pupae, with statistical differences in feeding rates between species. Preliminary data show that carabids [*Pseudophonus rufipes* (Duftschmid) and *Harpalus distinguendus* (Degeer)] were the most voracious species, consuming more than one pupa per day followed in order of importance by Cicindelidae, earwigs and spiders.
Population dynamics of *Ceratitis capitata* on citrus in northeast Spain: the influence of adjacent host fruit trees

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**Abstract:** The population of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), was studied from April 2003 to December 2004 in the citrus grove area of Catalonia, northeast Spain. Tephri-trap traps were used baited with the male specific pheromone trimedlure and with the female target attractants ammonium acetate, putrescine and trimethylamine. A total of 96 traps were placed on clementine trees, distributed at 23 observation points in different parts of the citrus area. Two main adult population peaks were observed in each year; one in summer and the other in autumn. From November, when T°C dropped below 15°C, populations drastically decreased. From mid December to mid May no females were captured, and from early March to mid April no adults, either male or female, were captured. No long distance movements were detected in the area. Adult females appeared in spring throughout the study area, but with no definite direction. The population dynamics and abundance of adult females in the citrus groves studied seemed to be related to the availability of alternative host fruits in the surrounding area. The most important alternative host fruits prior to population developments on citrus appear to be peaches in early summer and fig trees and *Ziziphus jujuba* in late summer and autumn.

**Key words:** *Ceratitis capitata*, Mediterranean Fruit Fly, dynamics, citrus, host trees

**Introduction**

*Ceratitis capitata* is one of the most serious pests in the world, as it is highly polyphagous and attacks more than 300 different species. In citrus, *C. capitata* mainly causes damage to Clementine varieties. As these fruits generally start to ripen in mid September, it is from this moment on that they are sensitive to *C. capitata* attacks.

The northern limits of citrus growth in Spain are in Catalonia, where approximately 10,000 ha of citrus are cultivated. This citrus area is largely located along the banks of the river Ebro, which is a traditional citrus growing area. Groves can also be found in southern Catalonia, in the area bordering on Castellón (in the Autonomous Community of Valencia), which has become a new zone of expansion for citricola production. Clementines are the major varieties grown and represent more than 50% of this area’s citrus production. These varieties are very sensitive to attacks from *C. capitata*. Where the citrus production area finishes in the north, peaches are cultivated. *C. capitata* females have a marked preference for this host plant, which is very sensitive to their attacks. From June onwards, peaches are susceptible to attacks and these normally continue until the end of August when the peach harvest comes to end. It would seem natural to suppose that at this moment there would be a migration from this zone towards the south and the citrus area, because at this time some Clementine varieties start to change colour and are particularly susceptible to attack.
Materials and methods

The study was conducted in the citrus grove area of Catalonia, in northeast Spain, from April 2003 to December 2004. Twenty-three commercial citrus groves, with mature trees of the Clementine variety (Citrus reticulata Blanco) were selected for study. These groves were distributed throughout the total citrus growing area.

C. capitata adults were monitored using Tephri-trap (Kenogard) traps baited with male specific parahemerone trimedlure (Aralure™, Agrisense) and with female target attractants: ammonium acetate, putrescine and trimethylamine (Tripack™, Kenogard). DDVP (Econex) was used as a toxicant in all traps. Trimedlure plugs were changed every 90 days and female target attractants and DDVP were replaced every 45 days.

Four traps, two of each kind, were placed on Clementine trees in each grove at a height of 1.5-2m above the ground. They were positioned facing south, in a shaded part of the canopy, and placed ~50m from each another in order to avoid interferences. The traps were checked once a week, with all of the captured C. capitata adults being counted and separated according to sex. Adults were removed after each revision.

Prior to the assay, the areas surrounding the groves were examined for other isolated fruit trees that might act as hosts to C. capitata. Other characteristics considered included wind speed, grove size, the presence of over-ripened oranges on trees, and general C. capitata management. Meteorological data were obtained from a station at Amposta, a town located in the middle of the southern section of the studied area.

Results

The number of males and females captured in the two years of the study were significantly different (Table 1). In 2003, 54,652 males and 15,191 females were captured, whereas in 2004, the total number of males and females captured was 41,316 and 7,455 respectively. High temperatures until September in 2003 and higher relative humidity throughout the year seemed to explain the increase in the C. capitata population.

Statistical analysis showed a significant month to month variation in the number of adults captured per trap and day (Table 1). September and October were the months with the greatest abundance of C. capitata captures, while from January to May, the number of adults captured per trap and per day was very low (Table 2).

Table 1. Analysis of variance of adults of Ceratitis capitata captured in traps hanged on Clementine citrus trees. Data were transformed to sqrt (x+0.05) prior to analysis.
Table 2. Adults of *C. capitata* captured per trap and day. Data were transformed to sqrt \((x+0.05)\) prior to analysis. Equal letters in columns indicate equal means (Duncan’s Multiple Rang Test \((P < 0.05)\)).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>0.020 ± 0.005 e</td>
<td>0.005 ± 0.002 d</td>
<td>0.025 ± 0.005 f</td>
</tr>
<tr>
<td>February</td>
<td>0.008 ± 0.003 e</td>
<td>0.001 ± 0.001 d</td>
<td>0.009 ± 0.003 f</td>
</tr>
<tr>
<td>March</td>
<td>0.002 ± 0.001 e</td>
<td>0.000 ± 0.000 d</td>
<td>0.002 ± 0.001 f</td>
</tr>
<tr>
<td>April</td>
<td>0.001 ± 0.001 e</td>
<td>0.001 ± 0.001 d</td>
<td>0.002 ± 0.001 f</td>
</tr>
<tr>
<td>May</td>
<td>0.030 ± 0.005 e</td>
<td>0.006 ± 0.002 d</td>
<td>0.036 ± 0.005 f</td>
</tr>
<tr>
<td>June</td>
<td>0.559 ± 0.098 d</td>
<td>0.174 ± 0.025 c</td>
<td>0.733 ± 0.100 d</td>
</tr>
<tr>
<td>July</td>
<td>3.590 ± 0.230 b</td>
<td>0.970 ± 0.083 a</td>
<td>4.560 ± 0.250 b</td>
</tr>
<tr>
<td>August</td>
<td>2.170 ± 0.150 c</td>
<td>0.460 ± 0.050 b</td>
<td>2.630 ± 0.170 c</td>
</tr>
<tr>
<td>September</td>
<td>5.430 ± 0.460 a</td>
<td>1.160 ± 0.110 a</td>
<td>6.590 ± 0.480 a</td>
</tr>
<tr>
<td>October</td>
<td>4.100 ± 0.330 b</td>
<td>1.200 ± 0.100 a</td>
<td>5.300 ± 0.340 a</td>
</tr>
<tr>
<td>November</td>
<td>2.350 ± 0.180 c</td>
<td>0.310 ± 0.030 b</td>
<td>2.660 ± 0.180 c</td>
</tr>
<tr>
<td>December</td>
<td>0.300 ± 0.050 d</td>
<td>0.040 ± 0.008 d</td>
<td>0.340 ± 0.050 e</td>
</tr>
</tbody>
</table>

The most significant source of variation was the bait used (Table 1). Traps baited with Trimedlure (TM) captured significantly higher numbers of *C. capitata* adults than those baited with Trypack (TP), with a TM / TP ratio of 70%. TM captured more males than females, with a general ratio of 99%. This ratio varied according to the month considered and was directly related to the abundance of adults captured in each month \((y = 0.0017 \ln(x) + 0.9804, R^2 = 0.5688, n = 16)\), with the ratio oscillating between 98.5% and 100%. Conversely, TP captured significantly higher numbers of females than males, with a general ratio of 63% (Table 3). This ratio also varied according to the month considered and oscillated between 41% to 76%. This ratio was directly related to the abundance of *C. capitata* in each month \((y = 0.0339 \ln(x) + 0.3606; R^2 = 0.4246; n = 17)\). Subsequently, the more abundance, the higher the female/male ratio captured in TP.

Table 3. Average number of adults of *C. capitata* per trap and day on traps with each kind of bait. Equal letters in columns indicate equal means (Duncan’s Multiple Rang Test \((P < 0.05)\)).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypack</td>
<td>0.6002 ± 0.0321 b</td>
<td>1.0185 ± 0.0448 a</td>
<td>1.6187 ± 0.0720 b</td>
</tr>
<tr>
<td>Trimedlure</td>
<td>3.8447 ± 0.1617 a</td>
<td>0.0196 ± 0.0015 b</td>
<td>3.8643 ± 0.1626 a</td>
</tr>
</tbody>
</table>

As TM mainly captured males and TP mainly captured females, the temporal patterns of adults were studied separately, based on each kind of baited traps. Average numbers of females captured per trap and day in TP and average numbers of males captures per trap and day in TM for all groves in 2003 and 2004 are shown in Fig 1. Dynamics were similar for males and females. Throughout the year, it was possible to observe two peaks for male and female captures: one occurred in summer and the other in autumn. In the case of females, the summer 2004 peak took place 3 weeks later than in 2003 and the autumn peak occurred 2 weeks later. For males, only the summer peak occurred later in 2004. This phenomenon was associated with temperature and relative humidity (Fig. 2): until September, average monthly
temperature was higher (about 2 °C on average) in 2003 than in 2004, and relative humidity was also higher in 2003 than in 2004.

Figure 1. Number of adult males and females of *Ceratitis capitata* captured per trap and day. Males were captured in baited Trimedlure traps and females in baited Trypack traps.

In 2003, we started the study at the end of April and by May we had already captured *C. capitata* adults. Although adult captures were very limited in winter, throughout almost the whole study period adults of *C. capitata* were captured. From 8/12/03 until 13/1/04 (~ 1 month) and from 17/2/04 to 4/5/04 (~ 3.5 months) no females were trapped in any of the groves. From 10/3/04 to 7/4/04 (~ 1 month) no females or males were trapped anywhere. These periods corresponded to the months with the lowest temperatures in the year. The first consistent captures of females started in May, when average temperatures reached 15°C. Similarly, adult captures drastically decreased in November 2003 and 2004, when temperatures fell below 15°C.

The grove selected had a significant effect on total numbers of *C. capitata* captured (Table 1). All of the groves in question were of Clementines. None of the characteristics considered for each grove was found to be related to *C. capitata* abundance, but the presence of alternative tree hosts near to these groves did prove significant. On some occasions, the presence of over-ripe fruit on trees, in some occasions determined high *C. capitata* populations. The number of *C. capitata* captured did not depend on the different management techniques used in the grove to control this pest (aerial treatments or mass trapping), because in both cases we found both large and small populations. Positive interaction between grove and month (Table 1) suggested that captures varied according to the presence of mature fruit on other host trees.
The dynamics and abundance of adult insects varied from grove to grove. In the whole study area, some groves showed population peaks only in summer. Other groves showed peaks only in autumn, and in others, the two peaks were observed, randomly in the studied area. These peaks were not related to the locations of the groves in question, but to host trees in their vicinity. Summer peaks were related to the presence of mature fruits such as loquats, apricots and peaches near the grove, while autumn peaks were associated with the presence of mature fruits of figs, Indian figs, or jujubes near the citrus grove. Of the 23 groves studied, 12 registered low levels of adult captures throughout the year, with maximum captures of fewer than 2 females per trap and day. Nine of these 12 groves were relatively isolated: at least, we could not detect any alternative host trees other than citrus. Eight of the
23 groves registered large adult populations, with peaks of between 4 and 12 females captured per trap and day. Fifty per cent of these groves had potential host trees in their immediate vicinity. Finally, 3 of the 23 groves had very high adult populations, which exhibited peaks of between 12 and 25 females per trap and day. All of these citrus groves had host trees nearby.

Discussion

It was possible to find adult insects flying in the study throughout the year: most of these were males. Both TM and TP baited traps showed similar capture tendencies over the year, both for males and females, and also registered the same peaks. TP baited traps captured both female and male adults. However, the number of females captured was always higher than the number of males in these traps, and this sex ratio was higher when general population was.

The population trends found in our citrus zone were similar to those reported for other Mediterranean countries or regions, with only relatively minor variations. In the Comunidad Valenciana (Spain), the two population peaks are also observed, but the summer peak tends to be much more copious than the autumn peak (Muñoz, A. and García-Mari, F., 2003). On the island of Chios (Greece), the population peaks in August, September and November (Katsoyannos et al., 1998). In northern Greece, adult populations are generally very low in June, July and December, moderate in August and September and very high in October and November (Papadopoulov et al., 2001). On Crete, the adult population shows one peak during the period June-August and another in September (Michelakis, 1991). In Muravera (Sardinia), the two peaks for the adult population occur in July and November (Delrio, G., 1986). In Israel, Israely et al. (1997) flies were continuously caught from May until mid-December, showing three waves of population in May, July and August-September.

In our study zone, adult captures were very low from January to May, but adults were captured throughout the year with the exception of March, when neither males nor females were detected in any trap. Other authors reported similar data in the Mediterranean area. In Italy, the presence of adults is observable from July to December (Ortu et al., 2005). On Chios, captures were low in June and for most of July and also in December and January, with no captures from February to May. On Crete, adult flies were detected from the end of autumn to the beginning of summer, with numbers decreasing from a very high registers in November-December to very low ones at the end of March, and then again rising to relatively high catches by the end of May (Mavrikakis et al., 2000). In Israel, Israely et al. (1997) reported that no C. capitata adult male were caught between January and April. In Granada, in southern Spain, C. capitata adults were observed throughout the year (Ros, 1975).

The evolution of weekly captures for the whole citricola zone failed to show any cases of long distance migration by C. capitata adults. In winter, when populations are very small, the first foci were detected in several different groves, but were independent of grove location. There was not a definite tendency for them to appear in some groves before or after in others. When the adult population started to rise, in spring, they could be found in all groves at the same time. This demonstrated that C. capitata hibernated throughout the whole citricola area, probably remaining inside oranges of late-ripening citrus varieties. Ros (1975) reported a gradual increase in trap catches with the first adults of the year being caught in southern Spain. This suggested migrations from coastal zones towards the interior.

When temperatures started to increase, C. capitata adults became more active and looked for susceptible fruits to attack. Host trees, including apricot, apple, fig, loquat, peach, jujube, and Indian fig, could be found throughout the study area. Peaks in early summer were related to the presence of mature loquat, apricot, and peach fruits near the grove, while peaks in late summer and autumn were related to the presence of mature figs, Indian figs and jujubes near
citrus groves. Fig fluids represent an important source of exogenous nitrogen for adult *C. capitata* (Hendrichs and Hendrichs, 1990). The fecundity and longevity of flies feeding on figs has been shown to be relatively high (Hendrichs et al., 1991). In addition, *C. capitata* larval development time on fig fruits is relatively fast and larval survival rates are moderate to high (Rivnay, 1950, Carey, 1984). Thus, the presence of these fruits together with jujube trees in our study zone provides an exceptional potential breeding site for generations that will later attack Clementine varieties in September.

In Spain, spray management of *C. capitata* is based on adult captures in traps hung on citrus trees. As previously demonstrated, adult captures are related to other host fruit trees in the areas surrounding the citrus groves. As a result, records from individual traps are only valid for the particular groves in which these trap are hung, but not for a larger area. In our study, all of the traps were hung from Clementine trees, but each grove showed different dynamics, which were determined by the presence or absence of other host fruit trees in the vicinity of the citrus grove in question.

Eighty-nine percent of isolated groves showed lower insect populations than those with neighbouring host fruit trees, with female adult peaks of fewer than 2 females per trap and day. In groves in which there was evidence of nearby host trees, 88% showed peaks of > 2 females per trap and day and 75% showed peaks of >4 females per trap and day, with a maximum of up to 25 females per trap and day in one case.

**Acknowledgments**

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**References**


The population dynamics and damage caused by the Mediterranean fruit fly (*Ceratitis capitata* Wied.) (Diptera: Tephritidae) in orange groves on Terceira Island, Azores

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Abstract: Given the dispersal capabilities and damage known to be caused by *Ceratitis capitata* (Wied.) it is important in a particular location to evaluate the areas and fruit hosts that are most affected by this pest, the local population dynamics and its seasonal presence in orchards. The work reported in this paper was one part of a wider integrated investigation developed in the INTERFRUTA project that had as one goal the use of GIS, especially ESRI software, ArcView 3.2, for the study of *C. capitata* dispersion in the tree fruit areas. To achieve better knowledge of the biology and also to monitor *C. capitata* population changes in each grove, a network of traps (Jackson with sexual attractant for males and Tephri with a three component food attractant for females) was installed in three fruit production areas (Biscoitos, Terra-Chã/Angra and S. Sebastião) on Terceira Island, Azores in orange groves using GPS. The results of spatial analysis in ArcView show some apparent dispersion within the three zones, with concentration of the pest population below 100 meters of altitude. With a three-dimensional analysis it was also possible to see that *C. capitata* has a preference for areas characterised by topographic depressions that may provide some climate protection which improves survival. The greatest utility of this type of analysis using GIS software is the possibility to define and apply a qualitative scale previously established for damage caused by this pest and to get maps of damage distribution in the different orange groves in order to provide consistent and more efficient control. It could also be useful to understand the relationship of population and infestation with factors such as altitude, topography, type and phenologic development of the surrounding vegetation and weather conditions. In the orange groves it could be seen that in the vicinity of Angra there are two periods that registered the greatest amount of adult *C. capitata* captures. A low first peak was in June and the second, higher peak was in September. In Biscoitos and S. Sebastião the greatest adult captures were registered in a single peak in September. The small early peak in Angra coincided with the maturity of some other nearby hosts. The September peak of trap catches throughout the island preceded the subsequent high infestation rates in oranges from October to November.

Key words: *C. capitata*, Mediterranean fruit fly, Medfly, GIS, fruit damage, population dynamics, Azores
Introduction

This work was one part of a wider integrated investigation carried out in the INTERFRUTA project, an Interregional Cooperation Project that includes Azores, Madeira and Canary Islands, financed by FEDER under the EC Program INTERREG III-B (Lopes, 2005a).

This project had as one goal to demonstrate the use of GIS, especially ESRI software, ArcView 3.2., as a tool that can be useful in the investigation of *C. capitata* adult dispersion and damage in commercial fruit areas (Nunes *et al.*, 2004; Lopes *et al.*, 2005b, 2005c; Pimentel *et al.*, 2005). *C. capitata* is an important pest throughout the Mediterranean area. In the Azores islands, as in Madeira, it is also a great menace to production in several species of fruit trees (Carvalho & Aguiar, 1997). Monitoring *C. capitata* adult dispersion is very important because it is a polyphagus pest causing severe losses on many hosts, and particularly on citrus.

Knowing *C. capitata* adult behaviour and its dispersal capabilities, it is also important to evaluate the areas which are more affected by this pest, its population dynamics and its seasonal presence in the different orange groves of the island (Bodenheimer, 1951; Leonardo, 2002). Estimating the period of greatest fruit damage and losses, and the most seriously affected kinds of fruit, are important aspects when implementing an integrated pest management program, especially on an island with diverse climatic and vegetation zones (Rossler, 1988; Carvalho & Aguiar, 1997).

Material and methods

Production areas of Terceira Island

At the beginning of the work in the INTERFRUTA project it was necessary to make surveys of the fruit producers registered with the Regional Agricultural Services and the local fruit producers’ association FRUTER, in order to have a better understanding and definition of the fruit production areas. To achieve that, 160 fruit producers from Biscoitos; Porto Judeu; São Brás; Lajes; Vila Nova; São Sebastião; and Angra were surveyed (Lopes, 2005a). ArcView software, with digital photos, was used to build a map of the distribution of the production areas around the island. Some experimental orchards were selected by choosing among the most productive orchards and the most influential and interested producers of each village and these fields were identified with plaques at the sites.

Monitoring Ceratitis capitata adults

To monitor the *C. capitata* adult population and also to get information on its biology, a network of traps was set up in the three most important orange areas of Terceira Island (Biscoitos, Terra-Chã/Angra and S. Sebastião) (Lopes, 2005a). The installation of this network followed IAEA standard methodology (IAEA, 2003). It was integrated into the general monitoring program within the INTERFRUTA project and provides better knowledge of the Medfly population development and dispersion by using GIS. The traps used in the network were Jackson and Tephri (Jackson with sexual attractant for males and Tephri with a three component food attractant for females) (IAEA, 2003).

In the first phase of this work, it was necessary to identify the locations in which to install traps for the network. Using ArcView 3.2, a grid with one square kilometre points was created over the three fruit production areas of Terceira Island (Biscoitos, Terra-Chã/Angra and S. Sebastião) in orange, apple and peach orchards. After that, the traps were installed using a GPS at the spots previously determined.
**GIS use in the definition of C. capitata adult population dynamics**

The second phase of work analysed the trap records in ArcView, performed a spatial analysis on the data from the network traps, using the spatial analysis extension and applied the Inverse Distance Weighting (IDW) method with a grid of 20m. It also applied a digital model of the terrain in order to cross-reference information from the network traps and the topographic information.

**Fruit Damage**

In the third phase of work, fruit damage caused by the Mediterranean fruit fly on orange fruits was analysed. Every two weeks, fruits were collected in the field and put into transparent boxes so that the *C. capitata* larvae that emerged from each fruit could be recorded. After determining the number of larvae in each kind of fruit, the number of larvae per kilo of fruit, and the number of pupae that developed were recorded.

**Results and discussion**

**Production areas of Terceira Island**

The surveys conducted with the fruit producers and the use of ArcView with digital photos provided the information and tools to build a map of the distribution of the different production areas around Terceira Island (Figure 1). This work also was useful later to select experimental orchards to study infestations and populations.

![GIS map showing the distribution of fruit production areas on Terceira Island.](image)

**Monitoring Ceratitis capitata adults**

A network of traps (Jackson and Tephri) was installed in the three largest orange growing areas of Terceira Island (Biscoitos, Terra-Chã/Angra and S. Sebastião) using a GPS. The data from each trap were used to build a GIS map (Figure 2) of captures, which were inserted into
an Excel spreadsheet table that could be used for spatial analysis in ArcView. The results (Figure 3) show some dispersion within the three areas studied, with a concentration of *C. capitata* adult captures below 100 meters of altitude.

![Figure 2 - GIS map showing the distribution of the Mediterranean Fruit Fly traps on Terceira Island.](image)

In the orange groves we can see that in Angra there are two periods with greater captures (Figure 3). The first period was in June and the second occurred in September. In S. Sebastião and Biscoitos (not illustrated) only one peak period occurred, in S. Sebastião a long peak from September to November and in Biscoitos a short peak in September.
Figure 3 – Adult captures of *C. capitata* by altitude level from S.Sebastião (3a), Angra (3b) and Biscoitos (3c).

Over the whole island captures in the orange groves, mainly occurred in September (Figure 4). The initial rise in population in June is caused by the presence of other Medfly hosts fruits maturing in the vicinity of Angra at that time.
Figure 4 – Med-fly/trap per day during 2004 in oranges orchards in the three districts sampled on Terceira Island.

GIS use in the analysis of C. capitata adult population dynamics
With three-dimensional analysis (Figure 5), it is possible to see that C. capitata adults are found more commonly in topographic depressions and in areas with a higher vegetation density that could provide some climatic protection. This could be understood as a behavioural indicator for protection and a survival strategy of this insect.

Figure 5 – Three-dimensional GIS maps of adult C. capitata population dynamics.
This GIS software was very useful in demonstrating Medfly dynamics and providing new analyses. Problematic locations were identified and *C. capitata* dispersion behaviour was examined.

**Fruit Damage**
The greatest Medfly infestation in orange groves occurs from October to November, after the peak in trap catches. The peak generation of adults, represented by September trap catches, may produce the high larval populations that are seen in the infested fruits harvested in October and November, while the adult populations decline with the cooler weather and immature development stages become longer.

Even greater fruit damage was recorded on other fruits surrounding or in the orchard, such as figs and loquat (Table 1 and Figure 6) that are present in the orchards in the study. It is possible that the high infestations in these nearby fruits, with greater susceptibility to Medfly, could influence the traps catches more than do the infestation in orange within the orchards.

![Figure 6](image_url)  
Figure 6. Results of the distribution of fruit damage caused by Mediterranean Fruit Fly in the three areas studied in each fruit.
Table 1. Results of the evaluation of the distribution of fruit damage caused by Mediterranean Fruit Fly in the three areas studied.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>March</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ameixeira</td>
<td>Prunus domestica L.</td>
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<tr>
<td>Ananas annosera</td>
<td>Anona communica L.</td>
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<tr>
<td>Araçáloiro</td>
<td>PSicum guineense S. &amp; P. cattleyanum S.</td>
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<tr>
<td>Barbeiro</td>
<td>Cynosopus laevispatum Forster</td>
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<tr>
<td>Cafézegro</td>
<td>Coffea arabica L.</td>
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<tr>
<td>Camélia jaunecoia</td>
<td>Camelia japonica L.</td>
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<tr>
<td>Citríos pequenos</td>
<td>Citrus nobilis e Citrus reticulata Blanca</td>
<td></td>
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**Total of 3 Zones**

- 0 Larvae/kg fruit
- 0.01 - 10 Larvae/kg fruit
- 10 - 50 Larvae/kg fruit
- 50 - 100 Larvae/kg fruit
- > 100 Larvae/kg fruit

Acknowledgements

The Azores Regional Government and the INTERREG III-B European Community Program funded all the investigation done in the INTERFRUTA Project (MAC/3.1/A1). Our thanks are expressed to all of the partners and their investigators that worked in this INTERFRUTA project. We thank the fruit producers on Terceira who cooperated and allowed us to work in their orchards.

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Ceratitis capitata Wiedman (Diptera: Tephritidae) na Ilha Terceira (Açores). V Congresso
Ecological based control of the Mediterranean fruit fly through a novel technology

Nimrod Israely  
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Current control methods of *Ceratitis capitata* (Wiedemann) use repeated sprays of pesticides to protect the crop against it. Although high level of protection is achieved, the side effects of such procedure are undesirable. Recent large-scale ecological study, by Israely et al., give evidence that flies do not survive in the same orchard year-to-year, but instead they continuously change their spatial position, searching and attacking available hosts. Hence, when hosts are becoming sensitive to attack, the source of flies must be external to the plot. Using this information together with thorough acquaintance with *C. capitata*'s biology and behavior, the Biofeed, a suspended device, have been developed and tested in Israel during the last four years. The Biofeed is made out of a yellow, two dimensional, shape cloth (30X30 cm) with a red circle on its lower side and a pocket stitched to its upper side, into which a bait container is inserted. The bait diffuses out of the pocket, during an 8 weeks period to the devices' surface. Attracted flies land on the Biofeed's surface, feeds on it, and die due to a poison mixed in the bait. Due to the constant reinvasion of flies to the orchards, placement of Biofeed is dancer in the orchards' perimeter, to hinder invading flies. The Biofeed's efficiency was compared to alternative methods in 13 different hosts' species, in over a hundred plots and thousands of dunams throughout Israel. Results show that control achieved by Biofeed is as good as alternative methods used in Israel. It is concluded that the Biofeed is a viable cost-effective alternative for conventional and organic farming and has a potential for use in conjunction with SIT projects.
Four years of medfly control using chemosterilization: results and performance possibilities

Vicente Navarro-Llopis, Javier Domínguez, Juan Sanchis, Eduardo Primo and Jaime Primo

*Ceratitis capitata* (Wiedemann), medfly, is the major pest in the Mediterranean Spanish citrus area and control measure still consist of several malathion aerial applications over large areas. A four-year field trial was carried out in Valencia, Spain, in order to test efficacy of chemosterilization against medfly compared with malathion treatments. Chemosterilization was applied hanging 24 bait stations per hectare in an 80 Ha citrus plantation. Bait stations were hung in May and remained active in the field during the whole year. Each trap contains a protein bait with lufenuron and male and female attractants. Lufenuron prevents egg hatching from females that ingest the bait or females that mate with males which have ingested the bait. In this way, the population is reduced, generation after generation, affecting medfly fertility during the whole year. A check field was located 1 km away from the field treated with chemosterilant and was treated 4 to 8 times per year with aerial applications of malathion and protein bait. The efficacy of the new medfly control method was evaluated as adult medfly population reduction, and measured as weekly catches in a monitoring trap grid over the two areas. Results show a continuous medfly population reduction since the first year till the fourth year of chemosterilization application. 60% reduction of medfly population in chemosterilant areas was achieved in the fourth year when compared with malathion treatments. This medfly population reduction suggests that chemosterilization would allow the suppression of the aerial treatments and the reduction of terrestrial pesticide application in a sustainable way.
Alternative methods for mass trapping of Medfly, *C. capitata* (Diptera: Tephritidae), in Algarve

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The control of the common Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) through the use of conventional pesticides is still the most used process in citrus orchards in Algarve region. Claiming for alternative solutions, without pesticide residues, trials based on mass trapping have been conducted in citrus orchards of AAZAP’s farmers.

The high cost of conventional traps, like the McPhail type, led the technicians to try a different kind of trap. This trap consists of a plastic bottle (used in commercial water) with a yellow waist-band and four small holes bellow (0.8 cm diameter). Two different baits where tested: a commercial protein hydrolysate (*Endomosyl*, 600 g/l of protein) in a water dilution of 9 %; and a commercial three-component trimethylamine + putrescine + ammonium acetate (*Starce*) in a water dilution of 1 %. A pottery detergent at 1 % was added to solution baits.

In one experimental unit (five rows of trees) in the citrus orchard, the protein hydrolysate (*Endomosyl*) was placed in a proportion of one trap per tree (HP 1/1). In a second experimental unit, the three-component (*Starce*) was placed in a proportion of one trap per every two trees (ST 1/2). In a third experimental unit, the same tri-compost was placed in a proportion of one trap per every three trees (ST 1/3). Two types of observations were made in each experimental unit: number of captured flies (males and females) in six traps and number of damaged oranges on six marked citrus trees. The observations were carried out once a week from September to November of 2003.

In spite of a high growth rate of flies, as usual in this period, results were promising, with only a few damage fruits. The results suggest that a lower trap density may be enough. The costs of this method were lower comparing with other traps and baits. The study is continuing with the same traps and baits, and including a new one, but with a variable number of traps according to the dynamics of medfly population.
Using proprietary adhesive powders as carriers of active ingredients in advanced control of the Mediterranean fruit fly and other important pests

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Abstract Using pheromones and/or food baits as attractants, specific insect pests can be lured into baiting stations containing a mixture of adhesive powder formulated with an active ingredient (i.e. pheromones, slow-acting pesticides, or biologicals). Insects lured into the bait station pick up the powder, which adheres to the insect cuticle, thus ensuring that the insect leaves the station carrying the active ingredient. Consequently, insects are automatically turned into vectors of the active ingredient. Thus as part of an integrated program, male insects can become carriers of synthetic analogues of female-produced pheromones in a mating disruption system. The same system can be used in a lure and kill system to contaminate insect pest individuals, which later through mating, gregarious/social behaviour, and/or cannibalism, transfer the active ingredient to conspecifics. In this article, we describe parts of the initial research towards development of a system that involves a baiting station, attractants, active ingredients and adhesive powder, for control of the Mediterranean Fruit fly, *Ceratitis capitata*.

Introduction

The concept of using electrostatically charged powders as a delivery system of pheromones, slow-acting pesticides, or biologicals was described by Howse and Underwood (2000). Exosect Limited in Southampton United Kingdom holds the exclusive rights to this novel technological approach in insect pest control. Currently, Exosect has on-going research trials in more than 20 countries regarding the development of delivery systems for over 25 different insect pest species. Exosect invites colleagues with both research and commercial interests in this type of delivery system to contact us directly and/or obtain more information through our website (www.exosect.com).

Insect cuticles carry a small electrostatic charge (McGonigle and Jackson, 2002; McGonigle et al., 2002), therefore powders that can be electrostatically charged will adhere to the insect cuticle and can act as particle carriers for active ingredients. Compared to conventional pesticide applications, the main advantages of this system are that: 1) the insect themselves are responsible for disseminating the active ingredients to oviposition sites, refuges, resting places and other microhabitats where conspecifics aggregate and which may be difficult to treat in conventional pesticide applications, 2) much smaller total amounts of active ingredient are required, because surfaces and/or volumes of air space with no insect activity are not being treated (active ingredients will only be deposited where insect activity occurs), and 3) compared to conventional broadcast applications, non-target insect pests (e.g. beneficials) are less likely to be affected by treatments. Here, we present results from an on-going research project that focuses on developing a lure and kill system for the Mediterranean Fruit fly (medfly for short), *Ceratitis capitata* (Weidemann) (Diptera: Tephritidae), which is one of the world's most destructive fruit pests (Papadopoulos et al. 2003) and is known to attack more than 260 different fruits, flowers, vegetables and nuts – most of which are of high economic value (Weems 1981, Liquido et al. 1991). This project is funded by the BBSRC
In general terms, the Exosect delivery systems consist of components: 1) a baiting station, 2) an attractant, and 3) a proprietary adhesive powder, and 4) one or more active ingredients. All 4 components require some level of species-specific adaptation to a given insect pest and/or cropping system. Regarding the mixture of adhesive powder and one or several active ingredients, a number of factors are crucial in the analysis of the efficacy of this technology, including: 1) to what extent the active ingredient affects the electrostatic characteristics of the adhesive powder, 2) how much powder mixture is picked up by insects while visiting the bait station, 3) where the powder mixture is deposited on the insect body, and 4) to what extent the powder-uptake by insects affect their flight and mating behaviour. In this article, we present results from laboratory studies on the behavioral effect of powdering medflies with a proprietary electrostatically charged powder, Entostat. We examined to what extent the powder was retained on their bodies over time and whether it affected their flight behaviour. We also quantified the amount of Entostat transferred from powdered males to unpowdered conspecific females. The results presented here are part of an on-going research effort towards development of a powder-based lure and kill system for medflies.

Materials and methods

Medfly colony
The medflies were received on Jan 2005 from a culture maintained by Tracey Chapman at UCL, and originated from the Moscamed mass rearing factory strain in Guatemala, established in 1984 (Rendon 1996). They have since been cultured at the quarantine facility at the University of Southampton at 25°C, 60 % relative humidity and 14:10 L:D. Adults were kept in Perspex cages (32 cm × 20 cm × 22 cm) and fed on a 75 % sugar and 25 % yeast paste. Water was provided in a pot with a sponge wick. Virgin flies were obtained by storing 7-day pupae in a fly cage with a water pot containing a sponge wick. Any flies that emerged overnight were sexed and male and females were transferred to separate Perspex cages with food and water.

Entostat
The powder used in this study, Entostat, is composed of highly refined carnauba wax obtained from the leaves of the Brazilian wax palm, Copernica cerifera. For the purpose of this study, Entostat was mixed with a fluorescent dye, Glo-Brite (Himar, Bradford, UK) (10% w/w), which was added for quantification purposes to determine how much powder was retained and/or taken up by medflies (see below). A powder batch of 30 g was prepared by dissolving 3 g of Glo-Brite in 10 ml of 95 % ethanol and poured over 27 g Entostat. The mixture was continually stirred under an airflow until all of the ethanol had evaporated. The dyed powder was left for 24 h to completely dry and then ground using a pestle and mortar to return it to a fine consistency.

Fluorometric analysis
The excitation and emission wavelength of Glo-Brite were determined by scanning with a fluorometer (Perkin Elmer Luminescence Spectrometer LS5OB). Glo-Brite is excited at wavelength 385 nm and emits fluorescence at a wavelength of 450 nm. Before samples were run, a blank cuvette containing 95 % ethanol was tested and set as a background reading. This value was automatically subtracted from the readings of all subsequent samples measured. Also before analysing samples from medflies, the emission of six different concentrations of
dyed powder in ethanol were measured to make a calibration graph: 0.1 µg, 0.2 µg, 0.4 µg, 0.8 µg, 1.5 µg and 1.9 µg of dyed powder per 1 ml 95% ethanol.

Experiment 1 Powder retention by males and females
In separate 20 ml glass vials, 40 6-day old male and 40 female medflies were coated in dyed Entostat by shaking them for 10 s in 0.3 g of the powder. The flies were released into separate Perspex cages (32 cm × 20 cm × 22 cm) containing food and water. At various time intervals (0, ¼, ½, 1, 2, 24, 48, and 72 h), 5 medflies of each sex were sampled, transferred to individual Eppendorf tubes and rinsed in 1 ml 95% ethanol. Each Eppendorf tube was mixed on a vortex for 30 sec to remove the powder from the flies, and subsequently the medfly was removed with tweezers. Subsamples were transferred to fluorometric cuvettes and measured in the fluorometer. Using the equation of the calibration graph, the quantity of powder that was on each fly sample was calculated.

Experiment 2 Flight behaviour of powdered mixed sex medflies
A flight tunnel (150 long × 60 wide × 60 deep cm) was used for flight observations of 10 male and 10 female medflies (12 day old). Flights of control and treated flies were observed separately. The tunnel was made from glass on all sides with a push/pull system of variable speed fans, charcoal filtration system at each end and steel mesh mounted in both ends of the tunnel. The air flow was kept at 30 cm/s for all experiments. The tunnel was illuminated by a fluorescent tube (light intensity of 550 lux at the floor of the tunnel). Room temperature was maintained at 23-25°C and humidity at 48-55% RH. A yellow Exosect trap (foldable plastic, 15 cm long, 12 cm wide, 8 cm high and with a convex roof) was placed in the upwind section of the wind tunnel, 20 cm from the steel mesh and suspended 30 cm above the floor. In the center of the Exosect trap, 1.5 g of diet (75 % sugar and 25 % yeast paste) was placed as a lure and to stimulate anemotactic flight by the medflies. Treated flies were coated in undyed Entostat using a small plastic puffer bottle. Flies were released into a Perspex box (14 × 14 × 7 cm) with a hole in the lid. The box was inverted and the puffer was inserted in the hole. Entostat was then gently puffed over the flies five times. Control flies were puffed five times with an empty puffer bottle. The medflies were released in the downwind section, 20 cm from the steel mesh, and during each trial, the medflies were observed continuously for a total of 120 min. Each flight by individual medflies was recorded to determine the number of flights in 5 min intervals. For each 5 min interval, the number of flights by coated flies was transformed into percentage of number of flights by untreated flies.

Experiment 3 Powder transfer from powdered males to conspecific females
As described in Experiment 1, 40 6-day old male medflies were shook for 10 s in a 20 ml glass vial containing 0.3 g powder. After coating, males were transferred to a Perspex cage (32 cm × 20 cm × 22 cm). The males were given 1h to recover and then 40 6-day virgin female flies were introduced into the cage. The cage was observed for 5 h. Once a male and a female started mating, they were immediately separated from the cage and transferred into a plastic pot with lid (4 cm diameter wide and 2.5 cm deep). In addition, each time a mating pair was removed from the large cage, an unmated female and unmated male were also removed and transferred individually into plastic pots. Mating pairs were observed, the total time of mating was recorded and the flies were subsequently frozen. Unmated females and males that had been removed along with mating pairs were also frozen, when the mating pair stopped mating. The amount of dyed Entostat on medflies was extracted and quantified as described in Experiment 1.

Statistical analysis
In Experiment 1, a pairwise t-test was used to compare amounts of powder on males and females at the different time intervals. Also in Experiment 1, regression analysis was used to describe the exponential decay of dyed powder on male and female medflies. The average
amount of powder extracted immediately after coating the flies was set to 100 % and the subsequent extractions were transformed into a percentage of this amount. The following equation was used:

Equation 1: \( F(h) = \frac{1}{a + b \times h} \)

in which \( h \) is the number of hours after coating and \( a \) and \( b \) are fitted coefficients. In Experiment 3, we used a pairwise t-test to compare amounts of powder extracted from mated and unmated males and females.

**Results and Discussion**

**Experiment 1. Powder retention by males and females**

Immediately after coating, male medflies carried, on average, 190.9 µg ± 26.3 SE, while an average of 154.1 µg ± 18.9 was carried by females. After 1 h, males and females were, on average, coated with 57.5 µg ± 8.1 and 65.6 µg ± 3.3, respectively. After 72 h, males and females were, on average, coated with 1.3 µg ± 0.3 and 4.9 µg ± 1.8, respectively. Using pairwise t-test, we found no significant differences in amounts of powder extracted from males and females after the different time intervals (P > 0.05). Highly significant regression lines (P < 0.001) described the gradual decline in amounts of powder extracted from male and female medflies up to 72 h after coating with a marked decline in powder retention during the first 2 h, while about 5% (approximately 10 µg) of the initial amount of powder was retained on both sexes up to 47 h after coating (Fig. 1).

**Fig. 1. Exponential decay of powder retention**

The amount of powder extracted at ¼, ½, 1, 2, 24, 48, and 72 h after coating the flies were converted into percentage of the amount of powder extracted immediately after coating. “a” and “b” are fitted coefficients in Equation 1, which was used to describe the exponential decay of powder retention.
Conventional calculations of LD’s (lethal dosages) do not specify the required dosage to kill individual insects, but knowledge about the amounts extracted from medflies over time is crucial for future optimisation of the concentration of active ingredients in this delivery system. For comparison, Nansen and Phillips (2004) conducted a toxicity test in which either the tip of a leg or an antenna of a virgin *Plodia interpunctella* male was touched <3 sec into a dot of attracticide gel containing 0, 3, 6, 12, or 18 % (w/w) permethrin. The toxicity test showed that such brief and gentle contact of *P. interpunctella* males with attracticide gel containing 3-18% permethrin caused a significant reduction in mating and killed males moths within 24 h. Thus, it seems reasonable to assume that 10 µg of powder on medflies after 24 h: 1) is a high enough concentration to kill the contaminated medfly and 2) means that the contaminated fly retains enough powder to be able to transfer lethal dosages to conspecifics. A relevant topic for future research (i.e. through image analysis) is to determine whether powder deposited on certain body parts is retained better than at other body parts and how this information can be used to optimise a powder dispensing system so that medflies are likely to be coated on these body parts. Some preliminary work (unpublished data) with SEM images indicated that the powder is retained for longer on the underside of the fly and between intersegmental membranes, probably because these areas are more difficult to groom.

**Experiment 2 Flight behaviour of powdered medflies of mixed sex**

The main objective of this experiment was to determine whether medflies could recover and perform flights after heavy coating with Entostat. The amount of Entostat applied to medflies was obviously many times higher than what they would take up by visiting a baiting station, so this experiment represents “the worst case scenario”. Over a 2 h period, a total of 924 flights were made by control flies and about half as many flights, 440, were performed by coated medflies. For the first 15 min, coated medflies showed negligible flight activity but after that showed a steady increase in flight activity (Fig. 2). About 100 min after the onset of the experiment, coated and uncoated flies showed similar flight activity. Thus, it appeared that the flies needed approximately 100 min to remove excessive amounts of powder from antennae and other body parts before being able to perform normal flight behaviour. Using the exponential curve in Fig. 1, this means that the flies started flying when approximately 25 % (40-50 µg) of the initial amount of powder was still retained on their body.

![Fig. 2. Flight activity of coated medflies compared to uncoated flies](image-url)
Numbers of flights by 30 flies were recorded in 5 min intervals for both powdered and unpowdered (control) flies during 120 min. For each 5 min interval, the numbers of flights by powdered flies were transformed into percentage of unpowdered flies. The dotted line is a linear regression of the flight activity unpowdered flies, and 100% indicates that powdered and unpowdered flies had similar flight activity (about 100 min after coating).

**Experiment 3: Powder transfer from powdered males to conspecific females**

A total of 14 pairs of mating flies were reported over a 5 h period. The first mating was observed 30 min after the females had been introduced into the cage, and the last mating occurred 3 h 36 min after the females had been introduced. Mating duration was, on average, 101.5 min ± 13.1 (range 13 – 191 min). On average, 1.2 µg ± 0.3 was extracted from unmated females, whilst 3.8 µg ± 2.2 was extracted from mated females. Thus unmated females appeared to take up some powder, probably through contact with coated surfaces upon which male flies had been grooming, or transferred from males during unsuccessful mating attempts. However, about 3 times more powder was extracted from mated females compared to unmated females and that difference was found to be highly significant (t₁₃ = 4.43, P < 0.01). 3.8 µg powder carried by mated females, it is important to mention that males, on average, started to mate about 3.44 h ± 0.2 (3 hours and 26 min) after they had been coated with powder. According to Equation 1 and Fig. 1, males only carried 12.5% of the initial amount of powder (23.9 µg) 3.44 h after coating, which means that females acquired about 16% of the powder retained on the males’ body at the onset of mating.

White columns denote the amount of powder extracted from males after completing their mating with females. Grey columns denote the predicted amount of powder based Equation 1 with time from coating until completing mating as independent variable.

Although significantly more powder was extracted from mated females compared to unmated females, there was no significant difference in amounts of powder extracted from mated and unmated males (t₁₃ = 1.10, P = 0.29), which suggests that the transfer of powder from males to females did not affect the amount retained by males. For each of the 14 pairs of
mated flies, we examined the correlation between the amount of powder extracted from the male and the female after mating, but there was no significant correlation (P > 0.05). For each of the mated medfly males, we compared the amount of powder extracted from them after mating with the amount that, based on Equation 1, we would have expected to extract. That is, we knew how long males had been coated for in Experiment 3 before freezing (acclimation time before introduction of females = 1 h + time before mating + mating duration), and that total time was introduced into Equation 1 to determine how much powder should be on each male medfly. Unexpectedly, considerably more powder was extracted all 14 mated males than what was predicted based exclusively on time since coating (Fig. 3). The implications of this finding are not straightforward, but one possible explanation is that while mating, both males and females are sitting very still for, on average, 101.5 min, and that could explain why more powder is retained by these mating males compared to the predictions based on males separated from females. We conducted the same calculations with unmated males in Experiment 3, and again the predicted amounts of powder were markedly higher than observed. It is therefore possible that in the presence of conspecific females males were spending less time grooming so that the rate of loss of powder was reduced. More detailed studies are needed to address this important aspect of medflies’ behavioural responses to being coated with Entostat.

Final remarks

In experiments where males and females were kept separately, we showed that about 2 days after coating medflies with Entostat, as much as 10 µg is retained on their body, which underscores that a delivery system based on Entostat and a slow-acting pesticide or biological may have considerable potential. Additionally, we showed that the exponential decline in powder retention on medfly males appeared to be considerably slower when exposed to conspecific females than initially predicted. This type of detailed behavioural knowledge is critical to the successful development of insect control systems. In order for our system to be successful, it is important that powder-coated flies are able to behave normally in order to transfer powder to conspecifics. We showed that initially the flight behaviour by medflies is indeed affected by coating with Entostat, but that the flies recovered within 100 min after the coating. We also showed that after as little as 90 min after coating, male flies would start mating with female flies and that they acquired about 16% of the powder retained on the males’ body at the onset of mating.

Acknowledgement

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References


Effects of gamma-radiation on midgut proteases of Ceratitis capi
tata (Diptera: Tephritidae)

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The Mediterranean fruit fly or medfly, Ceratitis capi
tata (Wiedemann), has a negative economic impact on citrus crops in Spain due to direct damage to fruits and to quarantine restrictions. Biologically based control methods, such as the Sterile Insect Technique (SIT) is gaining an increasing role in the control of medfly populations. However, gamma-radiation might damage the midgut epithelium cells, causing a lowering of nutritive assimilation and result in a short lifespan of adults. The studies of radiation effects on digestive physiology are well established for a number of insect pests, but there is no information on medfly. Our aim was to determine the effects of radiation on C. capitata digestive protease activity. Both larvae and adults were found to use a similar proteolytic system based on aspartyl-, trypsin-, chymotrypsin-, amino peptidase- and carboxypeptidase A- and B-like activities. Male pupae of the tsl vienna-7 strain were irradiated at 70 and 140 Gy, two days before emergence, and the adults fed during 5 days with solid diet (sugar:protein, in a ratio of 4:1). Protease activity was measured in midgut extracts and compared with males non irradiated reared in the same conditions. The results showed that the radiation doses tested do not affect the digestive proteolytic activities of the tsl vienna-7 strain.
Identification and abundance of ants (Hymenoptera: Formicidae) in Citrus trees from Valencia (Spain)

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Abstract: Ten commercial citrus plantations from Valencia (Spain) were sampled fortnightly all along the year for three years (1999 to 2001) to determine the identity, abundance and seasonal trend along the year of the ants found on the canopy of the trees. Samples were collected by aspiration with an engine-powered suction-machine and each simple came from about 8,000 leaves. From 15,983 adults, thirteen species of Formicidae were identified. The most common species, *Lasius niger* (Linnaeus), included 65% of the ants. The second species in abundance, with 21%, was *Pheidole pallidula* (Nylander). Followed in abundance *Plagiolepis pygmaea* Latreille, *Pl. schmitzii* Forel, and *Formica gerardi* Bondroit, which included altogether 11% of the ants identified. *L. niger* and *P. pallidula* showed two annual peaks between May and September. *L. niger* reached high population levels in spring and summer and *P. pallidula* mostly in summer. There was apparently a competitive displacement between the two species of ants as we found a negative correlation between their abundance in the same orchards. Positive correlations appeared between the abundance of *Ph. pallidula*, *Plagiolepis schmitzii* and *Tapinoma erraticum* Latreille. *L. niger* was associated to orchards with abundant aphid populations.

Key words: Ants, citrus, formicidae, seasonal trend.

Introduction

Many ant species have been described in citrus crops all around the world. Haney (1988), in a revision of the ants present in citrus crops from 50 countries, includes 295 species. In South Africa there are 123 ant species in citrus, out of which 44 live on the tree canopy and 25 have been observed collecting honeydew produced by homopteran insects. From them, only three or four species are considered responsible for outbreaks of different insect pests (Samways et al., 1982; Samways et al., 1998). The influence of the argentine ant *Linepitema humile* in pest outbreaks has been studied in citrus orchards from California. Important negative effects were confirmed with this ant species as inducer of population increases of several arthropod pests of the crop (Haney et al., 1987; Moreno et al., 1987).

Several papers have also been published in the Mediterranean area dealing with the influence of ants in citrus pest outbreaks. In France, Panis (1981) shows that *L. humile* reduces the parasitism caused by *Metaphycus helvolus* (Compere) on the black scale *Saissetia oleae* (Olivier). In Israel, Rosen (1967) reports up to 13 ant species on citrus crops, most of them associated with pest outbreaks. Several ant species are also described in Italy causing pest outbreaks in citrus crops (Di Martino, 1957; Tumminelli et al., 1996). Some studies on ants in citrus have also been published in Spain. Recently, the identity and influence of ant communities was studied in the soil of citrus orchards in Tarragona and Valencia, including information on their biology, ecology and seasonal changes in abundance (Palacios et al., 1999; Urbaneja et al, 2006).
The objectives of our research work were the identification of the species of ants present on the canopy of citrus trees in the Valencia area, determining the relative abundance of different species along the year and analyzing the relationship in abundance between the ant species and with different arthropod pests of the crop.

Materials and Methods

Samples were collected in ten citrus orchards in the País Valencià, in five localities, Riola, Carlet, Godella, Quartell and Cheste, with two nearby orchards on each locality. All the orchards were located in an area of 50 km around the town of Valencia, at the center of the main Spanish citrus growing area. All orchards were commercial plantations subjected to the cultural practices usually applied in the area. This practices included in eight of the orchards one or two chemical sprays per year to control armoured scales or other pests (in most cases with organophosphates plus mineral oil). The two orchards from Riola were not sprayed with pesticides during the sampling period.

The orchards were sampled fortnightly all along the year for three consecutive years (1999-2001). In all, 594 samples were collected. Sampling was performed with an engine-powered suction machine, McCulloch model Mac 320 BV of 1.1 Kw, modified with a cylindrical plastic hose 30 cm in diameter and 30 cm long. The hose cylinder was applied on a group of 20-30 leaves for a few seconds, repeating the procedure 280 times to form a sample, all around the tree canopy on at least 30 different trees per orchard. In the laboratory, the collected material was sieved with different size meshes and conserved at -20ºC. The formicidae were identified with the keys of Collingwood (1978), Collingwood and Prince (1998), and Martínez et al. (1985). The identifications were confirmed by Dr. Alberto Tinaut Ranera, from the Universidad de Granada (Spain).

Results

Species identified

In all, 15,983 ants were identified, with 13 different species. Most of the specimens (10,455) belonged to the most abundant species, Lasius niger (Linnaeus). The second species in abundance, with 3,401 specimens, was Pheidole pallidula (Nylander). The three following species, Plagiolepis pygmaea (Latreille), Pl. schmitzii Forel, and Formica gerardi Bondroit, included together 1,775 specimens. The remaining eight species (Camponotus sylvaticus Olivier, C. foreli (Emery), L. humile (Mayr), Tapinoma nigerrimum (Nylander), T. erraticum (Latreille) y T. simrothi (Krausse), Cardiocondyla mauritanica Forel y Tetramorium semilaeve André) were much less abundant. L. niger was the most abundant species in seven orchards (Table 1), being also common in two of the remaining three orchards sampled.

The relative abundance of species was very similar in the three years sampled (1999 to 2001). There is a high correlation in the total number of each species of ant found per year between two consecutive years (r = 0.929 between 1999 and 2000, and r = 0.996 between 2000 and 2001) reflecting a stability in the composition and abundance of the ant fauna with time.

Association between ant species

When studying the correlation coefficient between the abundance of ant species per orchard and year, a negative association was found between L. niger and L. humile (r = -0.708; P < 0.01), especially during 1999, as L. humile was almost absent in 200 and 2001. A negative association was also found between the two most common species, L. niger and Ph. Pallidula (r = -0.558; P < 0.05). The two Riola orchards show the most original and differentiated ant
fauna. *Ph. Pallidula* y *Pl. schmitzii* are mostly found in these two orchards, showing these ant species a positive association in abundance (*r* = 0.806; *P* < 0.01).

Table 1. Number of formicids identified in ten citrus orchards sampled fortnightly all along the year for three years (1999-2001) in Valencia (eastern Spain).

<table>
<thead>
<tr>
<th>O R C H A R D</th>
<th>Carlet A</th>
<th>Carlet C</th>
<th>Catadau</th>
<th>Cheste navel</th>
<th>Cheste A</th>
<th>Godella A</th>
<th>Godella Caixo</th>
<th>Godella Font</th>
<th>Quaretell Peatje</th>
<th>Riola Jove</th>
<th>Riola Peatje</th>
<th>Vell Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lasius niger</em></td>
<td>796</td>
<td>753</td>
<td>1781</td>
<td>2147</td>
<td>232</td>
<td>1175</td>
<td>943</td>
<td>1830</td>
<td>767</td>
<td>31</td>
<td>10455</td>
<td></td>
</tr>
<tr>
<td><em>Pheidole pallidula</em></td>
<td>23</td>
<td>158</td>
<td>74</td>
<td>4</td>
<td>35</td>
<td>13</td>
<td>72</td>
<td>105</td>
<td>856</td>
<td>2061</td>
<td>3401</td>
<td></td>
</tr>
<tr>
<td><em>Plagiolepis pygmaea</em></td>
<td>1</td>
<td>9</td>
<td>20</td>
<td>1</td>
<td>307</td>
<td>30</td>
<td>498</td>
<td>14</td>
<td>6</td>
<td>22</td>
<td>908</td>
<td></td>
</tr>
<tr>
<td><em>Plagiolepis schmitzii</em></td>
<td>2</td>
<td>6</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>278</td>
<td>201</td>
<td>510</td>
<td></td>
</tr>
<tr>
<td><em>Formica gerardi</em></td>
<td>11</td>
<td>10</td>
<td>116</td>
<td>214</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>357</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Camponotus sylvaticus</em></td>
<td>9</td>
<td>93</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td><em>Linepithema humile</em></td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>40</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td><em>Tapinoma nigerrimum</em></td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>6</td>
<td>27</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td><em>Tapinoma erraticum</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>2</td>
<td>27</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td><em>Camponotus foreli</em></td>
<td>1</td>
<td>25</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td><em>Tapinoma simrothi</em></td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td><em>Cardiocondyla mauritanica</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Tetramorium semilaevae</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Total** | 845 | 1064 | 2015 | 2374 | 609 | 1220 | 1514 | 2004 | 1926 | 2412 | 15983 |

**Seasonal trend**

*L. niger* showed a population fluctuation in abundance similar in the three years, with two peaks, the first (in May-June), higher than the second (in July-August). Ants were almost absent from the tree canopies in the coldest months of the year, from November to March (Fig. 1). *P. pallidula* showed a rather different trend, appearing on the leaves two months later, with two annual peaks, the first (in May-June), lower than the second (in August-September) (Fig. 1). *P. pallidula* was clearly more abundant at the end of the year.

**Relationship with the abundance of pests**

We related the abundance of ants with the abundance in the same orchards and years of several species of phytophagous arthropods, considering that the existence of significant correlations is not necessarily proof of causality. We found a positive relationship between the abundance of *Lasius niger* and the abundance of aphids (*r* = 0.658; *P* < 0.05). The five orchards with the highest population of aphids were also the orchards with the highest level of the ant. *Pheidole pallidula* is usually found in orchards with high populations of diaspidid scales (*r* = 0.729; *P* < 0.01). Finally, a positive correlation was found between the presence of *Plagiolepis pygmaea* and *Icerya purchasi* (*r* = 0.598; *P* < 0.05).
Figure 1. Seasonal trend in the abundance of the two most common ant species found on leaves of ten citrus orchards from Valencia (eastern Spain), sampled fortnightly all along the year during three years (1999-2001).

**Discussion**

The species of ants found in our survey of citrus orchards in Valencia (Spain) differ from the species reported in other areas where similar surveys have been undertaken, as South Africa (Samways *et al.*, 1982; 1998), Florida (Haney, 1988) or California (Flanders, 1958; Haney 1988). Studies developed in the Mediterranean area like Israel (Rosen, 1967), Italy (Di Martino, 1957; Liotta, 1963; Tumminelli *et al.*, 1996) and France (Panis, 1981) yielded also a species composition rather different. Our two most common species, *Lasius niger* and *Pheidole pallidula*, are usually not reported in those studies. However, recent surveys carried out in the soil of citrus orchards in Tarragona (Northeast Spain) showed species composition and abundance similar to our results, with *L. niger* as the most abundant and *P. pallidula* as the second species (Palacios *et al.*, 1999).

*L. niger* is a species common in the mirmecofauna of many countries. It is reportedly associated with aphids, protecting them on the plants and in their nests. *L. niger* disturbs the biological control by attacking the natural enemies of aphids and other homoptera (Itioka and Inoue 1996a, 1996b). These authors demonstrated that *L. niger* displaced other ant species in
citrus orchards (Itioka and Inoue, 1999). *Ph. pallidula* is a species of tropical origin widely distributed in southern Europe and adapted to warmer climates than *L. niger*. We found *P. pallidula* to be especially abundant during the summer, whereas *L. niger* predominated during the spring. *P. pallidula* is omnivorous, including in its diet live or dead insects, seeds and honeydew produced by aphids and other homoptera (Bernard, 1968; Detrain, 1990). The apparent negative association that we found between *L. niger* and *P. pallidula* could be caused by a direct competitive displacement, but could also be produced by its preference for different environmental conditions on the citrus orchards. We have observed that apparently *L. niger* prefers orchards with drip irrigation and with bare soil, whereas *P. pallidula* appears in orchards with flood irrigation, with the soil covered by weeds (at least in spring) and not heavily sprayed with pesticides. However, more observations are needed to confirm or deny these preferences.

In summary, *L. niger* was the most abundant species found in the canopy of cultivated citrus trees in Valencia (eastern Spain), with two annual peaks in abundance, in spring and autumn. The second species in abundance was *P. pallidula*, which proliferates especially in summer. The ant fauna is rather similar along the years in the same orchards but can be very different in different orchards. A negative relationship was found between the two most abundant ant species, *L. niger* and *P. pallidula*.

An important aspect of the presence of ants in the citrus orchards is the relationship they show with different phytophagous arthropods. Positive significant correlations in abundance were found between *L. niger* and aphids, *Ph. pallidula* and diaspidids, and *Plagiolepis pygmaea* with *Icerya purchasi*. Our observations do not allow us to infer a causal relationship between the presence of ants and the proliferation of pests, but show some interesting correlations that deserve further research to clarify the role the different ant species are playing in the citrus agroecosystem as inducers of pest outbreaks.

**Acknowledgements**

The authors are indebted to Alberto Tinaut Ranera, from the University of Granada, for his help in the identification of the ants, to Carmen Marzal, Vicente Bueno, José Manuel Rodriguez, Andrés Alonso and Carlos Granda, for their help in the field and the laboratory, and to the farmers that allowed us to sample their orchards. Economic support was provided by the Conselleria de Agricultura de la Generalitat Valenciana and the Agencia Española de Cooperación Internacional (AECI).

**References**


Interactions between ground cover management, hedges and aphids in lemon orchards

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Abstract: An experiment was carried out in three lemon orchards of Mafra region, in Portugal, aiming at studying the influence of ground cover management and hedges on the diversity and abundance of aphids. Three different weed management systems were considered, i.e., ground cover with resident vegetation, ground cover by sowing a selected seed mixture and herbicide application. Samples were collected from January up to November 2003 on lemon trees, ground cover vegetation and hedges both by visual observation and using “Vortis” arthropod suction sampler. A total of 1945 specimens were identified including 44 species from 28 different genera. Among these species, two were reported for the first time in Portugal, \(\text{Illinoia goldamaryae}\) (Knowlton) and \(\text{I. morrisoni}\) (Swain), and one in Continental Portugal, \(\text{Atheroides serrulatus}\) Haliday. From the identified aphid species, all but \(\text{Toxoptera auranti}\) (Boyer de Fonscolombe) and \(\text{Aphis spiraecola}\) Patch are non-economic important for citrus crops in Continental Portugal. \(\text{Aphis gossypii}\) Glover was never observed during the study. \(\text{T. auranti}\) was only detected on three plant species in ground cover vegetation, i.e., \(\text{Erodium moschatum}\), \(\text{Senecio vulgaris}\) and \(\text{Trifolium campestre}\), and on \(\text{Pittosporum undulatum}\), in the hedges. The infestation level of aphids, mainly \(\text{T. auranti}\), on lemon trees was low in all three orchards. However, the average number of aphids was higher in plots where herbicide was used compared with plots with ground cover vegetation. The possibility of exploring the interactions between aphids and ecological infrastructures, such as ground cover vegetation and hedges, to promote conservation biological control is discussed.

Key words: aphids, citrus pests, habitat management

Introduction

Aphids are considered worldwide citrus pests. They originate direct damage as a result of sap sucking, leaf deformation, reduction of shoot growth and drop of flowers and young fruits. Indirect damage is associated with honeydew excretion resulting in the development of sooty mould and, most important, the transmission of virus diseases (Barbagallo & Pati, 1986; Katsoyannos, 1996; Smith et al. 1997; Bedford et al., 1998).

About 20 aphid species may injure citrus crops. However, only a few species are of economic importance (Barbagallo & Pati, 1986). In Portugal, from the 10 species reported in citrus (Ilharco, 1993; unpubl. data), namely \(\text{Aphis craccivora}\) Koch, \(\text{A. fabae}\) Scopoli, \(\text{A. gossypii}\) Glover, \(\text{A. nasturtii}\) Kaltenbach, \(\text{A. spiraecola}\) Patch, \(\text{Aulacorthum solani}\) (Kaltenbach), \(\text{ Macrosiphum euphorbiae}\) (Thomas), \(\text{Myzus ornatus}\) Laing, \(\text{M. persicae}\)
(Sulzer) and *Toxoptera aurantii* (Boyer de Fonscolombe), only three, *A. spiraecola*, *T. aurantii*, *A. gossypii*, are major citrus pests (Carvalho et al. 1997).

Aphids may also be considered as potentially beneficial in pest management (Ilharco & Vieira, 1992; Nentwig et al., 1998) in particular those species associated with ecological infrastructures (Boller et al., 2004), such as ground cover vegetation within the orchard, hedges and windbreaks, that don’t use citrus as host plants. In fact, these species may contribute to maintain and enhance natural enemies populations of citrus pests, in two different ways: 1) excreted honeydew can be used as food by adult parasitoid and predators of citrus pests; 2) aphid may constitute alternate host/prey for natural enemies, including parasitoid and predators of aphid pests of citrus and generalist predators.

In this paper we present the results of a study carried out in lemon orchards of Mafra, in the Oeste region of Portugal, aiming at identifying the aphid species associated with ground cover vegetation and hedges and studying the influence of the weed management system on the species composition and abundance of aphid fauna.

**Material and methods**

**Experimental plots**
The study was carried out in three 1-4 ha lemon orchards from Mafra, in the Oeste region of Portugal, ca. 30 Km north of Lisbon, namely Carrasqueira de Cima (CC), Casal Mato de Cima (CMC) and Pinhal de Frades (PF). During the study, farmers followed an IPM system under the technical supervision of Frutoeste, a farmers association of Oeste region.

**Ground cover management**
In each orchard, three weed management systems were installed in 2002: 1) inter-row ground cover with resident vegetation (RV), 2) inter-row ground cover by sowing selected species (S) and 3) inter-row herbicide application, i.e., diuron+glyphosate+terbutilazine (H). The sown species were *Lolium multiflorum*, *L. perenne*, *Medicago polymorpha*, *Trifolium fragiferum*, *T. incarnatum* and *T. resupinatum*. Each management system was installed in plots of five or six tree rows.

**Sampling methods**
The infestation dynamics of the aphids in lemon trees was monitored from January up to November 2003 every two to four weeks. A total of 50-100 young shoots (2-4 per tree) were observed per modality in order to estimate the infestation level.

Samples were also monthly collected in lemon trees, orchard hedges and ground cover vegetation, from March up to September 2003, using the “Vortis” suction sampler (Arnold 1994), following a sampling procedure previously defined (Rodrigues et al. 2003). Each sampling unit consisted of in suctioning the foliage with a 8 cm diameter flexible tube (estimated airflow = 34.8 m/s) in three different positions, during four seconds per position. Ten sampling units were collected per modality and orchard. In the case of ground cover, samples were collected only from March up to May, because since June the vegetation reduced significantly due to water stress.

Samples were studied in the laboratory under magnification for the separation of aphids that were kept in 70% ethyl alcohol for posterior identification.

**Results and discussion**

**Infestation dynamics**
The pattern of infestation dynamics of the aphids was similar in the three lemon orchards (Figure 1). Aphids, mainly *T. aurantii*, were detected for the first time in a few shoots in the end of January. However, the aphid infestation increased only since early April, attaining maximal level
(30% - 58% infested shoots) in the end of May – early June and decreasing sharply afterwards. Maximum infestation level was observed in the “H” modality in two of the three studied orchards.

Despite the different aphid density observed in the experimental plots, in average it was consistently higher in the “H” modality in all three lemon orchards (Figure 2).

**Figure 1.** Infestation dynamics of aphids in lemon trees in the three studied orchards, Casal Mato de Cima (CMC), Pinhal de Frades (PF) and Carrasqueira de Cima (CC), in function of the weed management system: ground cover with resident vegetation (RV), ground cover by sowing selected species (S) and herbicide application (H).

**Figure 2.** Mean aphid density registered in lemon shoots in the three studied orchards, Casal Mato de Cima (CMC), Pinhal de Frades (PF) and Carrasqueira de Cima (CC), in each of the weed management systems: ground cover with resident vegetation (RV), ground cover by sowing selected species (S) and herbicide application (H).

Data collected by suction sampling (Figure 3) corroborate the results from visual observation monitoring (Figure 1). The number of captured aphids deeply increased from March
up to May. No aphid was collect in June and July and just a few individuals were sampled in September. The maximal number of captures was registered in the “H” modality.

The observed differences on the infestation pattern and average level of aphids between “S”/”RV” and “H” weed management modalities are consistent with the hypothesis of an earlier colonisation of lemon trees by natural enemies as a result of the presence of ground cover vegetation.

Figure 3. Number of aphids collected per plot by suction sampling in lemon trees from March up to September 2003, in each of the weed management systems: ground cover with resident vegetation (RV), ground cover by sowing selected species (S) and herbicide application (H).

**Aphid species and host plants**

A total of 1945 specimens were identified including 44 species from 28 genera and four families, namely Aphididae (39), Drepanosiphidae (3), Lachnidae (1) and Penfigidae (1) (Table 1). *Illinoia goldamaryae* (Knowlton) and *I. morrisoni* (Swain), respectively collected on *Conyza albida* from ground cover vegetation and *Cupressus* sp. hedges, are reported for the first time in Portugal. *Atheroides serrulatus* Haliday is also a first record to continental Portugal.

None of the sampling methods was able to collect all the species. Suction sampling allowed the identification of 34 species (77%) and plant sampling the identification of 29 (66%). For example, two of the new recorded species, i.e., *I. morrisoni* and *A. serrulatus*, were collected by suction sampling only.

Based on suction sampling data, the number of aphid species sampled in lemon trees, ground cover vegetation and hedges was respectively 17, 23 and 14 (Table 2-3). In lemon trees, most of the identified species were not related with citrus and correspond to captures of few individuals occasionally sampled (Table 2). The majority of the captures consisted of *T. aurantii*. From the five aphid species recorded from lemon in Portugal, i.e., *A. gossypii*, *A. spiraecola*,
A. solani, M. euphorbiae and T. aurantii (Ilharco, 1996), only T. aurantii was captured in the present study.

As expected, in ground cover vegetation, the number of identified species was higher in the “S” modality (19), where plant diversity was also higher, in comparison to “RV” (16). The dominant species was Rhopalosiphum padi (Linné), an aphid associated with grasses. Other representative species include Acrithosiphon pisum (Harris), Metopolophium dirhodum (Walker), Sitobion fragariae (Walker), Macrosiphum euphorbiae (Thomas) and Aulacorthum solani (Kaltenbach) (Table 2).

In orchard hedges, samples were collected from giant reed, Arundo donax, Australian cheesewood, Pittosporum undulatum, and cypress, Cupressus sp. (Table 3). Melanaphis donacis (Passerini) was the only representative species collected in A. donax with maximum captures registered in April and May. I. morrinsoni was the main aphid species captured in cypress whose presence was detected in all samples from March up to September. T. aurantii was collected in small quantities from the Australian cheesewood from April up to June. This presence was confirmed by visual observation in the end of June, when T. aurantii was detected in 10% of the shoots.

A total of 24 aphid species were identified from direct collection in infested plant species of ground cover vegetation (Table 4). From the 25 host plants identified, 12 were associated with two or more aphid species. Among the host plants from which three or more aphid species were recorded are Bromus hordeaceus, Chenopodium album, Conyza albida, Lolium perenne and Senecio vulgaris. T. aurantii was collected from Erodium moschatum, Senecio vulgaris and Trifolium campestre. All but one sample, consisting of nymphs and winged forms collected from S. vulgaris, were composed of winged individuals of T. aurantii.

**Conclusion**

The diversity and abundance of aphid species in citrus orchards ecosystems is influenced by plant diversity in ecological infrastructures such as hedges and ground cover vegetation. By selecting or favouring the most adequate host plant diversity, through habitat management, it is possible to increase the abundance and diversity of aphid species, in particular non-citrus pests. Therefore, the manipulation of hedges and ground cover vegetation may enhance conservation biological control of citrus pests, as aphids constitute a potential source of honeydew and alternative host/prey for natural enemies.
Table 1. Aphid species identified in the three studied lemon orchards, by suction and plant sampling in lemon trees, hedges and ground cover vegetation.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Sampling method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>suction</td>
</tr>
<tr>
<td>Aphididae</td>
<td><em>Acyrthosiphon malvae</em> (Mosley) s. str.</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Acyrthosiphon pisum</em> (Harris)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Amphorophora rubi</em> (Kaltenbach)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Aphis arbati</em> Ferrari</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Aphis craccivora Koch</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Aphis fabae</em> Scopoli</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Aphis rumicis</em> Linné</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Aphis solanella</em> Theobald</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Aphis spiraecola</em> Patch</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Aphis umbrela</em> (Borner)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Aulacorthum solani</em> (Kaltenbach)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Brachycaudus cardui</em> (Linné)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Brachycaudus helichrysi</em> (Kaltenbach)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Capitophorus elaeagni</em> (Del Guercio)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Cavariella aegopodii</em> (Scopoli)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Dysaphis</em> spp.</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Hyadaphis coronadi</em> (Das)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Hyadaphis foeniculi</em> (Passerini)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Hyperomyzus lactucae</em> (Linné)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Illinoia goldamaryae</em> (Knowlton)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Illinoia morrisoni</em> (Swain)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Lipaphis erysimi</em> (Kaltenbach)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Macroaspis euphorbiae</em> (Thomas)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Macroaspis rosae</em> (Linné)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Megoura viciae</em> Buckton</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Melanaphis donacis</em> (Passerini)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Metopolophium dirhodum</em> (Walker)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Myzus ornatus</em> Laing</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Myzus persicae</em> (Sulzer)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Nasonovia ribisnigri</em> (Mosley)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Protaphis terricola</em> (Rondani)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Rhopalosiphum padi</em> (Linné)</td>
<td>x</td>
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<tr>
<td></td>
<td><em>Rhopalosiphum rafialdominalis</em> (Sasaki)</td>
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<tr>
<td></td>
<td><em>Sitobion avenue</em> (Fabricius)</td>
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<td></td>
<td><em>Sitobion fragariae</em> (Walker)</td>
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<td></td>
<td><em>Toxoptera auranti</em> (Boyer de Fonscolombe)</td>
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<tr>
<td></td>
<td><em>Uroleucon jaceae</em> (Linné) s. str.</td>
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<tr>
<td></td>
<td><em>Uroleucon mierae</em> Tizado &amp; Nafria</td>
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<tr>
<td></td>
<td><em>Uroleucon sonchi</em> (Linné)</td>
<td>x</td>
</tr>
<tr>
<td>Drepanosiphidae</td>
<td><em>Anoecia corni</em> (Fabricius)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><em>Atheroides serrulatus</em> Haliday</td>
<td>x</td>
</tr>
<tr>
<td>Lachnidae</td>
<td><em>Theroaphis trifolii</em> (Monell)</td>
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</tr>
<tr>
<td>Penfigidae</td>
<td><em>Cinara cupressi</em> (Buckton)</td>
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</tr>
<tr>
<td></td>
<td><em>Tetraneura caerulescens</em> (Passerini)</td>
<td>x</td>
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</tbody>
</table>
Table 2. Aphid species collected by suction sampling in lemon trees and ground cover vegetation, in function of the weed management system, i.e., ground cover with resident vegetation (RV), ground cover by sowing selected species (S) and herbicide application (H), and the period of capture (March up to September).

<table>
<thead>
<tr>
<th>Aphid species</th>
<th>Lemon trees</th>
<th>Ground cover</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S RV H S RV</td>
<td>S RV</td>
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<tr>
<td>A. pisum</td>
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<td>A. corni</td>
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<td>A. arbuti</td>
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<td>A. fabae</td>
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<tr>
<td>A. solanella</td>
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<tr>
<td>A. serrulatus</td>
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<td>A. solani</td>
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<tr>
<td>B. helichrysi</td>
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<tr>
<td>C. elaeagni</td>
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<tr>
<td>H. lactucae</td>
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<tr>
<td>I. goldamarie</td>
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<tr>
<td>L. erysimi</td>
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<tr>
<td>M. euphorbiae</td>
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<tr>
<td>M. viciae</td>
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<td>M. donacis</td>
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<td>M. dirhodum</td>
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<td>P. terricola</td>
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<tr>
<td>R. padi</td>
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<tr>
<td>R. rufiabdominalis</td>
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<td></td>
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<tr>
<td>S. avenae</td>
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<td></td>
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<tr>
<td>S. fragariae</td>
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<td></td>
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<tr>
<td>T. trifolii</td>
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<td></td>
</tr>
<tr>
<td>T. caerulescens</td>
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<td></td>
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<tr>
<td>T. aurantii</td>
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<td></td>
</tr>
<tr>
<td>U. jaceae</td>
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<td></td>
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<tr>
<td>U. mierae</td>
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<td></td>
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<tr>
<td>U. sonchi</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: Number of captured aphids
- < 10
- 11-30
- 31-50
- 51-100
- > 100

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Table 3. Aphid species collected by suction sampling in lemon orchards hedges and respective period of capture (March up to September).

<table>
<thead>
<tr>
<th>Aphid species</th>
<th>Arundo donax</th>
<th>Pittosporum undulatum</th>
<th>Cupressus sp.</th>
</tr>
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<tr>
<td>A. rabi</td>
<td>M</td>
<td>A</td>
<td>M</td>
</tr>
<tr>
<td>A. fabae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. spiraeola</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A. solani</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C. cupressi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysaphis spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. morrisoni</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. donacis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. dirhodum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. ornatus</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>M. persicae</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P. terricola</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>R. padi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. rufiabdominalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. aurantii</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Host plants and aphids collected in ground cover vegetation.

<table>
<thead>
<tr>
<th>Plant host</th>
<th>Aphid species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andryala integrifolia</td>
<td>M. euphorbiae</td>
</tr>
<tr>
<td>Arctotheca calendula</td>
<td>A. solani</td>
</tr>
<tr>
<td>Atriplex nodiflorum</td>
<td>C. aegopodii, H. foeniculi</td>
</tr>
<tr>
<td>Briza minor</td>
<td>S. avenae, S. fragariae</td>
</tr>
<tr>
<td>Bromus hordeaceus</td>
<td>M. dirhodum, S. avenae, S. fragariae</td>
</tr>
<tr>
<td>Calendula arvensis</td>
<td>M. euphorbiae</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>A. fabae, R. padi, U. Sonchi</td>
</tr>
<tr>
<td>Conyza albida</td>
<td>B. helichrysi, M. euphorbiae, M. dirhodum, I. goldamaryae</td>
</tr>
<tr>
<td>Crepis versicolor</td>
<td>N. ribisnigri</td>
</tr>
<tr>
<td>Dactyliis glomerata</td>
<td>S. fragariae</td>
</tr>
<tr>
<td>Daucus carota</td>
<td>H. coriandri, H. foeniculi</td>
</tr>
<tr>
<td>Erodium moschatum</td>
<td>A. malvae, T. aurantii</td>
</tr>
<tr>
<td>Hordeum marinum</td>
<td>M. viciae, S. avenae</td>
</tr>
<tr>
<td>Lavatera cretica</td>
<td>A. umbrelia</td>
</tr>
<tr>
<td>Lolium multiflorum</td>
<td>S. avenae</td>
</tr>
<tr>
<td>Lolium perenne</td>
<td>B. helichrysi, M. euphorbiae, H. lactucae, S. fragariae</td>
</tr>
<tr>
<td>Pseudognaphalium luteo-album</td>
<td>B. helichrysi</td>
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<tr>
<td>Rumex crispus</td>
<td>A. fabae, A. solanella</td>
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<td>Senecio vulgaris</td>
<td>A. solani, B. cardui, M. euphorbiae, M. ornatus, T. aurantii</td>
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<td>Sonchus asper</td>
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<tr>
<td>Trifolium campestre</td>
<td>R. rufiabdominalis, T. aurantii</td>
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<tr>
<td>Trifolium incarnatum</td>
<td>A. pisum</td>
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<td>Trifolium resupinatum</td>
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<td>Vicia sativa</td>
<td>M. viceae</td>
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<tr>
<td>Vulpia spp.</td>
<td>S. fragariae</td>
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</tbody>
</table>

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Acknowledgements

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References

Developing a mating disruption tactic for pest management of citrus flower moth

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Abstract: The citrus flower moth (CFM), *Prays citri* (Millière), is a key-pest of lemon orchards in several citrus production areas including the Oeste region of Portugal. The management of CFM is actually dependent on chemical control. Up to 12 insecticide treatments may be carried each year in this region. The dependence on chemical control constitutes a major constrain to the development of IPM strategies.

Mating disruption may constitute an alternative tactic for changing the pest status of CFM, in a compatible way with IPM. In order to evaluate the feasibility of using mating disruption to control the CFM in lemon orchards a project was initiated in 2002 (PO AGRO DE&D nº30), by studying the male flight activity of CFM and the dynamics of injury on flowers in 13 lemon orchards in the Oeste region of Portugal. Mating disruption experiments were carried out in 2003 and 2004, using Isonet-CFM, an experimental pheromone dispenser formulation developed by Shin-Etsu (Japan) in collaboration with CBC (Europe) and Biosani (Portugal). The results showed a clear “shutdown” effect on male captures in pheromone traps installed in mating disruption plots for both pheromone doses tested, i.e., 1000 and 2000 dispensers/ha. Further experiments are being conducted in 2005.

Keywords: citrus flower moth, lemon, mating disruption

Introduction

The citrus flower moth (CFM), *Prays citri* (Millière), is a key-pest of lemon orchards in several citrus production areas in the Mediterranean basin including the Oeste region of Portugal (Katsoyannos 1996; Carvalho et al., 1997). The management of CFM is actually dependent on chemical control. Up to 12 insecticide treatments may be carried out against CFM each year in this region. Until recently, phosphamidon was the only active ingredient registered in Portugal to control this pest. This situation contributed to the use by the growers of non-registered insecticides. A recent survey of the active ingredients used to control the CFM in the lemon production area of Mafra (Oeste region) suggested that more than 20 different insecticides have been used in the last 10-15 years and that deltametrin, lambda cyalotrin and luphenuron were among the most used active ingredients in the region. However, since 2003 luphenuron became the only registered insecticide for the control of CFM.

The dependence on chemical control is a major constraint to the development of IPM strategies. Mating disruption may constitute a sustainable alternative tactic for changing the pest status of CFM, in a compatible way with IPM. Despite promising results have been
reported in preliminary field trials in Italy (Capizzi et al., 1987), no commercial formulation is available for mating disruption of CFM.

In order to evaluate the feasibility of using mating disruption to control the CFM in Oeste region, a project was initiated in 2002 (PO AGRO DE&D nº 30) in collaboration with Frutoeste, a farmers’ association of Oeste region, aiming to: 1) characterise the infestation dynamics (seasonal male flight activity and dynamics of injury intensity) of the CFM in lemon production area of Mafra; 2) evaluate the experimental pheromone dispensers Isonet-CFM, developed by Shin-Etsu (Japan) in collaboration with CBC (Europe) and Biosani (Portugal) for mating disruption of CFM.

Material and methods

Citrus flower moth dynamics

In 2002, 13 lemon orchards were selected in the lemon production area of Mafra, in the Oeste region of Portugal, in order to characterize the infestation dynamics of CFM. In each experimental plot, the male flight activity of CFM was monitored using two pheromone delta traps (15 cm × 15 cm sticky plates) activated with 1 mg of the sexual pheromone. Male captures were weekly counted and pheromone dispensers were substituted every four weeks.

The injury intensity on lemon flowers was estimated by fortnightly sampling of 150 flowers per plot, collecting 1-2 flowers per tree in the phenological stage C-D (EPPO, 1984). Flower samples were isolated in plastic bags and transported to the laboratory where the presence of CFM eggs, larvae or injuries was determined under magnification, after flower dissection.

Mating disruption experiments

Experimental plots. In 2003, two orchards were selected for mating disruption experiments, Casal do Zambujeiro and Ponte de Cuco. In 2004, the mating disruption experiment was repeated in Ponte de Cuco and in another orchard, Quinta do Micharro (Table 1). The plot of Casal do Zambujeiro used in 2003 for mating disruption was monitored in 2004 in order to evaluate the existence of pheromone release of the dispensers one year after being set up.

Table 1. Experimental plots installed in the selected lemon orchards from Mafra region for the mating disruption experiments of citrus flower moth, *Prays citri* (Millière), in 2003 and 2004.

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Area (ha)</th>
<th>Year of plantation</th>
<th>Distance between rows (m×m)</th>
<th>Experimental plot</th>
<th>Year of experiment</th>
<th>Nºtrees/area (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casal do Zambujeiro</td>
<td>5.62</td>
<td>1996, 1991</td>
<td>5.0 × 5.0</td>
<td>Mating disruption Control</td>
<td>2003</td>
<td>414/1.04, 238/0.60</td>
</tr>
<tr>
<td>Ponte de Cuco</td>
<td>3.75</td>
<td>1978, 1990, 1995</td>
<td>5.5 × 5.0</td>
<td>Mating disruption Control</td>
<td>2003</td>
<td>425/1.17, 430/1.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mating disruption Control</td>
<td>2004</td>
<td>535/1.45, 560/1.50</td>
</tr>
<tr>
<td>Quinta do Micharro</td>
<td>0.63</td>
<td>1991</td>
<td>6 × 4.5</td>
<td>Mating disruption</td>
<td>2004</td>
<td>203/0.63</td>
</tr>
</tbody>
</table>
Casal do Zambujeiro and Ponte de Cuco are located in an area with other neighbouring lemon orchards, while Quinta do Micharro is a small and isolated orchard surrounded by pine and eucalyptus stands.

**Experimental design.** In all lemon orchards but Quinta do Micharro two experimental plots were defined, one for mating disruption and a control. In the case of Quinta do Micharro, all the orchard was allocated as a mating disruption plot due to its small dimension (Table 1).

In Casal do Zambujeiro, the control plot (1.04 ha, 414 trees) was isolated and ca. 100 m apart from the mating disruption plot (0.60 ha, 238 trees) (Table 1). There were no other citrus orchards in a distance of at least 100 m around both plots.

In Ponte de Cuco the orchard, with a slope of ≈ 5%, was divided in two contiguous plots. In both 2003 and 2004, the upper plot was allocated for mating disruption (Table 1). In 2003, the control plot was contiguous to the mating disruption plot. In 2004, it was installed in the lower part of the orchard, with an intermediary plot (1 ha, 395 trees) separating from the mating disruption plot. Contiguous to the lower part of the orchard there is another lemon orchard belonging to another grower and no other lemon orchards are known in the neighbourhood. The allocation of the mating disruption plot to the upper part of the orchard was decided to minimize the possibility of migration of fecundated females from the neighbour lemon orchard, contiguous to the lower part of the orchard.

**Mating disruption dispensers.** The pheromone dispensers used in mating disruption experiments, Isonet-CFM, consist of a ca. 20 cm length polyethylene extruded single tube (capillary) filled with 100 mg of CFM pheromone. Based on the specific molecular weight and volatility of the pheromone and the characteristics of the dispenser it was estimated that the dispensers would not last longer than three months and two applications of dispensers were considered in order to cover the spring and summer period of crop susceptibility. Despite its low intensity the flowering period of mid summer is responsible for the setting of the most valuable fruits that will mature in the following summer. In all applications but the first of 2003, where two pheromone doses were tested, i.e., 1000 and 2000 pheromone dispensers, the number of installed dispensers was 1000 per hectare (Table 2). The dispensers were set up in March-April for the first application and in July for the second (Table 2).

The pheromone dispensers were distributed according a previously defined spatial pattern, based on the dimension and configuration of each plot and using higher dispenser density in the plot edges where the loss of pheromone was expected to be higher.

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Year</th>
<th>Number of pheromone dispensers applied per hectare and date of installation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casal do Zambujeiro</td>
<td>2003</td>
<td>1000 (April 16th) + 1000 (July 29th)</td>
</tr>
<tr>
<td>Ponte de Cuco</td>
<td>2003</td>
<td>2000 (April 16th) + 1000 (July 29th)</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>1000 (March 23rd) + 1000 (July 20th)</td>
</tr>
<tr>
<td>Quinta do Micharro</td>
<td>2004</td>
<td>1000 (March 31st) + 1000 (July 20th)</td>
</tr>
</tbody>
</table>

**Monitoring.** Two pheromone delta traps (15 cm x 15 cm sticky plates) were installed in both mating disruption and control plots for the monitoring of CFM male flight activity and to test
the occurrence of mating disruption through the shutdown effect on male captures in pheromone traps activated inside mating disruption plots. In the case of Ponte de Cuco, a total of 7 (in 2004) to 9 (in 2003) pheromone traps were installed along a transect within the orchard from the mating disruption plot, in the upper part of the orchard, up to the control plot, in the lower part of the orchard, to allow the spatial analysis of pheromone effect on the male captures. Male captures were counted weekly and pheromone dispensers were renewed every four weeks.

The injury intensity caused by CFM was estimated by fortnightly to monthly sampling of 195 to 375 flowers per plot, collecting 3 or 5 flowers per tree. Flower samples were isolated in plastic bags and transported to the laboratory where the presence of CFM eggs, larvae or injuries was determined under magnification, after flower dissection.

**Insecticide treatments.** As the intervention threshold was exceeded in both years, one to three insecticide sprays with luphenuron were needed to prevent yield losses. Despite that, it is expected the observed differences between plots will reflect mainly the result of mating disruption effect, as the chemical interventions were the same in both control and mating disruption plots.

**Release rate of the pheromone.** The release rate of pheromone from Isonet-CFM dispensers was estimated by determining through HPLC analysis the remaining amount of pheromone in the dispensers. With this purpose samples of five dispensers were fortnightly collected in each orchard for both applications of 2003 and maintained at 5°C for posterior analysis.

**Results and discussion**

**Citrus flower moth dynamics**

In average the male flight activity of CFM in the studied lemon orchards was very low from January up to March (Figure 1). It rapidly increased since April reaching maximal level between June and August (30-50 males/trap/day). The results show that despite the several insecticide treatments (from two up to eight) applied during the season the CFM was not effectively controlled, as it is clear from the dynamics of injury intensity on lemon flowers.

Based on the observed dynamics of CFM we suggested the existence of two geographically delimited categories of lemon orchards in the region (Table 3). Category I consists mainly of contiguous lemon orchards, located in the southern part of the lemon production area of Mafra, the region where the climate is more favourable, the crop has longer history (at least since the 1940's), the dynamics of CFM is expected to be influenced by immigrant moths from neighbouring orchards and often more than three insecticide sprays are carried out each year to control the CFM. On the other hand, category II includes relatively isolated lemon orchards with recent history, located in less climatically favourable zones for lemon production (e.g., frost problems during winter), where the dynamics of CFM is expected to be mainly dependent on local individuals. These results were taken in consideration in the selection of the lemon orchards used in mating disruption experiments. Ponte de Cuco is a category I orchard and Casal do Zambujeiro and Quinta do Micharro are category II orchards.
Figure 1. Dynamics of male flight activity and injury intensity of citrus flower moth, *Prays citri* (Millière), in 13 lemon orchards from the lemon production area of Mafra, in the Oeste region of Portugal, in 2002: grey line- average male capture in pheromone traps of the 13 orchards; black line - male capture in each plot; black bars - % of infested flowers; crosses - insecticide sprays carried out against the citrus flower moth.

Table 3. Orchard categories in function of citrus flower moth (CFM), *Prays citri* (Millière), dynamics.

<table>
<thead>
<tr>
<th>Orchard category</th>
<th>Male captures (% observations higher than average)</th>
<th>Flower injury (% observations higher than average)</th>
<th>Number of insecticide sprays against CFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>≥ 50</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>≥ 50</td>
<td>-</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>II</td>
<td>&lt; 50</td>
<td>&lt; 50</td>
<td>≤ 3</td>
</tr>
</tbody>
</table>

**Mating disruption experiments**

A clear “shutdown” effect of male captures in pheromone traps was observed for both tested pheromone doses, i.e., 1000 and 2000 dispensers per ha, and in all plots, in 2003 and 2004 experiments (Figure 2 and 3). In 2003, the male captures of CFM were almost nil (average of 0.1 males/trap/day in both orchards) during all period in mating disruption plots, compared
with an average of 4.3 males/trap/day (0-20) and 13.9 males/trap/day (0-40), in control plots, in Ponte Cuco and Casal do Zambujeiro, respectively (Figure 2). Similar results were observed in 2004 (Figure 3). The average male captures in mating disruption plot of Ponte Cuco was 0.2 males/trap/day, compared with 10.5 males/trap/day (0.1-25.0) in the control plot. Therefore, in comparison to the control there was a reduction of 95-99% in the male captures registered in mating disruption plots.

Further, comparing with the average male captures observed in Ponte de Cuco, in 2002 (Figure 1), one year before the beginning of mating disruption experiments, i.e., 17.2 males/trap/day (1.3-43.6), the reduction registered in 2003 was of 99%.

Figure 2. Comparative trends of male captures of citrus flower moth, *Prays citri* (Millière), in mating disruption and control plots, in Ponte de Cuco and Casal do Zambujeiro (Mafra, Portugal) lemon orchards, in 2003.

Figure 3. Comparative trends of male captures of citrus flower moth, *Prays citri* (Millière), in mating disruption and control plots, in Ponte de Cuco and Quinta do Micharro (Mafra, Portugal) lemon orchards, in 2004.
In the case of Ponte de Cuco, the “shutdown” effect is also clearly showed by the trend of CFM male captures in the delta traps distributed along the orchard (Figure 4). Data also evidenced the existence of a gradient in male captures downwards the mating disruption plot. The average male captures of CFM increased exponentially with the distance to the lower edge of the mating disruption plot (Figure 5). These results suggest that male captures in the pheromone traps installed in the lower part of the orchard, including the control plot, were reduced as a result of a descendent pheromone flow from the mating disruption plot, in the upper part of the orchard. Based on the average captures of CFM males registered in the pheromone traps installed in the lower limit of the orchard we can have a conservative estimate of the level of interference registered in male trapping originated by the descendent pheromone flow (Figure 6), i.e., a reduction on male captures between 13% and 75%, depending on the distance to the lower limit of the mating disruption plot and on the experiment (2003 or 2004).

According to Howse et al. (1998), a reduction of 98%-100% on the male captures must occur for the mating disruption to be effective. Values equal or lower than 95% are considered to reveal the occurrence of mating events within the mating disruption plots. The analysis of CFM male captures registered in mating disruption plots in our experiments shows that the effectiveness criteria of Howse et al. (1998) was verified 100% and 74% (23 out of 31) of the weekly samples, in 2003 and 2004, respectively, in Ponte de Cuco, and in 92% of the samples, in Casal do Zambujeiro. However, in the case of Ponte de Cuco, we have to consider that the values are underestimates (particularly, in 2004) of the reduction level on male captures because they were determined in relation to the captures registered in the pheromone trap installed in the lower limit of the orchard. As already explained (Figures 4-6), this part of the orchard was influenced by the descendent flow of pheromone from the mating disruption plot (slope ≈ 5%).

Figure 4. Average male captures of citrus flower moth, Prays citri (Millière), in each of the pheromone delta traps installed in a transect along the orchard slope, in Ponte de Cuco (Mafra, Portugal), in 2003 and 2004: mating disruption traps - grey bars, control plot - black bars, outside control plot - white bars.
The registered high level of trap “shutdown” suggests the occurrence of sufficient release and enough concentration of pheromone. Therefore, if dispensers are applied properly it is expected stable efficacy of mating disruption in order to reduce population levels of CFM.

Figure 5. Average male captures of citrus flower moth, Prays citri (Millière), in function of the distance to the lower edge of mating disruption plot, in Ponte de Cuco (Mafra, Portugal) lemon orchard, in 2003 and 2004.

Figure 6. Reduction rate of average citrus flower moth, Prays citri (Millière), male captures the traps outside the mating disruption (MD) plot, in function with the distance to the lower edge of mating disruption plot, at Ponte Cuco (Mafra, Portugal) lemon orchards, in 2003 and 2004.

The estimated rate of pheromone diffusion from Isonet-CFM dispensers presented a maximum of 73 mg/ha/h for the dose of 1000 dispensers/ha, just after the setting up of dispensers. It decreased to 25 mg/ha/h one month latter and stabilized afterwards (Figure 7). The residual amount of pheromone in the dispensers exponentially decreased with time of
exposition. An average of 28% of the pheromone load was still remaining in the dispensers 82 days after the setting up (Figure 8). Based on the adjusted exponential model it was expected that the time needed for the dispensers to deliver the total pheromone load would be longer than one year. This hypothesis seems to be corroborated by the data collected in Casal do Zambujeiro, in 2004. The reduction on the registered male captures in the mating disruption plot, in comparison with the control plot, one year after the experiment, is possibly the result of a residual releasing rate of pheromone from the remaining dispensers in the plot (Figure 9).

Figure 7. Trend of the average rate of pheromone diffusion from Isonet-CFM dispensers used in the experiments on mating disruption of citrus flower moth, *Prays citri* (Millière), in lemon orchards from Mafra (Portugal), in 2003, after the (a) first (17th April) and (b) second (29th July) application, for the dose of 1000 dispensers/ha.

![Figure 7](image_url)

Figure 8. Trend of the residual amount of pheromone in Isonet-CFM dispensers used in the experiments on mating disruption of citrus flower moth, *Prays citri* (Millière), in lemon orchards from Mafra (Portugal), in 2003, after the (a) first (17th April) and (b) second (29th July) application, for the dose of 1000 dispensers/ha.

![Figure 8](image_url)
However, the dispensers of the second application showed a faster reduction of the pheromone load that attained 15%, 70 days after the setting up (Figure 8), as a result of a higher rate of pheromone diffusion during the first month after the application of dispensers (Figure 7), in relation to the higher average temperatures registered in this period.

![Figure 9. Comparative trends of male captures of citrus flower moth, Prays citri (Millière), in the mating disruption and control plots, in Casal do Zambujeiro, in 2004 (Mafra, Portugal), one year after the mating disruption experiment.](image)

**Conclusion**

The consistent “shutdown” effect registered in the male captures in mating disruption plots in all experiments suggests that Isonet-CFM dispensers present the technical characteristics to be used in mating disruption of CFM. Further experiments are needed to evaluate the cumulative effect of mating disruption during several years in the same plot as well as in larger plots. With this purpose another experiment is being carried out in 2005 in Ponte de Cuco using the total area of the orchard, i.e., ca. 4 ha.

The fast pheromone release observed during the summer as a result of higher temperatures may imply higher pheromone dose in order to achieve enough pheromone concentration in the field, during this season. For a transitional phase between the actual chemical control approach and the application of mating disruption of CFM, the possibility of combining the setting up of Isonet-CFM dispensers in the spring with one or two insecticide sprays in the summer must be considered. The integration of mating disruption with other tactics, namely conservation biological control, through habitat management, constitutes also a scenario to be explored.

**Acknowledgments**

This study would not be possible without the support of Shin-Etsu (Japan) that in collaboration with CBC (Europe) Ltd and Biosani (Portugal) accepted to develop and provide the pheromone dispensers Isonet-CFM used in mating disruption experiments. We thank Vittorio Veronelli (CBC) for comments on the manuscript. Thanks are due also to Manuel
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References

Molecular discrimination of Tetranychidae mite species present in citrus orchards in Eastern Spain

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_Tetranychus urticae_ Koch (Acari: Tetranychidae) is a cosmopolitan and polyphagous mite which can be an important pest of citrus worldwide. This mite can be found feeding on many plant species occurring in the citrus agrosystem, moving freely from weeds to trees. However, high densities on cover plants do not always preclude high densities on the trees, and vice-versa, and this raises questions about the genetic structure of populations of _T. urticae_ in citrus groves, and the nature of exchanges between mites from different host plants in the orchard. Because field samples consist of a mixture of different Tetranychidae species, as a first step necessary to further implement intraspecific characterization of _T. urticae_, species-discriminating criteria based on molecular techniques are needed. In this study, the nucleotide variation of the Internal Transcribed Spacer 2 (ITS2) fragment of nuclear ribosomal DNA (rDNA) of _P. citri, T. evansi, T. ludeni, T. turkestani_, and _T. urticae_, have been determined. The high homogeneity of the ITS2 sequence observed among the specimens of _T. urticae_ obtained makes this DNA-sequence an excellent tool for species discrimination. We also tested the potential of PCR-RFLP analysis of the ITS2 for a quick screening of high numbers of field samples for species discrimination and estimation of species abundance in the different host plants.
Modified performance of *Tetranychus urticae* on NaCl-stressed citrus plants

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*Tetranychus urticae* is an important pest of citrus, especially lemon and mandarin under Mediterranean climate. Factors leading to this problem are poorly understood, but saline stress is suspected to contribute to spider mite outbreaks. In this study, the effect of NaCl concentration (0 to 60 mM NaCl) in nutritive solutions used to water potted young mandarin trees on population growth of *T. urticae* reared on leaf discs obtained from these plants was investigated. Although the differences observed between treated and control groups were in most cases not significant, when all biological parameters calculated were combined to obtain demographic parameters (R₀, T and r_m), remarkable differences appeared, and a concentration-dependent effect was detected. Although high salt concentrations negatively affected *T. urticae*, at the lowest concentrations tested the r_m values were significantly higher than control and this may contribute to the observed field explosions of *T. urticae*. 
Mites, lemon trees and ground cover interactions in Mafra region

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Abstract: Monthly samples were collected from April 2002 to March 2003 in three lemon orchards of Mafra (Oeste region of Portugal) aiming at identifying the species diversity and abundance of mites in both lemon trees and ground cover vegetation. Three modalities of ground cover management were installed in each of three orchards: resident vegetation, sowing of selected plant species and herbicide application. Low mite populations were observed on lemon trees, namely of the phytophagous species Aceria sheldoni (Ewing), Panonychus citri (McGregor) and Polyphagotarsonemus latus (Banks). Phytoseiids, especially Amblyseius stipulatus Athias-Henriot, and the tydeids Orthotydeus californicus (Banks) and Tydeus formosus (Cooreman) were the most common mites. The population levels of phytoseiids and tydeids on lemon trees were lower in herbicide, in comparison with both ground cover modalities. From the 208 plant species identified in ground cover vegetation, 33 were host plants of mites with agricultural interest, namely Solanum nigrum L., Rubus ulmifolius Schott, Conyza bonariensis (L.) Cronquist, Lavatera cretica L. and Convolvulus arvensis L. Tetranychus spp. and phytoseiids, especially A. stipulatus, were the predominant mites. Lists of phytophagous, predators and indifferent mite species associated to plant species of ground cover vegetation are given, as well as some acquirements on the three different groups and the relationship existing between ground cover vegetation and lemon trees mite populations.

A. stipulatus, the most widespread phytoseiid mite in Portuguese citrus, was the predominant phytoseiid species found on lemon trees and ground cover vegetation in the three studied lemon orchards.

Keywords: mites, lemon, citrus, ground cover vegetation, weeds

Introduction

Ground cover management is an important component of conservation biological control strategies, especially in perennial cropping systems. The interest of ground cover vegetation as potential reservoir of beneficial organisms is increasing in integrated pest management.

Contrarily to citrus mites, weed mites are still poorly studied in citrus orchards. In Spain, Pascual & Ferragut (1999) and Aucejo et al. (2003) studied the mite fauna associated to weeds in citrus, essentially tetranychids and phytoseiids.

Aiming at studying the interaction between mites’ fauna of both lemon trees and ground cover vegetation and their potential contribution to enhance conservation biological control of pests, a one-year experiment was carried out in three lemon orchards of Mafra, in the Oeste region of Portugal, where three modalities of weed management were compared. The results of this study are presented and discussed in this paper.
Materials and methods

Experimental plots
The study was carried out in three 1-4 ha lemon orchards from Mafra, in the Oeste region of Portugal, ca. 30 Km north of Lisbon, namely Carrasqueira de Cima (CC), Casal Mato de Cima (CMC) and Pinhal de Frades (PF). During the study, farmers followed an IPM system under the technical supervision of Frutoeste, a farmers association of Oeste region.

Ground cover management
In each orchard, three weed management systems were installed in 2002: 1) inter-row ground cover with resident vegetation (rv), 2) inter-row ground cover by sowing selected species (s) and 3) inter-row herbicide application, i.e., diuron+glyphosate+terbutilazine (h). The sown species were *Lolium multiflorum* Lam., *Lolium perenne* L., *Medicago polymorpha* L., *Trifolium fragiferum* L., *Trifolium incarnatum* L. and *Trifolium resupinatum* L. Each management system was installed in plots of five or six tree rows.

Sampling methods
Samples were monthly collected in lemon trees and ground cover vegetation from April 2002 to March 2003. A total of 100 leaves (4 per tree), 25 shoots (1 per tree) and 25 young fruits (1 per tree) were randomly collected from each modality and orchard. Samples from ground cover vegetation consisted of one plant per plant species present in each modality and orchard. Each plant was isolated in plastic bags and transported to the laboratory. Samples were further studied in laboratory and collected mites prepared for identification.

Results and discussion

The mite fauna in lemon trees
Eight mite species were identified on lemon trees in the studied orchards: three phytophagous, three predators and two indifferent species (Table 1). *Typhlodromus doreenae* Schicha is reported for the first time in Portugal. Other mites with little importance were also detected, such as *Petrobia* sp., *Tarsonemus* sp., Acaridae, Glycyphagidae, Parsitidae and Saproglyphidae.

The citrus red mite, *Panonychus citri* (McGregor), the citrus bud mite, *Aceria sheldoni* (Ewing), and the broad mite, *Polyphagotarsonemus latus* (Banks), are the three major mite pests of lemon trees in Portugal but there are many species of predators and indifferent mites associated to Portuguese citrus (Ferreira & Carmona, 1990; Carvalho et al., 1999; Ferreira, 2000; Silva & Ferreira, 2002).

Low mite populations were observed on lemon trees in the three orchards, namely of the phytophagous species *P. citri*, *A. sheldoni* and *P. latus*. Only symptoms of the presence of *A. sheldoni* and *P. latus* were detected in most of the samples (Table 1).

The tydeids *Orthotydeus californicus* (Banks) and *Tydeus formosus* (Cooreman) and phytoseiids, especially *Amblyseius stipulatus* Athias-Henriot, were the most common mites on lemon tress. The population level of both phytoseiids and tydeids on lemon trees was consistently lower in herbicide modality, in comparison with ground cover vegetation modalities, in the three studied orchards (Figures 1 and 2).

Phytoseiid mites are important biological control agents of phytophagous mites. Among the many phytoseiid species associated to citrus in Mediterranean basin, *A. stipulatus* is the most widespread species (McMurtry, 1977). It is considered the predominant phytoseiid species on citrus in Spain (Garcia-Mari et al., 1983, 1986, 1991; Ferragut et al., 1988), Sicily (Ragusa, 1986), Portugal (Ferreira & Carmona, 1990; Ferreira, 1992; Ferreira, 2000) and
Greece (Papaioannou-Souliotis et al., 1997). Tydeids may also contribute for biological control as they constitute alternative prey to phytoseiids.

Table 1. Mite species identified on lemon trees in the three studied orchards, Casal Mato de Cima (CMC), Pinhal de Frades (PF) and Carrasqueira de Cima (CC), in function of the weed management system: ground cover with resident vegetation (rv), ground cover by sowing selected species (s) and herbicide application (h).

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<tr>
<th>Family and species</th>
<th>CMC</th>
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<td><strong>Phytophagous</strong></td>
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<tr>
<td>Eriophyidae</td>
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<tr>
<td>Aceria sheldoni (Ewing)*</td>
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<td>Polyphagotarsonemus latus (Banks)</td>
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<td>Tetranychidae</td>
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<tr>
<td>Panonychus citri (McGregor)</td>
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<td><strong>Predators</strong></td>
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<tr>
<td>Phytoseiidae</td>
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<tr>
<td>Amblyseius stipulatus Athias-Henriot</td>
<td>x</td>
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<tr>
<td>Typhlodromus doreenae Schicha**</td>
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<tr>
<td>Typhlodromus rhenanoides Athias-Henriot</td>
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<td>Tydeidae</td>
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<tr>
<td>Orthotydeus californicus (Banks)</td>
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<tr>
<td>Tydeus formosus (Cooreman)</td>
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</table>

* species only observed in buds; ** new species for Portugal; ● only symptoms of the species were detected.

Figure 1. Density of phytoseiids on lemon trees in the three studied orchards, Casal Mato de Cima (CMC), Pinhal de Frades (PF) and Carrasqueira de Cima (CC), in function of the weed management system.
management system: ground cover with resident vegetation (rv), ground cover by sowing selected species (s) and herbicide application (h).

![Graph showing density of tydeids on lemon trees in three studied orchards](image)

**Figure 2.** Density of tydeids on lemon trees in the three studied orchards, Casal Mato de Cima (CMC), Pinhal de Frades (PF) and Carrasqueira de Cima (CC), in function of the weed management system: ground cover with resident vegetation (rv), ground cover by sowing selected species (s) and herbicide application (h)

**The mite fauna in ground cover vegetation**

Table 2. Identified host plants of mites in ground cover vegetation in the studied orchards.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
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<tbody>
<tr>
<td>Amaranthaceae</td>
<td><em>Amaranthus deflexus</em> L., <em>Amaranthus retroflexus</em> L.</td>
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<tr>
<td>Araceae</td>
<td><em>Arum italicum</em> Mill.</td>
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<tr>
<td>Chenopodiaceae</td>
<td><em>Chenopodium murale</em> L.</td>
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<tr>
<td>Convolvulaceae</td>
<td><em>Convolvulus arvensis</em> L.</td>
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<tr>
<td>Cruciferae</td>
<td><em>Sinapis arvensis</em> L.</td>
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<tr>
<td>Fagaceae</td>
<td><em>Quercus faginea</em> Lam.</td>
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<tr>
<td>Geraniaceae</td>
<td><em>Erodium moschatum</em> (L.) L’Hér., <em>Geranium dissectum</em> L.</td>
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<tr>
<td>Gramineae</td>
<td><em>Phalaris minor</em> Retz</td>
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<tr>
<td>Labiatae</td>
<td><em>Stachys arvensis</em> (L.) L.</td>
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<tr>
<td>Mavaceae</td>
<td><em>Lavatera cretica</em> L.</td>
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<tr>
<td>Papaveraceae</td>
<td><em>Papaver rhoes</em> L.</td>
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<td>Pittosporaceae</td>
<td><em>Pittosporum undulatum</em> Vent.</td>
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<tr>
<td>Plantaginaceae</td>
<td><em>Plantago major</em> L.</td>
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<tr>
<td>Polygonaceae</td>
<td><em>Rumex conglomeratus</em> Murray, <em>Rumex crispus</em> L.</td>
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<tr>
<td>Rosaceae</td>
<td><em>Rubus ulmifolius</em> Schott</td>
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<tr>
<td>Solanaceae</td>
<td><em>Datura stramonium</em> L., <em>Solanum nigrum</em> L., <em>Solanum tuberosum</em> L.</td>
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<tr>
<td>Umbeliferae</td>
<td><em>Ammi majus</em> L., <em>Oenanthe crocata</em> L.</td>
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<tr>
<td>Vitaceae</td>
<td><em>Vitis vinifera</em> L.</td>
</tr>
</tbody>
</table>
From the 208 plant species identified in ground cover vegetation, 33 were host plants of mites, including 19 botanical families (Table 2). Compositae was the most representative family, with nine different host species of mites, followed by Solanaceae, with three host species.

Many mite families were detected in ground cover vegetation: Acaridae, Bdellidae, Cunaxidae, Eriophyidae, Erythraeidae, Parasitidae, Phytoseiidae, Saproglyphidae, Stigmataceae, Tarsonemidae (Tarsonemus sp.), Tenuipalpidae, Tetranychidae, Trombiculidae and Tydeidae, but the most important were Eriophyidae, Tenuipalpidae, Tetranychidae, Phytoseiidae and Tydeidae, mainly the last three families.

Sixteen mite species were identified in ground cover vegetation in the studied orchards: eight phytophagous, five predators and three indifferent species (Tables 3 and 4). *Brevipalpus cuneatus* (Canestrini & Fanzago) is reported for the first time in Portugal. *A. stipulatus*, *O. californicus* and *T. formosus* were also identified on lemon trees.

Table 3. Mite species identified in ground cover vegetation in the three studied orchards, Casal Mato de Cima (CMC), Pinhal de Frades (PF) and Carraqueira de Cima (CC), in function of the weed management system: ground cover with resident vegetation (rv), ground cover by sowing selected species (s) and herbicide application (h).

<table>
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<th>Family and species</th>
<th>CMC</th>
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<tbody>
<tr>
<td><strong>Phytophagous</strong></td>
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<td>Eriophyidae</td>
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<tr>
<td><em>Aculops lycopersici</em> (Massee)</td>
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<tr>
<td>Tenuipalpidae</td>
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<tr>
<td><em>Brevipalpus cuneatus</em> (Can. &amp; Fanz.)*</td>
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<tr>
<td><em>Brevipalpus spinosus</em> (Donnadieu)</td>
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<tr>
<td>Tetranychidae</td>
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<tr>
<td><em>Bryobia rubrioculus</em> (Scheuten)</td>
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<tr>
<td><em>Tetranychus cinnabarinus</em> (Boisduval)</td>
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<tr>
<td><em>Tetranychus evansi</em> Baker &amp; Pritchard</td>
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<td><em>Tetranychus ludeni</em> Zacher</td>
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<tr>
<td><em>Tetranychus urticae</em> Koch</td>
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<tr>
<td><strong>Predators</strong></td>
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<td>Phytoseiidae</td>
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<tr>
<td><em>Amblyseius barkeri</em> (Hughes)</td>
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<td>Amblyseius graminis Chant</td>
<td>x</td>
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<tr>
<td><em>Amblyseius messor</em> (Wainstein)</td>
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<tr>
<td><em>Amblyseius stipulatus</em> Athias-Henriot**</td>
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<td><em>Phytoseiulus persimilis</em> Athias-Henriot</td>
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<td><strong>Indifferents</strong></td>
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<td>Tydeidae</td>
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<tr>
<td><em>Orthotydeus californicus</em> (Banks)**</td>
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<tr>
<td><em>Orthotydeus kochi</em> (Oudemans)</td>
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<td><em>Tydeus formosus</em> (Cooreman)**</td>
<td>x</td>
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</table>

* new species for Portugal; ** species also identified on lemon trees.
The genus *Tetranychus*, never identified on lemon trees in Portugal, represented by *Tetranychus cinnabarinus* (Boisduval), *Tetranychus evansi* Baker & Pritchard, *Tetranychus ludeni* Zacher and *Tetranychus urticae* Koch, and phytoseiids, especially *A. stipulatus*, were the predominant mites in ground cover vegetation in the studied orchards, followed by tydeids (Tables 3 and 4).

### Table 4. Mite and weed species relationship in the studied orchards, with indication of frequency and relative abundance: high [], medium [ ], and low [ ]

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<td>Arum italicum</td>
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<td>Sonchus asper</td>
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</table>

* phytophagous, ** predator, *** indifferent.

The most important host plants of *Tetranychus* spp. were *Solanum nigrum* L., *Lavatera cretica* L., *Rubus ulmifolius* Schott and *Convolvulus arvensis* L. (Table 4).
Among the tetranychid species identified in ground cover vegetation (Tables 3 and 4), only *T. cinnabarinus* or *T. urticae* may attain pest status on citrus crops. The two-spotted spider mite, *T. urticae*, a cosmopolitan and polyphagous mite, is considered an important pest of citrus in some citrus growing areas in the Mediterranean basin, namely Sicily (Ragusa, 1986), Greece (Papaioannou-Souliotis et al., 1997) and Spain (Aucejo et al., 2003). However, it seldom originates economic damage in citrus orchards in Portugal. It has been identified occasionally by the second author, only on orange trees.

Other phytophagous species identified in ground cover vegetation include *Aculops lycopersici* (Massee), a monophagous species associated to Solanaceae, *B. cuneatus* and *Brevipalpus spinosus* (Donnadieu) (Tables 3 and 4). These mite species do not constitute potential pests of lemon trees, as they do not feed on citrus. Instead, these phytophagous mite species or others not associated to the crop may have positive effect on biological control of mite pests, as they constitute alternative prey to predators.

*A. stipulatus* was the predominant phytoseiid species in both lemon trees and ground cover vegetation in the three orchards (Tables 1 and 3). It was identified in 13 different plant species (Table 4). Its most important host plants were *R. ulmifolius*, *S. nigrum* and *Chenopodium murale* L. In Spain, *A. stipulatus* was also reported to be the predominant phytoseiid species on citrus weeds (Pascual & Ferragut, 1999; Aucejo et al., 2003).

The most important host plants of tydeids were *R. ulmifolius*, *Conyza bonariensis* (L.) and *S. nigrum*. *Orthotydeus kochi* (Oudemans) was the predominant tydeid species. It was identified on six different host plants (Table 4).

Data suggest that ground cover vegetation may serve as reservoir for both phytoseiids and tydeids. These mites can move up to the trees and back down to ground cover, increasing their populations in crop and enhancing the impact of predators on pests.

**Acknowledgements**

Thanks are due to the lemon growers Virgínia Duarte, Carlos Batalha and Rosa Gomes that allowed us to carry out the experiment in their orchards. We thank also our project partners Rosário Antunes and Luís Aniceto (Frutoeste) for the logistic support. This is part of a research granted by Programme AGRO Medida 8.1.–DE&D (Project n.º 29 Management of ground cover and hedgerows in citrus orchards for biological control of pests).

**References**


Ground cover and weed management in citrus orchards

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Abstract: Wild plants in the understories of orchards can be managed as cover crops and contribute for both weed management and enhancing biological control of pests. Aiming at understanding the potential of cover crops to promote natural enemies of citrus pests and to control weeds in citrus orchards, the influence of three systems of ground cover management on flora composition and abundance was studied in one sweet orange orchard in Algarve and in three lemon orchards in the Oeste region of Portugal (Mafra). Two modalities of cover crops, i.e., resident vegetation and sowing of selected plant species, were compared with herbicide application (diuron+ glyphosate+ terbutilazine). Sown species include Lolium multiflorum, L. perenne, Medicago polymorpha, Trifolium fragiferum, T. incarnatum and T. resupinatum, in Oeste region, and Lolium multiflorum, Medicago polymorpha, M. scutellata, M. truncatula and T. resupinatum, in Algarve.

Key words: cover crops, weeds, lemon, sweet orange, citrus, orchards

Introduction

Plant and animal diversity of a high-stem orchard is influenced by site characteristics and management procedures. The connectivity with the landscape and the diversity and cultivation intensity of the habitats in the immediate neighbourhood of the orchard are of paramount importance (Kuchen et al., 1997).

The IOBC-WPRS guidelines for integrated production recommend that at least 5% of the farm surface must be designated and maintained as ecological infrastructures, aiming at supporting conservation biological control of crop pests by elements of functional biodiversity (Boller et al. 2004a,b). Ground cover with resident or sown vegetation may constitute an interesting ecological infrastructure for citrus orchards. It may be used both as a weed management tactic and to enhance beneficial arthropods in the orchards. Thus, knowledge on the diversity, species composition and phenology of flora associated with citrus orchard is much relevant.

In this paper we present and discuss the results of a study on the influence of ground cover management on flora diversity and abundance. The experiments were carried out in Algarve (Tavira) and in the Oeste region of Portugal (Mafra) aiming at understanding the potential of cover crops to promote natural enemies of citrus pests and to control weeds in citrus orchards.
Materials and Methods

Experimental plots
The study was carried out in three 1-4 ha lemon orchards from Mafra, in the Oeste region of Portugal, ca. 30 km north of Lisbon, namely Carrasqueira de Cima (CC), Casal Mato de Cima (CMC) and Pinhal de Frades (PF). During the study, farmers followed an IPM system under the technical supervision of Frutoeste, a farmers association of Oeste region.

CMC and PF orchards have sloping topography while CC and Tavira orchards are plain. Oeste (Caldas da Rainha) and Algarve (Tavira) mean annual temperature was 15.3°C and 17.2°C and the total annual precipitation was 607mm and 545 mm with 3-4 months without rain, respectively.

Ground cover management
In each orchard, three weed management systems were installed in 2002: 1) inter-row ground cover with resident vegetation (RV), 2) inter-row ground cover by sowing selected species (S) and 3) inter-row herbicide application, i.e., diuron+ glyphosate+terbutilazine (H). The sown species were Lolium multiflorum, L. perenne, Medicago polymorpha, Trifolium fragiferum, T. incarnatum and T. resupinatum, in Mafra experimental plots, and Lolium multiflorum, Medicago polymorpha, M. scutellata, M. truncatula e T. resupinatum, in Tavira. Each management system was installed in plots of five or six tree rows.

Before the experiment all the selected orchards but CC were managed with herbicides. In the case of CC the resident vegetation in inter-rows was managed by regular mowing.

Sampling methods
Inter-row vegetation was monitored through regular sampling during experiment, by determining the number of plants and the biomass per m² for each species and modality. Each sample consisted of observing four sampling units of 0.25 m².

Results and discussion

Flora diversity
Plant diversity in ground cover vegetation varied in function of the weed management modality, orchard and year, attaining maximal values in spring or autumn (Fig. 1). The maximal number of species identified in each observation was 25.

In general, plant diversity was higher in S modality. In fact, the number of samplings with diversity higher than five species was 14 in H, 36 in RV and 43 in S. Considering the total number of samplings in the four experimental plots, plant diversity was higher in S and RV in 69% and 31% of the observations, respectively. The total number of identified plant species, during the study, was 91 in S, 79 in RV and 51 in H.

In some plots and samplings (Fig. 1), the registered number of species in S modality was higher than expected, i.e., higher than the sum of the number of species identified in RV and the number of sown species (5 or 6), suggesting that the sowing of a mixture of selected plant species may originate favourable conditions for the growing of certain resident plant species that are not able to compete with the dominant species in RV. Bidens aurea, Heliotropium europaeum, Silene gallica, Bromus hordeaceus, Chamamaelum mixtum, Chenopodium album, Coleostephus myconis, Fumaria spp., Geranium rotundifolium, Juncus bufonius, Lamium amplexicaule, Lamium purpureum, Lythrum hyssopifolia, Oxalis corniculata, Polygonum aviculare, Ranunculus muricatus and Rumex pulcher were among the species identified in S modality and not detected in RV.
Flora abundance

The levels of plant density and biomass were much different between experimental plots, mainly reflecting differences in soil characteristics, light and climate conditions (Fig. 2 and 3). Plant density varied between 0 and 944 plants/m$^2$ in H, between 22 and 8184 plants/m$^2$ in S, and between 8 and 5021 plants/m$^2$ in RV. Maximal values were registered in autumn (Fig. 2). Considering the total number of samplings, maximal plant density was registered in 61% and 37% of the observations, in S and RV modalities, respectively.

Plant biomass varied between 0 and 852 g/m$^2$ in H, between 14 and 1537 g/m$^2$ in S, and between 3 and 1537 g/m$^2$ in RV. In general, maximal values were observed in spring (Fig. 3). Considering the total number of samplings, maximal plant biomass was registered in 54% and 44% of the observations, in S and RV modalities, respectively.

The 10 most abundant plant species in each weed management modality were determined based on the sum of plant density in all experimental plots and samplings (Fig. 4). *Poa annua* was the most abundant species in both S and RV. Besides sown species, *Polycarpon tetraphyllum* was among the most abundant species in S modality. Data suggest that the density of three of the most abundant species in RV, *Oxalis pes-caprae*, *Hordeum murinum* and *Arctotheca calendula*, was much reduced in S, possibly as result of competition with a higher number of plant species. In CC, ground cover vegetation was dominated by *A. calendula* before the experiment. The abundance of this weed was strongly reduced in the S modality by selective mowing (height cutting control) after *A. calendula* flowering in the spring of 2002. In this way, the resident population of *A. calendula* was reduced by allowing germination (seedbank decrease) and preventing reproduction, as plants were cut before seed production. Furthermore, posterior seed germination from the seedbank was controlled through competition with the already installed sown plant species.

*Portulaca oleracea*, a summer species, was the most abundant species in H, followed by *Arctotheca calendula*, *Cynya albida*, *Poa annua*, *Amaranthus deflexus* and *Oxalis pes-caprae* (Fig. 4). However, only *P. oleracea*, *C. albida* and *A. deflexus* showed higher density in H. This was possibly due to the open space (lack of competition) and the presence of spots of soil moisture originated by irrigation water.

Flora dynamics

The dynamics of ground cover vegetation was conditioned by the lack of rain during summer. During this period water stress originated vegetation dry in ground cover. After the first rains in the beginning of autumn, seed germination occurred followed by a fast increase of plant density (Fig. 2). Plant biomass increased during autumn-winter attaining maximal values in spring (Fig. 3).

Conclusion

Ground cover vegetation may be used as an ecological infrastructure in habitat management to increase flora diversity and enhancing conservation biological control in citrus orchards. Maximal plant diversity was attained by sowing a mixture of *Lolium-Medicago-Trifolium* species.

Beneficial effects of ground cover vegetation in the activity of natural enemies (e.g., source of nectar and pollen, alternative hosts and prey) are expected to be mainly originated by the presence of flowering plant species during autumn-spring as vegetation dries in summer in the Mediterranean climate of the southern region of Portugal.

The possibility of negative effects on citrus production as a result of competition between ground cover vegetation and citrus during spring must be evaluated. Delimiting the surface
occupied by ground cover vegetation within inter-rows of citrus trees and controlling vegetation growth through adequate mowing may minimise these negative effects.

Acknowledgements

We thank Manuel Cariano for the help in field work. Thanks are due to the lemon growers Virginia Duarte, Carlos Batalha and Rosa Gomes that allowed us to carry out the experiment in their orchards. This was part of a research granted by Programme AGRO Medida 8.1.–DE&D (Project nº 29 Management of ground cover and hedgerows in citrus orchards for biological control of pests).

References

Figure 1. Plant diversity in function of ground cover modality and orchard: S - sowing; RV - resident vegetation; H - herbicide application.
Figure 2. Plant density in function of ground cover modality and orchard: S - sowing; RV - resident vegetation; H - herbicide application.
Figure 3. Plant biomass in function of ground cover modality and orchard: S - sowing; RV - resident vegetation; H - herbicide application.
Figure 4. The 10 most abundant plant species in each ground cover modality: S - sowing; RV - resident vegetation; H - herbicide application.
Citrus phytosanitary survey project in the Comunidad Valenciana
I: cultivated areas

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Following the Act 120/2004 of 16 July from the Consell de la Generalitat Valenciana, a citrus phytosanitary survey project was established on the citrus crops of the Comunidad Valenciana with two main objectives, to determine and report on the citrus pest situation along the year, and to detect new exotic pests that could arrive from abroad. A Survey Net was established with that purpose.

The Project was initiated on October 2004. The Citrus acreage from the Comunidad Valenciana was partitioned in 100 areas of 25 km² (5 x 5 km). One fixed and three mobile sampling points were established on each area. Five (for mobile points) to ten (for fixed points) trees were sampled per sampling point, with four branches observed per tree. All pests present were determined and quantified following a numeric scale from 0 to 3. Else, 10 different types of insect traps were placed on each fixed point.

Half the areas are sampled weekly, so that a complete vision of the phytosanitary situation of the orchards is obtained every two weeks. This information is placed in the web page of the Conselleria de Agricultura, Pesca y Alimentación. For each pest, a distribution or extension map and an abundance or intensity map are provided, together with a histogram with the number of orchards included in the four levels of abundance considered (from 0 to 3). Else, a short comment on the general pest situation and changes occurred in the last week is included. When an exotic species is provisionally detected, a quarantine area of 1 km and a special searching area of 3 km in all directions are established, following predefined specific protocols for each pest. If the presence of the exotic pest is confirmed, different measures are undertaken following the Sanidad Vegetal Act, as the official declaration of the new pest, application of phytosanitary measures, compulsory chemical sprays, elimination of fruits or trees, establishment of areas quarantined and compensations to the farmers.
Citrus phytosanitary survey project in the comunidad Valenciana II: Packinghouses and commercial outbuildings

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Following the Act 120/2004 of 16 July from the Consell de la Generalitat Valenciana, a citrus phytosanitary survey project was established on citrus packinghouses and commercial outbuildings of the Comunidad Valenciana with the objective of detecting new exotic pests that could arrive from abroad. The project was initiated in July 2004.

To inspect the fruits in a packinghouse, the citrus commercial operator must report on the address of the packinghouse, design a contact person, keep the fruit isolated and not manipulated and facilitate the inspection. The fruits should be inspected in the three days following the report. Inspectors check carefully 5 fruits per box or 70 fruits per bin, thus making a total of 210 fruits per pallet. If the inspection is favourable, a certificate is issued stating that the commodity is free of exotic pests. If the inspection is unfavourable, a certificate is issued stating the quarantine organisms found and immobilizing the commodity. After confirmation of the identity of the exotic organism, fruits are disinfected, re-exported or destroyed.

Up till now 369 inspections have been carried out and 12 of them have been found contaminated with exotic pests. This represents 3.25% of the inspected commodities. The exotic organisms found were Elsinoe spp, Guignardia citricarpa, Pseudomonas axonopodis pv. citri and Crytophlebia leucotreta.
Residue analysis of Azadirachtin A, the main compound of NeemAzal-T/S in/on fruits and vegetables

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Residue analysis of foodstuff like fruits and vegetables is an important issue in terms of consumer protection and therefore for registration of a plant protection product. Azadirachtin A (AzA) is the analytical lead compound which is used for clarification of the residue situation in Neem products (active ingredient: Azadirachtin). Generally the crops were treated 3 times in weekly intervals with a 0.5% NeemAzal-T/S spraying solution in water. First sampling was carried out after drying of the spray film. The concentrations of Azadirachtin A directly after application is depending on the crop. Roughly the crops can be classified into two groups: 1. Fruity vegetables and fruits, small surface to the mass ratio (e.g. tomato, apple); 2. Leafy vegetables and herbs, large surface to the mass ratio (e.g. spinach, dill).

<table>
<thead>
<tr>
<th>Matrix</th>
<th>LOQ 1) [mg AzA / kg]</th>
<th>AzA concentration 2) [mg / kg]</th>
<th>DT 50 3) [d]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>0.10</td>
<td>&lt; 0.043</td>
<td>3</td>
</tr>
<tr>
<td>Strawberry</td>
<td>0.02</td>
<td>0.032</td>
<td>4.9</td>
</tr>
<tr>
<td>Peach</td>
<td>0.02</td>
<td>0.049</td>
<td>2.5</td>
</tr>
<tr>
<td>Cucumber, greenhouse</td>
<td>0.02</td>
<td>0.024</td>
<td>2.5</td>
</tr>
<tr>
<td>Cucumber, field</td>
<td>0.02</td>
<td>&lt; LOQ</td>
<td>-</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0.02</td>
<td>0.034</td>
<td>1.8</td>
</tr>
<tr>
<td>Orange, peel</td>
<td>0.02</td>
<td>0.055</td>
<td>7.6</td>
</tr>
<tr>
<td>Orange, pulp</td>
<td>0.02</td>
<td>&lt; LOQ</td>
<td>-</td>
</tr>
<tr>
<td>Spinach</td>
<td>0.10</td>
<td>1.01</td>
<td>2</td>
</tr>
<tr>
<td>Apple</td>
<td>0.10</td>
<td>&lt; 0.07</td>
<td>1</td>
</tr>
<tr>
<td>Potato</td>
<td>0.01</td>
<td>&lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Dill</td>
<td>fresh 0.02</td>
<td>0.70</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>dried 0.02</td>
<td>1.38</td>
<td>0.5</td>
</tr>
<tr>
<td>Savoir</td>
<td>fresh 0.02</td>
<td>1.43</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>dried 0.02</td>
<td>5.39</td>
<td>0.9</td>
</tr>
<tr>
<td>Parsley</td>
<td>0.02</td>
<td>2.75</td>
<td>2.3</td>
</tr>
<tr>
<td>Fennel seeds</td>
<td>0.02</td>
<td>&lt; LOQ</td>
<td>-</td>
</tr>
<tr>
<td>Lemon Balm, dried</td>
<td>0.02</td>
<td>6.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>

1) LOQ: limit of quantification
2) after recommended application; average of several tests
3) Disappearance time, where 50% of the initial AzA concentration is degraded
4) evaluation of the LOQ according to the analytical method
In the case of oranges peel and pulp were analysed separately. As expected, no Azadirachtin A was detected in the pulp.

Some herbs are sold in fresh and dry conditions. Therefore both products were analysed. During the drying process the AzA concentrations increase because of the loss of water.

**Conclusion**

We propose the following waiting periods on the basis of the residue data:

- Fruit and fruity vegetables: no waiting period.
- Leafy vegetables and herbs: 3 to 7 days, depending on the crop.
Survey of Carabidae, Staphylinidae and Cicindelidae in soil of citrus orchards in Spain

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A survey of coleopteran species belonging to Cicindelidae, Carabidae and Staphylinidae families was conducted in four citrus orchards with different types of crop management in Valencia, Spain. Sampling was performed from August 2003 to December 2004 by using 12 pitfall traps per orchard. Traps were changed every 14 days approximately. A total of 4,121 individuals were collected. Staphylinidae was the most abundant-active family with 2,567 individuals trapped, followed by carabids with 1,380. Only 162 individuals of Cicindelidae were found. *Anotylus inustus* (Gravenhorst), *Atheta* (*Xenota*) *mucronata* Kraatz and *Platystethus cornutus* Gravenhorst, 1802 represented 82% of the total number of staphylinids recorded. *Pseudoophonus* (s.tr) *rufipes* (Degeer) and *Harpalus distinguendus* (Duftschmid) were the prevalent Carabidae species on the four orchards sampled, representing about 86% of the total individuals recorded in this group. 99% of Cicindelidae collected consisted of *Cicindela campestris* Linné.
Abundance and diversity of spiders in lemon orchards with different weed management systems

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Abstract: In 2002 and 2003, a study was carried out in three lemon orchards from Mafra, in the Oeste region of Portugal, aiming at study the abundance and taxonomic composition of spiders under three different weed management systems, i.e., ground cover with resident vegetation, ground cover by sowing selected plant species and herbicide application. A total of 3429 specimens were collected using two sampling techniques, i.e., suction and beating. All specimens were identified at the family and genus levels except when not possible (e.g., damaged specimens or some immature stages). The Linyphiidae and Salticidae specimens were not identified at the genus level due to inherent identification difficulties. The collected specimens were distributed among 43 genera and 17 families. The Linyphiidae was the dominant family (23%). Among the identified genera, Cheiracanthium (Miturgidae), Araneus (Araneidae), Theridula (Theridiidae), Diaea (Thomisidae), Oxyopes (Oxyopidae), Synema (Thomisidae), Clubiona (Clubionidae) and Theridion (Theridiidae) are considered potential predators of citrus pests. Web spiders were more abundant than hunting spiders in our samples. Spider captures in lemon trees were higher in both ground cover systems, in comparison with the herbicide application. The beating technique was more efficient than suction, for spider sampling, on lemon trees.

Key words: spiders, citrus, habitat management

Introduction

Although spiders are ubiquitous, are entirely carnivorous, and prey predominantly on insects, their effect on pest population is poorly known. The secretive habits of many species, the difficulty of evaluating the impact of generalist predators on a give pest species, and the general lack of and/or interest in spiders are among the several factors contributing to this situation. Nevertheless, some studies suggest that spiders can be a significant mortality factor acting in some pest species (Hagen et al., 1999). It was also shown that spider numbers and diversity can be augmented through ground cover manipulation (Hagen et al., 1999; Riechert, 1998).

Few studies have been carried out on spiders associated to citrus orchards in some citrus growing countries such as Spain (Davila, 2003), Italy (Benfatto et al., 1994), South Africa (van den Berg et al., 1992), and USA, namely, Florida (Amalin et al., 2001b; Muma 1975; Mansour et al. 1982) and Texas (Breene et al. 1993). Spiders have been reported as predators of citrus pests, including fruit flies, aphids, whiteflies, scale insects and moths (Cherry & Dowell 1979; Mansour & Whitecomb 1986; van den Berg et al., 1992; Smith et al. 1997; Amalin et al. 2001a).

In Portugal, no study was conducted before on spiders associated to citrus orchards. In this paper we present and discuss the results of a study aimed at estimating and comparing the
abundance and diversity of spiders in different systems of weed management in lemon orchards from Mafra, in the Oeste region of Portugal.

**Material and methods**

**Experimental plots**
The study was carried out in three 1-4 ha lemon orchards from Mafra, in the Oeste region of Portugal, ca. 30 Km north of Lisbon, namely Carrasqueira de Cima (CC), Casal Mato de Cima (CMC) and Pinhal de Frades (PF). During the study, farmers followed an IPM system under the technical supervision of Frutoeste, a farmers association of Oeste region.

**Ground cover management**
In each orchard, three weed management systems were installed in 2002: 1) inter-row ground cover with resident vegetation (RV), 2) inter-row ground cover by sowing selected species (S) and 3) inter-row herbicide application, i.e., diuron+glyphosate+terbutilazine (H). The sown species were *Lolium multiflorum*, *L. perenne*, *Medicago polymorpha*, *Trifolium fragiferum*, *T. incarnatum* and *T. resupinatum*. Each management system was installed in plots of five or six tree rows.

**Sampling methods**
Samples were collected from lemon trees between July 2002 and December 2003 using two sampling techniques, i.e., beating and suction. Suction sampling was only carried out in 2003 and was applied to both the crop and ground cover vegetation. Beating samples were the result of sampling two branches per tree in 25 trees per modality and orchard. Suction samples were obtained using the “Vortis” suction sampler (Arnold 1994), following a sampling procedure previously defined (Rodrigues et al., 2003). Each sampling unit consisted of suctioning the foliage with a 8 cm diameter flexible tube (estimated airflow = 34.8 m/s) in three different positions, during four seconds per position. Ten sampling units were collected per modality and orchard.

Samples were studied in the laboratory under magnification for the separation of spiders that were kept in 70% ethyl alcohol for posterior identification. All collected specimens were identified to the family level except those damaged and the immature stages. The genus was also determined for all specimens but Linyphiidae and Salticidae.

**Results and discussion**

**Spider diversity**
A total of 3429 specimens of spiders were collected, distributed among 43 genera and 17 families (Table 1). All the identified specimens belong to the order Aranaea, suborder Araneomorphae (Labidognatha). The non-identified specimens, including imatures (7.8%) or damaged specimens, correspond to 18.3% of the total. Most of the captured specimens were juveniles (78.4%).

Linyphiidae was the dominant family, representing 22.5% of the identified specimens, followed by Dictynidae (19.7%), Theridiidae (14.5%), Thomisidae (14.4%), Araneidae (8.4%), Anyphaenidae (5.1%), Tetragnathidae (5%) and Clubionidae (3.2%). The other nine families only represent 7.4% of the total (Table 1). Some of this families, namely Theridiidae, Thomisidae, Araneidae, Tetragnathidae, Anyphaenidae and Clubionidae, were also reported as abundant in citrus orchards in other countries (Davila, 2003; Benfatto et al., 1994; van den Berg et al., 1992; Breene et al., 1993; Muma, 1975). However, in the present study the Salticidae was not as abundant as reported in other regions, which may be related to the
applied sampling methods. According to Marc & Canard (1997), the beating method underestimates this family.

Table 1. Spider genera identified in three lemon orchards from Mafra, in the Oeste region of Portugal, and respective abundance.

<table>
<thead>
<tr>
<th>Family</th>
<th>No. genera</th>
<th>juveniles</th>
<th>females</th>
<th>males</th>
<th>Total</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araneidae</td>
<td>12</td>
<td>191</td>
<td>25</td>
<td>20</td>
<td>236</td>
<td>8.4</td>
</tr>
<tr>
<td>Theridiidae</td>
<td>11</td>
<td>274</td>
<td>84</td>
<td>48</td>
<td>406</td>
<td>14.5</td>
</tr>
<tr>
<td>Thomisidae</td>
<td>7</td>
<td>377</td>
<td>19</td>
<td>8</td>
<td>404</td>
<td>14.4</td>
</tr>
<tr>
<td>Lycosidae</td>
<td>2</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>0.3</td>
</tr>
<tr>
<td>Ageleidae</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Amaurobiidae</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Anyphaenidae</td>
<td>1</td>
<td>127</td>
<td>4</td>
<td>13</td>
<td>144</td>
<td>5.1</td>
</tr>
<tr>
<td>Clubionidae</td>
<td>1</td>
<td>79</td>
<td>6</td>
<td>4</td>
<td>89</td>
<td>3.2</td>
</tr>
<tr>
<td>Dictynidae</td>
<td>1</td>
<td>489</td>
<td>40</td>
<td>22</td>
<td>551</td>
<td>19.7</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td>1</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>35</td>
<td>1.3</td>
</tr>
<tr>
<td>Miturgidae</td>
<td>1</td>
<td>31</td>
<td>4</td>
<td>1</td>
<td>36</td>
<td>1.3</td>
</tr>
<tr>
<td>Oxyopidae</td>
<td>1</td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>37</td>
<td>1.3</td>
</tr>
<tr>
<td>Philodromidae</td>
<td>1</td>
<td>21</td>
<td>1</td>
<td>2</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Tetragnathidae</td>
<td>1</td>
<td>98</td>
<td>8</td>
<td>30</td>
<td>136</td>
<td>5</td>
</tr>
<tr>
<td>Zoridae</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Linyphiidae</td>
<td>-</td>
<td>378</td>
<td>103</td>
<td>147</td>
<td>628</td>
<td>22.5</td>
</tr>
<tr>
<td>Salticidae</td>
<td>-</td>
<td>43</td>
<td>6</td>
<td>6</td>
<td>55</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>2194</td>
<td>301</td>
<td>302</td>
<td>2797</td>
<td>100</td>
</tr>
</tbody>
</table>

The 10 most abundant genera represent 82.2 % of the specimens (Table 2). The majority corresponds to web building spiders, i.e., *Nigma, Anelosimus, Anyphaena, Theonoae, Tetragnatha* and *Araniella*, and some hunting spiders, i.e., *Ozyptila, Clubiona, Diaea* and *Synema*. Among the captured genera, those that are known as predators of citrus pests include *Cheiracanthium, Araneus, Theridula, Diaea, Oxyopes, Synema, Clubiona* and *Theridion*.

The identified spiders present representatives of the eight guilds proposed by Uetz et al. (1999). The web builders were more abundant (70%) than hunting spiders (30%) (Table 3).

The influence of weed management system

As expected, the number of spiders was consistently higher in both ground cover vegetation modalities (RV and S) in comparison to the herbicide (H) (Figure 1).

Considering the 10 most abundant genera of spiders (Table 4), there is no consistent pattern in what concerns the influence of the weed management system. However, in the case of the genera *Tetragnatha* and *Araniella*, the mean number of spider captures in the “RV” and “S” modalities was about two times higher that of “H”.

The aerial web builders showed a consistent pattern (Figure 2). The spider captures registered in these guilds were higher in both ground cover modalities than in “H”. No clear differences were observed between modalities in the other guilds. Apparently, the aerial web builders were the guild most favoured by the ground cover vegetation. The higher abundance of this guild on the crop may be related to the higher prey availability in consequence of higher activity of flying insects attracted by the nectar and pollen of the different flowering plant species in ground cover vegetation.
Table 2. Number and relative abundance of the captured specimens per identified genus in three lemon orchards from Mafra, in the Oeste region of Portugal.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>No specimens</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agelenidae</td>
<td>Teextrix</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Amaurobiidae</td>
<td>Amaurobius</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Anyphaenidae</td>
<td>Anyphaena</td>
<td>144</td>
<td>6.8</td>
</tr>
<tr>
<td>Araneidae</td>
<td>Agalanatea</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Araneus</td>
<td>30</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Araniella</td>
<td>132</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>Cyclosa</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Cytarachne</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Gibbaranea</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Larinioides</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Mangora</td>
<td>12</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Nuctenea</td>
<td>17</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Singa</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Zilla</td>
<td>8</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Zygiella</td>
<td>21</td>
<td>1.0</td>
</tr>
<tr>
<td>Clubionidae</td>
<td>Clubiona</td>
<td>89</td>
<td>4.2</td>
</tr>
<tr>
<td>Dictynidae</td>
<td>Nigma</td>
<td>551</td>
<td>26.1</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td>Drassodes</td>
<td>35</td>
<td>1.7</td>
</tr>
<tr>
<td>Lycosidae</td>
<td>Pardosa</td>
<td>8</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Trabaeae</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Miturgidae</td>
<td>Cheiracanthium</td>
<td>36</td>
<td>1.7</td>
</tr>
<tr>
<td>Oxyopidae</td>
<td>Oxyopes</td>
<td>37</td>
<td>1.8</td>
</tr>
<tr>
<td>Philodromidae</td>
<td>Philodromus</td>
<td>24</td>
<td>1.1</td>
</tr>
<tr>
<td>Tetragnathidae</td>
<td>Tetragnatha</td>
<td>136</td>
<td>6.4</td>
</tr>
<tr>
<td>Theridiidae</td>
<td>Anelosimus</td>
<td>162</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>Coscinida</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Dipoena</td>
<td>27</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Enoplognatha</td>
<td>15</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Keijia</td>
<td>19</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Paidiscura</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Robertus</td>
<td>14</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Steatoda</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Theoneo</td>
<td>148</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Theridion</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Theridula</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Thomisidae</td>
<td>Diaea</td>
<td>88</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Misumena</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Ozyptila</td>
<td>206</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>Runcinia</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Synema</td>
<td>83</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Thomisus</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Xysticus</td>
<td>19</td>
<td>0.9</td>
</tr>
<tr>
<td>Zoridae</td>
<td>Zora</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2114</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Figure 1. Abundance of spiders in three lemon orchards from Mafra, in the Oeste region of Portugal, in 2002 and 2003, based on beating sampling and in function of weed management system: inter-row ground cover with resident vegetation (RV), inter-row ground cover by sowing selected species (S) and inter-row herbicide application (H).

Table 3. Number and relative abundance of the different spider guilds captured in three lemon orchards from Mafra, in the Oeste region of Portugal.

<table>
<thead>
<tr>
<th>Guilde (Family)</th>
<th>No specimens</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space web builders (Dictynidae, Theridiidae)</td>
<td>957</td>
<td>34,2</td>
</tr>
<tr>
<td>Wandering sheet/tangle weavers (Linyphiidae)</td>
<td>628</td>
<td>22,5</td>
</tr>
<tr>
<td>Ambushers (Philodromidae, Thomisidae)</td>
<td>428</td>
<td>15,3</td>
</tr>
<tr>
<td>Orb weavers (Araneidae; Tetragnathidae)</td>
<td>372</td>
<td>13,2</td>
</tr>
<tr>
<td>Foliage runners (Anyphaenidae, Clubionidae, Miturgidae)</td>
<td>269</td>
<td>9,6</td>
</tr>
<tr>
<td>Stalkers (Oxyopidae, Salticidae)</td>
<td>92</td>
<td>3,3</td>
</tr>
<tr>
<td>Ground runners (Gnaphosidae, Lycosidae, Zoridae)</td>
<td>48</td>
<td>2,0</td>
</tr>
<tr>
<td>Sheet web builders (Agelenidae, Amaurobiidae)</td>
<td>3</td>
<td>0,1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2797</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

The maximal captures of spiders registered by suction sampling in the lemon trees occurred in July, in all three modalities of weed management, when most of the ground cover vegetation was already dry (Figure 3).

Considering the most abundant spider guilds, both on lemon trees and ground cover vegetation, the results show that the relative abundance of the wandering sheet/tangle weavers and ambushers in ground cover vegetation was maximal in the period when this habitat was sampled, i.e., March and May (Figure 4). The other guilds were relatively more abundant on the crop, in the same period, in function of the different modalities.
Table 4. Number of spiders (± standard deviation) captured in lemon trees, by the beating method, in each modality, in three lemon orchards from Mafra, in the Oeste region of Portugal: inter-row ground cover with resident vegetation (RV), inter-row ground cover by sowing selected species (S) and inter-row herbicide application (H).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Family</th>
<th>H</th>
<th>S</th>
<th>RV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigma</td>
<td>Dictynidae</td>
<td>46,7±15,2</td>
<td>56,7±19,3</td>
<td>45,0±14,7</td>
</tr>
<tr>
<td>Ozyptila</td>
<td>Thomisidae</td>
<td>17,3±1,5</td>
<td>13,7±5,5</td>
<td>18,0±5,2</td>
</tr>
<tr>
<td>Anelosimus</td>
<td>Theridiidae</td>
<td>16,3±11,5</td>
<td>10,3±5,0</td>
<td>20,7±16,2</td>
</tr>
<tr>
<td>Anyphaena</td>
<td>Anyphaenidae</td>
<td>16,0±8,5</td>
<td>10,3±2,5</td>
<td>16,7±13,4</td>
</tr>
<tr>
<td>Theonoe</td>
<td>Theridiidae</td>
<td>8,7±7,2</td>
<td>10,7±3,2</td>
<td>26,3±9,9</td>
</tr>
<tr>
<td>Tetragnatha</td>
<td>Tetragnathidae</td>
<td>8,0±5,2</td>
<td>18,0±3,6</td>
<td>14,7±6,5</td>
</tr>
<tr>
<td>Araniella</td>
<td>Araneidae</td>
<td>7,3±5,9</td>
<td>14,3±9,9</td>
<td>14,0±12,5</td>
</tr>
<tr>
<td>Clubiona</td>
<td>Clubionidae</td>
<td>9,0±2,6</td>
<td>7,3±4,2</td>
<td>4,0±2,0</td>
</tr>
<tr>
<td>Diaea</td>
<td>Thomisidae</td>
<td>11,7±15,9</td>
<td>5,7±7,4</td>
<td>9,3±11,8</td>
</tr>
<tr>
<td>Synema</td>
<td>Thomisidae</td>
<td>9,7±7,0</td>
<td>6,0±1,0</td>
<td>8,3±6,1</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>15,1±8,1</td>
<td>15,3±6,2</td>
<td>17,7±9,8</td>
</tr>
</tbody>
</table>

Figure 2. Abundance of the spider guilds captured in lemon trees, by beating sampling, in three lemon orchards from Mafra, in the Oeste region of Portugal, in function of weed management system: inter-row ground cover with resident vegetation (RV), inter-row ground cover by sowing selected species (S) and inter-row herbicide application (H).

**Sampling methods**

The number of captures obtained with the beating method was more than four times that obtained by suction in the lemon trees (Figure 5). However, the relative abundance of spiders
registered in the three modalities of weed management (Figure 6) and the relative importance of the different guilds was similar in both sampling methods (Figure 7).

Figure 3. Number of spiders captured by suction sampling in the lemon trees and ground cover vegetation, in three lemon orchards from Mafra, in the Oeste region of Portugal: inter-row ground cover with resident vegetation (RV), inter-row ground cover by sowing selected species (S) and inter-row herbicide application (H).
Figure 4. Relative abundance of the major spider guilds, capture by suction sampling, both in lemon threes and ground cover vegetation, in three lemon orchards from Mafra, in the Oeste region of Portugal, 2003: a) space web builders; b) wandering sheet/tangle weavers; c) ambushers; d) orb weavers; e) foliage runners.
Figure 5. Abundance of spiders collected by suction and beating sampling in lemon trees, in three lemon orchards from Mafra, in the Oeste region of Portugal, 2003: ground cover with resident vegetation (RV), ground cover by sowing selected species (S) and herbicide application (H).

Figure 6. Relative abundance of spiders collected by suction and beating sampling in lemon trees, in three lemon orchards from Mafra, in the Oeste region of Portugal, 2003: ground cover with resident vegetation (RV), ground cover by sowing selected species (S) and herbicide application (H).

Figure 7. Relative importance of the spider guilds identified by suction and beating sampling, in lemon trees, in three lemon orchards from Mafra, in the Oeste region of Portugal.

**Conclusion**

It was shown that spiders are relatively abundant and diverse in lemon orchards, in the Oeste region of Portugal, presenting representatives of all the guilds proposed by Uetz et al. (1999). The results agree with the hypothesis that habitat manipulation of ground cover vegetation within
lemon orchards may contribute to increase the abundance of spiders in lemon tree canopy. Apparently, the most favoured guild consisted of the aerial web builders.

The beating method showed to be more efficient for sampling spiders in lemon trees in comparison to suction.

Acknowledgements

We thank Elsa Borges da Silva, Manuel Cariano, Ana Baptista, Ana Passarinho, Pedro Rodrigues and Nuno Grade for the support in field and laboratory work. Thanks are due to the lemon growers Virginia Duarte, Carlos Batalha and Rosa Gomes that allowed us to carry out the experiment in their orchards. We thank also our project partners Rosário Antunes and Luis Aniceto (Frutoeste) for the logistic support. The research was part of the Project nº 29 PO AGRO Medida 8.1. DE&D.

References


Raquel Abad, Pedro Castañera and Alberto Urbaneja
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The two-spotted spider mite, *Tetranychus urticae* Koch, is an important citrus pest in Spain and elsewhere in the Mediterranean areas. In the last few years, *T. urticae* has become one of the main pests in the clementines of the region of La Plana (Castellón), where more than 90% of Spanish mandarin is produced. *Panonychus citri* (McGregor) is maintained under a satisfactory control in all Spanish citrus areas by the phytoseid *Euseius stipulatus* Athias-Henriot. In contrast, predatory mites do not control *T. urticae*. As a first step to explain the inefficient biological control of *T. urticae*, a survey was carried out to compare the natural enemies related to the abundance of each mite in citrus orchards in the “Comunidad Valenciana”. A similar number of phytoseids per infested leaf was associated to both spider mites species, though the species composition of phytoseids was different. Thus, *Euseius stipulatus* was the most abundant on citrus groves infested with *P. citri*. In contrast, the abundance of *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor) was higher on those infested with *T. urticae*. Differences on the abundance of other natural enemies were also found, being *Stethorus punctillum* Weise and *Contwenzia psociformis* Curt. the two prevalent species for both spider mites.
Quality control in *Aphytis melinus* mass rearing for the biological control of *Aonidiella aurantii* *

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Abstract: Laboratory trials were conducted to test the quality of the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), the main bio-control agent of the California Red Scale *Aonidiella aurantii* Maskell (Homoptera: Diaspididae), reared in the insectary of the Sicilian Regional Phytosanitary Service. The trials were conducted following the guidelines of the IOBC working group “Quality control of mass reared arthropods”, under the following conditions 25±2°C, 60±10% RH, 16L:18D. The parameters analyzed were the number of adults as specified on the releasing container, the sex-ratio (100 adults from bulk material), survival in transport (1000 adults maintained at 17±2°C for 5 days in a container with honey on the lid) and fecundity (observed on 30 females placed individually in contact for 5 days with 100 specimens of *Aspidiotus nerii* Bouché (Homoptera: Diaspididae) on squash). The data on the number of adults confirmed the quantities indicated on the releasing container, the sex-ratio (100 adults from bulk material), survival in transport (1000 adults maintained at 17±2°C for 5 days in a container with honey on the lid) and fecundity (observed on 30 females placed individually in contact for 5 days with 100 specimens of *Aspidiotus nerii* Bouché (Homoptera: Diaspididae) on squash). The data on the number of adults confirmed the quantities indicated on the containers and averaged 5000 ± 500 (n= 3). The percentage of females was 70.8 ± 4.6% (n= 5) and the percentage of mortality after 5 days was 9.3 (n= 5). The mean progeny produced per single female was 39.67 ± 10.63% (n= 3).

Key words: Citrus, California Red Scale, augmentative releases, Aphelinid

Introduction

The California Red Scale *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) is considered one of the main key pests of citrus in the Mediterranean basin. The Diaspid completes 3 to 4 generations per year, it infests trunks, twigs, leaves and fruits. The control of this pest is mainly carried out using chemicals which often induce resistance in the scale populations therefore leading to increases in the infestation levels (Grafton-Cardwell & Vehrs, 1995; Grout & Richards, 1992; Martinez Hervas et al., 2005). The natural control of the California Red Scale is performed by a complex of entomophagous among which a central role is played by *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), widely distributed in all Italian citrus growing areas (Longo et al., 1995).

*A. melinus* and *A. lignanensis* were introduced in Italy in the 60’s and since then *A. melinus* parasitization rates in the field averaged 20-30% (Benfatto & Cucinotta, 1991; Longo et al., 1994; Siscaro et al. 1999, Siscaro & Mazzeo, 2003; Mazzeo et al., 2004). Biological control of the California Red Scale using augmentative releases of *A. melinus* is widely and

* The Authors equally contributed to the work.
positively employed in several citrus growing areas around the world, such as California (Luck et al., 1997) and recently Morocco (Rizqi et al., 2001; 2004); A. lignanensis is instead used in Australia (Papacek & Smith, 1992). In the last decade, several trials have been carried out in Italy to evaluate the efficacy of augmentative releases of A. melinus (often produced in Californian commercial insectaries) in integrated and organic citrus orchards. The results of such trials were not uniform and didn't manage to totally convince about the effectiveness of biocontrol of the Red Scale by means of A. melinus. Trials carried out in Sicily showed increases in the parasitization rates on the fruits (Tumminelli et al., 2000); but subsequent experiments, conducted in 2002-03 using locally produced A. melinus releasing around 90,000 adults/ha starting from the end of the winter, showed a significant difference in the percentage of infested fruits between the treatment and the control. However, these results were not confirmed in the second year of the trial (Tumminelli et al., 2004). Further field trials were jointly conducted in 2000-2003 in Italy (Sicily and Calabria) (Mazzeo et al., 2004); in the framework of this three-year biocontrol project, 120,000 adults A. melinus/ha were released but no statistically significant differences were recorded in the parasitization rates and in the infestation levels on the fruits between the treatment and the control.

The explanation of the data reported in this short review of the latest biological control programs of A. aurantii in Southern Italy, might be found in the biological intrinsic limits of the parasitoid which is unable to keep the scale populations under the economic injury level, in the methodology of release and in the difficulty to involve entire regions in the releases. In order to better evaluate the technical and economic validity of biocontrol by means of augmentative releases in the framework of integrated pest management, autoctonous strains of A. melinus should be reared and released on time and throughout large citrus growing areas, having large amounts of parasitoids available from the end of the winter and when the presence of susceptible instars of the scale is maximum in the field. Besides, further investigations should be carried out to better define the number of parasitoids to release per hectare also in relation to the role played by A. melinus in the natural enemies complex of the California Red Scale (Forster & Luck, 1997, Siscaro et al. 1999, Siscaro & Zappalà, 2005). This would help to better plan the releases and to optimize the rearing techniques and investigating the opportunity to rear and release also other natural enemies of the scale.

Another key factor in explaining the above mentioned inconsistent results might be the low quality of the parasitoid mainly due to storage conditions when the rearing is carried out in foreign commercial insectaries (Tumminelli et al., 2004; Mazzeo et al., 2004).

The importance of quality control in relation to biological control was highlighted only in the mid-1980s and from that moment on the topic gradually gained more interest (van Lenteren, 2003). Nowadays quality assessment of entomophagous arthropods used in augmentative biological control is one of the main concerns after their mass production (Grenier & De Clercq, 2003). Several examples of poorly functioning mass-reared arthropods in the absence of quality evaluation are known in the literature (Calkins & Ashley, 1989; Leppla, 2003) and the same kind of consideration can be made for inferior natural enemies which can result in failures in biological control (Bigler, 1994; van Lenteren et al., 2003). The aim of quality control protocols is to verify whether the overall quality of a beneficial is maintained during and after its rearing process. For this reason, biological and behavioral characteristics, quantifiable and relevant for the field performance of the species, are identified and become part of the quality control guidelines.

In this context, continuing previous studies on the main biological and behavioral features of field, commercial and locally reared A. melinus strains (Raciti et al., 2003; Raciti & Saraceno 2004), tests on the quality of the strain produced in an experimental insectary were conducted and are here presented.
Material and methods

Insect rearing

*Aphytis melinus* was reared on the Oleander scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae) on squash (var. Butternut) in the insectary of the Sicilian Regional Phytosanitary Service (SRPS), following the technique described by DeBach and White (1960) with subsequent minor modifications (Raciti et al., 2003) aiming at optimizing the rearing process by increasing the production levels although containing the costs. The initial *A. melinus* strain originated from a commercial insectary and it has since then been periodically crossed with field-collected material.

Testing methods

The tests on the parasitoid were conducted in 2004 following the procedures described in the guidelines developed by the IOBC working group on Quality Control of Mass-Reared Arthropods (AMRQC), the International Biocontrol Manufacturers Association (IBMA) and the Association of Natural Bio-control Producers (ANBP) and recently published (van Lenteren et al., 2003). The biological features used as quality control factors were quantity of adults in a releasing container, sex-ratio, survival of adult parasitoids at 17 ± 2°C for 5 days and mean fecundity and longevity per female.

The test on quantity (replicated 3 times) was conducted counting the specimens present in a standard releasing container (150ml) after having kept it for 10 minutes at -6°C. The trial was carried out 3 times on packages prepared to be released in the field. The percentage of females in the reared strain was evaluated observing 100 adults, randomly collected from bulk material, under binocular microscope for sex determination. This observation was repeated 5 times during the main releasing period (March-October). The capacity of the adults to survive standard storage and transport temperatures was estimated calculating the mortality rates on 1000 adults kept in a 150ml container for 5 days at 17 ± 2°C, provided with food (honey). This test was replicated 5 times in the same time interval of the previous one. The parasitic activity (intended as the offspring per single presumably mated female) was observed 3 times (from March to October) on 30 females confined individually in a plastic container (45ml, ∅ 35mm) with a male and provided with food (honey). The container was affixed, using atoxic plasticine, to the surface of a squash infested with *A. nerii*. Each female had access to 100 scales on average, the supernumerary specimens were eliminated using a pin. The parasitoids were removed after 5 days, the dead specimens were scored and the rate of parasitism was evaluated recording the progeny produced per female (number of emerged adults). All the tests (except for the survival at storage and transport temperature) were conducted at 25°C, 60% R.H., a 16L:8D light regimen and the specimens tested were always collected from the rearing units anesthetizing the parasitoids using CO₂.

Results and discussion

The results of the laboratory experiments conducted during 2004 proved that the *A. melinus* strain subjected to quality control tests totally fulfills the standard quality requirements fixed by scientists and the main producers of beneficials.

The parasitoid in particular perfectly survived the tested temperatures (at 17°C more than 90% of the specimens were alive after 5 days) which simulated storage and transport conditions (Table 1).

The mean progeny produced per single female (39.67 emerged adults) was higher than the inferior limit fixed as quality requirement (20/female). Besides, in none of the replicates
the mortality level was higher than 20%. This result fits the criteria fixed in the guidelines and can be used as parameter to evaluate the activity of the parasitoid in the field.

Table 1. The biological features tested, the limits fixed as quality control criteria and the results obtained in the tests carried out in 2004.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quality control criteria</th>
<th>Results (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity</td>
<td>As reported on the releasing container</td>
<td>5000 ± 500 specimens/container</td>
</tr>
<tr>
<td>Sex-ratio</td>
<td>70 ± 10% females</td>
<td>70.8 ± 4.6% females</td>
</tr>
<tr>
<td>Survival at 17 ± 2°C</td>
<td>≥80%</td>
<td>90.8%</td>
</tr>
<tr>
<td>Fecundity</td>
<td>≥20 offspring/5days/female</td>
<td>39.67 ± 10.63 offspring/female</td>
</tr>
</tbody>
</table>

Considering the biology of the parasitoid, which feeds on the hosts to acquire proteins useful to continue egg production, it might be useful to include also host-feeding in these quality control criteria, in order to better evaluate the total parasitic potential of the Aphelinid.

The procedures followed in this work are applied by a number of companies that mass-produce natural enemies in Europe and North-America and are used as well to compare performance of the same species of natural enemy by different companies. In any case these are product-control guidelines aiming at defining quality standards that need to be performed by the tested natural enemy and are intended to be conducted by the producers but can also be used by distributors, pest-management advisors and farmers (van Lenteren et al., 2003). Although they are intended to provide control on the product, these procedures might give useful suggestion to standardize the rearing process with specific reference to the adults collecting methods, host densities, food supply and number of specimens per releasing container. The tested *A. melinus* strain, which can therefore be considered as conform to quality requirements.

**Conclusive remarks**

The worldwide diffusion of commercial insectaries underlines the growing importance of biocontrol programs but at the same time stimulates reflection on the quality of the natural enemies produced. This parameter should actually be considered of central importance as it might result in failure of biological control.

The use of beneficials in biological or integrated pest control programs is politically and economically supported with special funding in most of the countries with modern agriculture, but it also became a commonly used practice in rationally managed farms. Farmers are no longer guided only by economic support nor by the necessity to find a technique to control pests that had become resistant to pesticides (van Lenteren, 2003). The market of natural enemies has been characterized up to now by a low competitiveness, but the increasing demand of beneficials is transforming the competition among the always more numerous commercial insectaries, focusing more on the costs and the volumes of production. A producer undoubtedly conducts a very careful costs vs. benefits analysis but often obtaining beneficials at a competitive cost doesn’t correspond to quality standard principles.

Leaving out the scientific aspects of quality control, which cannot be thoroughly investigated in this context, it is important to keep in mind that the main concern for mass production of natural enemies and subsequent augmentative releases is the control of a pest.
In this perspective, the purpose of quality control is to verify whether a mass produced beneficial is still able to control that pest (van Lenteren, 2003). Quality control procedures should not aim at obtaining maximal or optimal quality, but rather acceptable quality. This might be affected by the rearing conditions and by the handling before the release (removal from the plant or from the host, counting, packaging, gluing to substrate, induction of diapause, transport and release methods, etc.): the more artificial the rearing conditions become and the more the beneficials are manipulated before use, the larger the amount of tests that will have to be performed (van Lenteren, 2003).

The field evaluation of the produced material becomes therefore essential in the quality assessment process which shouldn’t be limited to laboratory trials but should become part of a total quality control system (TQC) (Leppla, 2003). In this perspective, the data obtained in the present work will be employed in field trials in order to evaluate its performances in biocontrol programs of the California Red Scale by means of augmentative releases in the Mediterranean citrus growing areas. The results of these trials would provide further elements to better define the role of the parasitoid in keeping A. aurantii populations under control.

Acknowledgements

The authors wish to thank Dr. Rosario Finocchiaro, Dr. Rosario Maugeri and Dr. Antonio Strano (Microbios s.r.l.) for their precious support in the rearing procedures.

References


Side effects of five acaricides on the predator Cryptolaemus montrouzieri (Coleoptera: Coccinellidae)

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The effects of 5 acaricides (fenazaquin, clofentezin, tebufenpirad, fenbutestan and mineral oil) on biological parameters (survival, fecundity, fertility and developmental time) of different stages of the mealybug destroyer, Cryptolaemus montrouzieri Mulsant (Col.: Coccinellidae), were evaluated under laboratory conditions by: (1) direct contact - topical application on larvae, pupae and adults; and (2) ingestion - feeding adult and larva with the citrus mealybug [Planococcus citri (Risso) (Hom.: Pseudococcidae)] previously treated by the correspondent acaricide. The five acaricides resulted harmless for adults and pupae of C. montrouzieri. All acaricides tested were slightly harmful to larvae of C. montrouzieri, except fenbutestan, which did not show any effect.
New records of hymenopteran parasitoid species from citrus orchards in Terceira Island (Azores, Portugal)

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Abstract: Samples of parasitized aphids and scale insects were collected in different plant hosts nearby citrus orchards in three different places, in Terceira Island (Azores), namely, Biscoitos, S. Sebastião and Terra-Chã, aiming at finding plant species that can provide alternative hosts for parasitoids of citrus pests. Emerged parasitoids consisted mainly of braconids, i.e., Lysiphlebus fabarum (Marshall), and Lysiphlebus testaceipes (Cresson) from Aphis fabae Scopoli in Pittosporum undulatum and Banksia sp., and Aphidius funebris (Mackauer) from Uroleucon sonchi (Linnaeus), in Sonchus sp. One specimen of both Aphelinus chaonia Walker and Microterys nietneri (Motschulsky) from Coccus hesperidum in Hedera sp. Scutellista obscura emerged from Parasaissetia nigra (Nietner) in P. undulatum and also Moranilla californica (Howard). Secondary parasitism of the pteromalid Pachyneuron aphidis (Bouché) was also collected from Aphis solanella, in Solanum nigrum L. These parasitoid species are reported for the first time from the Azores.

Keywords: Aphids and scales parasitoids, Aphelinus chaonia, Aphidius funebris, Lysiphlebus fabarum, Lysiphlebus testaceipes, Microterys nietneri, Moranilla californica, Pachyneuron aphidis, Scutellista obscura, Terceira Island, Azores.

Introduction

The natural control of aphid and scale pests through parasitoid activity is an important tool for pest management. The knowledge of the tritrophic plant-pest-parasitoid relationship can give the information to select the vegetation that support the multiplication of the most suitable parasitoids to increase natural control of pests and consequently reduce the input of chemical control (Tizado & Núñez-Perez, 1992, Cecílio et al., 1998).

Previous observations gave evidence of the absence of parasitism activity in the citrus orchards surveyed in Terceira Island. The lack of natural enemies is commonly associated to a high level of chemical control.

To get information on the biodiversity of parasitoids of aphids and scale pests a study was carried out aiming at finding plant species that can provide alternative hosts for parasitoids of aphid and scale insect pests of citrus orchards in Terceira Island, Azores.

Material and methods

Samples of parasitized aphids and scale insects were collected from June up to October of 2004 in different plant hosts nearby citrus orchards, in three different places, namely,
Biscoitos, S. Sebastião and Terra-Chã. The collected plants were studied in the laboratory, under magnification, and identified. Then, the plants with parasitized aphids and scale insects were placed in emergency cages for collecting the parasitoids. The emerged parasitoids were preserved in 70% ethanol for posterior identification.

Results and discussion

A total of 454 parasitoid specimens were collected from 13 plant species, including four hymenopteran families, namely Aphelinidae (1), Braconidae (172), Encyrtidae (1) and Pteromalidae (280) (Table 1). Seven parasitoid species are reported for the first time from Terceira Island, Azores: Aphelinus chaonia Walker, 1839, Aphidius funebris (Mackauer, 1961) Lysiphlebus fabarum (Marshall, 1896) Lysiphlebus testaceipes (Cresson, 1880) Microterys nietneri (Motschulsky, 1859), Pachyneuron aphidis (Bouché, 1834), Scutellista obscura (Foster, 1878). Pteromalid Moranila californica (Howard, 1881) is observed and already known from Terceira Island (Oliveira, 2002, Borges et al. 2005).

The association list of the tritrophic relation plant-host-parasitoid is referred for the first time for Azores Archipelago, at Terceira Island (Table 1).

Table 1. Plant - host – parasitoid relationship identified for Terceira Island.

<table>
<thead>
<tr>
<th>PLANT HOST</th>
<th>PARASITOID FAMILY</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banksia sp. Aphis fabae</td>
<td>Lysiphlebus testaceipes* Braconidae</td>
<td>33</td>
</tr>
<tr>
<td>Dahlia sp. Unidentified species</td>
<td>Pteromalidae</td>
<td>52</td>
</tr>
<tr>
<td>Foeniculum vulgare Miller</td>
<td>Lysiphlebus fabarum* Braconidae</td>
<td>39</td>
</tr>
<tr>
<td>Hedera sp. Coccus hesperidum</td>
<td>Aphelinus chaonia* Apheilidae</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Microterys nietneri* Encyrtidae</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Moranila californica Pteromalidae</td>
<td>1</td>
</tr>
<tr>
<td>Hibiscus rosa-sinensis L. Aphis gossypii</td>
<td>Lysiphlebus fabarum* Braconidae</td>
<td>21</td>
</tr>
<tr>
<td>Pittosporum undulatum Vent. Aphis fabae</td>
<td>Lysiphlebus fabarum* Braconidae</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Lysiphlebus testaceipes*</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Lysiphlebus testaceipes*</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Moranila californica Pteromalidae</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>Scutellista obscura*</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Parasaissetia nigra</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aphis spiraecola</td>
<td>Lysiphlebus testaceipes* Braconidae</td>
</tr>
<tr>
<td></td>
<td>Aphis gossypii</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aphis fabae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aphis solanella</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aphis fabae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aphis gossypii</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uroleucon sonchi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solanum nigrum L.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aphis gossypii</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sonchus sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uroleucon sonchi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zea mays L.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhopalosiphum padi</td>
<td></td>
</tr>
</tbody>
</table>

*species identified for the first time

The majority (62%) of the collected parasitoids belong to Pteromalidae (Table 1). Among these, Moranila californica was the dominant species contributing with 40% of total specimens. Pachyneuron aphidis was the second most representative pteromalid and Scutellista obscura the last. All specimens of S. obscura emerged from Parasaissetia nigra.
(Nietner, 1861) in *P. undulatum*, but one specimen of *M. californica*, emerged from *Coccus hesperidum* in *Hedera* sp. *M. californica* was collected by Oliveira (2002) and identified later by Borges *et al.*, (2005), without referring the host insect and the host plant.

*M. californica* is known from the Mediterranean Region as a parasitoid of *Saissetia oleae* (Oliver, 1791), a common citrus pest (Carvalho & Aguiar, 1997).

Braconidae was the second most abundant family, with *Lysiphlebus fabarum* as dominant species. This parasitoid was mainly collected from *Aphis fabae* Scopoli, 1763, on different host plants, such as *P. undulatum*, *Foeniculum vulgare* Miller and *Rumex* sp. *L. fabarum* also emerged from *Aphis gossypii* Glover, 1877 on *Hibiscus rosa-sinensis* and on *Taraxacum* sp. *A. gossypii* is a citrus pest. It can both originate crop injury and is considered a potential vector of CTV (Citrus Tristeza Virus) mild strains (Ilharco, 1992; Gariido & Rius 1993; Aguiar *et al.*, 1994).

The braconid *Aphidius funebris* (Mackauer, 1961) is a common species parasitizing *Uroleucon sonchi* (Linnaeus, 1767) on *Sonchus* sp.

*Lysiphlebus testaceipes*, was collected from four aphid species and five host plants, namely, *A. gossypii* on *Rosa* sp., *Aphis solanella* Theobald, 1914, on *Solanum nigrum* L., *Aphis spiraecola* Patch, 1914, on *Prunus lusitanica* L. ssp. *azorica* (Mouilefert) Franco and from *A. fabae* on *P. undulatum* and *Banksia* sp.

*L. testaceipes* is an active species parasitizing aphids of the genera *Aphis* and other aphids of citrus in continental Portugal (Cecílio, 1994; Cecílio *et al.*, 1998).

The braconids *Lysiphlebus fabarum* and *Aphidius funebris* are common species of the Mediterranean fauna. *Lysiphlebus testaceipes* was introduced in the Mediterranean region around (1973-74) and it is known from Continental Portugal since 1985 and from Madeira Island since 1990 (Cecílio, 1994, Starý *et al.*, 1996). *L. fabarum* and *L. testaceipes* were recommended as valuable biocontrol agents of several aphid pests (Starý *et al.*, 1996).

The seasonal pattern of the collected parasitoid species appear to show a clear trend with a dominance of aphid parasitoids in June, such as *L. fabarum* and *L. testaceipes* collected from *A. fabae* respectively on *F. vulgare* and *Rumex* sp., that is followed by an increasing of scale insect parasitoids late in the summer, such as *M. californica* sampled from *P. nigra* on *P. undulatum* (Figure 1). Although based on one year of observations, this pattern appears to follow the population dynamics of the aphid and scale hosts.

The absence of parasitism on the studied orchards can be justified by the application of blind chemical control on fruit trees. However, the population of aphid and scale parasitoids observed on shrubs and herbaceous vegetation, nearby citrus orchards shows that there is a population of natural enemies of aphid and scale pests with good potential for natural control.

Furthermore a programme of Integrated Plant Protection in orchards should be implemented associated to a further study of the diversity and abundance of natural enemies of the pests in order to clarify the insect and plant hosts suitable for a sustainable management of the ground cover and hedges of the orchards.
Figure 1. Emergence of parasitoid species by locality and month.

The parasitism of *A. spiraecola* in the Mediterranean region by braconids is made by different species, meanwhile, in Italy, Tremblay *et al.* (1983) said that this aphid it’s not common parasitized, and it is one of the most important pest on citrus orchards.

*A. spiraecola* is not commonly parasitized, even by indigenous parasitoid *L. fabarum*, and that’s the reason that is one of the most important pests in citrus orchards (Tremblay *et al.*, 1980).

The inventory of other useful plant species should show the interest of their preservation as useful on multiplication of natural enemies, predators and parasitoids, important for pest control.

**Acknowledgements**

Thanks are due to George Japoshivilli for the identification of encyrtids, aphelinids and some pteromalids.

**References**


Time allocation, predation and gut capacity of eleven phenotypes of *Adalia decempunctata* L. (Col., Coccinellidae) on black citrus aphid *Toxoptera aurantii* B. (Hom., Aphididae)

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Time allocation, predation behaviour and effectiveness and gut capacity were studied in the laboratory with eleven phenotypes of the aphidophagous coccinellid *Adalia decempunctata* preying on *Toxoptera aurantii*, the most abundant aphid on citrus in Morocco. The response of the various adult phenotypes towards these parameters was assessed and discussed. The results showed that all phenotypes allocated a large portion of their time to resting. Significant differences were noted among phenotypes with respect to their time allocations to walking, resting, feeding, predation, degree of prey consumption, and grooming. The total predation rate was higher for phenotypes P1 (71.6%), P11 (70.3%), P9 (64.3%) and P4 (45.3%) than for others, and no selectivity was observed to the specifics of *T. aurantii* parts. The relative gut capacity of *A. decempunctata* is low and varied among phenotypes (0.154 10^-3 to 0.267 10^-3). Knowledge of the interactions of these aphidiphagous ladybirds with their prey is important for their employment as indigenous natural enemies in the context of IPM. *A. decempunctata* is an autochthonous predator of which only the P1, P11, P9 and P4 morphs could be effective against aphids, particulary *T. aurantii*. It should be released and studied on a large IPM schedule on citrus orchard.
Parasitoid complex of citrus leafminer on lemon orchards in Portugal

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Abstract: The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was detected for the first time in Portugal during the summer of 1994 and rapidly dispersed throughout the citrus growing area. Since its introduction several parasitoid species have been reported including indigenous species that added CLM to their host range and, recently, exotic species, despite no classical biological control programme has been carried out in the country. During the summer of 2003, the rate of parasitism of CLM and the emerged parasitoids were studied in three lemon orchards from Mafra region, a major lemon production area, ca. 30 Km north of Lisbon. Eight parasitoid species were identified including the eulophids *Semielacher petiolatus* (Girault) (25% of the specimens), *Pnigalio pectinicornis* L. (23%), *Cirrospilus pictus* Ness (17%), *C. vittatus* Walker (13%), *Citrostichus phyllocnistoides* (Narayanan) (12%), *C. brevis* Zhu (8%), *Neochrysocharis formosa* (Westwood) (2%) and a pteromalid, *Pteromalus* sp. (0,4%). *C. phyllocnistoides*, *N. formosa*, *P. pectinicornis* and *S. petiolatus* are reported for the first time from Portugal. Considering that *S. petiolatus* and *C. phyllocnistoides* were introduced in Spain during 1997-1999, data suggest that the presence of this two exotic parasitoid species in Mafra is the result of natural geographical expansion from Spain.

Key words: *Phyllocnistis citrella*, parasitoids, citrus pests, biological control

Introduction

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an Asian native pest of citrus and a few closely-related Rutaceae. Since the 1990’s it has spread rapidly to all citrus-growing regions of the Mediterranean basin and America, attaining major pest status (Knapp et al., 1995; Argov & Rössler, 1996). In Portugal, the CLM was detected for the first time in Algarve, in the summer of 1994 (Carvalho et al., 2000).

After the invasion of CLM, several native parasitoids tended to explore the new host in each region. Almost 80 parasitoid species have been reared from the CLM throughout the world (LaSalle & Peña, 1997). In some regions, native parasitoids were able to control CLM populations (Vercher et al., 2003). In others, efforts had been made to implement programs of classical biological, through introduction of host-specific parasitoids from the native region of the CLM (García-Marí et al., 2004). The five parasitoid species considered as more efficient biological control agents and more frequently selected for classic biological control of CLM are the eulophids *Cirrospilus quadristriatus* (Subba Rao & Ramamani), *Citrostichus phyllocnistoides* (Narayanan), *Quadrastichus sp.*, *Semielacher petiolatus* (Girault) and the encyrtid *Ageniaspis citricola* Logvinovskaya (Heppner, 1993; Ujiye & Adachi, 1995; Vercher et al. 2003; García-Marí et al., 2004).

The first introductions of CLM parasitoids in the Mediterranean were conducted in Israel, in 1994-1995, including six exotic species (Argov & Rössler, 1998). Later, other countries, such as Algeria, France, Greece, Morocco, Italy and Spain have also developed programs of classical biological control (García-Marí et al., 2004).
In the Iberian Peninsula the programs of classical biological control of CLM were initiated in Spain in 1996. Up to 1999, the parasitoid species *A. citricola*, *Quadrastichus* sp., *S. petiolatus* and *C. phyllocnistoides* were imported and released. As a result *A. citricola* was able to establish only in Canary Islands; *Quadrastichus* sp. in Valence; *S. petiolatus* in Andalusia and Balear Islands; and *C. phyllocnistoides* dispersed throughout Continental Spain and Balear Islands (Vercher et al., 2000, 2003; García-Marí et al., 2004).

In Portugal, except for Madeira Island where *A. citricola* was imported in 1998 from Israel and Spain and released (Brazão, 1999), no classical biological control programme was carried out to control the CLM. Data on the parasitoid complex of CLM were presented by Naves et al. (1999), Soares et al. (2000) and Brazão (2002), including at least 10 species, from Madeira and the continent.

In this paper we present the results of a study carried out in 2003, in lemon orchards from Mafra, in the Oeste region of Portugal, aiming to collect information on the parasitoid complex associated with CLM in the region and to estimate the level of parasitism in relation to the infestation dynamics of the CLM.

### Material and methods

#### Experimental plots

The study was carried out in three 1-4 ha lemon orchards from Mafra, ca. 30 Km north of Lisbon. During the study, farmers followed an IPM system under the technical supervision of Frutoeste, a farmers association of Oeste region.

#### Infestation dynamics of the citrus leafminer

The infestation dynamics of CLM were monitored from January up to November 2003. Samples of 150 young shoots (two per tree) were observed every two weeks in each orchard to estimate the infestation level.

#### Citrus leafminer parasitism

The CLM parasitism was studied from August 20th up to October 28th 2003, by sampling one or two infested shoots per tree, in 75 trees per orchard. Sampled shoots were transported to the laboratory in plastic bags and dissected under magnification. The number of alive/death individuals, the respective developmental stage and the causes of death were determined by sampling 300 CLM larvae or pupae, per orchard.

The sampled shoots were maintained in 15 cm x 15 cm x 8 cm translucent plastic boxes, with etamine cover, in laboratory conditions (21-22ºC) for the emergence of CLM adults and parasitoids. Observations were carried out weekly during one month after sampling. The emerged parasitoids were preserved in 70% ethylic alcohol for posterior identification.

#### Parasitoid identification

The emerged specimens were studied under magnification and grouped according to morphotypes. Identification to the genera or species level was then carried out based on Boucek (1968), Gonzalez et al. (1996), Cancino et al. (2001) and Urbaneja (2003). Selected specimens of each morphotype were sent to Dr. Alberto Urbaneja (IVIA, Spain) and Dr. Maria Jesús Verdú (IVIA, Spain), who kindly confirmed the identifications at the species level.

### Results

#### Infestation dynamics of the citrus leafminer

In average the infestation level of the CLM was very low from January up to May (Figure 1). A fast increase was then registered from June up to September, reaching a maximum of 100%
of infested shoots. The CLM infestation level decreased rapidly afterwards. Between July and October the CLM presented a mean density of 1 larva or pupa/shoot: 0.2 in July, 2 in September and 0.9 in October.

Parasitism
The global parasitism of the CLM inflicted by the complex of parasitoids in Mafra was very low, reaching a maximum of 18%, in one lemon orchard (Figure 2).

Figure 1. Average infestation dynamics of citrus leafminer, *Phyllocnistis citrella* Stainton, in lemon orchards from Mafra (Portugal), in 2003.

Figure 2. Parasitism rate (%) of citrus leafminer, *Phyllocnistis citrella* Stainton, in lemon orchards from Mafra (Portugal), in 2003 (n= 248).
Parasitoid species

A total of 248 parasitoid specimens were collected, including seven species of Eulophidae, and one Pteromalidae, namely the ectoparasitoids Pteromalus sp. (0.4%), C. brevis (8%), C. pictus (17%), C. vittatus (13%), C. phyllocnistoides (12%), Pnigalio pectinicornis L. (23%) and S. petiolatus (25%) and the endoparasitoid Neochrysocharis formosa (Westwood) (2%) (Figure 3). Four species were identified for the first time in Portugal: C. phyllocnistoides, N. formosa, P. pectinicornis and S. petiolatus. Thus, the actual list of referenced parasitoids of CLM in Portugal increases up to 14 species (Table 1).

The two exotic species, S. petiolatus and C. phyllocnistoides, represented between 12% and 25% of the total parasitoids in each sampling period (Figure 4). S. petiolatus reach its maximum relative abundance in September decreasing afterwards. On the other hand, C. phyllocnistoides increased its relative abundance from September up to October.

Figure 3. Number of specimens collected for each of eight parasitoid species of citrus leafminer, Phyllocnistis citrella Stainton, identified in lemon orchards from Mafra (Portugal) in 2003.

Discussion

Comparing with the data presented by Naves et al. (1999), the results suggest a change in the parasitoid complex associated with the CLM in Mafra, between 1999 and 2003, particularly in what concerns species composition and relative abundance. The global parasitism was not significantly modified. Naves et al. (1999) reported five native unidentified parasitoid species of the genera Pnigalio (1 sp.), Symphiesis (1 sp.), Pteromalus (1 sp.) and Cirrospilus (2 sp.). Cirrospilus spp. represented 60% of the parasitoid complex. In the present study, we identified seven species, including six native species of the genera Pteromalus (1 sp.), Cirrospilus (3 sp.), Neochrysocharis (1 sp.) and Pnigalio (1 sp.) and two exotic species, C. phyllocnistoides and S. petiolatus (Table 1). The native species represented 63% of the parasitoid complex of CLM. However the dominant species was S. petiolatus (25%).
The actual parasitoid complex of CLM in Mafra seems to result from the restructuring (Ehler, 1994) of the indigenous parasitoid complex through classical biological control. In fact, the presence of *C. phyllocnistoides* and *S. petiolatus*, reported for the first time from Portugal, is apparently the result of fortuitous introduction from Spain, as no classical biological control programme was carried out in continental Portugal to control the CLM. These two parasitoid species were introduced and successfully established in Spain. They were released between 1997 and 1999 and up to 2001 *S. petiolatus* established in Andalusia and Balear Islands and *C. phyllocnistoides* expanded to all citrus grown in continental Spain and the Balear Islands, becoming the most abundant parasitoid in all the orchards, and displacing native and other introduced parasitoids (Vercher et al., 2000, 2003; García-Marí et al., 2004).

Besides Iberian Peninsula, *C. phyllocnistoides* was also introduced and established in several other citrus growing areas in the Mediterranean basin, e.g., Cyprus, Greece, Israel, Italy, Morocco (Siscaro et al., 2003; Rizqi et al., 2003). For example, Siscaro et al. (2003) reported that the parasitoid was released in Sicily, between 1990 and 1999 and in 2002 it represented up to 90% of the parasitoid complex of the CLM. Spontaneous dispersion of *S. petiolatus* from other Mediterranean countries where this parasitoid was introduced was also reported in Italy, Algeria and Jordan (Siscaro et al., 2003).

Our observations are consistent with the progressively displacement of native parasitoid species after the establishment of *C. phyllocnistoides* and *S. petiolatus* reported by other authors (Liotta et al., 2003; Márquez et al., 2003; Siscaro et al., 2003; García-Mari et al., 2004). Considering that the relative abundance of *C. phyllocnistoides* registered in 2003 in
Mafra was still low (12%), which suggests a recent colonization of the region, and based on the results obtained in Spain, and other Mediterranean countries, where *C. phyllocnistoides* became the dominant species and was responsible for a significant increase of the percentage of CLM parasitism and of damage decrease (García-Mari et al., 2004), we expect that a similar trend will occur in the Oeste region of Portugal. Further studies are needed to confirm this hypothesis.

Table 1. Parasitoid species of citrus leafminer, *Phyllocnistis citrella* Stainton, reported from Portugal.

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>References</th>
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<tbody>
<tr>
<td><em>Apotetraristichus flavifrons</em> (Walker)</td>
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<td><em>Chrysocharis gemma</em> (Walker)</td>
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<tr>
<td><em>Chrysocharis</em> sp.</td>
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<tr>
<td><em>Cirrospilus</em> spp.</td>
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<tr>
<td><em>Cirrospilus</em> sp. near <em>lyncus</em> Walker =</td>
<td>Algarve</td>
<td>Gravanita (1998)</td>
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<tr>
<td>= <em>C. brevis</em> Zhu, La Salle &amp; Huang</td>
<td></td>
<td>according to Zhu et al. (2002)</td>
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<td></td>
<td></td>
<td>Present study</td>
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<tr>
<td><em>Cirrospilus pictus</em> (Nees)</td>
<td>Algarve</td>
<td>Coelho (1997)</td>
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<td></td>
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<tr>
<td><em>Cirrospilus vittatus</em> Walker</td>
<td>Madeira</td>
<td>Brazão (2002)</td>
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<tr>
<td><em>Citrostichus phyllocnistoides</em> (Narayanan)*</td>
<td>Mafra</td>
<td>Present study</td>
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<td></td>
<td></td>
<td>according to Noyes (2003)</td>
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<tr>
<td><em>Neochrysocharis formosa</em> (Westwood)*=</td>
<td>Mafra</td>
<td>Present study</td>
</tr>
<tr>
<td>= <em>Closterocerus formosus</em> Westwood</td>
<td></td>
<td>according to Noyes (2003)</td>
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<tr>
<td><em>Pnigalio</em> sp.</td>
<td>Algarve</td>
<td>Soares et al. (2000)</td>
</tr>
<tr>
<td><em>P. mediterraneus</em> Ferriere &amp; Delucchi</td>
<td>Algarve</td>
<td>Coelho (1997)</td>
</tr>
<tr>
<td><em>P. pectinicornis</em> (L.)*</td>
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<td>Present study</td>
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<tr>
<td><em>Pteromalus</em> sp.</td>
<td>Mafra</td>
<td>Naves et al. (1999)</td>
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<td></td>
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<td>Present study</td>
</tr>
<tr>
<td><em>Semielacher petiolatus</em> (Girault)*=</td>
<td></td>
<td>Present study</td>
</tr>
<tr>
<td>= <em>Semielacher petiolata</em> (Girault)</td>
<td></td>
<td>according to Noyes (2003)</td>
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<tr>
<td><em>Sympiesis</em> sp.</td>
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<td>Naves et al. (1999)</td>
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<td></td>
<td></td>
<td>Present study</td>
</tr>
<tr>
<td><em>Sympiesis gregori</em> Boucek</td>
<td>Algarve</td>
<td>Soares et al. (2000)</td>
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</table>

* species reported for the first time from Portugal

Acknowledgements

We thank Manuel Cariano for the help in field and laboratory work. Thanks are due to the lemon growers Virginia Duarte, Carlos Batalla and Rosa Gomes that allowed us to use their orchards as experimental plots. We also thank Alberto Urbaneja and Maria Jesús Verdú for the help in the identification of parasitoids. This work is part of a research granted by Programme AGRO Medida 8.1.-DE&D (Project nº 29 Management of ground cover and hedgerows in citrus orchards for biological control of pests).
References


Parasitism of *Diachasmimorpha tryoni* on Mediterranean fruits infested with *Ceratitis capitata* larvae in the laboratory

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**Abstract:** *Diachasmimorpha tryoni* (Cameron) (Hymenoptera, Braconidae) was imported from Hawaii to the insect facilities of the Instituto Valenciano de Investigaciones Agrarias in August 2002, in order to study its use in the biological control of the Medfly *Ceratitis capitata* (Wiedemann) (Diptera, Tephritidae). Since then, it has been maintained in laboratory conditions in a successful rearing. Several experiments are being carried out in laboratory with the aim to know its potential as an effective biological control agent in the Valencian Community. One of these experiments is studying the attraction of different Mediterranean fruits infested by *C. capitata* and offered to females of the parasitoid by analyzing emergence and parasitism rates in choice and non-choice trials. In this work, loquat, plum, nectarine and apple were the selected fruits, obtaining a good parasitism in all cases. The knowledge of these biological parameters about parasitism in fruits is very interesting to determine the potential action of this species when it will be released in plots of sweet fruits and citrus of the Mediterranean area.

**Key words:** Tephritidae, Braconidae, Medfly, *Ceratitis capitata*, *Diachasmimorpha tryoni*, parasitism, Mediterranean fruits

**Introduction**

*Diachasmimorpha tryoni* (Cameron, 1911) (Hymenoptera: Braconidae) was imported from Hawaii to the insect facilities of the Instituto Valenciano de Investigaciones Agrarias (IVIA) in August 2002, in order to study its potential use in the biological control of the Medfly *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) in Spain. Since then, this parasitoid has been successfully maintained in laboratory conditions.

Several experiments are being carried out in our laboratory to study its possibilities as an effective biological control agent against the Medfly in the Valencian Community. One of the experimental lines deals with the study of the attraction of different Mediterranean fruits infested by *C. capitata* and offered to the parasitoid by analyzing several biological parameters such as the emergence and parasitism rates in choice and non-choice trials.

The knowledge of these biological parameters about parasitism in fruits in laboratory conditions is very interesting to determine the potential action of *D. tryoni* when it will be released in plots of sweet fruits and citrus of the Mediterranean area to try to control the pest.

**Material and methods**

**Parasitoid rearing**

The rearing of the parasitoid *D. tryoni* has been developed on the Medfly *C. capitata* as host material, following the process previously recorded by Falcó *et al.* (2003): larvae of third
instar of *C. capitata* are exposed to adult parasitoids on the mesh of the upper side of a cage; the host continues its development in a plate with 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.

**Parasitism on fruits with inoculated medfly larvae**
Several varieties of mediterranean fruits attacked by medfly larvae have been selected for the purpose of knowing its attractiveness to the parasitoid. The emergence rate (proportion of emerged adult parasitoids obtained from host puparia respect to total formed puparia) and the parasitism rate (proportion of emerged adult parasitoids plus juvenile non emerged parasitoids inside the host puparia respect to total formed puparia), were analyzed.

The tested fruits are several common varieties produced along the year in the Mediterranean agricultural areas of the Valencian Community (east coast of Spain) where *C. capitata* is pest of citrus and other fruits. The fruits are apples (*Pyrus malus*), loquat (*Eryobotrya japonica*), apricot (*Prunus armeniaca*), plum (*Prunus domestica*), nectarine (*Prunus persica* var. *nectarina*) and peach (*Prunus persica*).

These fruits were inoculated with larvae of second instar (L2): two larvae were placed into a bored hole. Five holes were made per fruit, which represents ten larvae per fruit. Twenty inoculated fruits and five control fruits were carried out per test. Inoculated fruits were placed inside plastic bottles or cages with water and honey. When medfly larvae reach the third instar (L3), two 4-5 days old female parasitoids per fruit are introduced into the bottle or the cage for 24 hours; after this time, parasitoids are eliminated. Puparia are collected and the emerged medflies and parasitoids counted; the failed puparia are opened to discover the non emerged juvenile insects.

Two kind of tests were carried out: parasitism of *D. tryoni* in non-choice trials (two female parasitoids into a bottle with only one inoculated fruit; separated experiments with all the tested fruits) and parasitism of *D. tryoni* in choice trials (14 female parasitoids into a metacrilate cage with seven inoculated fruits of different types).

**Results and discussion**

In non-choice trials, the emergence of *D. tryoni* from host puparia of inoculated fruits with medfly larvae is highest in peach and in the two varieties of apple, and, in minor level, in loquat. These four kinds of fruits also show the maximum parasitism rates. The emergence rate and the parasitism rate are statistically similar in the case of the apples, but for loquat and peach the parasitism rate is significantly higher compared with the emergence rate on each fruit; therefore, the mortality of juvenile instars of the parasitoid is higher in loquat and peach (Table 1, Figure 1).

In choice trials, the emergence of *D. tryoni* from host puparia of inoculated fruits with medfly larvae is highest in peach, while apricot and nectarine show high levels of emergence but not statistically different from the other types of fruits. The parasitism rate is statistically similar for all the tested fruits; the parasitoids do not show a preference for searching larval hosts when all types of fruits are offered together. The parasitism rate is always significantly higher than the emergence rate on each one of the fruits tested; perhaps the number of female parasitoids used in this experiment is the factor responsible for the high parasitism in all the fruits and for the mortality of juvenile instars observed (Table 2, Figure 2).
Table 1. Emergence and parasitism rates of *D. tryoni* in non-choice trials. Means followed by the same letter are not significantly different (P > 0.05) with Tuckey’s procedure.

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Emergence rate</th>
<th>Fruits</th>
<th>Parasitism rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apricot</td>
<td>30,80 a</td>
<td>Nectarine</td>
<td>48,45 a</td>
</tr>
<tr>
<td>Nectarine</td>
<td>35,90 a</td>
<td>Plum</td>
<td>53,05 a</td>
</tr>
<tr>
<td>Plum</td>
<td>36,65 a</td>
<td>Apricot</td>
<td>55,65 a</td>
</tr>
<tr>
<td>Loquat</td>
<td>40,40 ab</td>
<td>Apple-Golden</td>
<td>59,15 ab</td>
</tr>
<tr>
<td>Apple-Golden</td>
<td>50,30 bc</td>
<td>Apple-Fuji</td>
<td>60,80 ab</td>
</tr>
<tr>
<td>Apple-Fuji</td>
<td>51,65 bc</td>
<td>Loquat</td>
<td>73,85 b</td>
</tr>
<tr>
<td>Peach</td>
<td>55,85 c</td>
<td>Peach</td>
<td>74,90 b</td>
</tr>
</tbody>
</table>

Figure 1. Emergence rate and the parasitism rate of *D. tryoni* on different types of fruits in non-choice trials.

Table 2. Emergence and parasitism rates of *D. tryoni* in choice trials. Means followed by the same letter are not significantly different (P > 0.05) with Tuckey’s procedure.

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Emergence rate</th>
<th>Fruits</th>
<th>Parasitism rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple-Fuji</td>
<td>37,1 a</td>
<td>Apricot</td>
<td>67,6 a</td>
</tr>
<tr>
<td>Apple-Golden</td>
<td>38,7 a</td>
<td>Loquat</td>
<td>71,9 a</td>
</tr>
<tr>
<td>Plum</td>
<td>41,1 a</td>
<td>Peach</td>
<td>72,9 a</td>
</tr>
<tr>
<td>Loquat</td>
<td>43,4 a</td>
<td>Apple-Fuji</td>
<td>73,3 a</td>
</tr>
<tr>
<td>Apricot</td>
<td>45,1 ab</td>
<td>Plum</td>
<td>73,6 a</td>
</tr>
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<td>Nectarine</td>
<td>49,3 ab</td>
<td>Nectarine</td>
<td>73,9 a</td>
</tr>
<tr>
<td>Peach</td>
<td>57,9 b</td>
<td>Apple-Golden</td>
<td>77,0 a</td>
</tr>
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</table>

Wong *et al.* (1984) reported the relative percentage of parasitoids emerged from two species of tephritid pupae (one of them *Ceratitis capitata*) collected from infested fruits in Hawai. Their study showed that *D. tryoni* was one of the most abundant parasitoids against *C. capitata* in the field, being the preferred fruits loquats and peaches, with a level of parasitism of 7.2-7.8%. These results are in accordance with the level of parasitism found in our experiments with the same fruits.
D. tryoni is able to locate and parasite the mature larvae of Ceratitis capitata on all tested Mediterranean fruits. The highest parasitism rate of D. tryoni was observed on loquat, peach and apple. We can conclude that D. tryoni offers interesting possibilities to be included in integrated control programs against C. capitata in Spanish citrus and stone fruits as it is able to act on a large diversity of fruit species attacked by the Medfly throughout the year, in the agricultural ecosystems of the Valencian Community.

Acknowledgements

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References


Laboratory experiments with *Fopius arisanus*, an exotic egg-pupal parasitoid of *Ceratitis capitata*

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**Abstract:** Since it was imported to the IVIA from the USDA/ARS in Hawaii (USA) in August 2002, the braconid *Fopius arisanus* (Sonan), an egg-pupal parasitoid of tephritid fruit flies, has been kept in laboratory conditions on *Ceratitis capitata* (Wiedemann) as host, with a continuous improvement of the rearing. To investigate the feasibility to use this insect in the biological control of the Medfly, several experiments were performed in laboratory to analyze their biotic potential. A methodology was developed to study adult longevity and reproduction in the parasitoid by using fruits and Petri dishes artificially infested with eggs of *C. capitata* as egg-laying units. The parasitoid produced a very high mortality on eggs offered to parasitization due to the ovipositional female attempts. Results showed that fertility in females was quite irregular and it was necessary to work with more than one couple of the parasitoid as reproductive units. More experiments are needed to analyze this behaviour.

**Key words:** *Ceratitis capitata*, biological control, *Fopius arisanus*

**Introduction**

*Fopius arisanus* (Sonan) (=*Opius oophilus* Fullaway) (Hymenoptera, Braconidae, Opiinae) is an egg-pupal parasitoid of tephritid fruit flies native to the Indo-Pacific Region, originally described from Taiwan (http://hymenoptera.tamu.edu/paroffit). It was successfully introduced to Hawaii for the biological control of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), and the Medfly, *Ceratitis capitata* (Wiedemann), and has confirmed its importance in controlling both pest species (Wong & Ramadan, 1987; Harris *et al*., 2000; Vargas *et al*., 2001). After the success in rearing this parasitoid species in captivity, developing a laboratory-adapted strain, the possibilities of using the insect for augmentative biological control of fruit flies have increased (Bautista *et al*., 1999).

In 2002, a laboratory-adapted strain of *F. arisanus* was imported from the U.S. Pacific Basin Agricultural Research Center (USDA-ARS) at Hawaii to the I.V.I.A. (Montcada, Valencia, SPAIN), to establish a culture of the insect in order to attempt the biological control of the Medfly, *C. capitata*, a very serious pest in citrus crops in the Valencian Community. In this work we report the improvement in the rearing technique to keep a laboratory strain of *F. arisanus* and some observations on the parasitic behaviour of this insect.

**Material and methods**

*Insect laboratory rearing*

Since the arrival of individuals to the scientific facilities at the I.V.I.A., the rearing method chosen to initiate the laboratory rearing of insects was the procedure developed by Calvitti *et al.* (2002), using as oviposition unit a plastic bottle with several holes on it and a thin yellow
sponge fixed to the inner surface of the bottle where the eggs of *C. capitata* can be placed with a micropipette.

The parasitoid adults are kept inside transparent plastic cages (50 x 40 x 25 cm) and supplied with food (honey and sugar) and water at 26±1ºC, 60-70% RH (16h light) and 22±1ºC, 70-80% RH (8h dark). Parasitoid eggs from the sponge are removed with water and placed into an artificial diet for the development of *C. capitata* larvae (Falcó et al., 2003). Pupation takes place inside plastic boxes containing vermiculite as substrate. Then, pupae are transferred to plastic transparent cages (50 x 21 x 50 cm) for emergence of parasitoid adults and also fruit fly adults from the not parasitized pupae. Newly emerged adults of *F. arisanus* are moved to an adult cage to produce a new generation of parasitoids.

Along the 3 years since the starting of the rearing, the continuous improvement of the technique, especially by determining the optimal time for oviposition unit exposure to parasitoid adults and the best medfly egg/female parasitoid ratio has been for us the main challenge.

**Experimental procedures**

Fecundity and fertility in *F. arisanus* were analysed by using artificial egg-laying units. Two different units were tested: Petri plates and fruits. In both cases, medfly eggs were deposited in oviposition units with a brush and, after some time exposed to female parasitoids, were removed and transferred to small containers with an artificial medfly diet for the development of larvae. Else, the parasitic activity of *F. arisanus*, either in isolated couples or on small groups (5 pairs of couples), has been studied. In all cases, plastic cylinders (16 x 12∅ cm) with a provision of honey and water were used to confine adults. 25 medfly eggs per parasitoid female were offered for parasitization to 7-day-old adults in oviposition units for 24 hrs, three days per week, along a period of three weeks.

**Isolated couples:** Fertility was estimated as the number of parasitoid adults emerging from exposed eggs. Ten replicates and two controls (cages without parasitoids) were used. Mortality from egg to pupa in replicates was compared to that in controls to determine the effect of female oviposition on medfly development.

**Small groups:** Fertility and fecundity were calculated. The latter was assessed by using the method proposed by Moretti & Calvitti (2003): the dechorionation of *C. capitata* eggs which allows to see inside the eggs of *F. arisanus*. Four (fertility) and one (fecundity) replicates with two controls for Petri oviposition units, and 8 - 2 replicates with two controls for fruit oviposition units, have been analysed.

**Results and discussion**

**Rearing methodology**

Since the importation of the parasitoid strain from Hawaii, a total of 25 generations of *Fopius arisanus* have been obtained in the laboratory of Entomology at the IVIA. In figure 1 the number of adults per generation is shown. In general, the sex ratio in all generations was around 1:1 (male:female).

After some problems in the rearing until the 20th generation (a failure in the climatic chamber, an inappropriate introduction of plants with insecticide residues, ..) , all the process has been standardized as follows:

- A maximum of 400 adults are held in each adult cage. The optimal host clutch size has been determined as 4000 medfly eggs (in one bottle-oviposition unit) per 60-70 parasitoid females. This represents approximately a 60:1 host egg to female parasitoid ratio. Some authors have pointed out that 20:1 is the critical ratio because it means a plateau for the rate of parasitization by *F.arisanus* (Harris & Bautista, 1996; Bautista et al., 1998; Harris & Bautista...
Nevertheless, we have found that it is better to increase that ratio in order to avoid the negative effect of host mortality associated with the insect parasitization which can endanger the continuity of the rearing.

- 24 hrs-old medfly eggs are offered in the oviposition units for parasitization during 24 hrs, as it is done by other authors (Bautista & Harris, 1996; Harris & Bautista, 2001; Zenil et al., 2004). Reproducing adults (at least 7-day-old) are held for 2 weeks for breeding purposes.

- The mortality from egg to pupa in the parasitoid rearing was compared to that in the medfly rearing in order to know the mortality produced by the oviposition activity of F. arisanus. On average, 76% of eggs do not arrive to the pupal stage in the parasitoid rearing, compared with 14% in the medfly rearing. It is well known the latent death of developing hosts induced in the parasitization by F. arisanus. Harris & Bautista (2001) found a host mortality of around 70% when the parasitoid developed on Bactrocera dorsalis (Hendel), with a mean parasitoid recovery of 23.6%. That host mortality seems to occur during oviposition by female parasitoids: oviposition punctures on eggs, injury from oviposition trauma, microbial contamination, … (Bautista et al., 1999; Harris & Bautista, 2001; Calvitti et al., 2002).

The next step in our work on F. arisanus rearing will be the adaptation of this standardized methodology to the mass-rearing of the insect in order to allow its use in field experiments.

Parasitism by Fopius arisanus

Isolated couples: No progeny was found in any of the replicates. When comparing mortality egg-pupa in replicates to controls, it was similar and around 30%. This result suggests that females do not exhibit an oviposition activity on medfly eggs in these conditions. The mortality was a little larger than those occurred in the medfly and parasitoid rearings but it could be due to the more accurate number of initial eggs in this experiment.

According to the selected bibliography, not much work has been done using isolated couples of the parasitoid (Ramadan et al., 1992; Quimio & Walter, 2000; Vargas et al., 2002). In those works, parasitism is obtained but problems in adult mating resulting in the production of males only in the progeny appear. It is known that unmated females laid fewer eggs than mated ones (Wang & Russell, 2003). The same authors indicate that there is a relatively large
variation in egg-load among individual females. In any case, we must reconsider our experimental procedure in order to know its effect on positive parasitism. 

Groups of five couples: In both experimental lines, Petri and fruit oviposition units, progeny was found but not in all the replicates. A rough estimate of the percent fertility in females (number of parasitoids emerging) was fixed at 5%. This small percentage is similar to that found in other experiments by Bautista et al. (1999) and Zenil et al. (2004). The mortality due to parasitoid oviposition activity in replicates where progeny was obtained is around 60-80%, a value similar to that in the parasitoid rearing. Nevertheless, in controls and in replicates with no progeny, that mortality egg-pupa was smaller (20-30%) and similar to the mortality obtained in the medfly rearing. This fact suggests that in replicates with no offspring, parasitoid females have not exhibited an oviposition activity.

Egg-laying of females was only observed for dechorionation of medfly eggs on one of the three replicates. A total of 204 eggs per 5 females in 9 days were counted, and superparasitism (2 parasitoid eggs into the same medfly egg) was observed, although Wang & Messing (2003) have found that female wasps are able to avoid superparasitism.

In general, we have found that the parasitic activity in F. arisanus seems to be quite erratic when small numbers of individuals are confined in containers for reproductive experiments. This fact could explain the small number of experiments found in bibliography with this experimental procedure. F. arisanus is a synovigenic species and female wasps emerge with a small complement of eggs and mature more eggs over time. Wang & Messing (2003) indicate that oviposition in F. arisanus stimulates egg maturation and reduces resorption: increased oviposition activity increases egg-load. More work has to be done in the future on the effect of grouping and learning in F. arisanus female oviposition behaviour.

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References


Side effects on natural enemies of bait insecticide applications for the control of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae)

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Laboratory studies were performed to assess the side effect of three baited insecticides (spinosad, malathion and fenthion), used for the control of *Ceratitis capitata* (Wiedemann), on beneficial insects. The non-target species tested were four hymenopterans (*Citrostichus phyllocnistoides* (Narayanan) (Eulophidae), *Diachasmimorpha tryoni* (Cameron) (Braconidae), *Diglyphus isaea* (Walker) (Eulophidae) and *Anagyrus pseudococci* (Girault) (Encyrtidae)) and two heteropterans (*Orius laevigatus* (Fieber) (Anthocoridae) and *Nesidiocoris tenuis* Reuter (Miridae)). No effects of spinosad bait were observed on the mortality of the hymenopteran adults, except for *C. phyllocnistoides*, whereas the mortality induced by malathion and fenthion was significant higher (even 100%) for the four species tested. On the other hand, the surviving *C. phyllocnistoides* and *D. tryoni* adults were used to assess the effect of bait insecticide applications on the level parasitism, fecundity and developmental time of the progeny. No significant differences were recorded between the control and spinoas. In general, heteropterans were more resistant to the bait insecticides than hymenopterans, being spinosad the more friendly insecticide for both *O. laevigatus* and *N. tenuis*. The greater selectivity of spinosad compared to malathion and fenthion suggests that this product could be an alternative for the control of *C. capitata* in citrus crops.
The Mediterranean Fruit Fly *Ceratitis capitata* Wiedemann (Diptera, Tephritidae), a new pest in Montenegro

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**Abstract:** The Mediterranean fruit fly *Ceratitis capitata* Wiedem. was detected for the first time in October, 2000, in several mandarin orchards, in the southeastern part of Montenegrin seacoast. In the following years different host plants have been detected. Economically speaking, the most important is the mandarin (*Citrus unshiu* Marc.), which, with its several varieties, makes over 85% of Montenegrin citrus orchards. The other hosts are: the fig (*Ficus carica* L.), the kaki (*Diospyros kaki* L.), the orange (*Citrus sinensis* L.), the lemon (*Citrus limon* L.), the grapefruit (*Citrus paradisi* Macf.), and the ziziphus (*Ziziphus* spp.). Different male and female attractants were being used for population monitoring during 2003 and 2004. Monitoring covered the entire Montenegrin seacoast and showed presence of *C. capitata* in almost the whole region. Population density varied among localities. In 2003, first flies were caught in August and population reached its peak during September and October, depending on the locality. In 2004, first flies were caught in September and peak was reached at the end of October up to the middle of November. In both cases last flies were caught from the middle till the end of December. In 2003, first infestations were detected in fig fruits (end of August, beginning of September) and in the earliest mandarin varieties (middle of September). In 2004, when *C. capitata* appeared later than in the previous year, first infestations were also detected in some fig fruits (end of September and in October), but almost without symptoms on early mandarin varieties and other hosts. More serious infestations of mandarin fruits were detected on the varieties which ripe last, during November.

**Key words:** The Mediterranean fruit fly, *Ceratitis capitata*, distribution, host plants, monitoring

**Introduction**

The Mediterranean fruit fly *Ceratitis capitata* Wiedem. (Diptera, Tephritidae) is one of the most destructive and adaptive agricultural pests infesting 374 different host plants, from 69 botanical families; 40 percent of all of them belong to 5 families: Myrtaceae, Rosaceae, Rutaceae, Sapotaceae and Solanaceae (Liquido et al., 1998). It originates from western Africa and from there it spread and has successfully established in every country surrounding the Mediterranean Sea, in Central and South America, western Australia, Hawaii and in some oceanic islands (USDA, 1983, according to Liquido et al., 1998). In Mediterranean countries it particularly damages citrus and peaches (www.eppo.org).

In the former Yugoslavia, *C. capitata* was detected around the city of Split (Croatia) for the first time in 1947, on apricot, peach and kaki fruits (Tominić, 1951). Later, after the fly had seriously damaged peach and pear fruits, as well as fig, kaki and apple fruits in Croatia in 1958 (Kovačević, 1960a, Kovačević, 1960b, Todorović, 1960), risk of its appearance and establishment in Montenegro, in the area around the Adriatic seacoast, was very possible, because of a favourable climate and potential host plants (Mijušković, 1959). After several years of monitoring on at least ten localities, its presence was not detected either in traps or in fruits of potential host plants since 1959, so subsequent monitoring was stopped (Mijušković,
2005, oral communication). There were not any signs of its presence in the following decades. However, the first detection of *C. capitata* in Montenegrin orchards was confirmed in October, 2000. Fruits were seriously damaged in several mandarin orchards around the cities of Bar and Ulcinj. In sampled fruits more than several larvae were present per each fruit. A lot of fruits fell off of trees, as well.

Some preliminary monitoring with angelica seed oil at 9 localities in 2002, suggested its wider presence. Since that period, its presence has been confirmed and detected on different host plants on almost the whole seacoast, with variability in population density and damages it caused.

Generally speaking, the Montenegrin seacoast can be observed as a favourable region for *C. capitata* presence and development, as a region where commercial citrus orchards, as well as mixed orchards are present, with particular emphasis on mandarin production.

**Material and methods**

The Montenegrin seacoast is 298 km long area along the Adriatic Sea, located between 41°52’ and 42°29’ NL. Due to its specific position it is marked as an area of a wider citrus producing areal, even though it is in the peripheral zone. The absolute minimum temperature (around -8°C up to -9°C) may be reached once every 25 to 30 years in this area (Radulović, 2000). Vegetation period usually lasts about 7 months (April-October), but with some variability depending on the year. In this area the citrus (besides the olive) has the most important economic significance. The most important is mandarin *Citrus unshiu* Marc. With its several varieties, it makes over 85% of Montenegrin citrus orchards. It is presented with approximately 380000 trees in 2005 (Radulović, 2005, oral communication). Oranges are presented with approximately 10% with var. *Washington Navel* as the most important. Other citrus are: lemon (var. *Lunario, Meyer*) and grapefruit sporadically. The main citrus producing area is located in the southeastern part, around cities Bar and Ulcinj, where commercial mandarin orchards are most common. Main orange orchards are also there. In the other parts (localities from Budva to Herceg Novi), citrus are also present, but with fewer number of trees. These are smaller orchards with up to a few hundred citrus trees. They are usually mixed with some fig and kaki trees, pomegranate, occasionally peaches and ziziphus, as well as some vegetables (tomato, cucumber, capsicum, rarely aubergine). Harvesting of all available fruits is finished until the end of November and the middle of December. The main harvesting period for mandarin fruit is October until the middle of November, whilst for oranges it is the middle of November, beginning of December.

Field trials were conducted in 2003 and 2004 along coastal area in orchards where citrus could be found (in commercial as well as mixed orchards). No chemical control of *C. capitata* was carried out during these trials. Different male and female attractants were used for population monitoring. Traps were set up at the southern side of the orchard, at an eye level (approximately 170-180cm high).

In 2003, angelica seed oil (*Angelica archangelica* L.) was used as a male attractant, beginning April 23rd, and a combination of vanilla essence, household ammonia (5% NH₄OH) and water (2.5ml imitation vanilla essence + 10 ml household ammonia + 500ml water) for female (according to: [www.agric.wa.gov.au/ento_fpellass/medflytable4.gif](http://www.agric.wa.gov.au/ento_fpellass/medflytable4.gif)), beginning June 4th. Both were set up at 17 localities. One more locality was included in 2004. Therefore, two previous attractants were present at 18 localities. Trimedlure was set up at 7 localities and Capilure in two, as new male attractants. Female attractant - combination of di-ammonium phosphate + hydrolyzed protein Buminal (4:1) in water solution, was present at 10 localities. Newly used attractants were positioned in those localities which had a high number
of captured flies in the previous year, and consequently infested fruits. Some traps were set up at localities where none of the flies was captured in 2003. In 2004, vegetation was delayed for about 20-30 days, due to a very cold and rainy March.

Glass McPhail traps were used for angelica oil, 'vanilla solution' and di-ammonium phosphate + Buminal. In several cases, the modified version was used in the absence of a sufficient number of McPhail traps. It consisted of plastic 1-liter bottles. The upper half of the bottle was cut off and the opening closed with a plastic lid. Five to seven fly entry holes, 1 cm in diameter, were made and positioned in a semicircular way, 5-6 cm from the bottom of the bottle. In cases of "vanila solution" and di-ammonium phosphate + Buminal, 50 ml of solutions per each trap was used. For angelica oil, 50 ml of water was poured into each of the traps and 5 drops of angelica oil were put on a cotton wick fixed onto the upper opening of the trap.

Transparent Jackson traps, coated with glue, were used for Trimedlure, and some modified version of McPhail trap for Capilure (similar as for the angelica oil).

In 2003 and 2004 traps with angelica oil and 'vanilla solution' were continuously being exposed in the field, as well as di-ammonium phosphate in 2004. Trimedlure and Capilure were placed in orchards in June, 2004. With the exception of the last two (which were renewed in four-week intervals), all others were renewed in 15-day intervals, and from the beginning of August, in 7-10-day intervals. Since a lot of orchards were mixed, and a suitable phase for C. capitata attack appeared successively, traps were hung on a new tree if it was considered to be at a more suitable fruiting stage.

At the same time, fruits of potential host plants were successively sampled on surveyed localities, starting from the point when the fruits started being suitable for the flies' attack (in the semi-ripe phase of development). All sampled fruits were inspected in the laboratory, for C. capitata larvae presence.

Results and discussion

The population density of C. capitata in 2003, which was determined by using McPhail traps is shown in figures 1a,b and 2a,b. In main citrus growing areas, at the 9 localities around cities Bar and Ulcinj, population levels were significantly lower than at the other 8 localities. That was unexpected since infestation of mandarin fruits was recorded in the two previous years, although based on scarce information.

Traps baited with angelica oil show that the highest number of captured males was recorded at the locality Baošići (around Herceg Novi). The total number of captured males was 386♂♂ in period from September 2nd to November 19th, or 34.21 male/trap/week. The maximum number of captured males was 87, at the locality Lastva Grbaljska (01) on September 24th (Fig. 1a). It ranked second with the total of 326 males captured, while at the other localities the number was significantly lower. At 5 localities (29.4%) none of the males were captured.

Traps baited with 'vanilla solution' show that the highest number of captured flies (mostly females) was recorded at locality Njivice (around Herceg Novi) with the total number of 298 flies (274 ♀♀+24 ♂♂) in the period from September 15th to November 20th. During this period 28.63 females/trap/week were captured. The maximum number of captured flies was 100 (81 ♀♀+19 ♂♂) at the locality Njivice, on September 24th (Fig. 2a). Apart from Baošići with the total of 261 flies captured (which was also the locality where the first fly (1 female ♀♀ was captured on August 6th), the number was lower at the other localities. At 7 localities (41.17%) no females were captured.
The period of considerable fly occurrence varied depending on the locality, but it was generally from the middle of September until middle of November, and reached the peak in the last days of September and during October. A reduction in the number of captured flies was noticed beginning the middle of November and last flies were caught from the middle till end of December.

The first ones infested were fig fruits at the end of August, and the earliest mandarin and some orange varieties until the middle of September. Kaki fruits were infested at the end of September and at the beginning of October. Although they weren’t present in a significant number, they played an important role for the fly population, as ‘crossbridges’ until the period when most of the mandarins were almost ripe (in October).

In Ulcinj citrus growing area (as one of the two main ones) almost no flies were detected in traps. Besides, there were not any signs of citrus fruit infestations at surveyed localities. In Bar growing area, some wider presence of fig and kaki trees in the vicinity of citrus orchards probably indicated a higher number of captured flies, and consequently some damage. Besides fig and kaki fruits, the first infested mandarins (some early varieties) were detected at the beginning of October. Infestations of the main, later mandarin varieties as well as oranges were not detected. The biggest damage on mandarin fruits and oranges was recorded at localities Lastva Grbaljska, Baošići and Njivice. Symptoms in lemon fruits (var. Lunario), grapefruit and ziziphus were also recorded there. The fruit infestation period at those localities lasted throughout September, October and November. Infestations of any vegetable were not detected.
In 2004, the number of captured males was low in traps with angelica oil. The maximum was reached in Lastva Grbaljska (01) with the total number of 86 captured males from September 10th to December 13th, or 6.33 male/trap/week. Furthermore, the maximum number of captured males (44♂♂) was detected at this locality on October 29th (Fig. 3a). At 9 out of 18 localities (50%) none of the flies were captured.

According to the number of captured females, traps baited with 'vanilla solution' were almost without any significance, which indicates that this solution is not potent enough in cases of low population density (Fig. 4a, 4b). During the examined period, the maximum number of captured females was 31♀♀ at locality Bar (02). At 12 out of 18 localities (66.6%) none of the females were captured.

Data regarding traps baited with water solution of di-ammonium phosphate+Buminal (DAPh+Buminal) prove this combination to be more potent for capturing of females rather than 'vanilla solution', even in cases of small population density, as it was in 2004. The maximum number of all captured flies was in Lastva Grbaljska (01), with 290 flies (280♀♀ and 10♂♂) from September 10th to December 13th, or 20.63 females/trap/week. Also at this locality, the highest number of captured females (104♀♀) was detected on November 11th (Fig. 5a). Locality Bar (02), ranked second, with 164 captured females from October 4th to December 10th. At this locality the maximum number of captured females was 86♀♀,
detected on November 12th (Fig. 5b). At 3 out of 10 localities (30%) none of the females were caught.

![Figure 5a. Population density of C. capitata at localities Budva–H.Novi (DAPh+Buminal)](image1)

![Figure 5b. Population density of C. capitata at localities Ulcinj–Bar (DAPh+Buminal)](image2)

Traps with Trimedlure recorded presence of flies at 7 out of 8 localities (85.8%). The maximum number of all captured males (129♂♂) was recorded at locality Lastva Grbaljska (01), from September 10th to December 13th with 9.5 males/trap/week. At the same locality, the maximum number of captured males (67♂♂) was reached on November 11th (Fig. 6a).

![Figure 6a. Population density of C. capitata at localities Budva-H. Novi (Trimedlure)](image3)

![Figure 6b. Population density of C. capitata at localities Ulcinj – Bar (Trimedlure)](image4)

Although Capilure was placed at only 2 localities, it proved to be very efficient male attractant used in this study. The maximum number of all captured males was 278♂♂, at locality Lastva Grbaljska (01), in the period from September 10th to December 13th, or 20.49 males/trap/week. At the same locality, the maximum number of captured males (143♂♂) was reached on October 29th (Fig. 7).

In 2004, considerable population density of C. capitata was noticed from the beginning of October until the middle of November. The first flies were caught at locality Lastva Grbaljska (01) at the beginning of the second week of September. The population peak was reached at the end of October. Reduction in the number of flies was noticed from the middle of November and last flies were caught from the middle till end of December. That year, the first fruit infestation was detected in fig fruits at locality Lastva Grbaljska (01) at the end of September, while the first kaki fruits infestations were at locality Bar (02) almost one month
later. We did not detect infestations in the earliest mandarin and orange varieties. The first sporadic infestations of mandarin fruits were recorded at locality Bar (02) at the end of October and more infested fruits were detected in the next 20 days. On the other hand, at localities Lastva Grbaljska (01), Baošići and Njivice the first mandarin fruits infestations were recorded during the first week of November. We did not detect symptoms of attack in other citrus fruits, zizuphus fruits, or in any other hosts.

![Graph](image)

Figure 7. Population density of *C. capitata* at localities Lastva Grbaljska and Baošići (Capilure)

According to the results of this work, it can be concluded that *C. capitata* is present in Montenegrin citrus growing area. Although its presence has still not been detected at some serious level in the main citrus growing area, where commercial mandarin and orange orchards are found, the possibility of its permanent establishment is very present. Moreover, small distances between this area and all other localities (from Budva to Herceg Novi) where mixed orchards are dominant and where its presence was in abundance (found in traps as well as in infested fruits: different citrus fruits, kaki, fig and ziziphus fruits), make this possibility a certainty.

Although data from 2003 and 2004 can not be compared in details, because we used 3 more attractants in 2004, it is obvious that synthetic male attractants, as well as combination of di-ammonium phosphate+Buminal give the best results and could detect *C. capitata* presence even in a year of lower population density. It is worth considering of they to be used not only for population monitoring, but also as a tool in mass trapping, as a possible new strategy of *C. capitata* control in Montenegro. All attention should be directed to "crossbridge" hosts (fig, kaki, early mandarin and orange varieties) in terms of early fly population control.

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Influence of juvenile hormones and protein on male Caribbean fruit fly (Diptera: Tephritidae) sexual success

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Juvenile hormone levels and adult diet have important effects on the attractiveness and competitiveness of the male Caribbean fruit fly. Since the success of the Sterile Insect Technique requires the release of males that can compete in the wild, these effects are of crucial importance. Laboratory (sexual success, performance in a life time basis and sexual performance in a daily basis) and field cage experiments were conducted to compare male \textit{Anastrepha suspensa} sexual performance when submitted to four different treatments: (A) Application of juvenile hormones (JH) and sugar and hydrolyzed protein as adult food (JH\textsuperscript{+}/P\textsuperscript{+}); (B) Application of JH and sugar as adult food (JH\textsuperscript{+}/P\textsuperscript{-}); (C) No application of JH and sugar and hydrolyzed protein as adult food (JH\textsuperscript{-}/P\textsuperscript{+}); and (D) No application of JH and sugar as adult food (JH\textsuperscript{-}/P\textsuperscript{-}). Methoprene, a synthetic juvenile hormone analog, was applied topically in the first 24 hours after adult emergence at a rate of 5\textmu g in an acetone solution per fly. The adult diet was composed of sugar and water \textit{ad libitum} in P\textsuperscript{-} treatments. In P\textsuperscript{+} treatments protein was added to the adult diet (mixed with sugar in a proportion of 3 parts of sugar and 1 part of protein). The insects tested were from a “semi-wild” colony, i.e. less than 2 years in domestication. Mating success among sexually mature males from the 4 treatments, in laboratory and in field cages, was observed. The percentage of matings was respectively for laboratory and field cages, in treatment A 55\% and 59\%, for treatment B 22\% and 18\%, for the treatment C 18\% and 20\% and for treatment D 5\% and 3\%. The results obtained from both the laboratory and field cage experiments, show a clear improvement of male competitiveness due to the hormone application, protein supply, and interaction of hormone and protein. At same time JH application causes an earlier maturation and protein supply a positive effect in male adult longevity. The sexual performance in a life-time basis show a positive effect of the protein diet on longevity and an better sexual performance when JH was applied. In a daily basis, treatment A males perform better and are capable in 10\% of the cases to mate 3 consecutive times in the same day.
A new method to determine TML release rate based on Thermal Desorption-Gas Chromatography-Mass Spectrometry

Cristina Alfaro, Javier Dominguez, Vicente Navarro and Jaime Primo

Many control methods of Mediterranean fruit fly, Ceratitis capitata, are based on Trimedlure (TML). It is very important to know the real TML release rates in order to assure that the dispensers are working adequately.

Our laboratory has developed a method to measure the TML release rate during a short time period based on thermal desorption and GC/MS. The dispensers are submitted to controlled air flow and temperature conditions and the released TML is adsorbed on a Tenax® trap as solid porous adsorbent. Once collected, the sample is thermally desorbed and analysed by a complete GC/MS system. An assay was carried out for two commercial dispensers. Release rates were determined for each dispenser type at several aging times.

The emission rates were related with field captures and with residual amount of TML in the dispensers quantified by solvent extraction and GC analysis. Preliminary assays showed marked differences in the amount of TML released between different types of commercial dispensers and at different aging times. The variation in release performance of commercial dispensers demonstrate the convenience of routine evaluation of them. This non-destructive method allows the quick and accurate evaluation of the current behaviour of dispensers along their useful life.
Evaluation of two trimedlure dispensers for Mediterranean fruit fly 
*Ceratitis capitata* (Wiedemann) in field tests

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Centro de Ecología Química Agrícola-Universidad Politécnica de Valencia. Laboratorio 111. Edificio 9b (4f). Camino de Vera s/n. 46022 Valencia, Spain e-mail: jadorui@ceqa.upv.es

Trimedlure (TML) is a synthetic lure attractive to males of the Mediterranean fruit fly. The life-time of TML dispensers changes according to the type of support. This study contrasts the effectiveness of TML in two controlled-release dispensers, Aralure® (polymeric plug) and EPA-1® (mesoporous dispenser), in a field assay from April to November. Insect captures and laboratory-measured release rates and residual contents showed that polymeric plug was ineffective after three months of field exposure whereas mesoporous dispenser extended the duration of effectiveness to six months. Field-measured emission rates at temperatures until July showed linear correlation of rate with average temperature for the polymeric plug dispenser ($R^2=0.927$). On the contrary, release rate in mesoporous dispenser did not show this correlation ($R^2=0.437$). This fact demonstrates a greater sensitivity of Aralure® to temperature and explains the increase of emission in the warmest months. Residual contents and insect captures suggest a minimum value of TML release rate of about 2 mg / day to optimal attraction.
Compared efficacy assay of different systems for trapping *Ceratitis capitata* Wied. adults

Jesús Olivero, Eva Wong, Ana L. Márquez & Emilio García

*Depto. Sanidad Vegetal. Camino Viejo de Vélez, 8. 29738-Rincón de la Victoria (Málaga), Spain*

**Abstract**

We made an assay of trapping systems for *Ceratitis capitata* in two Navel-Late orange groves in Malaga province (Spain). The traps tested were Tephri-Trap, Multilure, Probodelt and Easy traps. All of them were lured with trimethylamine hydrochloride, ammonium acetate and putrescine (tri-pack), which were installed together with a DDVP (toxicant) strip (Dichlorvos 20% w/w) to retain the insects inside (with the exception of the Probodelt trap, which holds its own retention means). We used three traps of each type in every grove, and weekly checked the captures from April to September 2004. Significant differences were observed among the efficacy of these traps for both male and female individuals. We got the most abundant captures with Multilure and Easy traps, followed by the Tephri-Trap. Probodelt was the least effective device, probably because of a retention problem when lured with a solid attractant.

**Key words**: traps, monitoring, Medfly, *Ceratitis capitata*, citrics.

**Introduction**

The Medfly (*Ceratitis capitata* Wied.) is a very damaging pest for a great number of fruit species, including citrus fruits (Gomez-Clemente, 1948), because the maximum host susceptibility is reached about two weeks before the harvesting time (Moner et al. 1988; Ros, 1988; 1999). This makes farmers need a very close monitoring of the Medfly populations and their damages in order to draw up the control measure strategy. Traps are very useful tools for this, and because of the low capture levels that indicate the need to treat, 0.5 individuals per trap and day according with the official Integrate Production Regulations in Andalucia (Spain) (Junta de Andalucia, 2000), it is very important to optimise the capture efficacy of the trap-attractant combination used. Many assays have been performed in the last years to select the best traps from the great diversity existing in the market (e.g. Alonso et al., 2002; Alonso & García-Mari, 2004; Ros, et al., 2002; 2004; Wong et al., 2003). Our aim with the present work is to compare the capture efficacy of four trapping devices for the monitoring of *Ceratitis capitata*, selected either among those considered best in previous works, or among those recently developed and with good results in preliminary assays.

**Material and methods**

The traps tested were Tephri-Trap (Utiplas S.L.; Ros, 1999; Lloréns & Lloréns, 2002), Multilure (Better World Manufacturing Inc.; Llorens & Lloréns, 2002), Easy trap (Utiplas S.L.; Ros et al., in press) and Probodelt (Probodelt S.L.; Probodelt, 2004) (Figures 1 and 2), all of them lured with the Tri-Pack of Suterra España Biocontrol, S.L. (Figure 3): a combination of putrescine, ammonium acetate and trimethylamine hydrochloride. The tri-pack was considered the best attractant for *Ceratitis capitata* in the assays performed by Ros et al., (1997; 2002; 2004; in press) and by Wong et al. (2003). Tephri-Trap, Multilure and Easy Traps also contained a DDVP toxicant strip (dichlorvos 20% w/w; Sanidad Agrícola Econex,
S.L.) as the retention system. With the Probodelt trap, the toxicant strip was substituted by an inward extension of the entrance holes to prevent the flies from escaping (Figure 2 C). The Tephri-Trap and the Multilure are traps whose good efficacy for the monitoring of important Tephritidae has been proved (Olivero et al., 2004 for Bactrocera oleae [Gmel.], Ros, 1999, and Alonso & García-Mari, 2004 for Ceratitis capitata Weid.). The Easy and the Probodelt traps are new systems that are showing good results in preliminary assays with both Ceratitis capitata and Bactrocera oleae (Probodelt, 2004; Ros et al., in press).

Figure 1: Tephri-Trap (A) and Multilure trap (B)

Figure 2: Easy trap (A) and Probodelt trap (B). C: Probodelt opened to show its retention system.

Figure 3: Suterra España Biocontrol S.L.’s tri-pack.
The assay was repeated in two different Navel-Late orange groves in Malaga (Spain), 1.5 and 1.3 hectare wide, respectively. For every assay we installed three traps of each type, and checked the captures weekly from April to September.

We analysed the data set using two-factor analysis of variance (the factors being the trap type and the grove), and LSD and Scheffé pair wise mean comparison tests. The whole data set was analysed jointly after it was typified to avoid bias due to oscillations in time.

Results and discussion

All the traps captured Ceratitis capitata adult individuals. A visual analysis of the charts in Figures 4 and 5 shows a clear advantage of Multilure trap in grove I, and of both Multilure and Easy traps in grove II. Although the capture numbers were always higher for females than for males, the curves were very similar for both sexes within every grove, which denotes a nearly constant sex ratio along the study period.

Figure 4: Mean daily male captures per trap in groves I (A) and II (B).

Figure 5: Mean daily female captures per trap in groves I (A) and II (B).

Significant differences were observed among the efficacies of traps for both males and females (Table 1).
Table 1: Analysis of variance of the typified mean captures per trap and day. Fs: Snedecor & Fisher F; p: statistical significance; ** p<0.01.

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<th>Fs</th>
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<tr>
<td>MALES</td>
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<td>**</td>
</tr>
<tr>
<td>FEMALES</td>
<td>31.31</td>
<td>**</td>
</tr>
<tr>
<td>TOTAL</td>
<td>39.99</td>
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According to the mean comparison tests (Table 2), male and total capture numbers were significantly higher with the Multilure than with the other traps (only the less conservative LSD test showed significant differences between the Multilure and the Easy traps, the two devices with the maximum efficacy); the Easy trap captured significantly more individuals than both the Tephri-Trap and the Probodelt trap, and the Tephri-Trap also performed significantly better than the Probodelt. The results for females were similar (Table 2), with the exception of the relation between the Multilure and the Easy traps, whose captures were not significantly different. So, we obtained the most abundant male and female captures with the Multilure and the Easy traps, with almost no differences among them, and the lowest efficacy with the Probodelt trap.

Table 2: Results of the pairwise mean comparison tests. L: significant differences according to LSD test; S: significant differences according to both the LSD and the Scheffé tests. Captures increase rightwards and downwards in the table. P: Probodelt; T-T: Tephri-Trap; E-T: Easy trap; M: Multilure

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<tr>
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The Probodelt trap has been found more effective than the Tephri-Trap, the Easy trap and the Multilure trap for the capture of the Tephritidae fly *Bactrocera oleae* (assays performed by the authors of this paper, still unpublished). The lure used in that assay was a liquid food attractant made of the protein suspension Nu Lure (9%) and stabilised with sodium tetraborate (3%). The assays showed in Probodelt (2004) also lured this trap with a liquid attractant. However, we chose the tri-pack for the present work because this lure was considered the best for *Ceratitis capitata* in recent articles (Ros et al., 1997; 2002; 2004; in press; Wong et al., 2003). We think that the Probodelt trap did not perform well for the
Medfly in our assay because of the solid nature of the tri-pack. The retention device used by Probodelt as a substitute of the toxicant strip bases its efficacy on the difficulties caused to flies in finding the trap exit. A delay in leaving the trap could finally end with the insects drowned within a liquid attractant. On the contrary, if the lure is solid, the flies could find the exit sooner of later not suffering damages at all.

Acknowledgements

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References


Efficacy assay of mass killing for the control of *Ceratitis capitata*

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**Abstract** We installed 100 Magnet MED traps per hectare in a 5-hectare Clementina de Nules mandarin orchard in Malaga province (Spain), lured with a sexual (trimedlure) and food (ammonium bicarbonate) attractant combination and impregnated with insecticide (lambda-cyhalothrin). In the remaining 12 hectares of the farm, *Ceratitis capitata* was chemically controlled with malathion and proteins. The aim of this work was to compare the effects of both control systems on the *Ceratitis capitata* adult populations and on the damages it caused to fruits. We monitored the adults with traps and took fruit samples weekly from September to November, when harvesting took place. Although the initial captures were high (20 to 35 adults per trap and day), the pest control was successful with both strategies, as the damaged fruit proportion at harvesting time was zero. When the adult captures and the proportion of damaged fruits were compared, we found no significant differences between the effect of mass killing and chemical treatment techniques.

**Key words:** mass killing, *Ceratitis capitata*, Magnet Med, citrics.

**Introduction**

In Spain, the standard control method for *Ceratitis capitata* populations has been chemical control (Planes, 1956; Sastre, 1999), with persistent but not penetrating insecticides such as malathion (Sastre, 1999), and preferably in combination with hydrolysed proteins that, working as attractants, allow to only treat patches of the grove (Moner et al., 1988; Junta de Andalucía, 2000). The sensibility of the public opinion in the last decades, in relation to environmental matters, has led to the search for alternative control methods. Among the techniques that have caused more expectancy are mass trapping and mass killing. With both of them we aim to reduce the insect-pest populations by attracting adult individuals, and then by capturing or directly killing them, respectively. Some assays have been performed up to now to develop a suitable method for some of the most damaging Tephritidae: e.g. Petacchi et al. (2003) and Al-Elimi (2003) for *Bactrocera oleae* (Gmel.), and Ros et al. (1996), Sastre et al (1999), Alonso et al. (2002), García et al. (2003) and Alonso & García-Mari (2004) for *Ceratitis capitata* Weid.

The mass killing techniques for *B. oleae* have been proved to be more efficacious than the use of larvicides (Petacchi et al., 2003), and the control of low density adult populations has required no support of other methods (Al-Elimi, 2003). When applied to *C. capitata*, mass trapping has appreciably reduced the adult populations (Sastre et al., 1999), and the control efficacies have been similar to those of conventional treatments (Alonso et al., 2002; Alonso & García-Mari 2004). A preliminary assay of mass killing using Magnet Med traps (AgriSense BCS LTD) as the control device was presented by us to the IOBC Meeting at Valencia (Spain) in 2002 (García et al., 2003). In it we detected more abundant trap adult captures using chemical treatments than with the mass killing traps. However, no testing on fruit damages was then performed because of their extremely low incidences in the orchards compared. With the present work we aim to test the mass killing option with Magnet Med
traps for *Ceratitis capitata*, by comparing the trap adult monitoring and the damage incidences in fruits of both a mass killing orchard and a grove submitted to a conventional chemical control with malathion and proteins.

**Material and methods**

The assay was performed in a Clementina de Nules mandarin orange orchard, in Malaga province (Spain). We installed a mass killing device in a 5.15 hectare surface, and the surroundings were chemically treated every week with the standard method: malathion and hydrolysed proteins. The “Magnet Med” traps used for the mass killing are a piece of laminated cardboard set up in a conical form with a tree branch as the central axis (Figure 1) (Lloréns & Lloréns, 2002; García *et al.*, 2003). We installed them in the south-east upper branches of the orange trees. The inside parts of the cones were oriented towards the inner part of the treetops. The lure dispensers were located in the inside face of the traps, and two kinds of attractants were combined as recommended by the distributor company, AgriSense BCS LTD (see Figure 1): one trap of each four was lured with the male sexual attractant trimedlure (2 g), and three traps of each four were lured with the food attractant ammonium bicarbonate (4 g), alternatively distributed (see Figure 2). Besides, the cardboards were impregnated with the insecticide lambda-cyhalotrin (15 mg). The arrangement of traps within the orchard was as seen in Figure 2, with a 100 trap per hectare density, and with reinforcement in the edges of the protected area and in the surroundings of other host trees (fig, pear, peach or other trees). We took care to avoid direct contact between traps and fruits.

![Figure 1: Magnet Med traps lured with trimedlure (left) and with ammonium bicarbonate (right). The triangles are to relate the different lures with the trap arrangement shown in Figure 2.](image)
We compared the control effect on *Ceratitis capitata* of both the mass killing and the chemical control methods with adult monitoring using traps, and with fruit samplings to evaluate the pest incidences. The adult monitoring was performed by weekly revisions of three Tephri-Traps lured with tri-pack (trimetilamine, putrescine, and ammonium acetate), and three Nadel traps with trimedlure (Figure 3). Nadel traps are the specified monitoring system for *Ceratitis capitata* within the Citric Exportation Program from Spain to the United States. As trimedlure is also one of the attractants installed in the Magnet Med traps, the mass killing device could interfere with the captures of Nadel traps. Because of this, we used Tephri-Traps to have a contrast measure less affected by the lure concentration in the atmosphere. Besides, with the tri-pack we got a direct measure about the female population of *Ceratitis capitata*, whereas trimedlure only provided information about males. Nevertheless, food attractants are not free from the possibility of interferences with the mass killing device. So, the final evaluation method for the compared efficacy of the biotechnic control was the
monitoring of damages in fruits. For this, we weekly examined 12 fruits (three for every geographical aspect) from each of 25 trees chosen at random.

We applied analysis of variance to the mean *Ceratitis capitata* adult numbers per trap and day, considering males and females separately. For each kind of trap, the whole data set was included in only one test, and so we previously typified the data to avoid the effects of mean capture oscillations in time. To analyse the incidence of fruits with damages we used two proportion comparison methods: the arcsine test and the variance test for the binomial distribution homogeneity, the latter based on the $\chi^2$ test (Sokal & Rohlf, 1979).

**Results and discussion**

Figure 4 A and B show the 10 approximately weekly treatments applied by the farmer in the chemical control orchard, and the two treatments performed in the mass killing orchard, respectively. In the first orchard, a standard schedule for chemical control of *Ceratitis capitata* was followed. In the biotechnically controlled orchard, the first treatment was motivated by the detection of a slightly increase of damages in fruits, just when the fruits were in the most sensitive phenologic state to *Ceratitis capitata*. However, the second treatment in that orchard was imposed by the crop purchaser, even after the pest damages had been successfully controlled. In general, the captures seem to have responded similarly to both types of control.

![Figure 4. Mean capture numbers per trap and day. A: Chemical control; B: Mass trapping. Arrows indicate the treatment dates.](image)

The variance analyses of the mean number of captures per trap and day showed no differences between control strategies according to the data provided by the Tephri-Traps lured with tri-pack (see Table 1). This applies to the captures of male, female and total individuals. However, the Nadel traps with trimedlure showed significant differences, with reference to males, that indicate a higher capture number in the chemically treated orchard than in the biotechnically protected one (Table 1). The different results of the analyses of male captures, depending on the type of trap used, may be due to possible interactions between the monitoring traps and the mass killing traps in the area with this kind of control method. Trimedlure was used in both the Nadel traps and the Magnet Med traps, and this may have caused confusion for males to find the Nadel traps. This would explain that only Tephri-Traps, not lured with the sexual attractant, have shown no differences between orchards in relation to the number of captured male individuals.
Table 1. Fisher & Snedecor F values and probabilities (p) obtained in the analyses of variance for the mean number of captures per trap and day. *: p<0.05; n.s.: p>0.05.

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<thead>
<tr>
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<th>Males</th>
<th>Females</th>
<th>Total</th>
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<tbody>
<tr>
<td>Tephri-Traps</td>
<td>F=2.92; p=n.s</td>
<td>F=0.71; p=n.s</td>
<td>F=2.22; p=n.s</td>
</tr>
<tr>
<td>Nadel traps</td>
<td>F=5.53; p=*</td>
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The proportion of damaged fruits was very low in both the mass killing and the chemically treated orchards, since they never showed values higher than 0.021 (from 0 to 1) (see Figure 5). Table 2 shows that differences between damage incidences were not significant most of the time. According with the \( \chi^2 \) test, no significant differences are detected between both control methods. The less conservative arcsine test reveals higher damages within the mass killing orchard only in two punctual sampling days. At the harvest time (November 28), the damage incidence was zero in both areas.

![Figure 5](image-url)  
Figure 5. Monitoring curves of the proportion of damaged fruits by *Ceratitis capitata* along the study period (the range of values is from 0 to 1).

Table 2. Values obtained for the arcsine and \( \chi^2 \) tests. p: probability (statistical significance). *: p<0.05; n.s.: p>0.05.

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The main conclusion of this assay is that we have got almost identical results in the control of *Ceratitis capitata* with both the weekly application of malathion and proteins and the biotechnical method using Magned Med traps for mass killing. So, our results are similar to those obtained by Sastre *et al.*(1999), Alonso *et al.* (2002), and Alonso & Garcia-Mari (2004) with mass trapping. We do not deal with an economical comparison of both systems, because the trapping device is up to now experimental, and so commercial prices are still
unknown. From the environmental point of view, however, it is remarkably profitable that mass killing has provided an 80% reduction in the number of chemical treatments. Nevertheless, as Al-Elimi (2003) suggested for abundant populations of Bactrocera oleae, a slight chemical support was required with our initially high abundance of Ceratitis capitata.

Acknowledgements

This work was made within the Integrate Production Agreement between the citrus cooperatives S.C.A.A. Malaca, S.C.A.A. Estepona, S.C.A. Agrolimón and S.A.T. Citrimasat and the Consejería de Agricultura y Pesca, Delegación Provincial de Málaga.

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Lethal time of toxic baits in *Ceratitis capitata* and *Anastrepha fraterculus* (Dip.: Tephritidae) in laboratory

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The use of bait sprays against fruit flies is a key component of IPM activities. Laboratory trials were conducted to measure the mortality caused by six organophosphates and two pyrethroids in insecticide baits against fruit flies. The protein BioAnastrepha® (5%) was added to all insecticides. Five females and five males of 2-4d-old medfly *Ceratitis capitata* (Wied.) and 4-5d-old South american fruit fly *Anastrepha fraterculus* (Wied.) were placed in each of ten replicate small cages. Aproximately 0.2 ml of bait was disposed through the Teflon® gutter situated on the upper third part of the cage. Evaluations of adult survival were carried out at 15, 30, 45, 60, 75, 90, 120, 150, 180 and 195 minutes after initial exposure. For medfly females, fenpropathrin (12.0 g IA/100 L) and trichlorfon (150.0 g IA/100 L) showed the lowest LT50 (<5.0 min) and chlorpyrifos (96.0 g IA/100 L of water) presented the highest LT50 (50.1 min). For *A. fraterculus* females, trichlorfon and dimetoato (200.0 g IA/100 L) showed the lowest LT50s (7.9 and 8.8 min, respectively). The highest value (71.1 min) was obtained for ethion (100 g IA/100 L). *C. capitata* was more tolerant to malathion (200g IA/100 L), chlorpyrifos and dimethoate than *A. fraterculus*. Opposite results were found for deltamethrin (1.25g IA/100 L), ethion, trichlorfon, fenthion (50 g. IA/100 L) and fenpropathrin for which *A. fraterculus* adults were more tolerant than *C. capitata*. Except for chlorpyrifos, no differences in susceptibility between females and males were detected for all tested compounds. For chlorpyrifos, *A. fraterculus* females were slightly more susceptible than males of this species.
Comparing the efficiency of cover and mechanical bait terrestrial treatments in the control of *Ceratitis capitata*

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*Ceratitis capitata* (Wied.) has an important negative economic impact in Spanish citrus production. The most common method to control this pest are based on cover malathion treatments. Less aggressive alternatives like terrestrial bait treatments need to be more extended since they are less contaminant and aerial treatments are unfeasible due to high dense population in citrus growing areas. However, terrestrial bait treatments can not be performed with conventional air assisted sprayers, and currently are manually applied because they require large droplets and very low volumes.

The aim of this work is to compare the efficiency of a new machine specially designed for automatic distribution of mechanical bait treatments (20l/ha), like spintor cebo®, against conventional practices in Spain, using malathion (1000l/ha). The efficacy of both treatments was assessed by comparing the evolution of captures in tephri-traps with tri-pack in two plots in the Valencia Region of Spain. We also estimated the amount of infested fruits at harvest time. Results showed that the efficacy of both applications was similar. Since the bait treatment was less hand labour, water and power consuming, the better efficiency of the developed machine has been proved.
First results of field trials on Bait Station Project in *Ceratitis capitata* (Wied.), in Algarve

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The objectives of this project are: the decrease of the population of *Ceratitis capitata* (Wied.) and reducing the number of chemical treatments needed to control this pest in commercial citrus orchards. The field trials started in June of 2004 in Faro and Tavira. Three kinds of traps were applied on the plots: traps with lufenuron, mass trapping and monitoring traps. The captures of *C. capitata* were controlled once a week (in all traps) between June and December. This project will last for three years to achieve the proposed objectives.
We have studied the influence of orange citrus variety on the fecundity of three diaspidid (Homoptera, Diaspididae) species. The considered scale species were *Cornuaspis beckii* and *Parlatoria pergandii*. Twenty-seven samples were taken from two orchards, one of Navel Lanelate and another of Valencia Late. Per sample, the egg number in 30 gravid females was counted. Eggs were classified into three categories: pre-laying, laying and hatching eggs. Three methods were undertaken to show the variety influence. 1) Two types of ANOVA, one-way (variety as factor) and two-way (variety and observation as factors). In both analyses, the factor variety was significant (P<0.01) on *C. beckii*, in the laying eggs and in the sum of laying plus pre-laying eggs. 2) Only the sum of prelaying and laying eggs was considered, and the evolution of the fecundity was studied by means of the apparent increase rate of the logistic function which relates eggs.day to degrees.day. 3) As in 2, but measuring the evolution of fecundity using the parameters of a sinusoidal function for adjusting eggs.day variation to degrees.day. The last two methods did not show significant differences (p<0.05) of the selected varieties on the scale fecundities.

Finally, three models for predicting the egg numbers were fitted. One of them is based on the estimation of the Weibull density function, the other two in regression and autorregressive models.
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Cellophane tape with adhesive on both sides to monitor the emergence of armoured scale crawlers in Eastern Sicily lemon orchards

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Abstract: Oleander scale, Aspidiotus nerii Bouché (Homoptera: Diaspididae), is a cosmopolitan pest mainly spread in the Mediterranean basin. This scale insect usually has three generations per year. This corresponds to three waves of emergence of mobile nymphs, which can be assigned to succeeding generations. In the spring-summer 2003, 2004 and 2005 in lemon cv Femminello siracusano orchards we tested transparent cellophane tape with adhesive on both sides to monitor the emergence of first instar scales, called crawlers. The crawlers were captured on the tape as they move across a twig or branch. These tapes were used to confirm the arrival of the new generation and the beginning of crawler activity and predict when treat the Oleander scale. The first generation started in March-April. Mobile nymphs then moved towards sheltered areas of the tree, settling preferably on the underside of foliage and fruits. This generation developed in 8 to 9 weeks. A second generation then occurred in June, taking a similar amount of time to develop. Insecticide mode of action and formulation are important because the armor covers and protects all stages but the crawler and the adult male. Contact insecticides target the crawler stage, such as more selective Horticultural Mineral Oils. Care must be taken to conserve natural enemies. Populations of other pests, such as whiteflies, may increase if insecticides kill their natural enemies. Spray schedules can be determined by presence of scales in the field rather than by the calendar dates.

Key words: development rates, Aspidiotus nerii, Citrus limon

Introduction

Oleander scale, Aspidiotus nerii Bouché [Hemiptera: Diaspididae], is a major pest of lemon (Citrus limon (L.) Burman f. [Sapindales: Rutaceae]), that growers have traditionally controlled with insecticides (Catara et al., 1994). Although armoured scale insects cause little or no physical damage to either the tree or the fruit, their presence on fruit at harvest can have serious implications for market acceptability. In years when biological control is less effective, this pest is managed with horticultural mineral oils (HMOs) as a part of an integrated pest management approach (Areddia et al., 2000). Contact insecticides target the crawler stage, such as more selective HMOs. In Mediterranean basin, there are three or four generations of Oleander scale each growing season. Reproducing females release first instar scales, called crawlers. The crawlers travel a short distance and settle onto twigs, leaves or fruit where they will remain until maturity. Populations of Oleander scale was monitored in field with pheromone traps (Garcia et al., 1999), but the pheromone traps were not commercially developed. A more reliable method of timing treatments is to monitor for crawlers by wrapping sticky tape around 1-year-old branches that have both grey and green wood and are infested with live female scales (Sanfilippo et al., 1999). We report the results of a three-year field experiment in which we put a cellophane tape with adhesive on both side to monitor the emergence of oleander scale crawlers. The aim was determine: when
emergence is occurring and when to time insecticide applications.

**Materials and Methods**

*Trees and assessments*

The experiment was undertaken from March 2003 to July 2005 in a five commercial lemon orchards near Siracusa, in the South Eastern province of Sicily. The lemon variety used was “Femminello siracusano”. The trees were more than 30 years old and 3 to 4 m high. They were planted at a spacing of 6 x 6 m in calcareous clay loam. Three orchards were organically managed, one was IPM managed and one was conventional managed. We selected one tree from each five central orchard area.

Beginning in March we changed weekly four transparent cellophane tapes (trade name 3M) per tree around 1-year-old branches (about 0.5 inch diameter) that have both grey and green wood and were infested with live female scales. The crawlers were captured on the tape as they move across a twig or branch. These tapes were used to confirm the arrival of the new generation and the beginning of crawler activity and predict when treat the Oleander scale.

Different treatments were applied. Organic farm 1: HMOs 2% (target: Citrus broad mite and Citrus bud mite; timing: Nov. 2004); organic farm 2: HMOs 2% (target: Two spotted spider mite, Oleander scale; timing: Aug. 2003); *Leptomastix dactylopii* 1200 wasp/ha + *Cryptolaemus montrouzieri* 300 mealybugs destroyet/ha (target: mealybugs; timing: Jun. 2004); Sulphur + sodium silicate (target: Two spotted spider mite and Oleander scale; timing: Jul. 2004); organic farm 3: *L. dactylopii* 1200/ha + HMOs 2% (target: mealybugs, thrips, Oleander scale and Two spotted spider mite; timing: Jun. 2003); HMOs 2% (target: thrips, Oleander scale and Two spotted spider mite; timing: Jun. 2003); organic farm 3: *L. dactylopii* 1200/ha + HMOs 2% (target: Oleander scale; timing: Jan. 2003); fenbutatin oxide (target: Two spotted spider mite; timing: Jun. 2003); HMOs. 2% (target: Oleander scale; timing: Jan. 2004); chlorpyriphos etil (target: (trunk) ant; timing: Jun 2004); IPM farm 4: fenbutatin oxide (target: Two spotted spider mite; timing: Jun. 2003); HMOs. 2% (target: Oleander scale; timing: Jan. 2004); chlorpyriphos etil + fenbutatin oxide (target: Two spotted spider mite and Oleander scale; timing: Jun. 2003); HMOs 2% (target: Citrus bud mite and Oleander scale; timing: Jan. 2003); chlorpyriphos metil + fenbutatin oxide (target: Two spotted spider mite and Oleander scale; timing: Jun. 2003); chlorpyriphos etil + fenbutatin oxide (target: thrips, Two spotted spider mite and Oleander scale; timing: Jun. 2004). Chlorpyriphos metil (target: Oleander scale; timing: Oct. 2004), chlorpyriphos etil + HMOs 1.25% (target: Two spotted spider mite and Oleander scale; timing: Jul. 2005)

**Results**

Crawlers of same generation emerged over a period of several weeks. There was little difference between the resulting curves (Figure 1). In the 2004 year, when four farms treated in June, there was timing difference. During the early spring 2005, crawlers were absent, indicating near or total absence of reproduction by gravid over-wintering females. Cold winter killed early developmental stages. The first generation started in March-April, peaking in May. Mobile nymphs then moved towards sheltered areas of the tree, settling preferably on the underside of foliage and fruits. This generation developed in 8 to 9 weeks. In field experiment adult males caught (Garcia *et al.*, 1999, fig. 2) confirmed this results. The figure 2 shows when the first male generation inseminate the third instar virgin females. Third instar female continue to grow and develop into gravid mature females with crawlers in July. Thus a second crawler generation occurred in mid-June, peaking in July (fig.1).
This experimental program using tape counts will be tested in some areas. The economic threshold for fruit infestation of lemons varies substantially with the market and crop yield, but it has been found to average 3 to 5% fruit infested with more than 10 scales late in the fall (Peherson et al., 1991).

If counts of the fruit infestation at harvest indicate that the threshold will be exceeded, monitoring crawler production the next spring beginning at bloom may be a good option. Visual inspection of the fruit is the most precise way to determine the need for treatment in July, when crawlers peak is coming. Biological control of Oleander scale can be significant in lemon trees. Treatment decisions and choice of materials should take the level of parasitism into account.
Conclusion

Optimal treatment timing varied from year to year because of temperature, but usually occurred in May (first crawler generation peak) or July (second crawler generation peak). Treatment around petal fall may increase fruit drop and be toxic to honey bees. The beginning of second generation crawler production usually occurs about to mid-June; about 2 to 4 weeks after the peak of the male flight (fig.2). Make an application, when a sharp increase in second crawler production begins, may be a viable option. Thus spray schedules can be determined by presence of scales in the field rather than by the calendar dates. Always back up cellophane tape count decisions with inspection of fruit for female and immature scale. Thus, these tapes can be used to confirm the arrival of the new generation.

References


Survey of Resistance of the Citrus Red Scale *Aonidiella aurantii* (Homoptera: Diaspididae) to Chlorpyrifos in Spanish Citrus Orchards

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**Abstract:** California red scale *Aonidiella aurantii* (Maskell) is an important pest of citrus orchards in Spain. It causes cosmetic damage to the fruit resulting in downgrading in the packinghouses. Several populations of *A. aurantii* in citrus orchards from Valencia were tested for resistance to the insecticide chlorpyrifos during 2003-2004. All tests were made by bioassays. Fruits infested with first immature stages were dipped for 10 seconds in different concentrations of the chemical between 50 and 20,000 ppm of active ingredient. Mortalities were assessed after 15 days, considering dead those insects that had not developed to the second stage. The estimated CL50 was used in order to compare the values of susceptibility. The results showed important differences between orchards. Susceptibility was usually correlated with the history of previous treatments made in the plot, with greater degree of resistance in orchards with more previous applications. Some plots showed a high degree of chlorpyrifos resistance, with CL50 values 250 times higher than those found in the most susceptible plots.

**Introduction**

California red scale *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) is considered an important economic pest in the majority of the citrus regions of the world. This species was first detected in Spain in 1985 and it is now present in almost all citrus grown in the country. It causes cosmetic damage to the fruit resulting in downgrading in the packinghouses. Several insecticides, mostly organophosphates, have been used successfully to treat this insect, but recently control failures have been observed. These failures were found in chemicals frequently used in the control of the pest, so development of direct or crossed resistances are likely. Our objectives were to determine if California Red Scale has developed resistance to organophosphates in Spain and to compare pesticide susceptibility among different field populations.

**Materials and methods**

The method used in this study to determine resistances was based on the collection of fruits with presence of *A. aurantii* from the field (Grafton-Cardwell and Verhrs, 1995). Infested fruits were kept in the laboratory with controlled temperature and humidity (28°C and 70-80% RH) to facilitate the evolution of the population and to obtain abundant first instar immatures. The susceptibility of the *A. aurantii* to the insecticide chlorpyrifos was measured by immersion treatments. Fruits infested with first immature stages were dipped for 10 seconds in different concentrations of the chemical between 50 and 20,000 ppm of active ingredient. Control fruits were dipped in distilled water. Mortalities were assessed after 15 days, considering dead those insects that had not developed to the second stage. The percentage of mortality was corrected for natural mortality by Abbot's formula and the results were compared by means of the LC50 determined by probit analysis.
Results and discussion

The presented trials were carried out in the summer and autumn of the years 2003 and 2004. In no case they could be carried out before the middle of August because fruits were too small and not adequate to perform tests on them.

The values of LC50 obtained in the different plots were very different. These values ranged from 16 to 4189 ppm of chlorpyrifos active ingredient (Fig. 1). The CL50 obtained on each orchard was usually closely correlated with the history of previous treatments made in the orchard. In several orchards laboratory estimated values of LC50 were far above the dose recommended for field treatments. Considering that laboratory treatments were made on the most susceptible stage (Abdelrahman, 1973; Walker et al., 1990), field populations of *A. aurantii* in these orchards should be considered highly resistant to chlorpyrifos and almost no mortality should be expected when sprayed with this chemical.

Our results show wide differences in susceptibility to chlorpyrifos between nearby plot in the same locality (Fig. 2). Apparently, the low mobility of the populations of *A. aurantii* allows for the development of localized resistant strains that do not disperse readily to adjacent orchards. This low mobility is possibly also a factor that encourages the development of resistant strains, as mixing with nearby susceptible populations should be low in field situations (Georghiou, 1986). The findings of our study should be taken into account for future chemical spray recommendations and programs of management of the Citrus Red Scale in Spanish Citrus orchards.

References


Figure 1. LC$_{50}$ obtained in different citrus orchards from Valencia (Spain) tested for chlorpyriphos resistance in *Aonidiella aurantii*.

Figure 2. Log Concentration - probit mortality lines for resistance of *Aonidiella aurantii* to chlorpyrifos, from two nearby located orchards in the same locality.
**Planococcus citri** on ornamental Citrus plants in central Italy

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**Abstract:** Research was carried out on the life cycle and population dynamics of *Planococcus citri* (Risso) in Tuscany (central Italy), at nurseries in the province of Pistoia where cultivations of potted ornamental citrus plants are very widespread. There, potted citrus are grown in greenhouses all year long or put outside during the summer. In two nurseries (Nursery 1 with plants in the greenhouse from October to May and in the open air during the summer; Nursery 2, in the greenhouse all year round) 10 shoots with 5 leaves were sampled every 2 weeks, and the males’ flights were monitored by means of pheromone traps. In addition, trials were carried out, in the greenhouse and in the open air, on potted lemon trees prevalently infested by 1st instar larvae, using synthetic active ingredients having low toxicity and persistence (imidaclorpid, thiamethoxam and chlorpyriphos methyl) or of natural origin (azadirachtin and *Beauveria bassiana*). Under both cultivation conditions, *P. citri* completed 4 generations, but with a much higher level of infestation in the greenhouse cultivation. In Nursery 1, the first peak of male emergence was registered at the end of May and the maximum number of males per trap was 116 in August. Instead, in Nursery 2 the flights began a month earlier, and the maximum number of males captured reached 2800/trap in June. During the year, there was the contemporary presence of all the stages for the partial overlapping of the cycles, with a prevalence of females and 1st instar larvae in the winter. Specimens of the Encyrtid parasitoid *Leptomastoidea abnormis* (Grtl.) and of the Coccinellid predator *Cryptolaemus montrouzieri* (Muls.) were sporadically isolated. In the greenhouse, the product that gave the best results was the chloronicotinyl thiamethoxam (91.9% C), followed by chlorpyriphos methyl (84.8% C). Indeed, in the open air test, the latter was found to be the only efficacious a.i. (99% C). The products of natural origin, namely azadirachtin and *B. bassiana*, produced no results in either of the tests.

**Key words:** ornamental Citrus, *Planococcus citri*, nursery, life cycle, control

**Introduction**

In Tuscany, the Pseudococcid *Planococcus citri* has been found to be among the most damaging insects to cultivations of ornamental citrus, which are very widespread in nurseries in the area of Pistoia, where potted plants are raised in greenhouses all year round or are put outside during the summer. In particular, densely-populated colonies of this homopteran have been found in cultivations of potted lemon and citron trees. *P. citri* is a species of Palaeartic origin that has been present in Italy ever since the end of the 19th century. It is a polyphagous mealybug that lives on many arboreous and herbaceous plants, both in the open air and in greenhouses: Citrus species, cultivars and hybrids are among the most economically important host plants.

The high costs of maintenance and the aesthetic requirements of ornamental *Citrus* plants mean that the economic thresholds for treatments valid for citrus plantations (5-15% of infested fruits, OEEP, 2004) are too high, since the sole presence of *P. citri* on the leaves, which is detected from waxy secretions and honeydew, is enough to devalue the plants and to justify the use of insecticides. It has, therefore, been considered necessary to make an in-depth study of the biology and population dynamics of the species, for the purpose of limiting its
infestations with means having low environmental impact: neonicotinoids and natural insecticides.

**Material and methods**

**Life cycle**

During 2004, in two nurseries under different growing conditions (N1 with plants in the greenhouse from October to May and in the open air during the summer; N2 in the greenhouse all year round), every two weeks 10 lemon shoots with 5 leaves were sampled, and the male flights were monitored by means of pheromone traps. Parasitized forms of the Pseudococcid and preimaginal stages of predators were raised to adults.

**Control**

Trials were carried out on potted lemon trees (2 plants x 4 repetitions/experimented product) infested prevalently by 1st instar larvae, in the greenhouse (Test A) and in the open air (Test B), using synthetic active ingredients commonly used on citrus for the control of *Phyllocnistis citrella* (Del Bene & Landi, 2000) and having low toxicity and persistence or else of natural origin, which could be used in Biological Agriculture (Del Bene *et al*., 2001). The aa.ii. tested were the two chloronicotinyls imidacloprid and thiamethoxam, the organophosphate chlorpyriphos methyl, azadirachtin and *Beauveria bassiana*:

- imidacloprid: Confidor 20 SL, 0.5 ml/l, 2 treatments every 7 days
- thiamethoxam: Actara 25 WG, 0.4 g/l, 2 treatments every 7 days
- chlorpyriphos methyl: Reldan 22 EC, 2.5 ml/l, 2 treatments every 7 days
- azadirachtin: Oikos 3 EC, 1.5 ml/l, 3 treatments every 7 days
- *Beauveria bassiana*: Naturalis 7.16 SC, 1.5 ml/l, 3 treatments every 7 days.

A week after the last treatment, living and dead *P. citri* were counted on 2 shoots with 5 leaves/plant. The survival percentages were calculated and compared with the Duncan test (at P=0.05 level) after angular transformation according to Bliss. The level of efficacy of the products was determined according to Abbott.

**Results**

**Life cycle**

Under both growing conditions, *P. citri* completed 4 generations, even if with a different degree of infestation: the attack was much higher in the greenhouse cultivation (N2). While in the N1 nursery the first peak of males’ emergence (Fig. 1) was registered on 21 May and subsequent ones at the end of June, end of August (max. 116 males/trap) and end of October, in the N2 nursery (Fig. 2) male flights began one month earlier, and their extent was much higher. In fact, in N2 the capture trend revealed male flights from April to November, with two main peaks on 27 April (1200 males) and 18 June (2800 males) and two secondary peaks on 29 August (120 males) and 25 October (113 males).

An analysis of *P. citri* populations on the plants (Fig. 3) displayed the year-long simultaneous presence of the different stages for the partial overlapping of the cycles, with females reaching a maximum in the winter, in April, June, and August, and 1st instar larvae (the most vulnerable stage) in the winter, end of April/May, July and September.

The level of infestations on branches and leaves (Fig. 4) was more or less constant from November to May (< 100 individuals/shoot); it reached its maximum at the beginning of June, (862 individuals/shoot) during development of the 1st generation, and then it decreased at mid summer.
Figure 1 – Number of *P. citri* males/trap on lemon trees cultivated in the greenhouse during the cold months and in the open air in summer (N1).

Figure 2 – Number of *P. citri* males/trap on greenhouse-cultivated lemon trees (N2).

In the summer, on the plants that had been put out in the open air, specimens of *Leptomastoidea abnormis* (Grtl.) (Hym. Encyrtidae) were sporadically reared from parasitized forms of *P. citri*, and the predator *Cryptolaemus montrouzieri* (Muls.) (Col. Coccinellidae) was also isolated.
Figure 3 - Percentage of *P. citri* stages (females and larvae) on greenhouse-cultivated lemon trees.

Figure 4 – Mean number of *P. citri*/shoot on greenhouse-cultivated lemon trees.

**Control**

Among the insecticides experimented in the greenhouse (Test A), (Tab. 1, Fig. 5), the active ingredient that gave the best results was the chloronicotinyl thiamethoxam (Actara, 91.9%C), followed by chlorpyriphos methyl (Reldan, 84.8%C). Imidacloprid (Confidor) guaranteed 58.7% control, while almost zero results were obtained with 3 treatments of azadirachtin (Oikos) and *B. bassiana* (Naturalis).
In the open-air test (Test B) (Tab. 2, Fig. 5), chlorpyriphos methyl was found to be the only efficacious chemical (99% C), while imidacloprid and thiamethoxam gave poor results (9.1% and 30.6% C, respectively). Azadirachtin and B. bassiana confirmed an absence of efficacy on the Pseudococcid.

Table 1 - Percentage of living P. citri and level of efficacy (% C) of the products experimented in the greenhouse (test A). Significantly different values are indicated by different letters.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>n. tr.</th>
<th>dose ml-g/l</th>
<th>n. living</th>
<th>n. dead</th>
<th>% living</th>
<th>% C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confidor</td>
<td>2</td>
<td>0.5</td>
<td>542</td>
<td>772</td>
<td>41.2 c</td>
<td>58.7</td>
</tr>
<tr>
<td>Actara</td>
<td>2</td>
<td>0.4</td>
<td>182</td>
<td>2063</td>
<td>8.1 d</td>
<td>91.9</td>
</tr>
<tr>
<td>Reldan</td>
<td>2</td>
<td>2.5</td>
<td>222</td>
<td>1247</td>
<td>15.1 d</td>
<td>84.8</td>
</tr>
<tr>
<td>Oikos</td>
<td>3</td>
<td>1.5</td>
<td>4878</td>
<td>259</td>
<td>94.9 b</td>
<td>4.9</td>
</tr>
<tr>
<td>Naturalis</td>
<td>3</td>
<td>1.5</td>
<td>3725</td>
<td>512</td>
<td>87.9 b</td>
<td>11.9</td>
</tr>
<tr>
<td>Check</td>
<td>4</td>
<td></td>
<td>4267</td>
<td>7</td>
<td>99.8 a</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5 – Level of efficacy of insecticides experimented against P. citri in the greenhouse (test A) and in the open air (test B).
Table 2 - Percentage of living *P. citri* and level of efficacy (%C) of the products experimented in the open air (test B). Significantly different values are indicated by different letters.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>n. tr.</th>
<th>dose ml·g/l</th>
<th>n. living</th>
<th>n. dead</th>
<th>% living</th>
<th>% C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confidor</td>
<td>2</td>
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<td>1665</td>
<td>175</td>
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<td>9.1</td>
</tr>
<tr>
<td>Actara</td>
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<td>549</td>
<td>246</td>
<td>69.1 b</td>
<td>30.6</td>
</tr>
<tr>
<td>Reldan</td>
<td>2</td>
<td>2.5</td>
<td>212</td>
<td>78</td>
<td>93.9 a</td>
<td>5.7</td>
</tr>
<tr>
<td>Oikos</td>
<td>3</td>
<td>1.5</td>
<td>1194</td>
<td>104</td>
<td>98 a</td>
<td>1.6</td>
</tr>
<tr>
<td>Naturalis</td>
<td>3</td>
<td>1.5</td>
<td>5117</td>
<td>25</td>
<td>99.6 a</td>
<td></td>
</tr>
</tbody>
</table>

The differences noted in effectiveness in the two neonicotinoids (imidacloprid and thiamethoxam) in the A and B tests can be attributed to the different conditions under which the tests were conducted: A - in a protected environment, and B - in the open air, thus with a greater exposure to abiotic degradation factors in the latter case.

**Discussion**

The surveys which we carried out on cultivations of ornamental citrus plants in Tuscany emphasized the fact that, also in central Italy, *P. citri* can achieve 4 generations/year, as in citrus plantations in the southern regions of Italy. Our data agree, therefore, with what was observed by Monastero & Zaami (1958) and Tremblay (1983) in Sicily, while surveys carried out by other authors reveal 3 to 4 generations in Sardinia (Ortu & Delrio, 1983), 3 generations in the Campania region (Viggiani & Battaglia, 1984), and 2 to 3 generations in northern Italy (Tremblay, 1983).

In the case that the economic threshold for treatments, estimated empirically on ornamental citrus plants as being close to zero, is reached, in the greenhouse it is possible to limit the populations of the mealybug with thiamethoxam and chlorpyriphos methyl. However, when plants are exposed to the open air in summer, the intensity of infestation decreases naturally. In fact, in the open air, the conditions of humidity typical of the protected environments, which notoriously favour the development of the insect, cease to exist, and the action of indigenous natural enemies can be sufficient to maintain the species within limits of non-harmfulness.

**Acknowledgements**

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Investigations on population dynamics and mortality of *Phyllocnistis citrella* in western Sicily (Italy)

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Abstract: Population dynamics and mortality of *P. citrella*, and its natural enemies with parasitism levels in two different citrus orchards, sprayed and unsprayed, were investigated. The effect of host density on percentage parasitism and mortality was calculated by regression analyses. In both sites, over the 3-years period from 2002 to 2004, *P. citrella* was detected during the summer months and it was not found during the first flush. Population levels were low, never exceeding 3.0/leaf. *P. citrella* began to increase from June in 2002–2003 and from July in 2004. The monthly percentage parasitism of *P. citrella* in sprayed orchard fluctuated from 0 to 19%, while in unsprayed from 0 to 30%. High mortality was found in both locations with maximum levels of 100%. No significant regression was found between CLM population and parasitism and mortality percentage. Small differences between results in two orchards were found.

Key words: pests, parasitism, orchards.

Introduction

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was first discovered in Italy during the autumn of 1994 (Ortu et al., 1995), showing a very rapid range expansion. The pest attacks all cultivars of citrus, related species within the Rutaceae family, and several ornamentals and it preferentially oviposits on young leaves located within the terminal 5 cm of new shoots.

Since the first occurrence of the citrus leafminer in Sicily, several indigenous natural enemies have been found which attack the pest but recently the most abundant species have been the exotic endoparasitoids, *Citrostichus phyllocnistoides* Narayanan and *Semielacherpetiolatus* (Girault) (García-Marí, 2004). In the last years, natural mortality and environmental conditions appear to regulate CLM population reducing its damage.

The objectives of our studies were to monitor population dynamics, percentage parasitism and mortality of *P. citrella* in two citrus orchards, with different pest management methods.

Material and methods

The research was conducted in two citrus orchards, one sprayed and one unsprayed, from June 2002 to June 2005 in western Sicily, Italy. In the 1st orchard, farmer sprayed only about two times a year, in July and in October. The main targets for spraying in this orchard were aphids, scales and mites. Dimethoate, Acephate and Methidathion were the most important insecticides used against these pests.

In the 2nd citrus orchard, no pesticides were applied. At each location, sampling was done by collecting every 15 days 100 young citrus shoots, which were placed in plastic bags and brought to the laboratory. For each orchard, 200 tender leaves were examined under a
stereomicroscope and for each leaf total number of *P. citrella* (live / dead leafminer larva, leafminer pupa and leafminer pupal cases) and parasitoids were recorded. Apparent percentage parasitism was calculated.

Mortality was calculated by dividing the number of hosts with parasitoid eggs, larva or pupa plus number of hosts killed by a parasitoid or by a predator including the unknown mortality with total number of CLM(living and dead) (Amalin, 2002).

**Statistical analysis**

We tested the effect of population dynamics on percentage parasitism and mortality by using regression analysis. Percentage parasitism and mortality (dependent variable) were regressed on population density (independent variable). For this analysis, percentages data were transformed using arcsine-square root method.

**Results and discussion**

The seasonal pattern of *P. citrella* populations (Fig.1) was almost similar in both orchards during these three years. In both sites, populations began to increase in June–July, reaching peak numbers from August to September, and declined in late fall. Numbers of *P. citrella* per leaf were always low, never exceeding 3.0/leaf, and the summer peaks decreased year by year. In 2004, CLM population began to increase in the mid-July, one month later compared with two years before (Fig. 1).

![Figure 1. Population dynamic of *P. citrella* in two citrus orchards, sprayed and unsprayed, in Sicily (Italy).](image)

In the unsprayed orchard, the highest percentages of parasitism, 30 %, was observed in November 2002, 25%, in January 2004, 20%, in January 2005. In the sprayed orchard, the percentage parasitism did not exceeded 20%, parasitism of 17.54 % was found in October 2002, 18.75% in January 2004, and 17.65% in December 2004 (Fig. 2).
Figure 2. Parasitism levels on *P. citrella* population in two citrus orchards, sprayed and unsprayed, in Sicily (Italy).

Overall, the highest percentage of parasitism, that some times appeared, in the unsprayed orchard compared with sprayed orchard, could be due to the fact that in this orchard no pesticides were applied. This suggests that pest management methods may influence the percentage of parasitism.

Figure 3. Regression of numbers of *P. citrella* hosts per leaf and percentage parasitism in two Citrus orchards in Sicily (Italy).

There was no significant linear relationship between the total parasitized *P. citrella* and the total population of *P. citrella* in unsprayed \( (F=0.85; df=1,40; P= 0.36; r^2=0.02) \) and sprayed orchard \( (F=0.12; df=1,42; P= 0.70; r^2=0.00) \) (Fig. 3). These observations are similar to those reported by Legaspi and French (2000).
Figure 4. Mortality of *P. citrella* population in two Citrus orchards in Sicily (Italy).

Those author reported that parasitoids of *P. citrella* are generalist and attack immature of other lepidopteran miners (Peña et al., 1996).

Figure 5. Regression of numbers of *P. citrella* hosts per leaf and percentage mortality in two Citrus orchards in Sicily (Italy).

In unsprayed orchard, the mortality rate ranged from 27.27% (June, 2002) to 100% (December, 2002), from 32.56% (June 2003) to 100% (January, 2004) and from 8.70 % (July, 2004) to 82%, 43% (August, 2004). In sprayed orchard, mortality rate was in the rate of 23.15% (July, 2002) to 63.13% (December, 2002), from 7.69% (July, 2003) to 100% (January, 2004) and from 3.85 (July, 2004) to 100% (January, 2005) (Fig. 4).

No relationship between mortality of host and relative host densities (nr. mines per leaf) was found between unsprayed (F=1.50; df=1,42; P=0.22; r²=0.03) (Fig.6) and sprayed orchard (F=0.04; df=1,41; P= 0.85 ; r²=0.00) (Fig.5). The high mortality rate observed during December and January could be related with low temperature, always with average temperatures under 15°C.
References


Application of biorational pesticides on nursery trees against
*Phyllocnistis citrella* Stainton in Sicily: the effects on different citrus species

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Abstract: In Sicily (Italy), citrus trees are cultivated in nursery conditions both for new plantation and for ornamental purposes. Since 1995, sicilian citrus nursery growers considered the citrus leafminer *Phyllocnistis citrella* Stainton [Lepidoptera: Gracillariidae] one of the most important pest in citrus nurseries, and normally about 20 chemical applications per year are conducted in order to maintain citrus leafminer populations at low level. In previous years, several biorational pesticides for the control of citrus leafminer *P. citrella* were compared for testing their compatibility with integrated pest management (IPM)-programs under nursery conditions in Sicily. Based on encouraging results, in 2002-2004, biorational pesticides were utilized, on large scale trials, for testing their effects on citrus plant growth. Repeated sprays of horticultural Narrow-Range oils and azadirachtin were applied on 4800 two-years lemon, orange, mandarin, kumquat and lime trees in replicated blocks, cultivated in protected conditions. The physiological effects on tree flushing pattern and growth was periodically monitored and compared with the presence of citrus leafminer mines. The presence of natural parasites was recorded with yellow sticky traps. On average, lemon trees resulted the most infested species compared with orange and kumquat; mandarin and lime were slightly infested. On average, the yearly mean percentage of flushing trees was greater in lemon, mandarin and orange, compared with kumquat and lime. In 2003, trunk diameter growth was reduced on kumquat. In 2002, percentage of leaf area damage, caused by *P. citrella*, was higher on lemon. The presence of Eulophid parasitoids was not excluded.

Key words: citrus leafminer, azadirachtin, Narrow Range spray oils

Introduction

In Sicily, citrus trees are cultivated in nursery conditions both for new plantation and for ornamental purposes. Since 1995, Sicilian citrus nursery growers considered the citrus leafminer *Phyllocnistis citrella* Stainton [Lepidoptera: Gracillariidae] the most important pest in citrus nurseries in Italy (Viggiiani, 1998). Plant growth and quality can be compromised if leafminer is not managed during ornamental citrus nursery tree production (Caleca et al., 2000). In the Mediterranean area the presence of *P. citrella* is not considered as a serious threat for mature citrus groves (Garcia-Mari et al., 2003; Ortu & Acciaro, 1998) differently from Florida and China where yield reductions are reported (Villanueva and Hoy, 1998a). Biological control plays an important role in many countries (Smith & Beattie 1996; Hoy & Nguyen, 1997). In Italy, the introduction of *Semielacher petiolatus* (Girault) and *Citrostichus phyloconstoides* (Narayanan), two indigenous Eulophids parasitoids (Mineo & Mineo, 1999), resulted in leafminer infestation levels lower than those found when the pest appeared initially in Sicily citrus (Conti et al., 2001; Siscaro et al., 2003). An important contribution is played by the native general predators (lacewings, spiders, ants, minute pirate bugs, etc.) to mortality
experienced by citrus leafminer in field (Jacas & Urbaneja, 2002). However, because other pests and diseases must be suppressed during nursery tree production in Sicily, it is unlikely that biological control of *P. citrella* can be achieved unless selective pesticides are used. The ornamental finality of the citrus trees cultivation rendered this objective more complex.

In an effort to develop an IPM program for citrus nurseries, various pesticides were selected for their selectivity to hymenoptera wasps and their efficacy in suppressing the leafminer according to preliminary test conducted in Sicily (Conti et al., 1998; Serges et al., 1998; Fisicaro et al., 2001) and according to international literature (Ripolles et al., 1996a, 1996b; Villanueva & Hoy, 1998b; Villanueva et al., 2000). Analysis of these products under field conditions was necessary to confirm their suitability for an IPM program. In 2001, in the second main flush of the season, mineral oil and azadirachtin reduced the number of live mines of *P. citrella* per leaf compared with the untreated control, but had more mines than imidacloprid. All treatments had fewer mines parasitized by parasitoids compared with the untreated control. Actual *P. citrella* infestation levels had no detectable effect on tree growth. The environmental impact of the pesticides was calculated and the mineral oil based strategies resulted not safe for the high rate of the formulation and the spray solution (Conti et al., 2004). These results, obtained on small plots trials, must be confirmed on large scale trials and the physiological effects on different citrus species must be measured. In the periods 2002-2004, repeated sprays of horticultural NR oils and azadirachtin, have been applied on 4800 two-years lemon, orange, mandarin, kumquat and lime trees in replicated blocks, cultivated in protected conditions. The presence of leafminer mines and the physiological effects on tree flushing pattern have been monitored weekly. Trunk growth and leaf area damage have been measured. The presence of natural parasites has been periodically recorded.

**Material and methods**

Various citrus trees (2 years old at the start of the project, obtained from La Palmarica nurseries, Terme Vigliatore, Messina-Italy) grafted on sour orange rootstock (*Citrus aurantium* L.), were cultivated in 8 litres plastic pots starting from April 2002. In the experimental plot, forty-eight rows of 100 trees each were arranged in group of 4 rows with 1.2 m between groups, 0.4 m between rows, and 0.2 m between trees in a row. This layout was distributed on two sides of a screenhouse protected with green nets (50%). The two sides were separated by a service passage. Drip irrigation located in each row watered trees when required. Fertilizer was applied to each tree with irrigation. Mowing, weeds suppression, and other cultural practices were done when needed. Citrus trees were pruned and shaped with the aim to produce ornamental effects. Several fruits were allowed on the trees. Weather data were obtained from the mechanical meteorological station located in the farm. A randomised block design with 5 treatments and 6 blocks (replications) was established in the experimental nursery. Experimental units (EU) consisted of 4 rows of 25 trees each (100 trees for each EU; for lemon 4 EU for each block have been considered). All measurement were obtained from the 10 central trees in each experimental unit. The experimental project was replicated for three years (2002-2004).

The five treatments were as follows: 1) Lemon (*Citrus limon* (L) Burm. f.) “Lunario” trees; 2) Mandarin (*C. reticulata* Blanco) “Avana” trees; 3) Orange (*C. sinensis* (L.) Osbeck) “Tarocco” trees; 4) Kumquat (*Fortunella* spp) trees; 5) Lime (*C. limetta* Risso) trees. For lemon 4 EU per block have been included, but only 1 EU data are reported in tables. All treatments were protected by the same strategy based on repeated sprays of azadirachtin or NR oils, as reported in Table 1. Pesticides were sprayed with a motorized handled sprayer distributing about 0.08 litres per tree.
Table 1. List of active ingredients, rates, number and period of applications, utilized on citrus nursery trees, in the experimental site.

<table>
<thead>
<tr>
<th>active ingredients</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n. of applications</td>
<td>period</td>
<td>n. of applications</td>
</tr>
<tr>
<td>Azadirachtin-3%</td>
<td>11</td>
<td>30-May to 25-Sept.</td>
<td>17</td>
</tr>
<tr>
<td>150 ml (trade mark) per 100 litres</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narrow range oils-96%</td>
<td>9</td>
<td>11-May to 25-Oct.</td>
<td>5</td>
</tr>
<tr>
<td>2000 ml (t.m.) per 100 litres</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrethrum-4%</td>
<td>3</td>
<td>9-June to 9-Oct.</td>
<td>7</td>
</tr>
<tr>
<td>150 ml (t.m.) per 100 litres</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300/500 ml (t.m.) per 100 litres</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* for the control of aphids and woolly whitefly; X for the control of mites

The objective of the experimental project was to protect all the young flushes that are susceptible to attack by *P. citrella* and other pests. Timing of sprays was based on growth flush patterns. In order to detect the physiological response of each citrus species during three years, weekly measurement of flushing trees were carried out on 10 central trees per experimental unit. Flushing cycles were classified according to the phenology of the leafminer (Knapp et al., 1996; Villanueva-Jiménez et al., 2000) on the 3rd oldest leaf of a new shoot. Category 1 flushes were defined as shoots with the 3rd oldest leaf (counted from the bottom) larger than 2 cm and suitable for leafminer oviposition, with only eggs or 1st instar larval mines. Category 2 flushes included those with the 3rd oldest leaf infested with or suitable for infestation with 2nd- to 4th-instar leafminer larvae (parasitized or unparasitized by Eulophidae). Category 3 flushes had completely expanded 3rd leaves containing or suitable for containing leafminer pupae. Category 3 flushes were fully extended shoots with all leaves at the tip of the flush at least partially expanded.

The decision to spray was based on a minimum proportion of tree flushing (> 25% of trees with at least 1 new flush identified as category 1 or 2), and a minimum average of *P. citrella* mines per leaf (> 0.2). If these two conditions were not both respected, the treatment was delayed ant the sampling repeated after 7 days according to a sequential sampling program (Caleca et al., 2002).

Every week the number of mines per leaf with live larvae of *P. citrella* were recorded from a leaf of the category 1 or 2 flushes on 10 central trees per experimental unit. Leaves in the appropriate stage to sample were not always available on all trees, and thus the number of leaves sampled varied from the potential total of 60 leaves per treatment, on each sampling date.

Trunk diameter was measured at ground level at the beginning of the experiment and after all pesticides sprays were conducted. Leafminer damage per tree was visually inspected after all pesticides applications (December). The proportion of leaf area mined was used to categorize the level of canopy damage, inspecting ten trees per experimental unit.
The presence of leafminer parasitoids was assessed by mean of a yellow sticky card (12 x 8 cm) hanged in the centre of the experimental plot. The card was weekly changed and examined under stereomicroscope.

All data were analysed as randomised block design. The weekly records of raw data were yearly averaged. To reduce heterogeneity of variance before analysis of variance (ANOVA), square root transformation was applied on $P. \text{citrella}$ larvae per leaf, and arcsin transformation was applied on percentage of trees with new flushes, but untransformed means are presented in the table. The Duncan’s multiple range test ($P = 0.05$) was performed after transformation.

Results and discussion

Citrus flushing pattern
For all citrus species, the 1$^{\text{st}}$ flush infested by $P. \text{citrella}$ was recorded during March-May, promoted by a favourable weather (Figure 1 and 2). This flush was followed by a 2$^{\text{nd}}$ flushing from June to August stimulated by drip fertirrigation. A 3$^{\text{rd}}$ flush occurred in September-November. In 2002, for kumquat and lime, 1$^{\text{st}}$ and 2$^{\text{nd}}$ flushes were not clearly separated, due to a continuous plants flushing (Fig 1).

![Figure 1: Citrus flushing pattern and leafminer mines per leaf based on all mines observed on the 3$^{\text{rd}}$ oldest leaf of category 1 & 2 flushes on Kumquat and Lime nursery trees in Sicily. (In 2002, cat. 1, 2 & 3 flushes were cumulated)](image-url)
For kumquat in 2002, 1st and 2nd flushes were greater than 3rd flush. In 2003 only 1st flush was detectable while in 2004 1st flush was not detectable at all, because plants were consistently fruits bearing (Fig. 1).

Figure 2. Citrus flushing pattern and leafminer mines per leaf based on all mines observed on the 3rd oldest leaf of category 1 & 2 flushes on Lemon, Mandarin and Orange nursery trees in Sicily. (In 2002, cat. 1, 2 & 3 flushes were cumulated)
For lemon, the three flushes, in terms of percentage of plants with new flushes (cat 1+2 flush), were comparable, as well as for lime. For orange and mandarin the 1st flush was greater than other flushes, mainly in 2003 and 2004 (Fig. 1-2).

The yearly mean percentage of citrus plants flushing was statistically comparable in 2002 than for all citrus species. In 2003 kumquat and lime resulted with a percentage of flushing trees lower than other species (table 2). In 2004 kumquat had less flushes than other species. On average, the yearly mean percentage of flushing trees was greater in lemon, mandarin and orange, compared with kumquat and lime (Table 2).

Table 2. Yearly mean percentage (± SD) of citrus plants flushing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon</td>
<td>63.8 ± 8.3  a</td>
<td>42.6 ± 2.5  a</td>
<td>35.5 ± 8.1  ab</td>
<td>47.3 ± 3.8  a</td>
</tr>
<tr>
<td>Mandarin</td>
<td>67.6 ± 4.7  a</td>
<td>39.0 ± 4.1  a</td>
<td>34.5 ± 5.8  ab</td>
<td>47.0 ± 2.3  a</td>
</tr>
<tr>
<td>Orange</td>
<td>65.8 ± 6.1  a</td>
<td>39.0 ± 3.0  a</td>
<td>41.9 ± 5.9  a</td>
<td>48.9 ± 2.5  a</td>
</tr>
<tr>
<td>Kumquat</td>
<td>59.1 ± 4.7  a</td>
<td>8.9 ± 1.8  c</td>
<td>14.7 ± 5.4  c</td>
<td>27.6 ± 2.5  c</td>
</tr>
<tr>
<td>Lime</td>
<td>67.1 ± 8.3  a</td>
<td>29.1 ± 4.1  b</td>
<td>29.5 ± 3.9  b</td>
<td>41.9 ± 4.2  b</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letters did not differ significantly: Duncan’s multiple Range test P>0.05

Citrus leafminer presence

On all citrus species, the mean number of *P. citrella* living larvae reached a peak of 0.2 mines per leaf (on lemon) and decreased to near zero at the end of the 1st flush (Fig.1 and 2). From the beginning of the 2nd flush, *P. citrella* larvae per leaf increased to a maximum of 1.2 ±1.5 mines per leaf (on lemon and orange respectively) during the growing season. Only few *P. citrella* mines per leaf were recorded for the 3rd flush. On all citrus species, repeated sprays of azadirachtin or NR-mineral oil (see table 1) resulted in a higher number of live larvae per leaf on the 2nd flush (summer one), compared with the 1st and 3rd flush (Fig. 1 and 2). The relative abundance of leafminer adults in summer, and the short-term persistence of non-synthetic compounds with high temperatures, might have accounted for the non positive response obtained in the 2nd flush.

In 2002, the yearly mean number of *P. citrella* live larvae per leaf was significantly greater on lemon (0.27) compared with other citrus species (Table 3). In 2003, kumquat trees resulted with significantly fewer mines (0.04) than lemon. In 2004, lime resulted significantly less infested than kumquat. On average, the yearly mean number of *P. citrella* live larvae per leaf was significantly greater on lemon (0.18) compared with other citrus species in the experimental site; orange and kumquat resulted significantly less infested than lemon, but with a significantly higher number of live larvae (0.15) than mandarin (0.11) and lime (0.10) as reported in Table 3.
Table 3. Yearly mean number of Phyllocnistis citrella live larvae per leaf (± SD)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon</td>
<td>0.27 ± 0.06 a</td>
<td>0.12 ± 0.07 a</td>
<td>0.15 ± 0.05 ab</td>
<td>0.18 ± 0.04 a</td>
</tr>
<tr>
<td>Mandarin</td>
<td>0.11 ± 0.04 c</td>
<td>0.07 ± 0.03 ab</td>
<td>0.16 ± 0.05 ab</td>
<td>0.11 ± 0.03 c</td>
</tr>
<tr>
<td>Orange</td>
<td>0.20 ± 0.05 b</td>
<td>0.07 ± 0.03 ab</td>
<td>0.18 ± 0.08 ab</td>
<td>0.15 ± 0.04 b</td>
</tr>
<tr>
<td>Kumquat</td>
<td>0.18 ± 0.04 b</td>
<td>0.04 ± 0.08 b</td>
<td>0.22 ± 0.13 a</td>
<td>0.15 ± 0.05 b</td>
</tr>
<tr>
<td>Lime</td>
<td>0.15 ± 0.02 bc</td>
<td>0.08 ± 0.04 ab</td>
<td>0.09 ± 0.04 b</td>
<td>0.10 ± 0.02 c</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letters did not differ significantly: Duncan’s multiple Range test $P > 0.05$

**Trunk diameter and leafminer damage per tree**

In 2002, mean percentage of trunk diameter increase on nursery citrus trees after pesticide applications was significantly higher on lime compared with other citrus species. In 2003, increase of trunk diameter was significantly lower on kumquat than other treatments. Percentage of leaf area damage caused by *P. citrella* in 2002 was significantly higher on lemon compared with kumquat and lime (Table 4).

Table 4. Mean (± SD) % of trunk diameter increase and leaf area damage on nursery citrus trees after pesticide applications in 2002 and 2003.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% trunk diameter growth ± SD</th>
<th>% of leaf area damage in 2002</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2002</td>
<td>2003</td>
</tr>
<tr>
<td>Lemon</td>
<td>9.38 ± 6.05 b</td>
<td>17.89 ± 3.2 a</td>
</tr>
<tr>
<td>Mandarin</td>
<td>8.69 ± 4.3 b</td>
<td>18.90 ± 2.3 a</td>
</tr>
<tr>
<td>Orange</td>
<td>10.01 ± 4.4 b</td>
<td>16.05 ± 5.3 a</td>
</tr>
<tr>
<td>Kumquat</td>
<td>10.60 ± 2.7 b</td>
<td>7.78 ± 1.2 b</td>
</tr>
<tr>
<td>Lime</td>
<td>19.64 ± 10.0 a</td>
<td>20.18 ± 4.7 a</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letters did not differ significantly: Duncan’s multiple Range test $P > 0.05$

**Citrus leafminer parasitoids**

In 2002, the activity of Eulophidae parasites (*S. petiolatus* and *C. phyllocnistoides*) was very intense. A peak of 90 adults of *C. phyllocnistoides* was detected in August.
In 2003 and 2004, the activity of parasitoids dramatically decreased, but *Citrostichus phyllocnistoides* remained the most represented species (Fig.3).

**Discussion**

Pesticides can be considered compatible for IPM programs if they are effective in suppressing the target pest and preventing its resurgence and outbreaks of secondary pests, selective for natural enemies and non phytotoxic for sprayed trees (Villanueva-Jiménez *et al.*, 2000). Narrow-Range oil and azadirachtin are considered effective in suppressing *P. citrella* populations (Beattie *et al.*, 2002; Immaraju J.A., 1998; Knapp *et al.*, 1995) and selective to many natural enemies of *P. citrella* as *Ageniaspis citricola* Logvinovskaya (Villanueva-Jiménez & Hoy, 1998a). In Italy, some concerns are reported about the negative impact of azadirachtin A on the vedalia beetle [*Rodolia cardinalis* (Mulsant)] (Bernardo & Viggiani, 2002), and Eulophids parasitoids (Conti *et al.*, 2004).

The large-scale experimental trials conducted in Sicilian citrus nurseries during 2002-04 confirmed that NR oils and azadirachtin have potential in controlling *P. citrella* population, but their efficacy is significantly lower in summer season when temperature can drastically increase and non-synthetic chemicals are rapidly dissipated.

Among the different citrus species, lemon trees resulted more susceptible to leafminer infestation than kumquat and orange, while lime and mandarin were modestly infested. Further, kumquat and lime resulted in a progressive reduction of percentage of trees with new flushes. Only kumquat was affected in trunk diameter growth. Damage to tree appearance caused by *P. citrella* in 2002 was significantly higher on lemon.

Some impact was evidenced on Eulophid populations but anyway the presence of parasitoids was allowed in the sprayed plots.

These field experiments confirm that biorational pesticide applications are compatible with IPM-program and tree growth of mandarin and lime in nursery cultivation. Orange and kumquat are more difficult to submit to frequent application of these compounds. For lemon, the exclusive or frequent use of biorational pesticides should be avoided.
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Survey of the Ants (Hymenoptera: Formicidae) in soil of citrus orchards in Spain

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A survey of the ants (Hymenoptera: Formicidae) was conducted in four citrus orchards with different types of crop management in the province of Valencia, Spain. The sampling period was extended from August 2003 to December 2004. A total of 12 pitfall traps were distributed per orchard, and they were changed around every 14 days. A total of 55,384 individuals of 13 species belonging to 12 different genera were collected. *Lasius grandis* Forel was the most abundant species with 21,472 individuals collected. *Pheidole pallidula* (Nylander) and *Linepithema humile* (Mayr.) with 16,545 and 11,275 individuals respectively, were the two following species in order of abundance-activity. These 3 species represented 89% of the total collected individuals. Data on abundance, biology, ecology and taxonomic keys for the species collected are also reported.
Thrips (Thysanoptera) associated to lemon orchards in the Oeste region of Portugal

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Abstract: Thrips were surveyed in three lemon orchards from Mafra, in the Oeste region of Portugal, each with three different weed management systems, i.e., ground cover with resident vegetation; ground cover with sowed selected plant species; and herbicide application. A total of 42 samples were collected, in 2002 and 2003, using two sampling techniques, i.e., suction and beating. Most of the collected thrips were Terebrantia (Aeolothripidae, Thripidae and Adiheterothripidae). The most abundant genera were Pezothrips (44%), Aeolothrips (30%) and Thrips (14%). Other identified genera include by decreasing order of abundance Neohydatothrips, Melanthrips, Anaphothrips, Limothrips, Frankliniella, Rhipidothrips, Chirothrips, Heliothrips, Isoneurothrips, Scirtothrips and Holarthrothrips. Four thrips species are reported for the first time from Portugal and 10 species are first records on citrus in the country, including Pezothrips kellyanus. Thrips diversity was apparently higher in ground cover modalities in comparison to the herbicide.

Key-words: thrips, Pezothrips kellyanus, predators, citrus

Introduction

At least about 40 thrips species are recorded from citrus in the world, including phytophagous and predators (Longo, 1986). In the case of phytophagous species, flowers are often favoured feeding sites, but thrips also feed on developing fruits and leaves. As a result of feeding activity citrus injury may occur, namely stem-end scarring, ring scarring and scurfing of young fruits, rind discolouration of mature fruits and rust marks between touching fruits, depending on thrips species (Smith et al. 1997; Gilbert & Bedford, 1998). Thrips injuries have been confused with fruit blemishes originated by other citrus pests and wind (Bedford, 1998), which may affect the actual perception of the importance of thrips as citrus pests.

Thrips are usually considered minor or occasional citrus pest in most of the citrus producing areas in the world (Longo, 1986; Katsoyannos, 1996; Smith et al., 1997), including Portugal (Carvalho et al., 1997). However, some species may attain major pest status in certain regions, e.g., Scirtothrips aurantii in South Africa (Gilbert & Bedford, 1998) and S. citri in California (Flint, 1991). Recently, Pezothrips kellyanus has emerged as a key thrips species of citrus in Southern Italy (Conti et al., 2003).

The economic importance of citrus thrips is mostly related with the cosmetic damage they originate on fruits, i.e., fruit discoloration and scarring, with a consequent downgrading for both export and domestic markets (Arpalia & Morse, 1991; Grout & Stephen, 1997; Froud et al., 2001; Conti et al., 2003).
Information on thrips as citrus pests in Portugal was published by Carvalho & Aguiar (1997), Carvalho et al. (1997), Cavaco et al. (2002) and Ramos & Fernandes (2002). However, thrips are still poorly studied in citrus agroecosystems. There is lack of knowledge on species composition of thrips fauna associated to citrus orchards and thrips importance as citrus pests.

In this paper we present the results of a survey carried out in 2002 and 2003 in three lemon orchards, from the Oeste region of Portugal, based on samples collected in an experiment aimed at studying the influence of weed management systems in the abundance and diversity of beneficial arthropods (Franco et al., 2005).

**Material and methods**

**Experimental plots**
The study was carried out in three 1-4 ha lemon orchards from Mafra, in the Oeste region of Portugal, ca. 30 Km north of Lisbon, namely Carrasqueira de Cima (CC), Casal Mato de Cima (CMC) and Pinhal de Frades (PF). During the study, farmers followed an IPM system under the technical supervision of Frutoeste, a farmers association of Oeste region.

**Ground cover management**
In each orchard, three weed management systems were installed in 2002: 1) inter-row ground cover with resident vegetation (RV), 2) inter-row ground cover by sowing selected species (S) and 3) inter-row herbicide application, i.e., diuron+glyphosate+terbutilazine (H). The sown species were *Lolium multiflorum*, *L. perenne*, *Medicago polymorpha*, *Trifolium fragiferum*, *T. incarnatum* and *T. resupinatum*. Each management system was installed in plots of five or six tree rows.

**Sampling methods**
A total of 42 samples were collected in lemon trees from July up to December in 2002 and from March up to December in 2003, using two sampling techniques, i.e., beating and suction. Beating samples were the result of sampling two branches per tree in 25 trees per modality and orchard. Suction samples were obtained using the “Vortis” suction sampler (Arnold,1994), following a sampling procedure previously defined (Rodrigues et al., 2003). Each sampling unit consisted of suctioning the foliage with a 8 cm diameter flexible tube (estimated airflow = 34,8 m/s) in three different positions, during four seconds per position. Ten sampling units were collected per modality and orchard.

Samples were studied in the laboratory under magnification for the separation of thrips, which were kept in 70% ethyl alcohol for posterior identification. All collected specimens were identified to genus level and specimens samples of Terebrantia were identified to species level.

**Results and discussion**
A total of 1397 thrips were collected, mostly Terebrantia (99%), including 19 species, 14 genera and three families (Figure 1, Table 1). Almost 90% of the specimens belong to the three most abundant genera, i.e., *Pezothrips* (44%), *Aeolothrips* (30%) and *Thrips* (14%) (Figure 1).

Four thrips species, *Neohydatothrips gracilicornis*, *Pezothrips kellyanus*, *Rhipidothrips brunneus* and *Holarthrothrips tenuicornis*, are reported for the first time from Portugal and 10 species, *Aeolothrips collaris*, *Melanthrips fuscus*, *Anaphothrips obscurus*, *Chirothrips aculeatus*, *Chirothrips manicatus*, *Frankliniella tenuicornis*, *Limothrips cerealium*, *Scirtothrips inermis*, *Thrips angusticeps*, *Thrips atratus* are first records on citrus in Portugal.
(Table 1). Therefore, the number of thrips species recorded from citrus in Portugal is updated to 19.

![Graph showing relative abundance of thrips collected in three studied lemon orchards from Mafra, in the Oeste region of Portugal.]

Figure 1. Relative abundance of thrips collected in the three studied lemon orchards from Mafra, in the Oeste region of Portugal.

Similarly to the results reported by Conti et al. (2003), from a survey carried out in Sicilian citrus orchards, *P. kellyanus* seems to be the dominant thrips species in the studied lemon orchards from Mafra, in the Oeste region of Portugal, considering that most of the collected *Pezothrips* specimens probably belong to this species (Figure 1 and Table 1). This is a known noxious species of citrus crops in other countries, e.g., Italy, Australia and New Zealand (Conti et al., 2003).

Most of the temperate area species of *Aelothrips* are flower living facultative predators. *Melanthrips* include flower feeding temperate species. Most of the Thripidae are phytophagous on higher plants (Hoddle et al., 2004). However, a few species are obligate predators, e.g., *Scolothrips sexmaculatus* is a known predator of the two-spotted mite, *Tetranychus urticae*, on citrus in Australia (Smith et al., 1997), and some polyphagous pest thrips may behave as facultative predators, e.g., *Frankliniella occidentalis* (Hoddle et al., 2004) and *T. tabaci* (Milne & Walter 1998).

The thrips captures, both in global terms and in the case of *Pezothrips*, the dominant genus, were mainly concentrated between May and September in correlation with the three main flowering periods of lemon in the region (Figure 2 and 3).

No apparent and consistent relationship was observed between thrips abundance and weed management systems (Table 2). However, the number of identified genera of thrips was consistently higher in both ground cover modalities, i.e., 10-13 genera in “S” and 8-10 genera in “RV”, in comparison to “H” (5-8 genera), in all there lemon orchards. This apparently higher thrips diversity is related with the higher plant diversity observed in the “S” and “RV” modalities of ground cover (Sousa et al., 2005).
Table 1. Thrips (Terebrantia) species identified from samples collected by suction and beating in the three studied lemon orchards from Mafra, in the Oeste region of Portugal.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeolothripidae</td>
<td><em>Aeolothrips collaris</em> Priesner *</td>
</tr>
<tr>
<td></td>
<td><em>Melanthuris fuscus</em> Sulzer *</td>
</tr>
<tr>
<td></td>
<td><em>Rhipidothrips brunneus</em> Williams</td>
</tr>
<tr>
<td>Thripidae</td>
<td><em>Anaphothrips obscurus</em> Muller*</td>
</tr>
<tr>
<td></td>
<td><em>Chirothrips aculeatus</em> Bagnall *</td>
</tr>
<tr>
<td></td>
<td><em>Frankliniella occidentalis</em> Pergande**</td>
</tr>
<tr>
<td></td>
<td><em>Frankliniella tenuicornis</em> Uzel*</td>
</tr>
<tr>
<td></td>
<td><em>Heliothrips haemorrhoidalis</em> Bouché**</td>
</tr>
<tr>
<td></td>
<td><em>Isoneurothrips australis</em> Bagnall**</td>
</tr>
<tr>
<td></td>
<td><em>Limothrips cerealium</em> Haliday*</td>
</tr>
<tr>
<td></td>
<td><em>Neo hydatothrips gracilicornis</em> Will.</td>
</tr>
<tr>
<td></td>
<td><em>Pezothrips kellyanus</em> Bagnall</td>
</tr>
<tr>
<td></td>
<td><em>Scirtothrips inermis</em> Priesner*</td>
</tr>
<tr>
<td></td>
<td><em>Thrips angusticeps</em> Uzel*</td>
</tr>
<tr>
<td></td>
<td><em>Thrips atratus</em> Haliday*</td>
</tr>
<tr>
<td></td>
<td><em>Thrips flavus</em> Schrank**</td>
</tr>
<tr>
<td></td>
<td><em>Thrips major</em> Uzel**</td>
</tr>
<tr>
<td></td>
<td><em>Thrips tabaci</em> Lindeman**</td>
</tr>
<tr>
<td>Adiheterothripidae</td>
<td><em>Holarthrothrips tenuicornis</em> Bagnall</td>
</tr>
</tbody>
</table>

* species reported from Portugal according to zur Strassen (2005), Guimarães (1980) and Guimarães & Lopes (1991); ** species reported from citrus, in Portugal, according to Ramos & Fernandes (2002)

Figure 2. Trend of thrips captures in the three studied lemon orchards, in 2002 and 2003, by beating (B) and suction (S) sampling.
Figure 3. Trend of *Pezothrips* captures in the three studied lemon orchards, in 2002 and 2003, by beating (B) and suction (S) sampling.

Table 2. Number of thrips collected, by beating (B) and suction (S) sampling, in the three studied lemon orchards from Mafra, in the Oeste region of Portugal, in 2002 and 2003, in function of the weed management system, i.e., ground cover with resident vegetation (RV), ground cover by sowing selected species (S) and herbicide application (H), and excluding *Tubulifera*.

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeolothrips</em></td>
<td>9</td>
<td>111</td>
<td>64</td>
<td>5</td>
<td>79</td>
<td>80</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td><em>Anaphothrips</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Chirothrips</em></td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td><em>Frankliniella</em></td>
<td></td>
<td></td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Heliophylops</em></td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>Holarthrothrips</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Isoneurothrips</em></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Limothrips</em></td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Melanthrips</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>Neohydatothrips</em></td>
<td>5</td>
<td>16</td>
<td>4</td>
<td>14</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td><em>Pezothrips</em></td>
<td>16</td>
<td>126</td>
<td>96</td>
<td>27</td>
<td>48</td>
<td>43</td>
<td>12</td>
<td>79</td>
</tr>
<tr>
<td><em>Rhipidothrips</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>Scirtothrips</em></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thrips</em></td>
<td>21</td>
<td>53</td>
<td>3</td>
<td>17</td>
<td>13</td>
<td>21</td>
<td>14</td>
<td>37</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>51</td>
<td>310</td>
<td>172</td>
<td>78</td>
<td>160</td>
<td>182</td>
<td>36</td>
<td>155</td>
</tr>
</tbody>
</table>
Acknowledgements

We thank Elsa Borges da Silva, Manuel Cariano, Ana Baptista, Ana Passarinho, Pedro Rodrigues and Nuno Grade for the support in field and laboratory work. Thanks are due to the lemon growers Virginia Duarte, Carlos Batalha and Rosa Gomes that allowed us to carry out the experiment in their orchards. We thank also our project partners Rosário Antunes and Luis Aniceto (Frutoeste) for the logistic support. The research was part of the Project nº 29 PO AGRO Medida 8.1. DE&D.

References

Study on Acari fauna of citrus orchards in southern Iran

Mohammad Khanjani and Majid Mirab Balou
Department of Plant Protection, Faculty of Agriculture, Bu-Ali Sina University, Hamadan, Iran, Khanjani@basu.ac.ir

There are 8821 hectares of citrus orchards in southern Iran (Khuzestan provinces) with fruit yield of 12.1 t/ha. Since citrus trees are evergreen they need more care throughout the year. Some mites are major citrus pests in this area. Amongst them *Eotetranychus orientalis* (Klein) is the dominant phytophagous species. Its damage on leaf cause necrotic spots and finally infested leaves turn brown and fall resulting in weakness of tree. In this study in addition to this mite, we have collected some predatory mites:

**Laelapidae**

*Hypoaspis* (*H. p.* *Hypoaspis* polyphyllae* Khanjani & Ueckermann

*Hypoaspis* (*H.* *H. aculifer*

**Phytoseiidae**

*Kuzinellus kuzini* Wainstein

*Paraseilus jirofticus* Daneshvar

*Typhlodromus* (*A.* *kettanehi* *Dosse*)

**Anystidae**

*Anystis baccarum* (*L.*)

**Parasitidae**

*Parasitus consanguineus*

**Ascidae**

*Laseius youcefi* McGroger

*Proctolaelas* sp.

**Tydeidae**

*Tydeus caryae* Khanjani & Ueckermann

*Typhlodromus* (*A.* *kettanehi* *Dosse*) was the dominant species in this area. Its natural efficiency was about 20%.
Population dynamics and specific composition of phytoseiid mites (Parasitiformes, Phytoseiidae) associated with lemon trees in three differently managed orchards in eastern Sicily

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Abstract: Surveys of phytoseiid mites were carried out in three lemon orchards in Sicily. The three orchards had different farm management and different environmental conditions: A) traditionally farm managed; B) organic farm managed; C) in state of neglect. The aim was to determine the specific composition of the above different types of orchards and to compare their population dynamics in order to obtain information on the influence of the different agricultural practices on the phytoseiid fauna. After two years of weekly samplings it can be assessed that no substantial differences were found in the phytoseiid specific composition among the three types of orchards. On the other hand, differences, sometimes remarkable ones, were found as far as concerns the role of the various species in the three orchards and in the various seasons. *Iphiseius degenerans* Berlese, *Amblyseius andersoni* (Chant) and *Euseius stipulatus* (Athias-Henriot) were present in the three orchards, *Typhlodromus cryptus* Athias-Henriot was found in orchards A and B, while *Cydnodromus californicus* (McGregor) was found only in orchard C. In orchard A the dominant species was *A. andersoni* (48%), followed by *I. degenerans* (44%) and *E. stipulatus* (7%). The first and the last species were present exclusively in the winter-spring period, while *I. degenerans* was the only species present in summer. On the contrary, in orchard B, the dominant species was *I. degenerans* (69%), present in all seasons, followed by *A. andersoni* and *E. stipulatus* (16 and 14% respectively). These latter ones were found exclusively in spring. In orchard C the dominant species was *E. stipulatus* (58%) mainly present in the hot seasons, followed by *I. degenerans* (38%) and *A. andersoni* (3%).

Key-words: Phytoseiidae, Citrus, traditional farm management, organic farm management

Introduction

In the second half of 20th century many scientists have dealt with phytoseiid mites because of their importance as biological control agents of phytophagous mites (Ragusa and Swirski 1975; De Moraes and McMurtry 1981; Janssen and Sabelis 1992; Argov et al., 2002). Studies carried out on *Citrus* showed that the rich phytoseiid fauna associated with this crop, brings to an effective control of tetranychid mites especially when no chemicals are applied (Ragusa, 1986). About twenty species of phytoseiids associated with *Citrus* are reported and they are mainly generic facultative predators (Ragusa, 1986; Papaioannou-Souliotis et al., 1994; Argov et al. 2002). Among these, *Euseius stipulatus* (Athias-Henriot) is the predominant one, in the mid-and-western Mediterranean basin (McMurtry, 1977; Garcia-Mari et al., 1984; Ragusa, 1986); however the specific composition and the role of the
different species inside the community may depend on both the environmental conditions (Tsolakis et al., 2000; Argov et al. 2002) and some agricultural practices (Marzâl et al., 1987). In this work we compare three lemon orchards, differently farm managed and at different environmental conditions, in Sicily.

The aim of the present paper is to determine the specific composition of each type of orchards and to compare its population dynamics in order to obtain information on the influence of the different farm managements on the phytoseiid fauna.

Materials and methods

Observations were carried out from April 16, 2003 to April 13, 2005 in a traditionally farm managed lemon orchard (A), in an organic farm managed lemon orchard (B) located in the province of Syracuse (South East of Sicily), and in a neglected lemon orchard (C) in the province of Messina (North East of Sicily). In the latter field samplings were started three months later, because of technical problems.

Surveys were carried out weekly on 9 plants (3 randomized blocks of 3 plant each) using the shaking method (Tsolakis & Ragusa, 1999). Phytoseiid mites, fallen on a black plastic table, were counted and thrown back on the plants, to prevent the fauna reduction. From the 4th plant of each block, mites were collected twice a month using a small mouth aspirator. Specimens were preserved in alcohol 70%, cleared in Nesbitt solution, mounted in Hoyer solution and observed under an interference microscope to identify the species and to ascertain the presence of different young stages.

In orchard A two sprayings were carried out during the observation period: the first on June 25, 2003 with Fenbutatin-oxide, to control a *Tetranychus urticae* Koch infestation, and the other on January 14, 2004 with mineral oil to control scales. In orchard B only one spraying was carried out on July 2, 2003 with mineral oil to control thrips, while no sprayings were carried out in orchard C.

Temperatures and relative humidity were recorded 24 hours per day using a mechanic thermohygrograph located in a meteorological box. As orchards A and B were only 300 m far one from the other, a single thermohygrograph was used for both fields. Rainfalls were also recorded 24 hours per day, using a pluviograph in each site.

The results obtained were statistically analyzed by Student *t* test and Analysis of Variance (ANOVA) followed by Tukey’s test when the ANOVA was significant (*P* < 0.05). The software “Statistica” from StatSoft Inc. was used.

Results

Four species of phytoseiid mites constitute the phytoseiid fauna in each orchard. Three of them, *Amblyseius andersoni* (Chant), *Iphiseius degenerans* Berlese and *Euseius stipulatus* (Athias-Henriot) were common in the three orchards, while *Cydnodromus californicus* (McGregor) replaced *Typhlodromus cryptus* Athias-Henriot in orchard C (table 1). In the latter, *E. stipulatus* was the most abundant species followed by *I. degenerans*. In orchard A the most abundant species was *A. andersoni* followed by *I. degenerans*, while the opposite happened in orchard B. The gap among these species was small in orchard A (4.1%) while it was greater in orchard B (53.8%) (table 1).

The trend of temperatures and of relative humidity are reported in figure 1. No statistical differences were found among the two sites if mean temperatures and relative humidity are considered (*F*=1.68; *df*=1, 63; *P*=0.19).
However, as we can see in figure 1, the trend of maximum and minimum values of temperatures and the relative humidity is different in the two fields. As a matter of fact, the gap of minimum-maximum temperatures and RH values is statistically different between the two sites ($F=54.66; df=1, 203; P=0.00$).

Table 1. Phytoseiid species and their frequency in the three orchards

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Frequency</th>
<th>%</th>
<th>Orchard</th>
<th>Frequency</th>
<th>%</th>
<th>Orchard</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. andersoni</td>
<td>47.9</td>
<td></td>
<td>I. degenerans</td>
<td>69.3</td>
<td></td>
<td>E. stipulatus</td>
<td>58.4</td>
<td></td>
</tr>
<tr>
<td>I. degenerans</td>
<td>43.8</td>
<td></td>
<td>A. andersoni</td>
<td>15.5</td>
<td></td>
<td>I. degenerans</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>E. stipulatus</td>
<td>7.3</td>
<td></td>
<td>E. stipulatus</td>
<td>14.4</td>
<td></td>
<td>A. andersoni</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>T. cryptus</td>
<td>1.0</td>
<td></td>
<td>T. cryptus</td>
<td>0.8</td>
<td></td>
<td>C. californicus</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

As far as the fluctuation of the predators’ population on the whole is concerned, a drastic reduction occurred after the 3rd decade of May 2003 in both orchard A and B (Fig. 2).

As far as the fluctuation of the predators’ population on the whole is concerned, a drastic reduction occurred after the 3rd decade of May 2003 in both orchard A and B (Fig. 2).

![Figure 1. Trend of temperatures (°C) and Relative Humidity (%) in the two areas.](image)

Afterwards, because of summer adverse climatic conditions (maximum temperatures up to 35°C and RH below 50%), phytoseiid fauna was maintained at a low level until October 2003 (Figs. 1, 2). It should be mentioned that the phytoseiid population was statistically lower in orchard A during the 2nd year, while a higher presence was recorded in orchard B in spring 2004.
No statistical differences were found from the quantitative point of view among the phytoseiid population in orchard B and C during the observation period.

On the other hand, as far as the specific composition of phytoseiid population is concerned, strong differences among the three orchards were found (Fig. 3 orchards A-C).

In orchard A, about 92% of phytoseiid population is represented by *A. andersoni* (47.8%) and *I. degenerans* (43.8%) but, as it can be noted in figure 3 (orchard A), the two species were present in the field in different seasons. *I. degenerans* was present mainly in late spring and summer, while *A. andersoni* was more frequent during autumn and winter. This preference of *A. andersoni* for cooler seasons is confirmed by data reported from the other two orchards, where it was found only during late winter and spring (Fig. 3 orchard B and C). On the other hand, the dominant species were *E. stipulatus* and *A. andersoni* in orchard B, during spring 2003, but these species were replaced by *I. degenerans* at the beginning of summer. The latter species became the dominant one during the remaining observation period, even if a remarkable presence of *E. stipulatus* and *A. andersoni* was noted during springtime 2004. In orchard C, *I. degenerans* was present during all year round, but its population was dominated by the more frequent *E. stipulatus* present in the same season. On the other hand in orchards A and B the latter was found mainly during spring and the beginning of summer (Fig. 3 orchards A and B).

**Discussion**

Differences between phytoseiid faunas found in eastern Mediterranean countries (i.e. Israel and Egypt) and the mid regions (i.e. Greece and Italy (Sicily)) and western regions (i.e. Spain) were reported in literature. From 9 to 16 phytoseiid species were found in these regions associated with *Citrus*, but only 2-3 of them were frequent.
Figure 3. Frequency of the various species found in the three orchards.
E. stipulatus is considered the most common species in all mid and western Mediterranean regions followed by A. andersoni, Euseius finlandicus (Oudemans) and I. degenerans in Greece, Typhlodromus exhilaratus Ragusa, Typhlodromus rhenanoides Athias-Henriot and Typhlodromus athenas Swirski & Ragusa in Sicily, Typhlodromus phialatus Athias-Henriot Cydnodromus californicus (McGregor) and T. rhenanoides in Spain. On the other hand Typhlodromus athiasae Porath and Swirski, Amblyseius swirskii Athias-Henriot and I. degenerans are considered the main predators on Citrus in Israel (Porath and Swirski, 1965; Ferragut et al., 1983; Ragusa, 1986; Papaioannou-Souliotis et al., 1994; Swirski et al., 1998; Argov et al., 2002).

A. andersoni and I. degenerans were reported as rare species for Sicilian Citrus orchards (Ragusa, 1986) and as occasional ones in Greece (Papaioannou-Souliotis et al., 1994) and in Israel (only I. degenerans) (Porath and Swirski, 1965). According to Gerson and Vacante (1993) the differences among the phytoseiid fauna present in the various Mediterranean regions might be ascribed to the sensitivity of phytoseiids to their environment. On the other hand, Argov et al. (2002) report that one of the reasons why E. stipulatus did not establish in Israel might be the competition with the local dominant A. swirskii. As a matter of fact, our results suggest that macroclimatic conditions may affect the composition of phytoseiid fauna on the whole, but that microclimatic conditions, cultural practices, the availability of food, could play a primary role on the ecological importance of each species inside the community. However, it should be said that some factors influencing the phytoseiid population are very difficult to explain. Thus, the absolute dominance of A. andersoni during autumn-winter 2003 and spring 2004, could lead to think that the cooler microclimate of orchard A favoured this species rather than I. degenerans, present all year around in orchard B, even if no climatic differences are noted between these orchards as only 300 m separate the two fields. On the other hand the crash of phytoseiid population at the end of May, cannot be related to the climatic conditions, as no rainfalls were recorded and temperatures of 24-26°C and relative humidity of 50-60% are not considered dangerous for phytoseiids. This reduction cannot be related to the agricultural practices either, as pesticides were applied one month later (July 2, and July 9, 2003 for acaricide and mineral oil respectively) (Figs 1, 2). However, the acaricidal spraying might have had an influence on the succeeding dynamics of species inside the community. Infact, even if fenbutatin oxide is considered slightly harmful to some phytoseiid mites (Reis & Sousa, 2001), it might have had a more negative impact on I. degenerans than on A. andersoni as various phytoseiid species respond to pesticides in a different way. It is also probable that the above mentioned cases could be ascribed to the availability of food or, considering the size of mites, to the microclimatic conditions created by the different canopy management.

On the other hand, the dominance of E. stipulatus in orchard C seems to be related more to the different microclimatic conditions recorded (Fig. 1) than to the absence of agricultural practices. In Spain this species presents two reproductive periods (spring and autumn) and reaches the pick in December while it is rare in summer (Garcia-Mari et al., 1984). On the other hand, Ragusa (1986) reports that the summer collapses of phytoseiid population, in particular of E. stipulatus, were caused by the hot wind “scirocco” blowing from Africa, especially when the peak of temperature is maintained for some hours.

The massive presence of I. degenerans in Sicilian citrus groves in the past years might also be related to the infestations of Pezothrips kellyanus (Bagnall), as this phytoseiid is considered a voracious predator of various thrips (van Rijn & Tanigoshi, 1999; Grafton-Cardwell et al., 1999; Faraji et al. 2002), and its population increases in the same period of P. kellyanus infestations (Conti et al., 2003). The latter Authors cannot find a positive
relationship between phytoseiid fauna (mainly *I. degenerans*) and the scar ranking but no investigations were carried out to relate the phytoseid populations to *P. kellyanus* populations. Further field and laboratory investigations are needed, to clarify the latter aspect in Sicilian citrus orchards.

**Acknowledgements**

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Sampling plans for *Tetranychus urticae* (Acari: Tetranychidae) for IPM decisions on Clementines in Spain

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*Tetranychus urticae* is a major pest in citrus. Clementine is the most affected citrus variety, where this mite can result in serious defoliation and fruit scarring. IPM requires pesticide application decisions to be based on estimations of mite density beyond the economic threshold. Several commercial Clementine groves in the provinces of Castelló and Tarragona (NE Spain) were sampled from 2001 to 2003. A total of 343 samples including random outer leaves, symptomatic leaves and fruit were taken. Because *T. urticae* showed an aggregated pattern of distribution both within and between-trees, betapic sampling methods have been developed. These consist of a random choosing of both the primary units (trees in the orchard), and the secondary units (leaves or fruit in each tree). Furthermore, based on the precise relationships found between the different variables considered, sampling plans that combine the observation of symptomatic leaves in a ring 0.25 m² in diameter and population density on leaves have been developed.
Efficacy assay of different phytosanitary chemicals for the control of *Eutetranychus orientalis* (Klein) (Oriental Spider Mite) on Fine lemon and Valencia-Late orange crops

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**Abstract:** The Oriental Spider-Mite (*Eutetranychus orientalis*) is one of the most important citrus pests due to its great colonisation ability. Several phytosanitary treatments per year are usually required for its control. In this work we test the efficacy of several chemicals for controlling *Eutetranychus orientalis*. We carried out two assays between September and November 2004, one of them on a Fine lemon crop and the other on a Valencia-Late orange crop. We used the following substances: Dicofol 48%, Propargite 57%, Hexitiazox 10%, Etoxazol 11% and Fenpiroximate 5%, at the commercial doses, and also considered a non-treated area. Each assay was repeated three times, each of them with three trees per product, and we weekly revised six leaves per tree at random to evaluate the compared responses of the Oriental Spider-Mite to the different theses. All the substances tested produced an effective control on *Eutetranychus orientalis*. Nevertheless, the compared efficacy showed differences as the assay progressed: Dicofol 48% and Fenpiroximate 5% caused an early and persistent decrease by nearly 100%. Hexitiazox 10% and Etoxazol 10% showed a 95% efficacy but it was observed three weeks after the treatment. Propargite 57% showed a good early impact, but its efficacy finally decreased.

**Key words:** *Eutetranychus orientalis*, spider-mites, chemical control, lemon, orange

**Introduction**

The origin of the Oriental Spider-Mite (*Eutetranychus orientalis*) is the Middle East and at present it is extended to the citrus crops of Africa, Southern and Eastern Asia and Australia (Bodenheimer, 1951; Jeepson, 1989 and Walter et al., 1995). In Spain, *E. orientalis* was observed for the first in Malaga province, in 2001, from where it extended towards nearby areas (García et al., 2003). The primary host of *E. orientalis* is *Citrus* spp., although it can cause damages to more than 50 plant species. It mainly colonizes the upper leaf surface, and those areas around the midribs. Its presence can be detected by discoloration of the leaves and pale-yellow streaks along the midribs and veins. It is one of the most important citrus pests due to its great colonisation ability. Several phytosanitary treatments per year are usually required for its control. In this work, we test the efficacy of several chemicals for controlling *E. orientalis*.

**Material and methods**

We carried out two assays in Malaga province (Spain) between September and November 2004, one of them on a Fine lemon crop and the other on a Valencia-Late orange crop. We used four substances in each assay. Dicofol 48% (Kelthane, Aventis), Propargite 57% (Omite New, Kenogad), and Hexitiazox 10% (Zeldox, Syngenta Agro) were used in both assays. Etoxazol 11% (Borneo, Kenogad) was used on Fine lemon trees, and Fenpiroximate 5%
(Flash, Sipcam Inagra) was used on Valencia-Late orange trees. All of the acaricides were used at the maximum commercially recommended doses (see Table 1).

### Table 1. Chemicals tested in the assays for the control of *E. orientalis*.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Effect type</th>
<th>Vulnerable stage</th>
<th>Security term (days)</th>
<th>Dosage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicofol 48% (T1) Kelthane (Aventis)</td>
<td>Contact</td>
<td>Mobile stages</td>
<td>28</td>
<td>0.15</td>
</tr>
<tr>
<td>Propargite 57% (T2) Omite New (Kenogad)</td>
<td>Contact and ingestion</td>
<td>Larvae and adults</td>
<td>14</td>
<td>0.05</td>
</tr>
<tr>
<td>Hexitiazox 10% (T3) Zeldox (Syngenta Agro)</td>
<td>Contact</td>
<td>Eggs and larvae</td>
<td>14</td>
<td>0.015</td>
</tr>
<tr>
<td>Etoxazol 11% (T4) Borneo (Kenogad)</td>
<td>Contact and ingestion</td>
<td>Eggs and larvae</td>
<td>14</td>
<td>0.2</td>
</tr>
<tr>
<td>Fenpiroximate 5% (T4) Flash (Sipcam Inagra)</td>
<td>Contact and ingestion</td>
<td>Larvae, nymphs and adults</td>
<td>14</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Each assay consisted of three blocks, each of them with three trees per acaricide. We weekly revised six leaves per tree at random to evaluate the compared responses of the Oriental Spider-Mite to the different theses. We also considered three non-treated trees per block as a control. The number of sampling days was one before the treatment application, and three after it.

The statistical processing was made using variance analyses (ANOVA) to detect possible differences among phytosanitary theses related with the *E. orientalis* living individual numbers per leaf. Once statistical differences were detected, we used the LSD and Scheffé mean comparison tests to define the pair-wise substance differences in control efficacy. If differences among trees existed previous to the treatment applications, then we included the initial data as a covariable in the ANOVA, to avoid bias not attributable to the nature of the acaricides. In such case, the *E. orientalis* individual numbers after the treatments was expressed as the adjusted mean, and the mean comparison tests were applied on the residuals coming from the consideration of the covariable.

### Results and discussion

**The assay in the Valencia-Late orange crop**

When the treatment was made (September 20), the percentage of affected leaves was 91.85%, and the mean number of mites per leaf was 11.35. We show the progression of the mean number of individuals per leaf along the study period in Figure 1.

We detected differences in infestation among trees assigned to different acaricides previous to the treatment (F=5.36, p<0.001). Differences were also detected in the latter sampling sessions, even after the initial data set had been considered as a covariable (F=28.28, p<0.001; F=54.35, p<0.001 y F=39.47, p<0.001, respectively).
The mean comparison tests showed that the significant differences found after the treatments pointed at a significantly higher mean mite number in the leaves treated with any of the chemicals tested with respect to the leaves of non-treated trees. In Table 2 we show the results of the mean comparison tests applied to the pair-wise comparison between chemicals, when we exclude the data of non-treated trees from the analysis.

Table 2. Pair-wise mean comparison tests. In bold are the differences according to the LSD and Scheffé tests; in normal are the differences according to the LSD test

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>28/9/04 Adjusted mean</th>
<th>Significant difference with:</th>
<th>5/10/04 Adjusted mean</th>
<th>Significant difference with:</th>
<th>13/10/04 Adjusted mean</th>
<th>Significant difference with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicofol (T1)</td>
<td>0</td>
<td></td>
<td>0.235</td>
<td></td>
<td>0.094</td>
<td></td>
</tr>
<tr>
<td>Propargite (T2)</td>
<td>0.249</td>
<td></td>
<td>1.347</td>
<td>T1</td>
<td>2.625</td>
<td>T1,T4</td>
</tr>
<tr>
<td>Hexitiazox (T3)</td>
<td>3.244</td>
<td>T1,T2</td>
<td>1.689</td>
<td>T1,T2,T4</td>
<td>1.148</td>
<td>T1</td>
</tr>
<tr>
<td>Etoxazol (T4)</td>
<td>3.489</td>
<td>T1,T2</td>
<td>0.262</td>
<td>T1</td>
<td>0.442</td>
<td></td>
</tr>
</tbody>
</table>

One week after the treatment, Dicofol 48% and Propargite 57% showed a significantly lower mean mite number per leaf with respect to Hexitiazox 10% and Etoxazol 11%. After the second week, leaves treated with Dicofol 48% continued showing the lowest damage levels, but this time followed by Etoxazol 11%. Both substances showed significant differences with respect to Propargite 57% and Hexitiazox 10%.

**The assay in the Fine lemon crop**
Before the treatment (October 25), the mean damage levels in the Fine lemon crop were 90% of the leaves with *E. orientalis* individuals and 42.02 individuals per leaf. Figure 2 shows the progression of the mean individual number along the study period.
As in the assay performed on a Valencia-Late orange crop, we detected initial significant differences in the damage levels (F=4.59, p<0.01) which had to be considered as covariables in the latter analyses. Also as in the orange crop, differences among *E. orientalis* occupation due to the effect of treatments were observed (F=10.35, p<0.001; F=57.30, p<0.001 and F=37.96, p<0.001 in the three weeks after the treatment, respectively). The mean comparison tests showed that these differences were especially significant in the comparison between the leaves submitted to any chemical and the non treated leaves. Table 3 shows the pair-wise comparison between the treatments, after excluding the data of non-treated trees from the analysis.

Table 3. Pair-wise mean comparison tests. In bold are the differences according to the LSD and Scheffé tests; in normal are the differences according to the LSD test.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>2/11/04 Adjusted mean</th>
<th>Significant difference with:</th>
<th>8/11/04 Adjusted mean</th>
<th>Significant difference with:</th>
<th>15/11/04 Adjusted mean</th>
<th>Significant difference with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicofol (T1)</td>
<td>0.912</td>
<td></td>
<td>1.071</td>
<td></td>
<td>0.291</td>
<td></td>
</tr>
<tr>
<td>Propargite (T2)</td>
<td>5.890</td>
<td>T1,T4</td>
<td>2.296</td>
<td>T3,T4</td>
<td>9.474</td>
<td>T1,T3,T4</td>
</tr>
<tr>
<td>Hexitiazox (T3)</td>
<td>7.583</td>
<td>T1,T4</td>
<td>0</td>
<td></td>
<td>1.163</td>
<td></td>
</tr>
<tr>
<td>Fenpiroximate (T4)</td>
<td>0</td>
<td>T1,T4</td>
<td>0</td>
<td></td>
<td>0.951</td>
<td></td>
</tr>
</tbody>
</table>

One week after the treatment, Fenpiroximate 5% and Dicofol 48% seemed to control *E. orientalis* significantly better than Propargite 57%. Two weeks after the applications, the trees treated with Hexitiazox 10% and Fenpiroximate 5% showed significantly fewer mite
individuals per leaf than those treated with Propargite 57%. Finally, trees treated with Propargite 57% showed significantly more *E. orientalis* individuals per leaf than with the other three substances.

**Efficacy**

All the acaricides included in the two assays have significantly reduced the populations of *E. orientalis* with respect to those observed in the non-treated trees. In Table 4 we show the relative efficacy of each treatment as measured one and three weeks after the applications.

Table 4. Efficacy of the different chemicals

<table>
<thead>
<tr>
<th>Substances</th>
<th>Valencia-Late orange crop</th>
<th>Fine lemon crop</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Dicofol 48%</td>
<td>100%</td>
<td>99.65%</td>
</tr>
<tr>
<td>Propargite 57%</td>
<td>98.85%</td>
<td>90.29%</td>
</tr>
<tr>
<td>Hexitiazox 10%</td>
<td>85.05%</td>
<td>95.75%</td>
</tr>
<tr>
<td>Etoxazol 11%</td>
<td>83.92%</td>
<td>98.37%</td>
</tr>
<tr>
<td>Fenpiroximate 5%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Dicofol 48% caused a decrease of nearly 100% from the first week after the treatment, in both assays, and its efficacy was persistent at least up to the third week. The same applies to Fenpiroximate 5% in the assay performed on Fine lemon trees. Propargite 57%, instead, showed a soon impact on *E. orientalis* in the first week, more important in the assay on orange trees, but its effect was not persistent, and so it lost between 10 and 15 percentage points of the initial efficacy three weeks after the treatment. Finally, Hexitiazox 10% and Etoxazol showed an efficacy of 80 to 85% in the first week, which increased to over 95% three weeks after the treatments. In previous chemical control assays, Dicofol was also the most efficacious acaricide (García & Márquez, 2004). This product has been one of the most frequently used for the control of Tetranychidae in citrics, as *Tetranychus urticae* or *Panonychus citri* (García-Mari et al., 1994).

All the acaricides included in the two assays have significantly reduced the populations of *E. orientalis* with respect to what was observed in the not-treated trees. Dicofol 48% and Fenpiroximate 5% caused an early decrease of nearly 100% that persisted throughout all the study period. These substances were significantly more efficacious than the other ones. Hexitiazox 10% and Etoxazol 10% showed a moderate efficacy one week after the treatment, but reached more than 95% after tree weeks. On the contrary, Propargite 57% showed a good early impact, but its efficacy decreased tree weeks after the treatment.

**Acknowledgements**

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Flora of lemon and sweet orange orchards in Portugal

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Abstract: Flora surveys were carried out in lemon orchards, in the Oeste region of Portugal, in 2002, and in sweet orange orchards, in Algarve, in 2004, aiming at studying the potential of resident vegetation of citrus orchards to be managed as cover crops in order to improve biodiversity and promote natural enemies of citrus pests. The results of these surveys, including the frequency and abundance of plant species observed in both regions, are presented and discussed. The main plant species were *Anagallis arvensis*, *Arisarum vulgare*, *Beta vulgaris*, *Bromus diandrus*, *Calendula arvensis*, *Convolvulus arvensis*, *Conyza albida*, *Cynodon dactylon*, *Erodium moschatum*, *Geranium aparine*, *Geranium dissectum*, *Lavatera cretica*, *Medicago polymorpha*, *Oxalis pes-caprae*, *Pieris echioides*, *Piptatherum miliaceum*, *Poa annua*, *Raphanus raphanistrum*, *Senecio vulgaris* and *Sonchus oleraceus*.

Key words: resident vegetation, weeds, citrus orchards, ecological infrastructures

Introduction

Ground cover vegetation within orchards constitutes a modality of ecological infrastructures that can be manipulated both for weed management and to enhance conservation biological control of crop pests. At least 5% of the farm surface must be designated and maintained as ecological infrastructures, according to IOBC/WPRS guidelines for integrated production (Boller et al. 2004a,b). Therefore, it is much relevant to get information on the diversity, species composition and phenology of resident vegetation of orchards.

The resident vegetation of the citrus orchards is relatively well studied in Portugal. Several flora surveys were carried out in sweet orange orchards in Central and Southern Portugal since the 70’s by Silva (1988), Sá & Fontes (1976), Carvalho et al. (1994) and Guerreiro & Martins (1994). However, significant alterations have been introduced on tree density, weed management and irrigation systems that can impact resident vegetation of citrus orchards. On the other hand, almost no information was available on citrus orchards from the Oeste region, the major lemon production area of Portugal.

In this paper we present and discuss the results of a flora survey conducted in lemon orchards in the Oeste region of Portugal and sweet orange orchards in Algarve aiming at understanding the potential of resident vegetation to be managed as ecological infrastructures in citrus IPM. Preliminary data were previously presented by Vasconcelos et al. (2003, 2005).
Material and methods

The flora of lemon orchards from Mafra in the Oeste region of Portugal was studied, in 2002. A total of 54 surveys were carried out in 50 lemon orchards, in each of two seasons, Autumn-Winter and Spring-Summer. In Algarve, the survey was conducted in 21 orange orchards in the Autumn-Winter of 2004.

An abundance index was attributed to the surveyed species, corresponding to the number of plants per square meter, using Barralis (1975) scale. Mean abundance of each species was determined based on Barralis (1976).

Results and discussion

Considering the lemon orchards from Mafra and the two studied seasons, a total of 202 species were identified, from 52 families (Table 1), including 136 species from 41 families in the Autumn-Winter and 146 species from 46 families in Spring-Summer (Vasconcelos et al., 2005). The predominant families were Asteraceae (18.4%), Poaceae (8.1%) and Fabaceae (7.4%), in Autumn-Winter (Table 2), and Asteraceae (16.4%), Poaceae (12.3 %), Polygonaceae (5.5 %) and Chenopodiaceae (4.8 %), in Spring-Summer (Vasconcelos et al. 2005).

In relation to sweet orange orchards from Algarve, a total of 127 species, from 33 families, were identified in Autumn-Winter. Asteraceae (16.5 %), Poaceae (15 %) and Fabaceae (7.9 %) were the predominant families (Table 2).

The most frequent species, with relative frequency equal or higher than 50%, include Amaranthus albus, A. deflexus, Anagalis arvensis, Chenopodium album, Convolvulus arvensis, Conyza albida, Erodium moschatum, Hedera helix, Lavatera cretica, Oxalis pes-caprae, Picris echioiodes, Poa annua, Portulaca oleracea, Rubus ulmifolius, Rumex crispus, Solanum nigrum, Sonchus asper, S. oleraceus and Urtica urens in Mafra, and Bromus madritensis, Hedera helix, Lavatera cretica, Medicago polymorpha and Oxalis pes-caprae in Algarve. A. arvensis, Arisarum vulgare, Beta vulgaris, Bromus diandrus, Calendula arvensis, Convolvulus arvensis, Conyza albida, Cynodon dactylon, Erodium moschatum, Galium aparine, Geranium dissectum, Lavatera cretica, Medicago polymorpha, Oxalis pes-caprae, Picris echioiodes, Piptatherum milieceum, Poa annua, Raphanus raphanistrum, Senecio vulgaris and Sonchus oleraceus were present in both regions (Table 1).

Daucus carota, Hordeum murinum ssp. leporinum, Medicago polymorpha, Polygonum aviculare, Sonchus oleraceus and Stellaria media are among the species reported as favourable for conservation biological control because constitute good sources of nectar or pollen or alternative hosts/prey for parasitoids and predators (UC SAREP, 2002).

Conclusion

The resident vegetation of citrus orchards constitutes a potential resource that adequately managed can be used as an ecological infrastructure to increment functional diversity within orchards and enhance conservation biological control of citrus pests.

Acknowledgements

We thank Paulo Forte for the help in field work. Thanks are also due to the lemon growers Virginia Duarte, Carlos Batalha and Rosa Gomes that allowed us to carry out the experiment in their orchards. This was part of a research granted by Programme AGRO Medida 8.1.–DE&D
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UC SAREP – University of California Sustainable Agriculture Research and Education Program 2002: UC SAREP Cover Crop Database http://www.sarep.ucdavis.edu


Table 1 - Relative frequency (RF), and mean abundance (MA*) of the species in Mafra (autumn-winter and spring-summer seasons) and Algarve (autumn-winter season) with a frequency higher than 10% at least in one region.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mafra</th>
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<td>- Victoria sativa L.</td>
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Table 2. Relative importance of the different botanical families based on the respective number of species identified in the flora surveys carried in Autumn-Winter in lemon orchards in Mafra (2002) and in sweet orange orchards in Algarve (2004).

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Molecular and morphological characterisation of *Colletotrichum* species involved in citrus anthracnose in Portugal

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(2) Warwick HRI, University of Warwick, Wellesbourne, Warwickshire CV35 9EF, UK

Abstract: Three forms of anthracnose diseases of citrus caused by *Colletotrichum* spp. are recognised: the post-bloom fruit drop and the Key lime anthracnose due to *C. acutatum* and the anthracnose caused by *C. gloeosporioides*, a common postharvest pathogen. In Portugal, in the last few years, a significant percentage of the citrus production has been rejected due to chlorotic or necrotic spots, which apparently do not decrease yield or juice quality but affect market appeal, resulting in important economic losses. Anthracnose lesions associated with intact or injured rind of fruits after harvest have been important in ‘Navel’ oranges and ‘Encore’ mandarins. Lesions on leaves, shoot dieback and blossom blight leading to significant loss of flowers and young fruits have also been recorded. However, the behaviour and the relative importance of *C. acutatum* and *C. gloeosporioides* in citrus remain unknown in Portugal. The main objectives of this study were to identify the pathogen(s) associated with these symptoms and investigate the diversity of *Colletotrichum* spp. populations in citrus orchards. A total of 57 isolates from several citrus varieties were characterised by molecular and phenotypic analysis along with reference isolates. Morphological and cultural features and the use of diagnostic PCR for *C. acutatum* and *C. gloeosporioides*, based on ribosomal DNA and the β-tubulin 2 gene, revealed *C. gloeosporioides* as the main *Colletotrichum* species present on citrus in Portugal, although more isolates from other regions have to be studied. Furthermore, the use of ISSR-PCR analysis revealed a limited degree of molecular diversity among these isolates.

Key words: *Colletotrichum gloeosporioides*, *C. acutatum*, PCR, ISSR, molecular diversity

Introduction

Infection of citrus by *Colletotrichum* species can result in post-bloom fruit drop (PFD) and in Key lime anthracnose (KLA), both due to *Colletotrichum acutatum*, and in postharvest anthracnose caused by *Colletotrichum gloeosporioides*.

PFD produces serious yield losses of sweet oranges and other citrus, affecting petals and inducing abscission of fruitlets and production of calyces (Timmer & Zitko 1995). *C. acutatum* isolates responsible for PFD are weakly pathogenic on the leaves and fruit of Key lime and are considered hemibiotrophic pathogens on citrus other than Key lime (Timmer et al. 1994). In contrast, KLA isolates are mostly necrotrophic, cause all symptoms of PFD on sweet orange flowers and also cause anthracnose on twigs, shoots, leaves and flowers of Mexican Lime (Brown et al. 1996). *C. gloeosporioides* is a postharvest pathogen that produces necrotic lesions on the rind of citrus varieties, causing fruit rotting, but also occurs commonly as a saprophyte (Brown et al. 1996).

Traditionally, the foregoing species of *Colletotrichum* have been discriminated by methods relied on colony colour, size and shape of conidia, optimal temperature, growth rate, presence or absence of setae, sensitivity to selected fungicides and existence of the teleomorph *Glomerella*. However, as both species exhibit considerable phenotypic plasticity
there has been a considerable interest in the development and use of molecular tools for rapid and reliable diagnosis of \textit{C. acutatum} and \textit{C. gloeosporioides} populations as well as for intra- and inter-species differentiation (Talhinhas et al. 2005). In fact, molecular approaches in conjunction with phenotypic analysis have been applied to correctly diagnose the pathogens involved in various diseases and understand their epidemiology to develop accurate control methods (Timmer & Brown 2000).

In Portugal, in the last few years, a significant percentage of the citrus fruits produced have been rejected due to superficial blemishes, appearing like chlorotic or necrotic minute spots, resulting in important economic losses particularly with ‘Navel’ oranges and ‘Encore’ mandarins. Moreover, lesions on leaves, shoot dieback and blossom blight leading to significant loss of flowers and young fruits have been recorded. The research carried out up to now has led to the conclusion that some of these symptoms may be attributed to infections caused by \textit{Colletotrichum} spp., among other biotic and abiotic disorders (Silva & Bernardes, 2000; Ramos et al., 2003). However, the behaviour and the relative importance of \textit{C. acutatum} and \textit{C. gloeosporioides} in citrus remain unknown in Portugal.

The main objectives of this study were the: (i) genotypic and phenotypic characterization of \textit{Colletotrichum} spp. populations associated with citrus anthracnose symptoms and (ii) to identify the key pathogen(s) present in citrus orchards in Portugal.

\section*{Material and methods}

\textbf{Fungal isolates}

The sources of isolates used in the study are listed in Table 1. A total of 43 \textit{Colletotrichum} spp. isolates were established from leaf, flower and fruit samples of citrus varieties, collected during 2004 from different citrus production areas in Portugal. In addition, 14 isolates were established from symptomatic mandarins from Argentina (isolates 437 to 454) and isolate 432 was obtained from symptomatic leaves of \textit{Mangifera indica}. All the isolates were monoconidial cultures and were stored as agar plugs in water at room temperature. Depending on the studies undertaken at least two isolates were used for comparison out of the eight reference isolates mentioned in Table 1.

\begin{center}
\begin{table}[h]
\centering
\caption{Details of \textit{Colletotrichum} spp. isolates used in this study and their identification}
\begin{tabular}{cccccc}
\hline
\textbf{Reference} & \textbf{Location} & \textbf{Collection date (mo-yr)} & \textbf{Host} & \textbf{Tissue} & \textbf{Species} \\
\hline
371 & Tavira, Portugal & Apr-04 & \textit{Citrus sinensis} 'Lane Late' & branch & CG \\
372 & Tavira, Portugal & Apr-04 & \textit{C. sinensis} 'Lane Late' & flower & CG \\
373 & Tavira, Portugal & Apr-04 & \textit{C. reticulata} 'Encore' & fruit & CG \\
374 & Tavira, Portugal & Apr-04 & \textit{C. reticulata} 'Encore' & fruit & CG \\
375 & Tavira, Portugal & Apr-04 & \textit{C. sinensis} 'Valencia Late' & fruit & CG \\
376 & Tavira, Portugal & Apr-04 & \textit{C. sinensis} 'Valencia Late' & flower & CG \\
377 & Tavira, Portugal & Apr-04 & \textit{C. sinensis} 'Valencia Late' & fruit & CG \\
378 & Tavira, Portugal & Apr-04 & \textit{C. reticulata} 'Ortanique' & flower & CG \\
379 & Tavira, Portugal & Apr-04 & \textit{C. sinensis} 'Valencia Late' & fruit & CG \\
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381 & Tavira, Portugal & Apr-04 & \textit{C. sinensis} 'Valencia Late' & flower & CG \\
382 & Tavira, Portugal & Apr-04 & \textit{C. sinensis} 'Valencia Late' & flower & CG \\
383 & Tavira, Portugal & Apr-04 & \textit{C. sinensis} 'Valencia Late' & flower & CG \\
384 & Loulé, Portugal & Apr-04 & \textit{C. reticulata} 'Ortanique' & fruit & CG \\
385 & Olhão, Portugal & Apr-04 & \textit{C. reticulata} 'Encore' & fruit & CG \\
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</tr>
<tr>
<td>CA397 g</td>
<td>USA</td>
<td>-</td>
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<td>-</td>
<td>CA</td>
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<td>V.V. Ródão, Portugal</td>
<td>Nov-01</td>
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<tr>
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<tr>
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<td>-</td>
<td>Citrus sp.</td>
<td>-</td>
<td>CA</td>
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a Species according to diagnostic PCR: CA, C. acutatum; CG, C. gloeosporioides; b reference isolate (Talhinhas et al. 2002); c data not available; d reference isolate (Sreenivasaprasad et al. 1992); e reference isolate (Sreenivasaprasad et al. 1996); f reference isolate (Talhinhas et al. 2005); g reference isolates from the collection held at the Queen’s University of Belfast, UK.
Assessment of morphological and cultural characteristics

For morphological observations and measurements and of conidia each isolate was grown on three plates (90 mm diameter Petri dishes) of Difco potato dextrose agar (PDA) under darkness at 25ºC. Data were analysed using analysis of variance and the Tukey honest significant difference test with Statistica software (StatSoft, Tulsa, OK, USA). Conidia were harvested after 10 days, mounted in lactophenol and measured at 40 x with the aid of a Leitz light microscope. For each isolate, the length and width of 50 conidia were measured and the shape was recorded. The colony diameter (six replicates; two measurements per replicate; statistic analysis as above) and the cultural characteristics (texture, density, colour [Saccardo 1891], zonation, nature of the growing, presence of conidial masses, and colour of the reverse side) were registered from 5-days-old cultures grown in conditions as above. The sensitivity of the isolates to benomyl was assessed by measuring colony diameter on PDA amended with 2 mg/dm³ benomyl (Benlate; Dupont, Delaware). Isolates CR20 and CA397 were used as references (Talhinhas et al. 2002).

Molecular analysis

For molecular analysis, fungal mycelium was grown in Petri dishes containing yeast extract (0.2% wt/vol) and glucose (1.0% wt/vol) liquid medium (Difco) for 4 days at 25ºC. DNA was extracted from freeze-dried mycelium using the DNeasy Plant Kit (QIAGEN, Hilden, Germany), according to the instructions provided by the manufacturer. The DNA concentration was evaluated both by agarose gel electrophoresis and by spectrophotometry.

Identification of pathogens using species-specific PCR primers

PCR primers CaInt2 specific for *C. acutatum* (Sreenivasaprasad et al. 1996) and CgInt specific for *C. gloeosporioides* (Mills et al. 1992) were each used in conjunction with the conserved primer ITS4 (White et al. 1990) for diagnostic PCR based on the rRNA gene-ITS region. Similarly, primers TBCA specific for *C. acutatum* and TBCG specific for *C. gloeosporioides* were each used in conjunction with the primer TB5 of the β-tubulin 2 gene (Talhinhas et al. 2005). PCR reactions and amplification conditions were done as described in Talhinhas et al. (2005). PCR products (10 µl) were visualised by electrophoresis on 1% (wt/vol) agarose gels (Roche Diagnostics GmbH, Mannheim, Germany). CA397 and PT111 were used as reference isolates.

ISSR analysis

ISSR profiles were generated for each isolate using five different primers: primers MR, GAC₅ and TCC₅ were used at 50ºC annealing temperature and primers CAG₅ and GACC₅ at 60ºC. Each PCR reaction (20 µl) contained 20 ng DNA, 20 µM of primer (Sigma-Genosys, S.A., Haverhill, UK) and 10 µl of ReadyMix RedTaq (Sigma-Aldrich, Gillingham, UK). Amplifications were performed with a thermal cycler (Proteus II; Helena Biosciences, Sunderland, UK) programmed for 1 cycle of 5 min at 94°C; 30 cycles, each of 1 min at 94°C, 1 min at appropriate annealing temperature, and 2 minutes at 72°C; and ending with 1 cycle of 5 min at 72°C. PCR products (10 µl) were visualised by electrophoresis on 1% (wt/vol) agarose gels. For each primer the presence or absence of bands in each isolate was visually scored. Data were set in a binary matrix and genetic similarities were calculated using the Dice coefficient. The dendrogram was obtained by clustering according to the UPGMA method and the cophenetic correlation coefficient was calculated using the NTSYSpc 2.01b Applied Biostatistics Inc.. Reference isolates were CR45, CG315, CA397, PT111, CS-1, DPI and Homestead.
Results and discussion

Diagnostic PCR

Amplification reactions were carried out for the 58 test isolates and the reference isolates using the specific primers for the ITS region of rRNA. DNA fragments of 450 bp and 490 bp each were amplified with CgInt/ITS4 and CaInt2/ITS4 primers, respectively. As for the fragment of the β-tubulin 2 gene, the amplified fragments, both with TBCG and TBCA primers, had about 330 bp. The size of the fragments was according to those of the reference isolates (isolates CR45, CG315 and PT111 for C. gloeosporioides; isolate CA397 for C. acutatum). Analysing the overall results, all test isolates belonged to C. gloeosporioides.

Cultural and morphological characteristics

Measurements of the mycelial growth on PDA (Table 2) distinguished two groups of isolates. The first group contained 3 isolates obtained from mandarins, from Argentina, and the reference isolate CA397 of C. acutatum, with a growth diameter of 37.6 to 39.3 mm. The second group comprised all the remaining isolates studied, including the reference isolate CR20 (C. acutatum), although with a significant higher growth than that of CA397. These observations vary from previous results of other authors such as Lardner et al. (1999) and Talhinhas et al. (2002), which refer to lower mycelial growth rates with C. acutatum compared to C. gloeosporioides. Nevertheless, 15 of the 58 Colletotrichum spp. isolates tested showed higher levels of mycelial growth after 5 days of incubation (for example isolates 387, 404 and 410). As to the benomyl sensitivity, as expected, CA397 exhibited only partial inhibition of growth on PDA amended with benomyl (Table 3). Within the group of isolates from Argentina, 447 (identified as C. gloeosporioides) was sensitive to benomyl, but the mycelial growth of isolates 449 and 453 was higher on PDA containing 2mg.dm⁻³ benomyl. A similar situation occurred with isolate 432 from Mangifera indica. Also isolates 443 and 450, identified as C. gloeosporioides, were not sensitive to benomyl under these conditions, showing a mycelial growth similar to that of CR20 and CA397, reference isolates of C. acutatum (Table 3). It appears that, in the case of isolates obtained from citrus, C. gloeosporioides cannot be separated from C. acutatum isolates based on mycelial growth on PDA containing 2mg.dm⁻³ benomyl, unlike previous results with these species from other hosts (Denoyes & Baudry 1995; Talhinhas 2002). This observation needs to be further investigated. The overall colony appearance of the 58 isolates on PDA was quite variable as has generally been noted previously (Sutton 1992). Thus, C. gloeosporioides from citrus produced light orange or grey-olive colonies to dark-grey colonies, with differences in the growth of sectors occurring erratically on some colonies. Some isolates (401 and 441 for example) had beige to grey colonies, but after 3 to 4 days of incubation the colony was covered with abundant orange masses of conidia. Evaluation of the conidial size and shape revealed high variability within each isolate and little statistically significant difference among isolates (data not shown). Conidial length was 15.0 to 25.7 μm and width was 4.3 to 11.4 μm. No clear grouping of the Colletotrichum isolates within citrus was observed, which along with reference isolates CR45 and PT111 had mainly round-ended conidia. Conidia of the reference isolates CR20 and CA397 of C. acutatum were fusiform.

ISSR – PCR analysis

ISSR-PCR analysis was performed with five primers to characterize 65 Colletotrichum isolates in all. These include 57 isolates from Citrus spp., one isolate from mango and seven reference isolates (four C. acutatum and three C. gloeosporioides). Primer MR amplified 1 to 12 bands per isolate and for the remaining primers, on an average, 2 to 10 bands were amplified per isolate; 13 to 23 characters were scored per primer with all isolates, none being monomorphic. Overall similarity values varied from 0.1 to 1.0. Cluster analysis (UPGMA) of
similarity matrix (Dice) generated from the DNA profile data with the five primers used in this study revealed two main clusters (Fig. 1), the first included the *C. acutatum* reference isolates and the second included all the *C. gloeosporioides* isolates. Thus none of the *Colletotrichum* spp. isolates being tested from citrus clustered together with *C. acutatum* reference isolates, which is in agreement with the diagnostic PCR analysis. Isolate CA397 (*C. acutatum*) from strawberry clustered with reference isolates CS-1, Homestead and DPI from *Citrus* sp., being the group supported by a bootstrap value of 65.4%. According to Sreenivasaprasad & Talhinhas (2005) the isolate CA397 broadly represents the homogenous group A2 within the species *C. acutatum*. Within the *C. gloeosporioides* group, isolate 432 from *Mangifera indica* exhibited only 43% similarity (Dice coefficient) with the remaining isolates including the references CG315 (from strawberry), PT111 (from olive) and CR45 (from citrus). The citrus isolates from Portugal and the majority of the isolates coming from Argentina cluster in a different group either from PT111 and CR45; yet the Argentinean isolates 437 and 451 showed some variation, with 65% similarity to the remaining *Citrus* isolates. Isolates 389, 395 and 398, from the south of Portugal, were very identical to 440, 441, 443, 450 and 453, from Argentina, with a genetic similarity of 95%. The results generated so far suggest that the *C. gloeosporioides* population does not exhibit specificity towards particular varieties of *Citrus* or an association with the geographic origin of the isolates.

**Conclusion**

Molecular and phenotypic characterization of 57 *Colletotrichum* spp. isolates associated with citrus anthracnose and comparative analysis with a selected set of reference *C. acutatum* and *C. gloeosporioides* isolates identified all the isolates as *C. gloeosporioides*. Cultural characteristics such as colony growth and benomyl sensitivity did not reveal any specific groups within the population studied. In fact, some results were different to the previously reported trends, which will be further investigated. Conidial morphology of the test isolates, although assessed from PDA medium recognized as producing more variability, was different to *C. acutatum*. Application of species-specific PCR using rRNA gene-ITS and tub2- based primers provided reliable diagnosis of *Colletotrichum* sp. isolates from citrus as belonging to *C. gloeosporioides*. The use of ISSR markers revealed that the vast majority of *C. gloeosporioides* from citrus displayed >75-80% similarity and separated these isolates from the reference *C. gloeosporioides* and *C. acutatum* isolates. In order to investigate the apparent genetic homogeneity among the citrus *C. gloeosporioides* populations, additional isolates will be collected and characterised and the pathogenicity of a representative set of isolates on citrus hosts will be tested to evaluate their host-preference/specificity.

**Acknowledgements**

We are thankful to Celestino Soares and Entrudo Fernandes for help in field surveys, and to Cecília Rego and Filomena Caetano for help in morphological characterization of cultures. Field surveys were supported by the Project AGRO 29, and other parts of this work were carried out at the Warwick HRI.
Fig. 1. Dendrogram showing the diversity and relationships among the *Colletotrichum* spp. isolates from Citrus based on cluster analysis (UPGMA) of similarity matrix (Dice) generated from ISSR-PCR profile data with five different primers. Bootstrap values in per cent are shown from a total of 1,000 data sets used. Cophenetic correlation coefficient $r = 0.98805$. Table 1 provides isolate details.
Table 2. Distribution of *Colletotrichum* spp. isolates in homogeneous groups according to the Tukey test (95% confidence) based on diameter of the colonies (mm) (PDA, 25°C, darkness, 5 days)

| Isolates | Mean  | Homogeneous Groups *
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<tr>
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<td>g h i j k l m</td>
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* Values labelled with the same latter are not significantly different at $\alpha =0.05$ using Tukey’s test.
Table 3. Distribution of *Colletotrichum* spp. isolates in homogeneous groups according to the Tukey test (95% confidence) based on percentage of growth of the colonies (PDA+benomyl, 25ºC, darkness, 5 days) [considering 100% growth on PDA]

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* Values labelled with the same latter are not significantly different at $\alpha =0.05$ using Tukey test.
References


Distribution patterns and sampling design for “Wrinkle Rind” or “Rumple” on lemon crops

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Abstract: In autumn 2002 and 2003, samplings of “Wrinkle Rind” of “Rumple” symptoms in several lemon crops of Malaga province (Spain) were carried out to analyse the distribution of damages within the crops and within the trees, and then to design a suitable sampling method. Our results suggest different distribution patterns for the symptoms named here as type I and type II. Within the crops, Rumple type I showed an aggregate distribution, whereas type II showed a regular but nearly random distribution. Within the trees, Rumple type I was more abundant in the medium-higher branches, whereas the frequency of type II damages was greater in the medium-lower ones. Besides, the outward-facing fruits and those facing east or south showed Rumple with the highest frequency. Our analyses determined that a two-stage fruit sampling is the most precise method. For type I damage, the best primary unit is depth (outside, medium and inside position within the tree top), and for type II the best primary unit is height (high, medium and low).

Keywords: Lemon, Rumple, Wrinkle Rind, distribution pattern, aggregation, sampling design

Introduction

“Wrinkle Rind” (Russo & Klotz, 1963), “Rumple” (Knorr, 1963) and “raggrinzimento della buccia” (Salerno, 1963) are names for a type of damage that is located on the external layer of lemon fruits. The symptoms described in the literature can be summed up as rusty stains with depressions (Knorr, 1963; 1973; Del Rivero, 1997). We will call Rumple type I to this kind of disorder, and Rumple type II to the lemon squashed surface in the areas exposed to sunshine that are often observed in Malaga (Spain), associated to the usual Rumple symptoms (Figure 1). Rumple does not cause damages to the juice, but it makes fruits lose their commercial value. At present, it is widespread in the Mediterranean basin. The causes of the symptoms described are unknown. A physiological origin has been postulated as the most probable, but the effect of genetic mutations, pathogens, arthropods and mechanic damages have also been proposed. However, up to now there is no generally accepted explanation for it (Knorr, 1973; Knor et al., 1963; Alarcón, 1996; Del Rivero, 1997; Valero et al., 1999; Alcaráz-López et al., 2004; Beltrán et al., 2004).

The aim of this work is to characterize the spatial distribution patterns of Rumple, paying attention to the aggregation of affected trees within the crops and to the position of damaged fruits within the trees. Then we take these patterns into account to determine an optimal sampling strategy for the incidence evaluation of symptoms, in relation with the suitable type of sampling units to be used. Our objective is to provide a tool that could contribute to future investigations about the causes of Rumple.

Material and methods

Rumple spatial aggregation
We used the m*/m patchiness ratio (aggregation index proposed by Lloyd, 1967) to analyse the spatial aggregation patterns within the three crops sampled in 2003. So, we defined the mean crowding m* as, for every damaged fruit, the mean number of other damaged fruits in the same tree:

\[ m^* = m + \frac{s^2}{m - 1} \]

where \( m \) is the mean number of fruits with Rumple in the sample, and \( s^2 \) is the variance related with such mean (Lloyd, 1967). A m*/m ratio higher than 1 indicates an aggregate distribution of damaged fruits; lower than 1 indicates a regular distribution; equal to 1 means a random distribution of fruits with Rumple within the crop.

**Rumple distribution within the tree**

We aimed to detect significant preferences for different parts in which the sampled trees were divided according with the following criteria: aspect, height and depth. For this purpose we used two factor analyses of variance (ANOVA) and pair-wise mean comparison tests (LSD and Scheffé) with the proportion of fruits with Rumple.

**Sampling strategy**

We used six Fine lemon crops in Malaga province: three in 2002 and three in 2003. In each crop, we randomly revised 20 trees, and took 36 fruits from each of them. For every fruit we noted down the presence or absence and the type of Rumple observed, and its position within the treetop: aspect (north, south, east or west), height (high, medium or low) and depth (inside, medium or outside, with the limits defined by the light and shadow parts of the tree).

We defined the best sampling strategy among five possibilities, namely a one-stage random fruit sampling, and four two-stage sampling designs with the fruit as the secondary unit, and with one of the following primary units: tree, aspect, height or depth. For this we considered the variance of the mean damaged fruit number (VM), computed for every unit combination, and then we chose the option with the lowest VM as the best sampling design.

For the two-stage unit combinations, we computed the VM as follows (Cochran, 1980):

\[ VM = \frac{1-f_1}{n} S_b^2 + \frac{f_1(1-f_2)}{mn} S_w^2 \]

where:

\( f_1 = n/N \)
\( f_2 = m/Mn = \) number of sampled primary units
\( m = \) number of sampled secondary units (fruits) per primary unit
\( N = \) total number of primary units in the crop
\( M = \) total number of fruits per primary unit

\( S_b^2 = \) variance between primary units in relation to the proportion of fruits with Rumple
\( S_w^2 = \) variance within the primary units in relation to the proportion of fruits with Rumple.

\( S_b^2 \) and \( S_w^2 \) were computed as follows:

\[ S_b^2 = \frac{\sum_{i=1}^{n}(P_i - \bar{P})^2}{n-1} \quad S_w^2 = \frac{m\sum_{i=1}^{n}P_iq_i}{n(m-1)} \]
where \( P \) is the mean proportion of damaged fruits per primary unit, \( p_i \) is the proportion of damaged fruits within the primary unit \( i \), and \( q_i = 1 - p_i \).

Several approximations were assumed to use the equations above: every crop was 4 hectare wide, the tree number per hectare was 270, and the number of fruits per tree was 750. To compute the VM for the one-stage fruit sample we used the following equation:

\[
VM = \frac{S^2}{n}
\]

where \( S^2 \) is the variance in relation to the proportion of damaged fruits within the complete data set, and \( n \) is the number of fruits revised.

**Results and discussion**

**Rumple spatial aggregation**

Rumple type I shows an aggregate distribution within the crop (\( m^*/m > 1 \)) (Table 1). The mean crowding values (\( m^* \)) are between 3.66 and 5.66, which means that those trees showing damaged fruits contained 4 to 6 fruits with Rumple type I in a sample of 20. For Rumple type II, an aggregate distribution was found in crop 1 (Table 1), whereas in crops 2 and 3 it showed \( m^*/m \) values lower than but very close to 1 (0.98 and 0.96, respectively). This could indicate a random distribution of fruits with Rumple II, with a trend towards a regular distribution. The number of damaged fruits of this type per affected tree is similar than for Rumple type I. Finally, when Rumple types I and II are considered together, we found an aggregate distribution with 8 to 10 damaged fruits per affected tree (Table 1).

**Table 1. Aggregation parameters for Rumple types I, type II and total in the tree crops sampled in 2003.** m: mean number of damaged fruits per tree; \( m^* \): mean crowding; \( m^*/m \): patchiness (aggregation index).

<table>
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<th>Crop 2</th>
<th>Crop 3</th>
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<tr>
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<td>Type II</td>
<td>Total</td>
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<tr>
<td>m</td>
<td>1.85</td>
<td>3.7</td>
</tr>
<tr>
<td>( s^2 )</td>
<td>6.13</td>
<td>5.91</td>
</tr>
<tr>
<td>( m^* )</td>
<td>4.17</td>
<td>4.3</td>
</tr>
<tr>
<td>( m^*/m )</td>
<td>2.25</td>
<td>1.16</td>
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**Rumple distribution within the tree**

According to the ANOVA results, differences in damage incidence are significant between aspects for Rumple type II, and between heights and between depths for Rumple types I and II (see Tables 2 to 4). The pair-wise mean comparison tests show a clearly higher Rumple type II and total Rumple incidence on fruits oriented south or east with respect to those oriented north or west.

Although total Rumple showed to be affected by height according to the ANOVA, the pair-wise mean comparison tests did not indicate significant differences related to it between the low, high and medium parts of the trees. This is because of a different trend showed by Rumple depending on the type: type I seems to be significantly favoured in the high and
medium heights, whereas type II shows the highest incidences in the lowest and medium branches of the trees.

Table 2. Analysis of the aspect. Results of the ANOVA, and significant pair-wise differences according to LSD and Scheffé tests (bold characters indicate differences in both tests; normal characters indicate differences only in the LSD test). N: north; S: south; E: east; W: west.

<table>
<thead>
<tr>
<th>Type</th>
<th>Type I</th>
<th>Type II</th>
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<th>Incidence</th>
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<td>North</td>
<td>0.0931</td>
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<td>South</td>
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<td>East</td>
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<tr>
<td>East</td>
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<td>N-W</td>
<td>South</td>
<td>0.1639</td>
<td>N-W</td>
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</table>

Table 3. Analysis of the height. Results of the ANOVA, and significant pair-wise differences according to LSD and Scheffé tests (bold characters indicate differences in both tests; normal characters indicate differences only in the LSD test). L: low; M: medium; H: high.

<table>
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<tr>
<td>Sig.</td>
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<td>0.0896</td>
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<td>Low</td>
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Table 4. Analysis of the depth. Results of the ANOVA, and significant pair-wise differences according to LSD and Scheffé tests (bold characters indicate differences in both tests; normal characters indicate differences only in the LSD test). I: inside; M: medium; O: outside.

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<td>M-O</td>
<td>Inside</td>
<td>0.0187</td>
<td>M-O</td>
</tr>
<tr>
<td>Medium</td>
<td>0.049</td>
<td>O-I</td>
<td>Medium</td>
<td>0.049</td>
<td>O-I</td>
<td>Medium</td>
<td>0.0969</td>
<td>O-I</td>
</tr>
<tr>
<td>Outside</td>
<td>0.1031</td>
<td>I-M</td>
<td>Outside</td>
<td>0.1656</td>
<td>I-M</td>
<td>Outside</td>
<td>0.2687</td>
<td>I-M</td>
</tr>
</tbody>
</table>
Significant differences are detected in Rumple incidence, whichever the type is, between the fruits located in the inside, medium and outside parts of the treetop. In all cases, the outside fruits showed the highest incidence and, at least for Rumple type I and for total Rumple, it was followed by the fruits in the medium parts.

**Sampling strategy**

Provided that the distribution of Rumple has showed to be slightly aggregated in some trees more than in others, and also to be significantly affected by the fruit position within the trees, a sampling design that takes tree, aspect, height and depth into account is recommended. To consider the four factors in only one sampling design is not practical from a fieldwork point of view. So, a good option is to choose which type of unit combination, among those considering the factors above, is the most precise, and then to propose it as the optimum sampling design. Table 5 shows the VM values obtained for five sampling designs, computed for Rumple types I and II and for total Rumple, in all of the crops sampled in 2002 and 2003. A two-stage fruit sampling is always the most precise method. For Rumple type I, the best primary unit is depth (outside, medium and inside position within the treetop). For Rumple type II and for total Rumple the best primary unit is height (high, medium and low).

Table 5. Mean variance (VM) for the five sampling designs tested. In bold we indicate the lowest VM values obtained for each sampling.

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<th>2002</th>
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<td>Type II</td>
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<td>Type I</td>
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<td>Fruit</td>
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<td>0.19</td>
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<td>0.000138</td>
<td>0.0000833</td>
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**Acknowledgements**

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References


Survey on the situation of citrus pest management in Mediterranean countries

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Abstract: A survey on the actual situation of citrus pest management practices, problems and constraints was carried out in Mediterranean countries based on a questionnaire sent to a list of experts in order to prepare a summary for the IOBC/WPRS meeting of the Working Group “Integrated Control in Citrus Fruit Crops” held in Lisbon, in 26th-27th September 2005. Data was collected from Algeria, Georgia, Greece, France (Corsica), Israel, Italy, Portugal, Montenegro, Morocco, Spain and Turkey. Results include statistics on IPM/IP situation and a list of pest problems and pest status.

Key words: citrus pests, IPM, Mediterranean

Introduction

In order to carry out a survey on the actual situation of citrus pest management in different Mediterranean countries, including Integrated Pest Management (IPM) and Integrated Production (IP) practices, problems and constraints, and prepare a summary for the IOBC/WPRS meeting of the Working Group “Integrated Control in Citrus Fruit Crops” held in Lisbon, in 26th-27th September 2005, a questionnaire was sent to experts on citrus IPM. The results obtained on the basis of the answers received from 11 countries are presented and discussed.

Material and methods

The questionnaire (Annex 1), consisting of a set of general questions on the IPM/IP situation and on the pest status of citrus arthropod pests and diseases in each country, was sent to a list of experts in different Mediterranean countries, namely, Algeria, Georgia, Greece, Egypt, France (Corsica), Georgia, Israel, Italy, Montenegro, Morocco, Portugal, Spain, Tunisia and Turkey.

Pest problems were classified according to their pest status: key (3), occasional (2), potential (1), or not reported pests (0). Key-pests impose the application of control measures most of the years because usually originate economic damage; occasional pests only occasionally reach economic injury level; and potential pests are always below economic injury level.

Results and discussion

Number of answers to the questionnaire

Answers to the questionnaire were received from 11 countries, namely Algeria (Yamina Guenaoui), Georgia (Valentina Yasnosh, Ekaterine Tabatadze), Greece (Alexandrakis
Venizelos, Kalaitzaki Argyro), Israel (Drishpoun Yoel), Italy (Gaetano Siscaro, Ernesto Raciti, Filadelfo Conti), France (JP Thermo, Gilles Tison, Olivier Pailly), Portugal (Celestino Soares, Entrudo Fernandes, Miriam Cavaco, José Carlos Franco), Montenegro (Tatjana Perovic), Morocco (Ahmed Mazih, Mohamed Besri), Spain (Ferran Garcia-Mari) and Turkey (Lerzan Erkilic).

Citrus pest management

The area of citrus production in each country ranged between 480 ha, in Montenegro, and 280,000 ha, in Spain (Table 1). In most of the countries, between less than 1% (France) and 30% (Turkey) of total citrus area was reported to be under IPM. IPM was considered to be the common practice in all citrus area, in Israel, and in most of citrus area, in Greece. No data was available for Algeria, Georgia and Montenegro.

IP was only reported in Spain, Italy, Portugal and France, ranging between 0.4% (Portugal) and 10% (Italy) of total citrus area. However, in the case of Italy, data correspond to organic farming (Table 1).

Table 1. Total area of citrus production and relative importance of integrated pest management (IPM) and integrated production (IP) in each country.

<table>
<thead>
<tr>
<th>Country</th>
<th>Citrus production area (ha)</th>
<th>IPM (%)</th>
<th>IP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>280,000</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Turkey</td>
<td>150,000</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Italy</td>
<td>132,500</td>
<td>10-20</td>
<td>10</td>
</tr>
<tr>
<td>Morocco</td>
<td>76,000</td>
<td>10-20</td>
<td>-</td>
</tr>
<tr>
<td>Greece</td>
<td>47,198</td>
<td>most of the area</td>
<td>-</td>
</tr>
<tr>
<td>Algeria</td>
<td>45,400</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>Portugal</td>
<td>27,755</td>
<td>14</td>
<td>0.4</td>
</tr>
<tr>
<td>Israel</td>
<td>17,300</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Georgia</td>
<td>11,000</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>France (Corsica)</td>
<td>1,800</td>
<td>&lt;1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Montenegro</td>
<td>480</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In Italy, France, Portugal and Spain, the citrus growers receive financial support for practicing IPM or IP as part of EU Agri-environmental measures. In Italy, the financial support the grower receives per ha is 600 euros for IPM and 800 euros for IP. In Portugal, it is dependent on the dimension of the citrus orchard: ≤ 5 ha = 409 euros, 5-10 ha = 327 euros, 10–25 ha =245 euros, > 25 ha = 164 euros, in IPM, and ≤ 5 ha = 549 euros, 5-10 ha = 399 euros, 10–25 ha = 299 euros, > 25 ha = 200 euros, in IP.

Among the requisites for a grower to receive financial support for practicing IPM or IP the following were reported, depending on the country: to be an associate of an IPM/IP farmers organization (Italy, Portugal); to attend a course on IPM/IP (Spain, Portugal); to follow official IPM/IP guidelines (Italy, Spain, Portugal); to keep accurate records of pesticide interventions and, in the case of IP, other cultural practices (Italy, Portugal).

IPM/IP guidelines are defined at regional level in Morocco and Algeria, at national level in Greece, Portugal and Turkey, and at both regional and national level in Israel, Italy and Spain. IPM/IP certification companies were reported to exist in Italy, Spain and Portugal.
The increased complexity in IPM strategies, the increased risk perception by the farmer, the lack of scientific based economic thresholds, the production costs, the lack of effective alternative tactics to pesticides and the lack of accurate and practical pest monitoring methods were among the considered major problems/constrains for the development of citrus IPM/IP (Figure 1).

Figure 1. Rank of major problems/constrains for the development of citrus integrated pest management and integrated production, based on an index of relative importance (sum of notations by the experts on the respective level of importance, i.e., low-1, medium-2 or high-3).

Up to 72 insecticides/acaricides, 16 fungicides and 60 herbicides are registered in each country. Except in Israel, insecticides/acaricides represent more than 40% of registered pesticides (Table 2).

Table 2. Number of pesticides registered for citrus pest management in Mediterranean countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Insecticides/acaricides</th>
<th>Fungicides</th>
<th>Herbicides</th>
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</thead>
<tbody>
<tr>
<td>Spain</td>
<td>72</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>Turkey</td>
<td>20</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Italy</td>
<td>41</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Morocco</td>
<td>&gt;60</td>
<td>&lt;10</td>
<td>?</td>
</tr>
<tr>
<td>Greece</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Algeria</td>
<td>&gt;25</td>
<td>&gt;10</td>
<td>?</td>
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<tr>
<td>Portugal</td>
<td>25</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Israel</td>
<td>35</td>
<td>16</td>
<td>60</td>
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<tr>
<td>Georgia</td>
<td>65%</td>
<td>30%</td>
<td>5%</td>
</tr>
<tr>
<td>France (Corsica)</td>
<td>9</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Montenegro</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Citrus pests and pest status
A total of 143 pest problems were reported, including 108 insects, 10 mites, 1 nematode, 14 fungi, 2 bacteria, and 8 virus and virus like diseases (Table 3). The insects include 2 Orthoptera, 66 Hemiptera, 8 Thysanoptera, 13 Coleoptera, 12 Lepidoptera, 1 Diptera, and 6
Hymenoptera. Scale insects are the most diverse taxonomic group representing 40% of citrus insect pests.

From the reported pest problems, 46 were considered key-pests in at least one country, namely 26 insects, 4 mites, 1 nematode, 1 bacterium, 9 fungi, and 5 virus and virus like diseases. Major key-pests, i.e., reported as key-pests in at least 50% of the countries, include the medfly, *Ceratitis capitata* (91% of the countries), the citrus red scale, *Aonidiella aurantii* (73%), the citrus leafminer, *Phyllocnistis citrella* (64%), and the citrus mealybug, *Planococcus citri* (64%). The citrus flower moth, *Prays citri* (45%), the citrus phytophthora gummosis (36%), and the citrus tristeza (36%) were reported as key-pests in 30%-50% of the countries (Table 3).

Quarantine pests recently introduced in the western Mediterranean area include the brown citrus aphid, *Toxoptera citricida*, the African citrus psylla, *Trioza erytreae*, and the citrus snow scale, *Unaspis citri*. These species are in the list of A1 pests regulated as quarantine pests in the EPPO region (EPPO, 2005). Special attention must be paid to *T. citricida* and *T. erytreae*, as they are efficient vectors of citrus tristeza (CTV) and citrus greening (*Liberobacter* spp.), respectively. *T. citricida* was reported for the first time from Madeira Island in 1994 and it was recently detected in the Iberian Peninsula, namely in the North of Portugal and in the Spanish province of Galicia (Ilharco, 2005). *T. erytreae* is actually present only in Madeira and Canary islands.

**Conclusion**

Despite some limitations on the accuracy of the collected information, as data are not equally available in all the countries and it was not possible to guaranty the homogeneity of criteria between experts, we believe this is a valuable preliminary contribution for the characterization of the actual situation of citrus pest management practices, problems and constraints in Mediterranean countries and will constitute a basis for more complete studies to be carried out in the future.

**Acknowledgements**

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**References**


Hemiptera

Aphididae

Cicadellidae

338

3
3

1
0
1
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2
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Turkey

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Spain

Anacridium aegyptium (L.)
Phaneroptera nana Fiebre
Aleurothrixus floccosus (Maskell)
Bemisia hancocki (Corbett)
Bemisia tabaci (Gennadius)
Dialeurodes citri (Ashmead)
Dialeurodes citrifolii (Morgan)
Parabemisia myricae (Kuwana)
Paraleyrodes bondari Peracchi
Paraleyrodes citricolus Costa Lima
Paraleyrodes minei Iaccarino
Aphis craccivora Kock
Aphis fabae Scopoli
Aphis gossypii Glover
Aphis spiraecola Patch.
Aulacorthum solani (Kaltenbach)
Macrosiphum euphorbiae (Thomas)
Myzus ornatus Laing
Myzus persicae (Sulzer)
Neomyzus circunflexum (Buckton)
Ropalosiphum maidis (Fitch)
Toxoptera aurantii (Boyer de Fonscolombe)
Toxoptera citricida (Kirkaldy)
Empoasca decedens (Paoli)
-

Portugal

Acrididae
Tettigoniidae
Aleyrodidae

Morocco

Orthoptera

Montenegro

Insects

Italy

Species

Israel

Family

Greece

Order

France
(Corsica)
Georgia

Group

Algeria

Table 3. Arthropod pests and diseases of citrus and their pest status in Mediterranean countries: 3- key pest, 2- occasional pest, 1- potential pest. 0- not
reported species.

0
0
2
0
2
1
0
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<th>Georgia</th>
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</table>

**Legend:**
- a- lemon, b- clementines, c- early maturing varieties, d- fortune, f- sour orange rootstock, g- young trees, h- nurseries, i – post-harvest, j- natural enemies disruption, k- Andalucia, l- Canary Islands, m- limited areas, n –Algarve, o – North of Portugal, p-Madeira Island, q- old trees, r-Northwest of Algeria, s- near olive groves, t- grapefruits, u- Oeste region, v- sweet orange, w- mandarins, x – mineole tangelo, y- none virulent strain, z- Azores, α- detected in some orchards, β – only *A. citri*
Annex 1 – Questionnaire.

1 – What is the citrus production area in your country (hectares)?

2.1 - What is the percentage of the total citrus production area applying Integrated Pest Management (IPM)?

2.2 – And Integrated Production (IP)?

3 – Do growers receive any financial support for practicing IPM or IP? Yes ☐ No ☐

If you answered NO go to question 6

3.1.- Is the financial support part of the agri-environmental measures of EU? Yes ☐ No ☐

4- What are the requisites for a grower to receive financial support for practicing IPM or IP?

- to be an associate of a IPM/IP farmers organization Yes ☐ No ☐
- to attend a course on IPM/IP Yes ☐ No ☐
- to follow official IPM/IP guidelines Yes ☐ No ☐
- other requisites (please specify):

5- What is the amount of financial support the grower receives per hectare (Euros):

5.1- For practicing IPM?

5.2- For practicing IP?

6- Are the IPM/IP guidelines defined at: national level? ☐ regional level? ☐ both? ☐

7 – Are there certification companies for citrus IPM/IP in your country? Yes ☐ No ☐

8- According to you, what are the major problems/constraints for the development of citrus IPM/IP in your country?

<table>
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<tr>
<th>Level of importance</th>
<th>Low</th>
<th>Medium</th>
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<tbody>
<tr>
<td>Lack of accurate and practical pest monitoring methods</td>
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<tr>
<td>Lack of scientific based economic thresholds</td>
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<tr>
<td>Pest resistance to pesticides</td>
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<tr>
<td>Lack of effective alternative tactics to pesticides (e.g., biological control, biotechnical methods)</td>
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<tr>
<td>Increased complexity in IPM strategies</td>
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<tr>
<td>Increased risk perception by the farmer</td>
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<td>New pest problems (introduction of exotic species)</td>
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<td>Production costs</td>
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<td>Other (please specify)</td>
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</table>

8- How many pesticide active ingredients are actually registered in your country for the control of citrus pests?

- Nº insecticides:
- Nº fungicides:
- Nº herbicides:
9- Please fill the following table by mentioning the citrus pests present in your country according to their pest status (key, occasional or potential pest, not described): usually, key-pests impose the application of control measures most of the years, occasional pests only occasionally reach economic injury level, and potential pests are always or almost always below economic injury levels. If needed, add the name of pest species not included in the list. Else, add a note if the pest differs in pest status according to citrus species or area (for example, *Tetranychus urticae* is a key pest in clementines and lemons, but only occasional or potential in other citrus species; *Chrysomphalus aonidum* is a potential pest in the Canary Islands, but is not present in the rest of Spain). **Please mark with an asterisk the exotic species introduced in the last five years.**