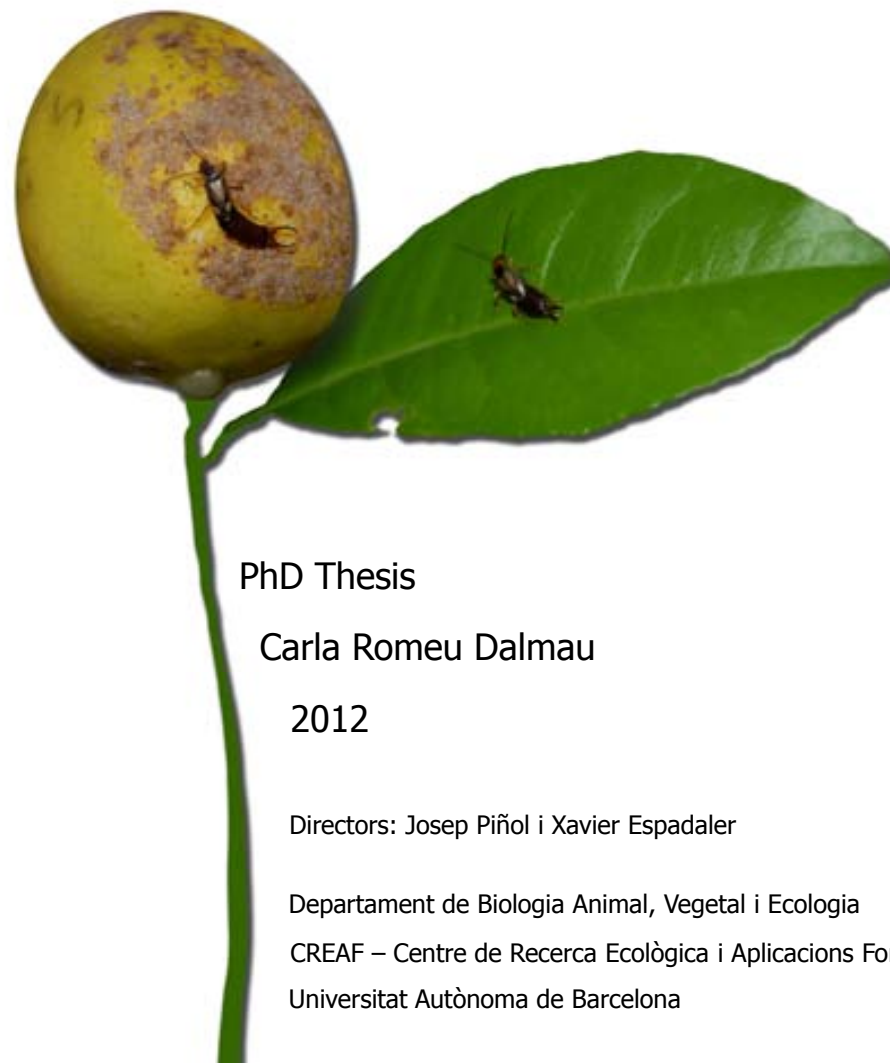# Friend or foe?
The relative role of earwigs as pests versus biocontrol agents in citrus canopies

PhD Thesis

Carla Romeu Dalmau

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Directors: Josep Piñol i Xavier Espadaler

Departament de Biologia Animal, Vegetal i Ecologia

CREAF – Centre de Recerca Ecològica i Aplicacions Forestals

Universitat Autònoma de Barcelona

CREAF

UAB
Universitat Autònoma de Barcelona

Departament de Biologia Animal,

de Biologia Vegetal i d'Ecologia,

Facultat de Biociències

Friend or foe? The relative role of earwigs as pests versus biocontrol agents in citrus canopies

Presentada per:

Carla Romeu Dalmau

Per optar al grau de Doctora

Amb el vist-i-plau de:

Josep Piñol i Pascual,

Catedràtic d'ecologia, UAB

Xavier Espadaler i Gelabert,

Catedràtic de zoologia, UAB

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Per tu, que m'ho has donat tot,

tot deixant-me volar….

Per tu mare

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Mil gràcies a tots i a totes!!

Primer de tot, i amb el cor a la mà, vull donar mil gràcies al Pep i al Xavi. Heu estat dos directors excepcionals, i com sempre dic a tothom que em vol escoltar, aquesta tesis no hauria estat tan genial sense vosaltres. Gràcies als dos per ensenyar-me tant, per fer-me evolucionar, i sobretot, per estar sempre allà, a punt. A tu Pep, gràcies per l'hospitalitat a la Selva, per la coca que no falta mai, i per les mandarines exquisides. A tu Xavi, gràcies per les mil converses, per descobrir-me les meravelles dels insectes, i per les dolces.

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També vull donar les gràcies a la família Cañellas per acollir-me a les seves terres. I en especial a la Nuri, per l'amabilitat i el caliu.

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Abstract

Earwigs (Insecta: Dermaptera) are common in agro-ecosystems. Most studies on earwigs have been conducted in USA, Oceania, and North-Central Europe, and in apple and pear orchards, but very little is known of earwigs in Mediterranean citrus orchards. Earwigs are key insects in orchards because they have been proven effective predators of several pests such as aphids, leafrollers and psyllids. However, as omnivores, earwigs can also have negative effects on plants by feeding on leaves, flowers and soft fruits. In this context, the main objective of this thesis was to study the relative role of earwigs as pests versus biocontrol agents in citrus canopies, and the specific objectives were: (i) to design a method to differentially exclude ants and earwigs from citrus canopies; (ii) to study the abundance, the interannual variation and the potential pest predator role of earwigs in citrus canopies; (iii) to detect aphid predation by earwigs using molecular markers; (iv) to study the earwig's relative role as pest versus predator in Mediterranean organic citrus trees; and (v) to study the earwig's role as pest and as predator in conventional citrus orchards in California. The European earwig, *Forficula auricularia* L., had a longer active period in the Mediterranean than in other colder regions, and it was proven to be an important aphid predator in citrus canopies. A negative relationship between aphid and earwig abundance was observed, suggesting a top-down control of aphids by earwigs. This control was most likely a consequence of European earwig early seasonal pressure on aphids, which would have had a disproportionate effect on the final aphid density. Another species of earwigs was found in citrus canopies, *Forficula pubescens* Gené, which is seldom cited in the literature. This species apparently did not regulate aphid populations, probably due to its late appearance in canopies. To study the relative role of earwigs as pests versus biocontrol agents, earwig contribution to pest management was studied while defining earwig damage to yield. The results showed that earwigs were beneficial insects in the studied Mediterranean organic citrus orchard, as they reduced aphid attack but did not affect the final fruit yield. In addition, when adult earwigs were offered both plant and animal material in greenhouse conditions, they preferred to feed on California red scale rather than on leaves. Thus earwig role as an insectivore appeared to be more important than earwig role as an herbivore, what concurred with other studies in millet and in apple orchards. However, earwigs cannot be considered beneficial insects in orchards as a rule, because as omnivores, earwig status as a pest or as a predator can vary depending on several factors. Thus, the earwig's relative role as pest versus predator needs to be measured in each particular agroecosystem before using earwigs in biocontrol programs. However, earwig omnivore habits should not undervalue earwig contribution to biological control.

Resum

Les tisoretes (Insecta: Dermaptera) són comunes en els sistemes agrícoles. La majoria d'estudis sobre aquest insecte han tingut lloc als EEUU, Oceania, i al nord i centre d'Europa, majoritàriament en cultius de pomes i peres, però hi ha poca informació publicada sobre les tisoretes en el Mediterrani i en conreus de cítrics. Les tisoretes són insectes clau en l'agricultura, ja que s'ha demostrat que són importants depredadores d'algunes plagues com els pugons, les arnes i els psyllids. No obstant això, com a omnívores, les tisoretes també poden tenir efectes negatius sobre les plantes ja que es poden alimentar de fulles, flors i fruits. En aquest context, l'objectiu principal d'aquesta tesis era estudiar el paper relatiu de les tisoretes com a plaga i com a agent de biocontrol de plagues en les capçades dels cítrics, i els objectius específics eren: (i) dissenyar un mètode per excloure diferencialment formigues i tisoretes de les capçades dels cítrics; (ii) estudiar l'abundància, la variació interanual i el potencial de les tisoretes com a depredador de plagues en les capçades dels cítrics; (iii) detectar la depredació de tisoretes sobre pugó utilitzant marcadors moleculars; (iv) estudiar el paper relatiu de les tisoretes com a plaga i com a depredador de plagues en les capçades d'un cultiu de cítrics ecològic al Mediterrani; i finalment (v) estudiar el paper de la tisoreta com a plaga i com a depredador en cítrics convencionals de Califòrnia. La tisoreta europea, *Forficula auricularia* L., va resultar tenir un període actiu més llarg al Mediterrani respecte altres regions més fredes, i també es va demostrar que aquesta espècie és un depredador important de pugó en les capçades dels cítrics. Es va observar una relació negativa entre l'abundància de pugó i la de tisoretes, el que suggereix un control de les tisoretes sobre el pugó. Aquest control probablement és el resultat de la pressió que exerceix la tisoreta sobre el pugó des del primer moment que la plaga arriba al cultiu, fet que tindria un efecte desproporcionat sobre la densitat final de pugó. L'altra espècie de tisoreta present a les capçades estudiades, *Forficula pubescens* Gené, és rarament citada en la literatura. Aquesta espècie aparentment no regula les poblacions de pugons, probablement degut a que arriba tard a les capçades. Per tal d'estudiar el paper relatiu de la tisoreta com a plaga i com a depredador, s'ha d'estudiar la contribució d'aquest insecte en la gestió de plagues així com definir el dany que pot causar sobre la producció. Els resultats mostren que les tisoretes són insectes beneficiosos en el cultiu ecològic de cítrics estudiat, ja que redueixen l'atac del pugó i no afecten la producció final de fruits. A més a més, quan es va oferir tant matèria animal com vegetal a les tisoretes en condicions d'hivernacle, van preferir alimentar-se del poll roig de Califòrnia abans que de fulles. Per tant, en ambdós casos el paper de les tisoretes com a insectívor sembla que va ser més important que com a herbívor, fet que coincideix amb altres estudis realitzats en mill i en pomeres. De totes maneres, les tisoretes no es poden considerar insectes beneficiosos com a norma, ja que com a omnívors, el seu estatus com a plaga o com a insecte beneficiós pot variar depenent de diversos factors. Per tant, el paper relatiu de la tisoretes com a plaga i com a depredador ha de ser mesurat en cada sistema agrícola abans d'utilitzar aquest insecte en programes de biocontrol. No obstant això, els hàbits omnívors de la tisoreta no haurien de menystenir la seva contribució en el control biològic de plagues.

Chapter 1

General introduction

*"Agroecology [...] can play a central role
in achieving the transition towards a
low-carbon, resource-preserving
type of agriculture"* De Schutter, 2010

The application of agroecological principles in organic farming

To feed a crowded world without compromising the future represents a big challenge today. Conventional agriculture will be hard pressed to rise to this challenge because its practices affect future productivity in many different ways (see Table 1 for further information; Gliessman 1998). By contrast, agroecology, which is defined as *the application of ecological concepts and principles to the design and management of sustainable agroecosystems* (Gliessman 1998), can play a central role (De Schutter 2010). Agroecology aims to maintain and/or enhance biodiversity in agroecosystems and to sustainably use water and soil while applying ecological methods to make agriculture economically viable (Gliessman 1998). Different technical scenarios can adopt agroecological principles, such as organic farming in industrial countries or traditional farming in developing countries (Altieri & Nicholls 2005).

Soil degradation	Salting, waterlogging, compaction, contamination by pesticides, decline quality of soil structure, erosion
Waste and overuse of water	Agriculture accounts for 2/3 of global water use while… only 50% of the water applied is used by the plants it is intended for
Pollution	Agricultural pollutants include pesticides, herbicides, fertilizers, salts
Dependence on external inputs	Many of the inputs are non-renewable and their supplies finite Farmers, regions and countries are vulnerable to supply shortages and market fluctuations
Loss of genetic diversity	The overall genetic diversity of domesticated plants has declined due to emphasis on short-term productivity gains, which mainly favors highly productive varieties
Global inequality	Productivity and yields have increased, but hunger persists Huge disparities in calorie intake and food security between countries

Table 1. Practices of conventional agriculture that tend to compromise future needs (Gliessman 1998)

Although much research is still needed, interest in organic agroecosystems has increased among scientists in order to achieve a greater understanding of the mechanisms that operate in such systems. There exist contradictory results as it is difficult to perform comparative studies between different farming techniques, but nowadays it is well accepted that organic farming, as compared to conventional farming, has positive effects on biodiversity (Mäder et al. 2002; Bengtsson et al. 2005; Hole et al. 2005; Macfadyen et al. 2009; Crowder et al. 2010), and that it conserves soil fertility and water resources (Stolze et al. 2000; Mäder et al. 2002; Pimentel et al. 2005). Moreover, even though some studies based on specific cases claim that conventional farms produce more than organic farms, several studies and reviews show that organic farming

may have the potential to produce at least as much as conventional farming (Pimentel et al. 2005; Badgley et al. 2007; Pretty et al. 2007; Perfecto & Vandermeer 2008).
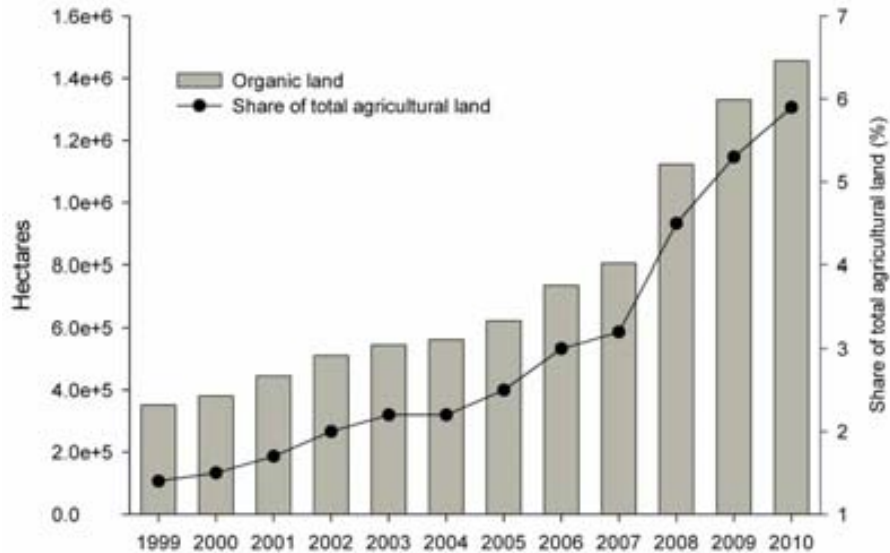

Fig. 1.1. Development of organic agricultural land in Spain from 1999 to 2010 (MARM 2011; www.marm.es/es/alimentacion/temas/la-agricultura-ecologica).

Currently, agroecology principles are supported not only by the scientific community but also by international organizations such as the United Nations Food and Agriculture Organization (FAO) and by diverse countries such as the United States, Brazil, Germany and France (De Schutter 2010). Organic agriculture has grown worldwide during the last two decades, and according to the latest survey, more than 37 million hectares are organic systems today, which represents 0.9% of total agricultural land (information available from 160 countries; Willer & Kilcher 2011). In Spain, organic areas increase year after year, and in 2010 almost 1.7 million hectares were organically managed which represents 5.9 % of total agricultural land (fig. 1.1). Organic citrus orchards occupy 5391 ha, 67% in Andalucía (Willer & Kilcher 2011).

Biological control of pests in citrus orchards

Organic farming, by increasing diversity among natural enemies, tends to improve the natural control of pests (Crowder et al. 2010; but see Macfadyen et al. 2009). Natural enemies are predators, parasites, parasitoids and diseases that exercise a natural control of an organism's population. When the target of such regulation is a pest, this natural control is called biological control (biocontrol) (Hajek 2004).

The first written records of biological control of pests come from citrus orchards. As early as in the fourth century, Chinese growers enhanced populations of the weaver ant, *Oecophylla smaragdina* (F.) to control citrus pests such as the stinkbug *Tessaratoma papillosa* Drury in citrus orchards (Urbaneja et al. 2008; Van Mele 2008). Moreover, at the end of the XIX century, the introduction of the Vedalia bettle *Rodolia cardinalis* (Mulsant) in California controlled cushion scale *Icerya purchasi* (Maskell) populations, and saved the citrus industry from this destructive pest (Hajek 2004). Since then this record is considered the biological control paradigm (Urbaneja et al. 2008).

In citrus orchards, neuropterans, coleopterans, heteropterans, dermapterans, spiders and parasitoids play an important role in naturally controlling many herbivores (Alvis et al. 2002; Soler et al. 2002; Ribes et al. 2004; Cañellas et al. 2005; Urbaneja et al. 2006; Urbaneja et al. 2008). More than 90 herbivores have been described so far feeding on citrus orchards, but not all of them are considered pests, as many are controlled by their natural enemies (Urbaneja et al. 2008). However –occasionally- some of these herbivores may not be properly regulated and become a pest. These are the so-called occasional pests, such as the woolly whitefly *Aleurothrixus floccosus* (Maskell), the citrus leafminer *Phyllocnistis citrella* Stainton, scales such as the Chinese wax scale *Ceroplastes sinensis* Del Guercio, and aphids such as the green citrus aphid *Aphis spiraecola* Patch and the cotton aphid *Aphis gossypii* Glover (Urbaneja et al. 2008). All of these pests are present in the Mediterranean clementine orchard under study in this thesis (see below), and *A. gossypii* and *A. spiraecola* are among the most abundant.

Even though aphids are not considered key pests in citrus, they can seriously affect citrus orchards by the indirect transmission of virus such as Citrus tristeza (CTV; Closteroviridae; genus *Closterovirus*), the most serious virus disease of citrus worldwide (Michaud 1998). The most efficient vector of CTV is the citrus brown aphid *Toxoptera citricida* (Kirkaldy), which is 25 times more efficient transmitting CTV than *A. gossypii*, the most efficient vector in the Mediterranean Basin (Belliure et al. 2008; Urbaneja et al. 2008). In Spain, *T. citricida* has already been found in the northwest (Hermoso de Mendoza et al. 2008), but there are no records of its presence in citrus most productive regions, such as Valencia. Today, CTV is under control in Spain as most rootstocks are CTV-tolerant to the isolates now present. However the brown citrus aphid may carry hazardous CTV isolates that may generate new problems to the Spanish citrus industry (Cambra et al. 2000). In this context, the integration of biological control techniques with cross protection (the protection of plants with mild CTV strains; Moreno et al. 2008) may have the potential to mitigate the CTV threat.

Finally, in citrus orchards there are other herbivores that are not significantly impacted by their natural enemies and may require chemical treatments for their regulation (Urbaneja et al. 2008). This is the case of the California red scale *Aonidiella aurantii* (Maskell), the red spider

mite *Tetranychus urticae* Koch and the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). The California red scale and the red spider mite are probably present in the organic citrus orchard under study in this thesis albeit in low numbers. The Mediterranean fruit fly is present in higher densities but it is not considered dangerous to the variety present at the study site, "Clemenules" clementines, as *C. capitata* mainly causes damage to early-ripening varieties (Martinez-Ferrer et al. 2011).

The study site

In this thesis, earwigs (Insecta: Dermaptera) were studied as natural enemies of pests in citrus canopies. Nearly all studies were performed in an organic citrus orchard located in Spain (La Selva del Camp, Tarragona, NE Spain; 41° 13' 7″N, 1° 8' 35″E; fig. 1.2a). This orchard consists of ca. 300 clementine trees grafted onto the hybrid rootstock Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb.). One study was located in a tango-mandarin orchard in California (Exeter, United States; 36° 22' 23″ N, 119° 03' 45″ O; fig. 1.2b). Both countries, Spain and US, are leaders in citrus production, along with Brazil, China, and Latin-American countries such as Mexico and Argentine (Spreen 2003).

This thesis is framed in an on-going broader project that started in 2002 in the above-mentioned clementine orchard. The main objective of this project was to study the ecological interactions that occur in a community of arthropods in citrus canopies.

a)

b)

Fig. 1.2. Study sites; (a) organic clementine orchard located in La Selva del Camp (Spain); (b) tango-mandarin orchard located in Exeter (California).

One of the first studies within this project aimed to use ant-exclusion methods to control aphid populations (Piñol et al. 2009a). As ants (Hymenoptera: Formicidae) are well-known aphid mutualists (Hemiptera: Aphidoidea), the hypothesis was that ant-excluded trees would have less aphids than control trees, but it turned out just the opposite. Piñol et al. (2009a) argued that the most plausible reason of this unexpected result was that the exclusion of ants from citrus canopies was concurrently excluding an important aphid predator: earwigs.

To further study aphid population dynamics, in Piñol et al. (2009b) a mechanistic model was developed and tested using the data obtained in the above-mentioned ant-exclusion experiments. The model considered two kinds of aphid antagonists: omnivorous or generalist predators that are always at the site (sedentary predators exemplified by earwigs) and specialists that arrive in important numbers only once the aphid population has already developed to some degree (non-sedentary predators such as coleopterans, heteropterans, dipterans and neuropterans). The model showed that even low densities of sedentary predators (earwigs) could have a disproportionate effect on aphid populations during the main spring peak, as they preyed on small populations and thus the per capita effect on aphid populations was higher. By contrast, the role of non-sedentary predators was secondary during this peak.

In Piñol et al. (2010), not only ants but also birds were excluded from canopies during one year to study ant versus bird exclusion effects on the arthropod assemblage. The results showed that both the exclusion of ants and birds affected the arthropod community, but that the exclusion of ants was far more important than the exclusion of birds. Almost all groups of arthropods had higher abundance in ant-excluded trees compared to control trees, whereas only earwigs were more abundant in bird-excluded than in control trees.

Finally, a fourth study has shown that in general, the exclusion of ants from tree canopies has positive effects on the arthropod assemblage (Piñol et al. 2012). However, they concluded that not only ants but also earwigs were affecting the arthropod community. They also highlighted the importance of long-term experiments, as they observed that the 8-year duration of the experiment could be divided into two periods with contrasting results probably due to differences in ant and earwig abundance.

All aforementioned studies revealed earwigs as key insects in this clementine orchard. In this context, this thesis was intended to study in detail earwig role in this particular agroecosystem.

Biology and ecology of earwigs

Earwigs (Insecta: Dermaptera) are common insects in agroecosystems. They are generally omnivores, feeding on both plant and animal material (Albouy & Caussanel 1990). Earwigs are essentially nocturnal; they forage at night and seek dry and cool places to hide during the day (Albouy & Caussanel 1990). The life cycle of earwigs has been studied thoroughly, mainly due to their trait as presocial insects. Most earwigs take care of their eggs and feed and protect early instars in their subterranean nests (Vancassel & Foraste 1980; Albouy & Caussanel 1990).

In the studied citrus orchard, five species of earwigs were found - all mentioned in the two reports on Spanish Dermaptera (Lapeira & Pascual 1980; Herrera 1999) -: *Euborellia moesta* (Gené), *Euborellia annulipes* (Dohrn), *Nala lividipes* (Dufour), *Forficula auricularia* and *Forficula pubescens* Gené (= *Guanchia pubescens*) (unpublished information). However, only two of these species are regularly found in citrus canopies: the European earwig *F. auricularia* and *F. pubescens*.

The European earwig, *Forficula auricularia*

Due to its worldwide distribution, the European earwig *Forficula auricularia* L. (fig. 1.3) is the best-studied earwig species. Many questions about its biology have been addressed in the literature. For instance, it is known that European earwig maternal behavior is crucial for increased larval survival (Kölliker 2007; Kölliker & Vancassel 2007) and that from the second instar onwards, nymphs leave the nest and start the free-foraging phase in the surface until they enter the soil again in autumn to overwinter as adults (Behura 1956; Vancassel & Foraste 1980). It has also been described that although some females die before their nymphs have matured, others lay a second batch of eggs one month after the first eggs have hatched (Fulton 1924; Crumb et al. 1941; Burnip et al. 2002), and that these two reproductive strategies probably belong to two different sibling species (Wirth et al. 1998). Moreover, other studies examined male forceps length dimorphism (Tomkins & Simmons 1998; Tomkins 1999; Forslund 2003; Tomkins & Brown 2004; Tomkins & Moczek 2009), and addressed different behavioral aspects such as aggregation (Hehar et al. 2008), aggression (Dobler & Kölliker 2009), reproduction (Forslund 2000; Walker & Fell 2001), and dispersion (Lamb 1975; Moerkens et al. 2010).

The European earwig, as an omnivorous insect common in orchards, is frequently studied as key predator of pests and/or as pest in its own right. As insectivore, the European earwig has been considered a key biological control agent for some important pests. For instance, it is an active predator of the woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Mueller et al. 1988; Nicholas et al. 2005), the leafroller *Epiphyas postvittana* (Walker) in apple orchards and vineyards (Suckling et al. 2006; Frank et al. 2007), and the pear psyllid *Cacopsylla pyri* L. (Jauset et al. 2005; Höhn et al. 2007). As herbivore, the European earwig is perceived as pest since it may feed on plant material (Brindley 1918; Fulton 1924; McLeod & Chant 1952; Grafton-Cardwell et al. 2003); however, no literature has been found that actually measures to what extent earwigs might affect the tree performance. An example of this dual perception of the European earwig in orchards is illustrated by the literature that screen insecticide effects on earwigs. While some studies screen insecticides to control earwig populations (Bradley & Mayer 1994; Colvin & Cranshaw 2009), others examine European earwig susceptibility to insecticides only with the purpose to protect this species (Sterk et al. 1999; Epstein et al. 2000; Nicholas &

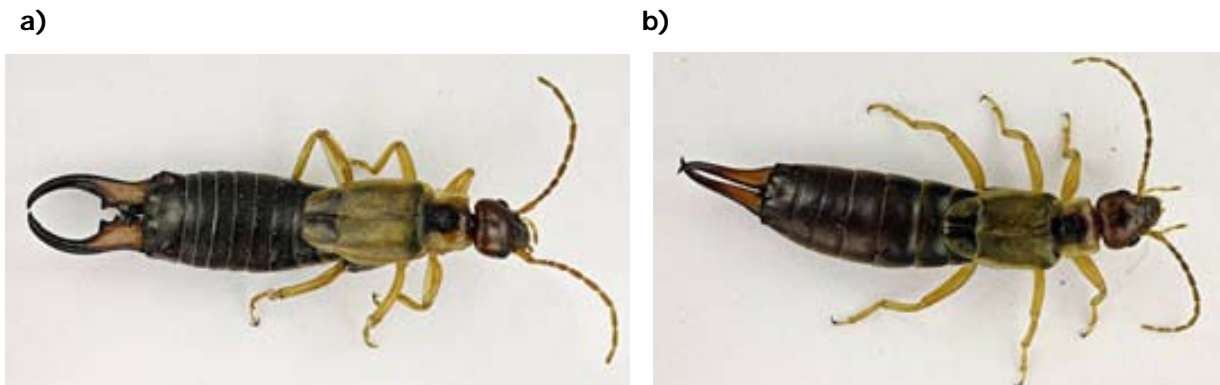Thwaite, 2003; Badji et al. 2004; Maher et al. 2006; Shaw & Wallis 2010).

a)   b)

Fig. 1.3. European earwig, *Forficula auricularia* L., (a) male, (b) female. Author: A. Viñolas.

Forficula pubescens Gené

Knowledge of other earwig species than the European is limited to their presocial condition (Kohno 1997; Vancassel & Foraste 1980; Suzuki et al. 2005; Matze & Klass 2005; Kamimura 2003) and to evolutionary aspects (Jarvis et al. 2005; Kamimura 2006; Tworzydio et al. 2010) and in general, little is known of most earwig species (see Earwig Research Center website, http://www.earwigs-online.de/, by Fabian Haas, for further information related to earwigs worldwide). This is the case of *Forficula pubescens* (fig. 1.4), which is hardly cited in the literature. Only four studies have been found that report on *Forficula pubescens*. Under laboratory conditions, Herter (1964) studied *F. pubescens* reproduction and Sauphanor & Sureau (1993) evaluated the aggregation behavior and the interspecific relationships between five earwig' species, being *F. pubescens* one of them. In the field, Debras et al. (2007) and Dib et al. (2010) studied both *F. auricularia* and *F. pubescens* as pear psylla *Cacopsylla pyri* and as rosy apple aphid *Dysaphis plantaginea* Passerini predators (respectively).

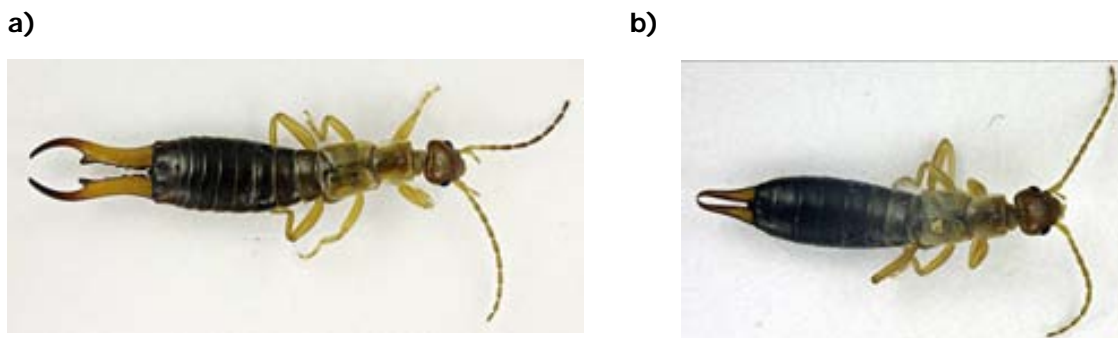a)   b)

Fig. 1.4. *Forficula pubescens* Gené, (a) male, (b) female. Author: A. Viñolas. Please note that *F. pubescens* size is smaller related to that of *F. auricularia* (for further information related to earwig sizes see Fig. s2.1 in Chapter 2)

Diet analysis

There are two main ways to study an animal diet: by direct observation or by gut-content analysis of field-collected individuals (Symondson 2002; Hardwood & Obrycki 2005). Direct observation is difficult in arthropods as they can be disturbed easily (Symondson 2002). Moreover, direct observation in the field of insects with nocturnal habits, such as earwigs (Albouy & Caussanel 1990), is far more complicated. As an alternative method, gut-content analysis can identify food remains in individuals collected in the field and thus provide more reliable information (Symondson 2002). Different methods have been used to identify food remains in animal guts, including dissection, radio-isotope labelling, stable isotopes, electrophoresis, chromatography, monoclonal antibodies and DNA-based analysis (reviewed in Symondson 2002, and in Hardwood & Obrycki 2005). Currently, the DNA-based approach, using the polymerase chain reaction (PCR) amplification of the DNA of food remains is the most common method for diet analysis (King et al. 2011). However, if not only an animal's diet is studied but also its trophic level, stable isotope analysis ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) is the tool to be used (Gannes et al. 1997). Both techniques (DNA-based analysis and stable isotope analysis) are today the most promising approaches to explore food webs (Carreon-Martinez & Heath 2010).

In this thesis the DNA-based approach was used to track aphid predation by earwigs.

Aims and outline of the thesis

As earwigs are omnivore insects commonly found in orchards, but very little is known of earwigs in the Mediterranean region or in citrus orchards, this thesis studied earwigs in Mediterranean organic citrus canopies with the aim to examine their relative role as pests versus biocontrol agents in this particular agroecosystem. In all chapters but one (Chapter 2) earwigs were the main characters. The scenario was a Mediterranean organic citrus orchard in all chapters but one, where earwigs were studied in conventional California citrus orchards (Chapter 6). Finally, all chapters were published or are intended to be published in peer-review journals, except for Chapter 6, which was published in a farmer's journal.

The specific objectives of the thesis were:

Chapter 2 - To design a method to differentially exclude ants and earwigs from citrus canopies

In previous studies it was not possible to empirically distinguish between earwig and ant effects on aphid populations, because the method used to exclude ants also excluded earwigs (Piñol et al. 2009a, 2009b, 2010 & 2012). In Chapter 2, a new method was presented that permits the differential exclusion of ants and earwigs. However, as ants are far more studied than earwigs, the method was published to be used for the differential exclusion of ants based on ant body size, and no references to earwigs were made. For this reason, a supplementary section was added at the end of this chapter to illustrate that the method published is also effective in excluding earwigs without excluding ants. This method was finally not applied in this thesis.

Chapter 3 - To study the abundance, the interannual variation and the potential pest predator role of earwigs in citrus canopies

In Chapter 3, the abundance of *F. auricularia* and *F. pubescens* in the organic citrus orchard under study was described, the interannual variation of earwig abundance during a five-year period was measured, and the interspecific association of both earwig species was examined. Finally, their potential role as pest predators was evaluated by exploring the relationship between aphid and earwig abundance and by comparing prey and predator abundance for other citrus pests.

Chapter 4 - To detect aphid predation by earwigs using molecular markers

In Chapter 4 aphid DNA was detected in earwigs to track aphid predation rates by the European earwig under field conditions during aphid main spring peak. Group-specific PCR primers for aphids were designed, and the detectability half-life of aphid DNA in earwigs was measured by laboratory feeding trials.

Chapter 5 - To study the earwig's relative role as pest versus predator in Mediterranean organic citrus trees

In Chapter 5 earwig-exclusion experiments were performed during two years to compare aphid attack, flower survival and fruit yield in trees with earwigs (control trees) with that in trees without earwigs (banded trees). However, as in banded trees not only earwigs but also all other crawling insects were excluded (as explained above), a third group of trees (earwig trees) was added where crawling insects were excluded but earwigs were periodically added to the canopy.

Chapter 6 - To study the earwig's role as pest and as predator in conventional citrus orchards in California

In Chapter 6 earwig-exclusion experiments were performed in the field during five months to study earwig feeding on leaves. Moreover, a set of greenhouse experiments were conducted to study earwig damage to seedlings and also to study earwigs as California Red Scale' predators. Finally, pesticide trials were also performed to determine which insecticides affect earwigs. Chapter 6 results - although interesting - are preliminary. As the manuscript was published in a farmer's journal, a supplementary section was added at the end of this Chapter to give further information on the methodology used and on the statistical outputs, which were not published.

A simple method to differentially exclude ants from tree canopies based on ant body size

Abstract

Ants are ubiquitous components of most terrestrial communities. Ant-exclusion from tree canopies using sticky barriers is frequently used to ascertain the ant's role in the community. This method is very effective but cannot differentiate between ant species, and excludes them all from the canopy. Here we describe a simple method which permits free access of small ants to the canopy, and excludes ants larger than a defined size. This method is flexible in the sense that can be adapted to decide to some degree which species of ants in each particular community are allowed access to tree canopies.

Introduction

Since the pioneer work of Paine (1966), the experimental exclusion of predators has been used as a major tool to reveal species interactions in ecological communities. One major drawback of this methodology is that a wide range of organisms is normally excluded. In terrestrial communities, the most frequently excluded predators are ants. In the meta-analytic study of Schmitz et al. (2000) of trophic cascades in terrestrial systems, 58% of the reported studies were ant-exclusion experiments. Ants are normally excluded from trees by placing sticky barriers (Samways & Tate 1985) or slippery substances (Lenoir 2003) on the trunk. These exclusion systems do not discriminate among ant-species but exclude them all. Occasionally, for some experiments it could be necessary to selectively exclude a group of ants rather than the entire ant community. The reason might be, for example, that some ant species are mainly arthropod predators, whereas others are hemipteran-mutualists or leaf-cutting species (Beattie 1985).

Here we describe a method intended to exclude only some ant species from tree canopies. The method is based on the body size of the ant species. We used the classical sticky barrier on the trunk, but underneath we placed two plastic tubes of a certain diameter which granted access to the canopies to a subset of small ant-species. The internal diameter of the plastic tube is clearly the key criterion. To decide the appropriate diameter, an a *priori* knowledge of the ant community of the study site is needed, with an accurate estimate of ant size within the community. We demonstrate the method with the ant-community of an organic citrus grove in the Mediterranean.

Materials and methods

We tested the method in an organic plantation of citrus trees located at La Selva del Camp (Tarragona, NE Spain; 41° 13' 7″N, 1° 8' 35″E). The climate is Mediterranean, with a rainy spring and autumn and a dry winter and summer. The plantation consists of ca. 320 Clementine trees grafted on the hybrid rootstock Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb.). More details about the study site can be found in Piñol et al. (2009a).

The experiment was conducted with 10-year-old trees separated in three rows of 23 trees each. Each row was divided into three blocks. In each block, three adjacent trees were randomly assigned to one of three treatments: control trees (n=9), with no trunk barriers; banded trees (n=9), where all ants were excluded using a polibutene-based sticky barrier (Rata Stop®) applied to an alimentary plastic sheet tightly attached over a padding cylinder in contact with the trunk; and tube-banded trees (n=9), with the same sticky barrier as banded-trees but with

two translucent plastic tubes (Neoplast Ultravinil®) of 3 mm of internal diameter underneath the padding cylinder (fig. 2.1). Treatments were established in April 2009 and trees were sampled in May, June, July and August. Twice a month we checked that the sticky surface of barriers had not become saturated with trapped insects that ants could use as stepping stones to reach the canopy. Also, tall grasses and forbs were trimmed so they would not act as bridges for ants to access the canopy.
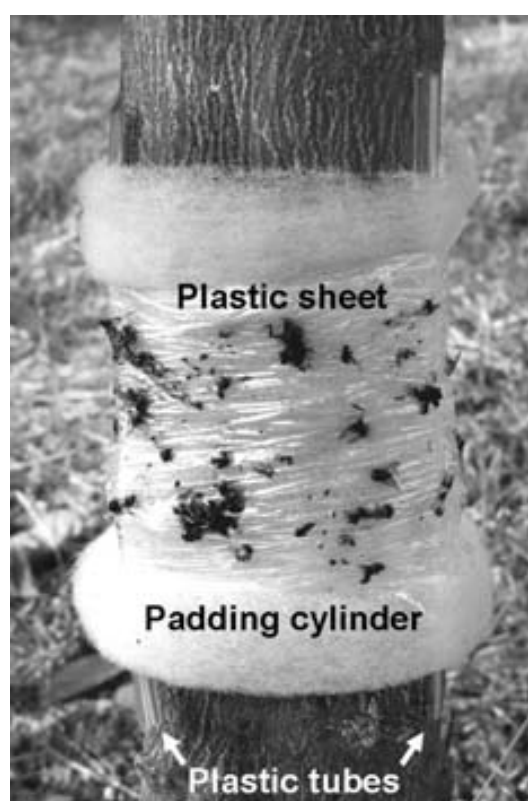

Fig. 2.1. Photograph of the tube-banded exclusion method. Two translucent plastic tubes (Neoplast Ultravinil®) of 3mm of internal diameter oppositely placed under a padding cylinder (covered with an alimentary plastic sheet thick with polibutene-based sticky barrier, Rata Stop®). Notice some dead insects stuck into the glue barrier.

Arthropods on the tree canopy were sampled once a month using beating trays (three vigorous hits to the tree crown in two opposite directions), captured with entomological pooters and preserved in 70% alcohol. Ants were counted and identified in the laboratory to species level.

To decide the internal diameter of the tubes inserted under the sticky barrier on tube-banded trees, we needed to know our ant community species composition, and the ant species' sizes. The prior knowledge of the ant community was from previous studies of this arthropod community (Piñol et al. 2009a). Ant width was estimated by measuring the scape length (SL)

and the distance between the center of antennal sockets or articular condyles (AC). The measure of ant size used was: 2*SL + AC (see inset in fig. 2.2). When walking, ants' scape is normally almost perpendicular to the body axis. We therefore used scape span as an estimate for body width. Ten workers of each ant-species in the community were measured. The use of a 3 mm tube of internal diameter was basically intended to allow access to the canopies for *Lasius grandis* Forel and to exclude *Formica* sp. (fig. 2.2). *L. grandis* is an important aphid-mutualist whose effectiveness is based on mass recruitment, whereas *Formica* sp. are also aphid-mutualists but they forage individually. *L. grandis* and *Formica* sp. are the dominant species in the community.
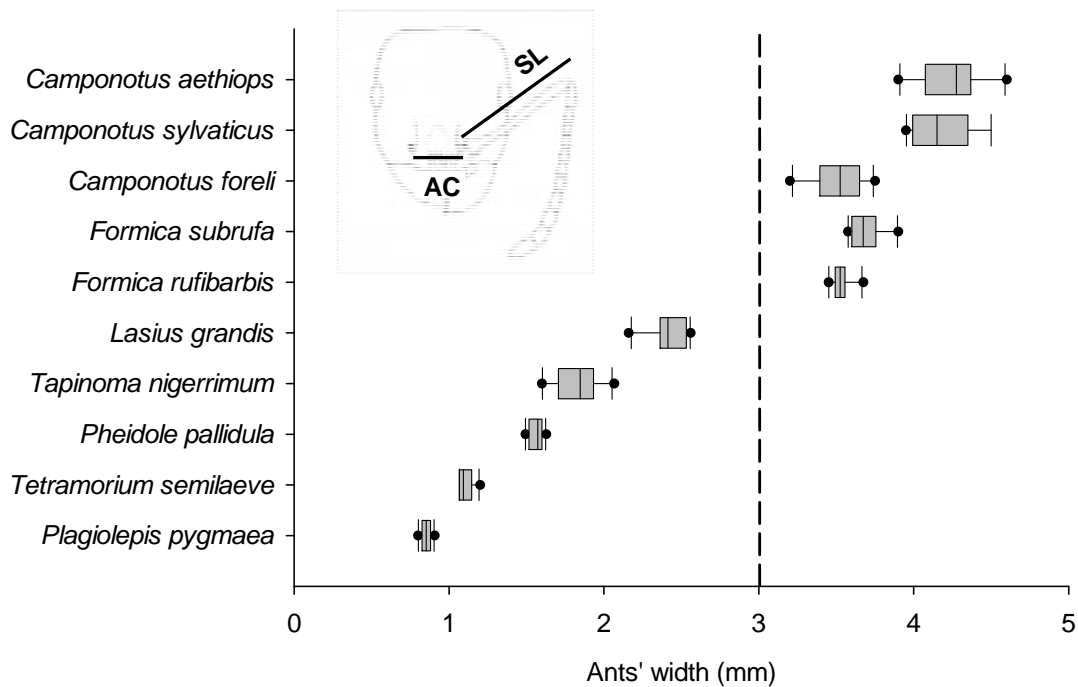

Fig. 2.2. Boxplots (median, 10th, 25th, 75th and 90th percentiles) of ten individuals' width of each ant species. The vertical dash line at 3 mm indicates the internal diameter of the tubes used in the experiment. A picture of a *Messor* sp. shows the distances measured to obtain a proxy of ants' width (width = 2 * SL + AC).

Data analysis

Ants were divided in two groups: large ants, which included *Formica* sp. and *Camponotus* sp., and the remaining small ants (fig. 2.2). We report cumulative abundance of both groups per tree by summing the abundance measured in each month. Log-transformed ant abundance of

each treatment was compared using one-way ANOVA's with treatment as fixed factor and block as a random factor. Post-hoc Tukey HSD tests were conducted following significant effects of treatment. The software used was SPSS 15.0.

Results

Nine species of ants were found in control trees, the most abundant being *Lasius grandis* (56%), *Tapinoma nigerrimum* (Nylander) (20%), *Formica rufibarbis* Fabricius (10%) and *Formica subrufa* Roger (8%). The other five less abundant species (from higher to lower abundance) were: *Plagiolepis pygmaea* (Latreille), *Camponotus sylvaticus* (Olivier), *C. aethiops* (Latreille), *C. foreli* Emery and *Tetramorium semilaeve* André. *Pheidole pallidula* (Nylander) was found in low numbers in tube-banded trees but not found in control trees. *Lasius grandis* comprised 69% of the small ants found on control trees, while 96% of the large ants' group were *Formica* sp.
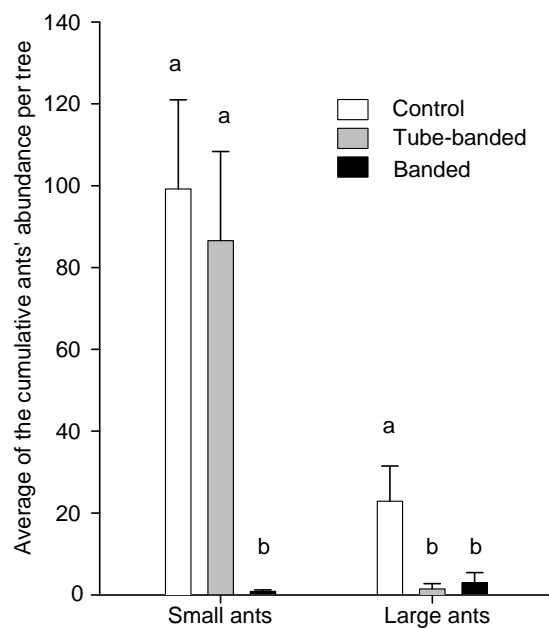
Fig. 2.3. Mean (+SE) values of the cumulative abundance of small and large ants sampled using beating trays (measured as the sum of abundance of workers per tree between May and August). Different letters on the top of columns indicate groups that were significantly different (Tukey HSD post-hoc test at *P*<0.0001).

Small ants' abundance differed significantly depending on the treatment ($F_{2,16}$ = 74.47, $P < 0.0001$). The post-hoc analysis revealed that control and tube-banded averages were not

significantly different (P = 0.69), indicating that small ants could overcome the sticky barrier by passing through the tubes (fig. 2.3). In tube-banded trees, *Lasius grandis* contributed 62% of the total number of small ants found. The effect on small ants abundance was already observable at the first sampling date, May 13th ($F_{2,16}$ =23.87, P<0.0001, and the post-hoc analysis revealed no differences between control and tube-banded trees, P=0.39). This result indicates that ants were able to find and use the tubes 25 days after establishing the experiment.

The treatment was also significant for large ants ($F_{2,16}$ = 19.95, P < 0.0001). Despite finding some large ants in both banded and tube-banded trees, their abundance was significantly lower than in control trees (P < 0.0001 for both banded treatments *vs.* control; Tukey HSD post-hoc test).

Discussion

In our experiment, we obtained trees with (1) small ants, especially *Lasius grandis,* but with almost no large ants (tube-banded trees), (2) trees with all ant species (control trees), and (3) trees almost with no ants (banded trees). These were the differences expected between treatments, and proved the size-selection efficacy of using a tube of 3 mm of internal diameter for the ant-community characteristics of our study site.

Tube diameter can be selected depending on the size of ant species in a particular community or on the objectives of study. If desired, more than three factor levels can be obtained by using tubes of different diameters. For instance, it would have been possible to provide trees with (1) all small ant species except *L. grandis* using tubes of 2 mm of internal diameter under the sticky barrier (fig. 2.2); (2) all small ant species up to *L. grandis*, using tubes of 3 mm under the sticky barrier; (3) no ants, using the traditional sticky barrier; and (4) all ant species (no treatment). The described method could also be useful in small exclusion plots in herbaceous communities that might use sticky barriers to keep ants away, such as those studied in Sanders & Platner (2007).

This differential ant exclusion system depending on body size might provide an opportunity to study the effect of individual or groups of ant species in comparison to other ant species, or to the entire ant community. In most habitats, the ant community shows a large range of body sizes. For instance, Mediterranean local ant communities have a range of 2.4 orders of magnitude (Gómez & Espadaler 2000). Across Central and Northern Europe, *Formica* and *Lasius* species normally coexist (Seifert 2007), albeit with different species sizes to the ones described here, but *Formica* always larger than *Lasius*. Savolainen et al. (1989) describe a taiga-inhabiting

ant community composed of (in decreasing size) *Camponotus, Formica,* and *Lasius*, which shows structure in its competition and hierarchy. Sanders & Platner (2007) studied the interaction between ants and spiders in grassland plots in Hesse (Germany). The community included *Myrmica sabuleti* Meinert (a predatory ant), *Lasius flavus* (Fabricius), and *L. alienus* (Förster) (two hemipteran-mutualists) as the dominant ant-species. In all these cases, the method described here could have helped to separate the effect of some or each ant species on other components of the ecosystem.

A potential problem with the method described is variability in size within polymorphic species, as small morphs could pass through the tubes but not the larger morphs. Approximately 85% of ant genera are monomorphic (figure 1.1 of Oster & Wilson 1978). In some of the remaining 15% genera, worker subcastes may be involved in largely different tasks, like in leaf-cutting ants, where very small workers are solely involved in tending the nest's fungal culture or the small brood (Wilson 1980). In dimorphic species, major (soldier) workers are specialized in defence or assault (Wilson 1976). For *Pheidole pallidula,* the only dimorphic species in the community studied, we used the AC of minor workers. Major workers of *P. pallidula* are only rarely seen in tree canopies, and none was found in this experiment.

In summary, the technique described here has potential useful application to many situations where there are ant species of different size (i.e. almost everywhere) and where there is the need to identify the specific contribution of individual ant species to some biological effect. In particular, it would be especially interesting to use differential exclusion of ant species based on their body-size when they belong to different functional groups.

Acknowledgements

Supplementary material

The method presented in this Chapter is also useful to differentially exclude earwigs and ants from tree canopies. As shown in both fig. s2.1 and fig. s2.2, adults of *Forficula auricularia* L. cannot access the canopy when the internal diameter of the tubes is 3 mm[1]. The other earwig species present in the study site, *Forficula pubescens* Gené, failed to access canopies through tubs even though it is capable according to its body size. One possible reason for this result might that there exist a negative interaction between ants and this earwig species. Einser (1960) reported ants as earwigs predators, showing that one earwig could be killed by a swarm of *Pogonomyrmex badius* (Latreille) workers. Ants, which are much more numerous than earwigs, might find the tubes sooner and when they are established, earwigs such as *F. pubescens* might not use this way to access canopies.
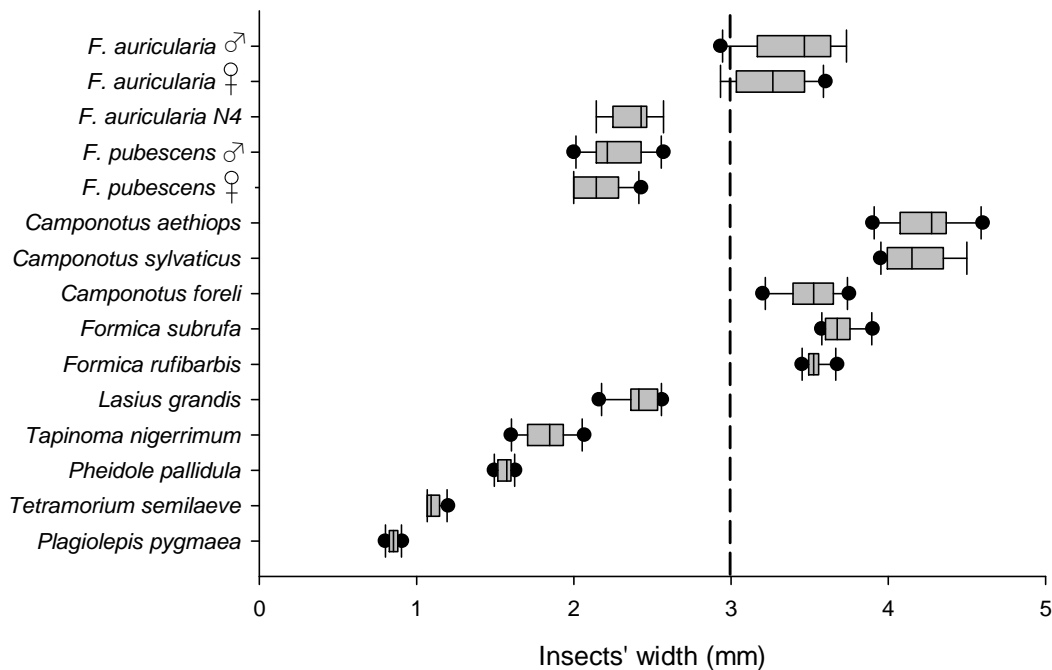
Fig. s2.1. Boxplots (median, 10th, 25th, 75th and 90th percentiles) of ten individuals' width of each species. The vertical dash line at 3 mm indicates the internal diameter of the tubes used in the experiment. 'N4' means fourth instar nymphs.

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[1] To measure earwigs width, the wider part of the individual was measured, which almost in all cases corresponded to the abdomen, although in a few male adults it corresponded to the forceps.
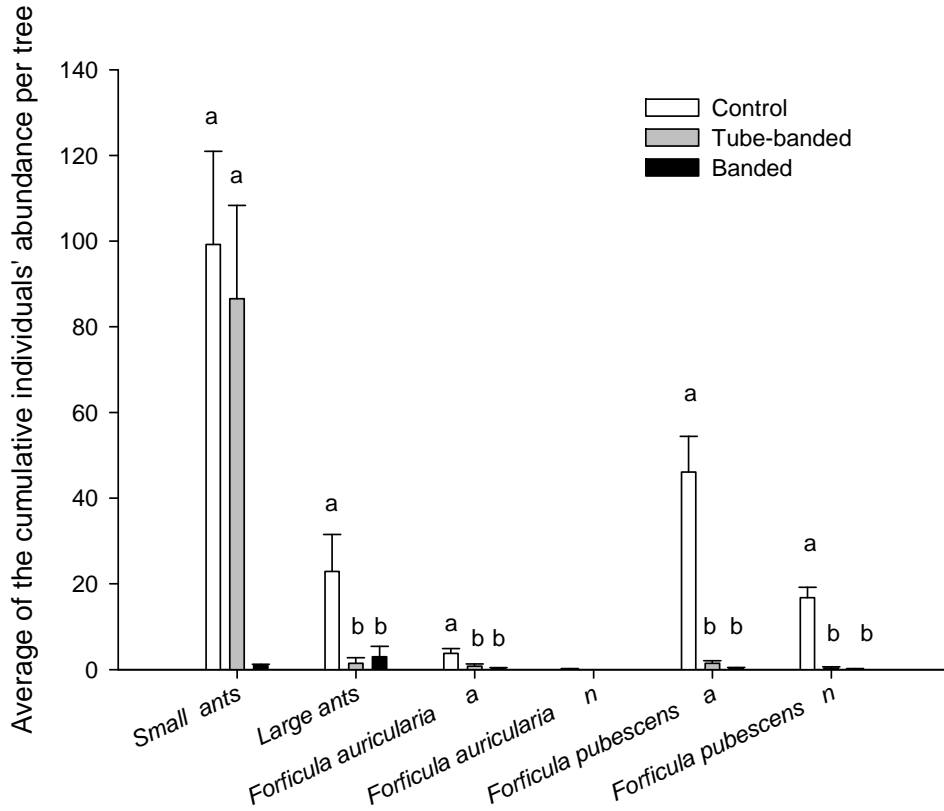
Fig. s2.2. Mean (+SE) values of the cumulative abundance of ants and earwigs sampled using beating trays (measured as the sum of abundance of individuals per tree between May and August). Different letters on the top of columns indicate groups that were significantly different (Tukey HSD post-hoc test at $P < 0.0001$). 'a' means adults and 'n' nymphs.

Chapter 3

Abundance, interannual variation and potential pest predator role of two co-occurring earwig species in citrus canopies

Abstract

Earwigs are usually considered pest predators in orchards. Due to its worldwide distribution, most research on earwigs focuses on the European earwig *Forficula auricularia* L. (Insecta: Dermaptera: Forficulidae). However, very little is known of this species in Mediterranean citrus orchards. Earwigs and aphids were collected monthly during five years (2006-2010) from citrus canopies. Two species of earwigs were found: *F. auricularia* and *Forficula pubescens* Gené (= *Guanchia pubescens*), with the latter seldom cited in the literature. The goals of this study were (i) to document the abundance of these two earwig species in Mediterranean citrus canopies; (ii) to determine whether they are positively or negatively associated with each other, or randomly distributed; (iii) to measure the interannual variation of the abundance of both species during a five-year period, and (iv) to evaluate the potential role of earwigs as pest predators in citrus canopies. As compared to colder regions, *F. auricularia* active period in citrus canopies in our study site lasted longer. Both species co-occurred randomly in canopies. In 2006 both species showed approximately the same abundance, but in 2010 *F. pubescens* abundance in canopies was 28 times greater than that of *F. auricularia*. The potential role of earwigs as pest predators is higher in the Mediterranean than in other colder regions due to the longer active period. *Forficula auricularia* is a sedentary generalist predator, already present in citrus canopies at the onset of most pest outbreaks, while *F. pubescens* arrived later to the canopies, but most likely was abundant enough to contribute in the control of citrus pests.

Introduction

Earwigs (Insecta: Dermaptera: Forficulidae) are common insects in agro-ecosystems. They are mainly omnivorous, feeding on both plant and animal material (Albouy & Caussanel 1990). Earwigs are essentially nocturnal; they forage at night and seek dry and cool places to hide during the day (Albouy & Caussanel 1990). The life cycle of earwigs has been studied thoroughly, mainly due to their trait as presocial insects. Earwig females take care of their eggs and feed and protect early instars in their subterranean nests (Vancassel & Foraste 1980; Albouy & Caussanel 1990). This maternal behavior has been proven crucial for increased larval survival (Kölliker 2007; Kölliker & Vancassel 2007).

Because of its worldwide distribution, the European earwig, *Forficula auricularia* L., is the best-studied earwig species. Many questions about European earwig biology, phenology, natural habitats, dispersal rate, food habits and genetic aspects have been addressed in the literature (i.e. Behura 1956; Albouy & Caussanel 1990; Wirth et al. 1998; Moerkens et al. 2009, 2010 & 2011). However, knowledge of other earwig species is limited to their presocial condition (Kohno 1997; Vancassel & Foraste 1980; Suzuki et al. 2005; Matze & Klass 2005; Kamimura 2003) and to evolutionary aspects (Jarvis et al. 2005; Kamimura 2006; Tworzydio et al. 2010). In general, little is known of the ecology of most earwig species.

Earwigs, as omnivorous insects often found in orchards, are frequently studied as key predators of pests and/or as pests themselves. As insectivores, earwigs have been considered key biological control agents for some important pests. For instance, the European earwig is an active predator of the woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Mueller et al. 1988; Nicholas et al. 2005), the leafroller *Epiphyas postvittana* (Walker) in apple orchards and vineyards (Suckling et al. 2006; Frank et al. 2007), and the pear psyllid *Cacopsylla pyri* L. (Höhn et al. 2007). European earwigs, along with *Forficula pubescens* Gené (= *Guanchia pubescens),* are also the predominant natural enemies of the rosy apple aphid *Dysaphis plantaginea* Passerini (Dib et al. 2010). The earwig *Doru taeniatum* (Dohrn) is one of the three most common predators of the fall armyworm *Spodoptera frugiperda* (Smith) in Honduran maize (Wyckhuys & O'Neil 2006). As herbivores, earwigs can have negative effects on plants by feeding on soft fruits and vegetative tissue (Brindley 1918; Fulton 1924; McLeod & Chant 1952; Grafton-Cardwell et al. 2003). An example of this dual role of earwigs in orchards is *Forficula senegalensis* Audinet-Serville. This species was considered a pest on millet in the Sudanese-Sahelian region of Niger. However, after analyzing the gut content of more than 500 individuals, it was observed that arthropods were an important part of its diet while plants were not. As a result, Boukary et al. (1997) concluded that its role as a pest should be reconsidered.

In the citrus orchard studied, two species of earwigs were present in tree canopies: the European earwig *Forficula auricularia* and *Forficula pubescens*. The former has been studied worldwide, but few references can be found in the literature for the latter. Research on European earwig biology has been conducted in the USA (Fulton 1924; Crumb et al. 1941), New Zealand (Burnip et al. 2002; Suckling et al. 2006), and North-Central Europe (Belgium: Gobin et al. 2008; UK: Behura 1956; the Netherlands: Helsen et al. 1998; and Czech Republic: Kocárek 1998), but we are not aware of any study in the Mediterranean Basin. The objectives of this study were: (i) to describe *F. auricularia* and *F. pubescens* abundance in Mediterranean citrus tree canopies; (ii) to determine whether they are positively or negatively associated with each other, as both species often co-occur in the same canopies, or otherwise randomly distributed; (iii) to establish the interannual variation in abundance during a five-year period, as previous work revealed important temporal changes in the entire arthropod community (Piñol et al. 2012); and (iv) to evaluate the potential role of earwigs as pest predators in citrus canopies by exploring the relationship between aphid and earwig abundance while also comparing prey and predator abundance for other citrus pests.

Materials and methods

Study site

The study was conducted in a citrus plantation at La Selva del Camp (Tarragona, NE Spain; 41° 13' 7″N, 1° 8' 35″E). The climate is Mediterranean, with a rainy spring and autumn and a dry winter and summer. The grove consisted of *ca.* 320 clementine trees grafted on the hybrid rootstock Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb.). The plantation complied with all organic agriculture standards during the whole studied period (2006-2010). Trees were regularly irrigated during dry periods.

Earwig and aphid sampling and classification

Trees were randomly selected each year from a subset of 69 individuals, all planted in 1999. Eight trees were sampled in 2006, 2007 and 2008 and nine in 2009 and 2010. No trees were sampled in two consecutive years. Earwigs and aphids in each canopy were sampled once a month using square beating trays of 0.50 m^2 (three vigorous hits of the tree canopy in opposite directions). This method is recommended by Albouy & Caussanel (1990) for earwig sampling. Insects were captured with entomological aspirators and immediately preserved in 70% ethanol. We counted the total number of aphids in each sample. Presence of wings in *F. auricularia* adults was used to distinguish this species from the wingless *F. pubescens*, while nymphs were differentiated by body color and size and by type of setae of the cerci: long and

erect in *F. pubescens* and short and decumbent in *F. auricularia*. Nymphal stage within each species was determined by size and number of antennal segments (Albouy & Caussanel 1990). Sex of adults was determined by dimorphism of the cerci. Sex-ratio of each species was analyzed separately. We used a paired t-test comparing the mean number of males and females per month and tree (data in supplementary material; Table s3.1). In this study, earwigs were sampled in tree canopies, so the abundance described is relevant only to populations from canopies.

In previous studies (Cañellas et al. 2005; Piñol et al. 2009a & 2009b & 2010) all earwigs present in citrus canopies were erroneously identified as *F. auricularia*. Thus future references to earwigs in those papers should be regarded as *Forficula* sp.

Interspecific association

Each sample (beating trays obtained per canopy per month) was classified into four categories according to earwig presence: (i) without earwigs, (ii) with both earwig species, (iii) with only *F. pubescens* and (iv) with only *F. auricularia.* Data from months in which samples only showed one species of earwig were excluded from the analyses. A contingency table was constructed for each year and the Pearson chi-square test was conducted using SPSS 15.0 (SPSS Inc. Chicago, Illinois, USA). Although Sauphanor & Sureau (1993) used the point correlation coefficient to study the interspecific association of two earwig species, their method is numerically equivalent to the Pearson chi-square test used here.

Interannual variation of earwig abundance

The abundance between years of each earwig species was compared using a univariate permutational ANOVA on the square root of the cumulative abundance per sample (beating trays per canopy per year). Year was considered a fixed factor and the Euclidean distance was used to calculate the dissimilarity among samples. Pair-wise tests (corrected with the Bonferroni adjustment) were conducted following significant differences between years. The software used was PRIMER v6 and PERMANOVA + (Anderson et al. 2008).

Relationship between earwig and aphid abundance

To study the relationship of each earwig species with aphids we used the sum of individuals present each year in each canopy during the main aphid attack (April to July). A power (log–log) function was fitted to these cumulative values of aphid and earwig abundance. A negative relationship between aphid and earwig abundance would suggest a top-down regulation of

aphids by earwigs. A positive relationship would suggest a bottom-up regulation of earwigs by aphids (McQueen et al. 1989; Worm & Myers 2003; Piñol et al. 2009a). Top-down regulation would probably imply that earwigs are sedentary predators already in canopies when aphid attack starts and, thus, able to control aphid population since the beginning (Piñol et al. 2009a).

Results

Earwig abundance in Mediterranean citrus tree canopies

Forficula auricularia was generally active in canopies from April to November (fig. 3.1A). On some occasions, *F. auricularia* was even found in tree canopies in January, March and December. Nymphs of *F. auricularia* were found in April, and in two of the five years they were also captured in December. First instars were never captured in canopies. Second instars were occasionally found in canopies, and the following stages were progressively more abundant until adults emerged (Table s3.1). There were no differences between male and female frequencies in canopies (Mean ± SE; n = 36 months; males 0.27 ± 0.23; females 0.26 ± 0.30; t = 0.23; P = 0.81).

Forficula pubescens did not arrive in canopies until May but remained there until December (fig. 3.1B). Nymphs of *F. pubescens* were only found once a year in May-June. First instars were never found in canopies. Male and female frequencies in canopies did not differed (Mean ± SE; n = 36 months; mean ± SE; males 1.89 ± 2.89; females 1.87 ± 2.64; t = 0.15; P = 0.87).

Interspecific association

The distribution in canopies of the two species appeared to be random (n = 48, χ^2 = 0.13, P = 0.72 for 2006; n = 56, χ^2 = 1.22, P = 0.27 for 2007; n = 56, χ^2 = 1.57, P = 0.21 for 2008; n = 72, χ^2 = 1.67, P = 0.20 for 2009; n = 36, χ^2 = 1.09, P = 0.30 for 2010). Data represented in the supplementary material (fig. s3.1).

Interannual variation of earwig abundance

The abundance of *F. auricularia* significantly changed during the studied five-year period (Pseudo-$F_{4,37}$ = 5.93, P < 0.001). The abundance of *F. pubescens* also varied during the studied period (Pseudo-$F_{4,37}$ = 29.10; P < 0.001), having its maximum in 2009. In 2006 and 2007 both species had approximately the same abundance, but from 2008 onwards there was a gradual increase of *F. pubescens* abundance and in 2010 it was 28 times more abundant in canopies than *F. auricularia* (fig. 3.2).
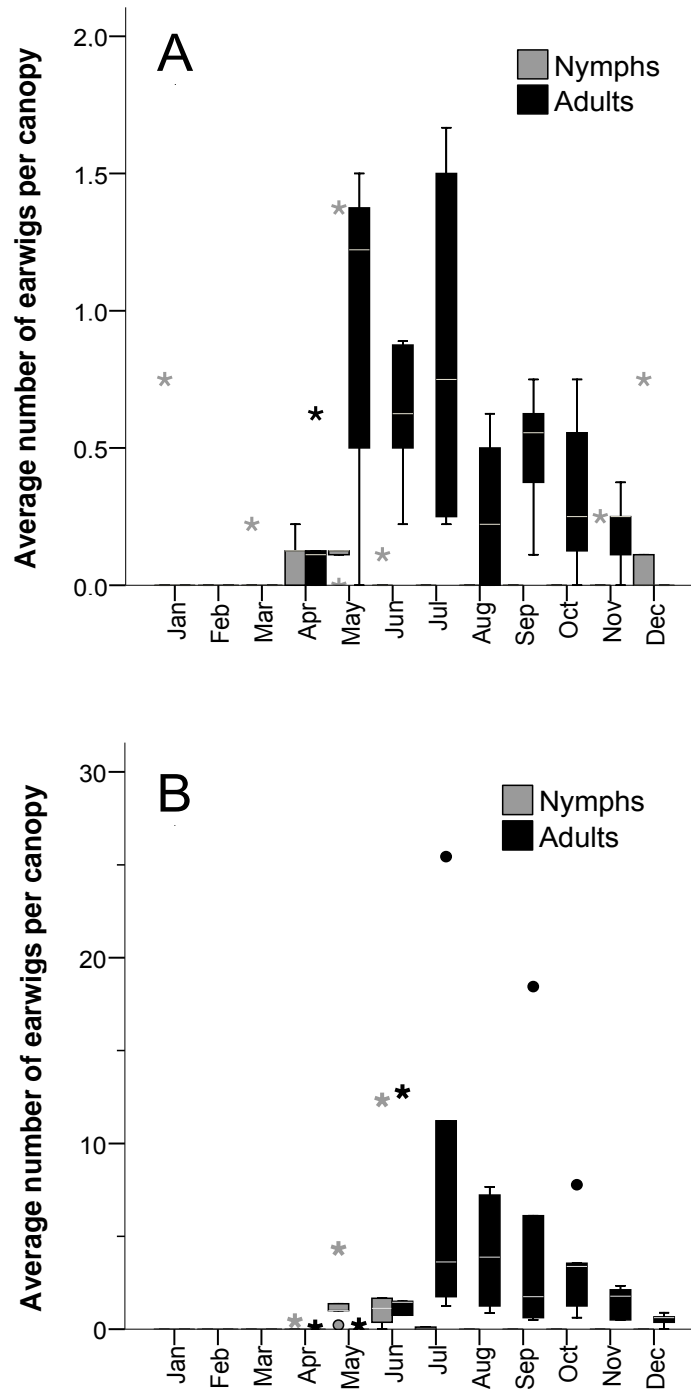
Fig. 3.1. Boxplot of the average number of earwigs captured per canopy per month during the studied period (2006-2010). The top of the box is the 75th percentile, the bottom the 25th percentile, and the middle line the median. In the absence of outliers and/or extreme values, the bars that extend out the top and bottom of the box represent the highest and lowest values. Outliers (values that are 1.5 to 3-fold the interquartile range) and extreme values (values that are more than 3-fold the interquartile range) are represented by circles and asterisks, respectively. (A) *Forficula auricularia*. (B) *Forficula pubescens*. Note that y-axis scales for both graphics are different.
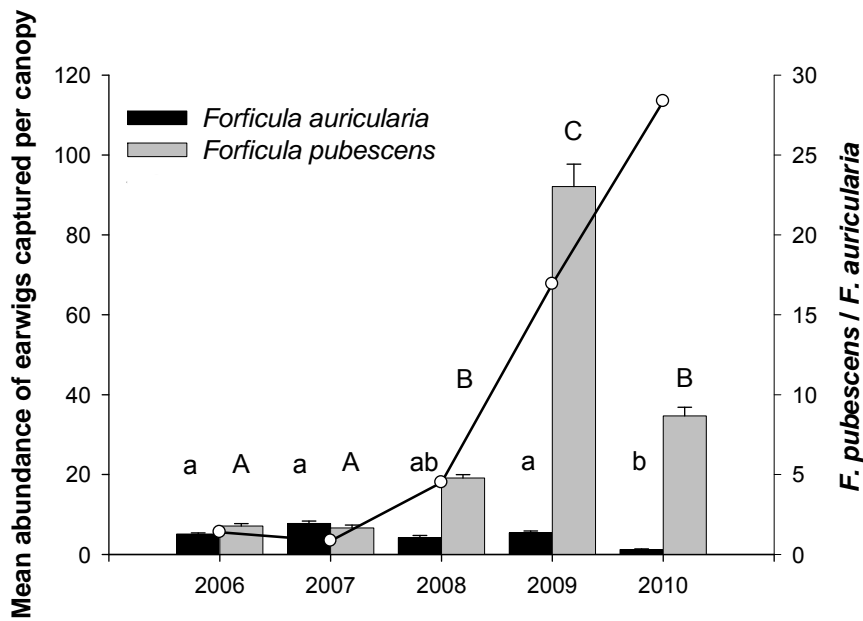
Fig. 3.2. Mean (± SE) abundance of earwigs, measured as the cumulative abundance (from January to December) per canopy for each year sampled (2006-2010). Different letters indicate years with significantly different abundance (Pair-wise test adjusted at *P* < 0.005). Lower case refer to *F. auricularia* and upper case to *F. pubescens*. The line is the ratio of *F. pubescens* to *F. auricularia* mean abundance.

Relationship between earwig and aphid abundance

There was a significant negative relationship between the cumulative abundance of aphids and *F. auricularia* (fig. 3.3A), but a non-significant one between aphids and *F. pubescens* (fig. 3.3B).
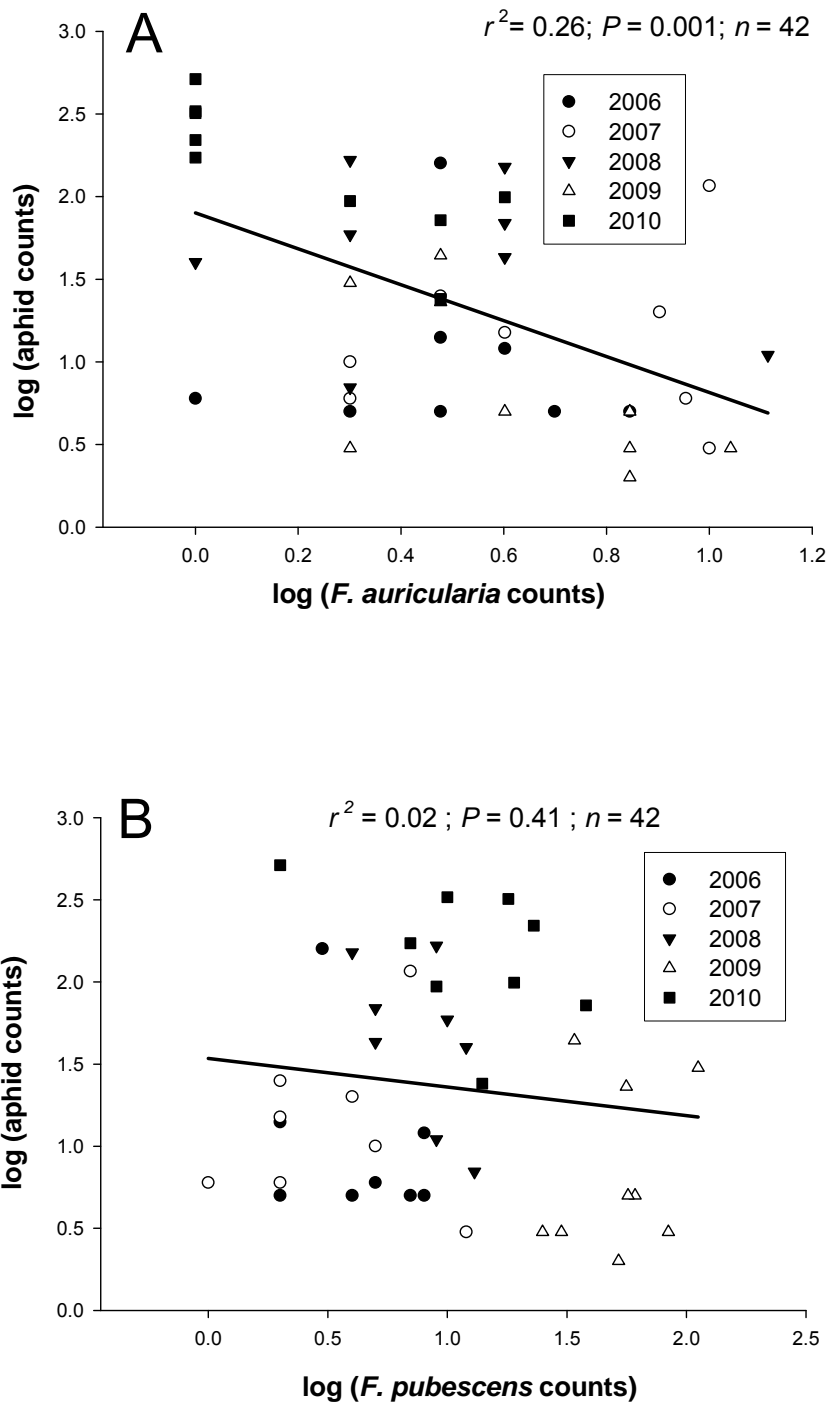
Fig. 3.3. Cumulative abundance of aphids *versus* cumulative abundance of earwigs captured per canopy per season (from April to July) for the five years sampled (2006-2010). (A) *Forficula auricularia vs.* aphids. (B) *Forficula pubescens vs.* aphids. For each diagram the total number of samples, the coefficient of determination and its statistical significance are given.

Discussion

Earwig abundance in Mediterranean citrus canopies

Forficula auricularia was generally present in canopies from April to November and sometimes until December. In colder regions, European earwig appears in canopies in May (reviewed in Moerkens et al. 2011) and moves back to the soil in October (Crumb et al. 1941; Behura 1956; Gobin et al. 2008; Moerkens et al. 2009). Thus the active period of *F. auricularia* in Mediterranean citrus canopies lasted longer than in colder regions. This is not surprising as it is well known that earwig activity is dependent on temperature (Crumb et al. 1941; Behura 1956; Helsen et al. 1998; Moerkens et al. 2011). First instars were not found in canopies probably due to maternal care taking place in the soil (Vancassel & Foraste 1980; Albouy & Caussanel 1990; Helsen et al. 1998; Kölliker 2007; Kölliker & Vancassel 2007). In the study site, *F. auricularia* had two reproductive periods per year. Nymphs were found active in April, earlier than in most regions studied (Moerkens et al. 2011). The abundance of males and females at the canopies was not different from a 50:50 sex-ratio. Brindley (1912) and Behura (1956; and included references) reached a similar conclusion on the proportion of sexes of European earwigs in the British Isles.

Forficula pubescens was usually found in citrus canopies from May to December. The species had one reproductive period per year, with nymphs present in canopies in May-June. Similarly to *F. auricularia*, first instars were never found in canopies. This might indicate that they remained in the subterranean nest with females, as a result of parental care of early instars (Herter 1964; Albouy & Caussanel 1990). Males and females were found in the proportion 50:50 in the canopies. We are unaware of published information on sex-ratio in *F. pubescens*, although the scarce data in Herter (1964) of captures in two years in Corsica (28 males, 23 females) indicate a similar situation.

Interspecific association

Forficula auricularia and *F. pubescens* co-occurred in time and in space in citrus tree canopies as they did in cardboard shelters in pear tree trunks (Debras et al. 2007) and in rolled up leaves (Herter 1964). However, both species were not in association. By contrast, Sauphanor & Sureau (1993) observed a high level of association of individuals of both species under laboratory conditions. These contrasting results may indicate that both species can coexist without having a negative effect on each other, and sometimes, as in the experiments of Sauphanor & Sureau (1993), even gaining benefits from each other.

Interannual variation of earwig abundance

The abundance of the two species of earwigs in canopies significantly changed over a 5-year period. In 2006, both species had similar abundance, but at a later point, *F. pubescens* gradually increased its abundance to the extent that by 2010 it was 28 times more abundant than *F. auricularia*. As both species were randomly distributed, strong interspecific competition can hardly be the cause of the observed temporal change in their relative abundance. Other factors such as climate, predation, reproduction success, and/or survival rate may have differentially influenced both species. Climate variables (such as temperature or wind velocity) were shown to significantly correlate with European earwig abundance (Chant & McLeod 1952). Predation could have also been differential since *F. auricularia* is nearly double in size than *F. pubescens*, and may have been subjected to a heavier predation rate, especially by birds, which are known to feed on European earwigs (Brindley 1918) and to significantly affect their abundance (Gunnarson et al. 2009; Piñol et al. 2010). Although egg number seems to be of a similar magnitude in both species –around 30 eggs/brood (Crumb 1941; Herter 1964)-, survival rate or pathogen, parasite or parasitoid loads might have also differed in both species. A similar significant interannual variation in the abundance of other insects has already been described in the citrus canopy arthropod community (Piñol et al. 2012). A consequence of these findings is that short-term studies can be misleading and that long-term monitoring should be conducted whenever possible.

Relationship between earwig and aphid abundance

The abundance of *F. auricularia* was negatively related to that of aphids. This suggests a top-down regulation of aphids by *F. auricularia* (McQueen et al. 1989; Worm & Myers 2003; Piñol et al. 2009a). A possible explanation for this regulation is the role of *F. auricularia* as a generalist sedentary predator (Piñol et al. 2009b). Since *F. auricularia* was already present in citrus canopies as early as April, it could feed on aphids from the very beginning. Early arrival of predators such as heteropterans and coccinellids has already been proved to be important in the biological control of aphid populations (Pons et al. 2009; Brown 2010). If predators are already present in canopies when pest population growth starts, they can prevent the outbreak; otherwise they can only help to reduce the attack when it has already taken place (Murdoch et al. 1985). The other species present in citrus canopies, *F. pubescens,* did not show a significant relationship with aphids, probably due to its late appearance in canopies (in May).

Potential role of earwigs as pest predators

The European earwig has been considered an effective biocontrol agent of aphids (Mueller et al. 1988; Nicholas et al. 2005) and midges (He et al. 2008) in apple orchards, psyllids in pear orchards (Höhn et al. 2007) and leafrollers in vineyards (Frank et al. 2007). In Mediterranean citrus orchards, *F. auricularia* was present in canopies in April, earlier than in colder regions (Moerkens et al. 2011) and prior pests major attack (fig. 3.4). Thus, as a sedentary predator with generalist feeding habits, *F. auricularia* may assist in controlling citrus pests since the onset of the infestation (Piñol et al. 2009b). *Forficula pubescens* apparently did not regulate aphid populations. However, we known that it did predate on aphids, as preliminary visual gut-content analysis of *F. pubescens* individuals showed aphid remains (legs and bucal siphons) in their stomachs. In fact, *Forficula pubescens* is considered an active predator of aphids in apple orchards (Dib et al. 2010), and of the psyllid *Cacopsylla pyri* in pear trees (Debras et al. 2007). Thus *F. pubescens* may also play its role as pest predator in citrus tree canopies, especially due to its high abundance. The following citrus pests co-occurred with both earwig species in our study site: the citrus leafminer, *Phyllocnistis citrella* Stainton, the woolly whitefly *Aleurothrixus floccosus* (Maskell), and soft scales such as the Chinese wax scale *Ceroplastes sinensis* Del Guercio and the cottony cushion scale *Icerya purchasi* Maskell. Although it remains unknown whether earwigs feed on these particular species, different studies indicate that earwigs are important natural enemies of Lepidoptera and non-aphid Homoptera (McLeod & Chant 1952; Badji et al. 2004; Suckling et al. 2006; Xiushan et al. 2006; Debras et al. 2007; Frank et al. 2007).
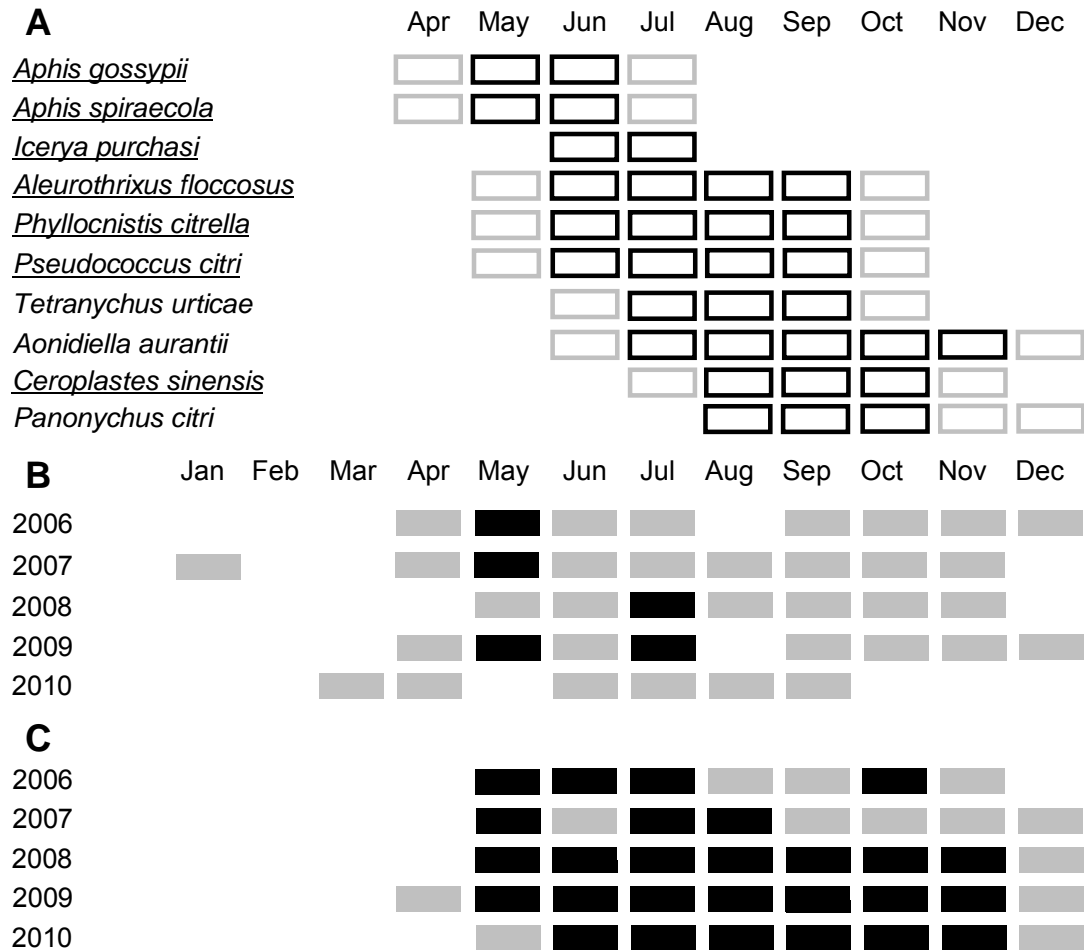
Fig. 3.4. (A) Months of medium (grey line) and major (black line) abundance of most important citrus pests in the Mediterranean, according to Garcia-Marí (2009) and to personal field observations. Underlined species are those observed in the study site. *Forficula auricularia* (B) and *F. pubescens* (C) monthly abundance in canopies each year. Months with low earwig abundance (less than 1 earwig captured per beating trays per canopy on average) are in grey.

Concluding remarks

(i) The active period of *F. auricularia* in Mediterranean citrus canopies was longer than in colder regions. (ii) *F. auricularia* and *F. pubescens* co-occurred randomly in citrus canopies. (iii) The relative abundance of both species changed during a 5-year period. In the first year, 2006, both species had similar abundance, but in 2010 *F. pubescens* became much more abundant than *F. auricularia*. (iv) The potential role of earwigs as pest predators in tree canopies is likely to be higher in Mediterranean than in colder regions due to a longer active period in the former. Our data suggested a possible top-down control of aphids by *F. auricularia*, probably as a consequence of their early presence in canopies, at the onset of aphid outbreak.

Acknowledgements

Supplementary material

Fig. s3.1. Percentage of samples (beating trays per canopy per month) classified into the following categories: (a) without earwigs, (b) with both earwig species, (c) with only *Forficula pubescens* and (d) with only *Forficula auricularia*.

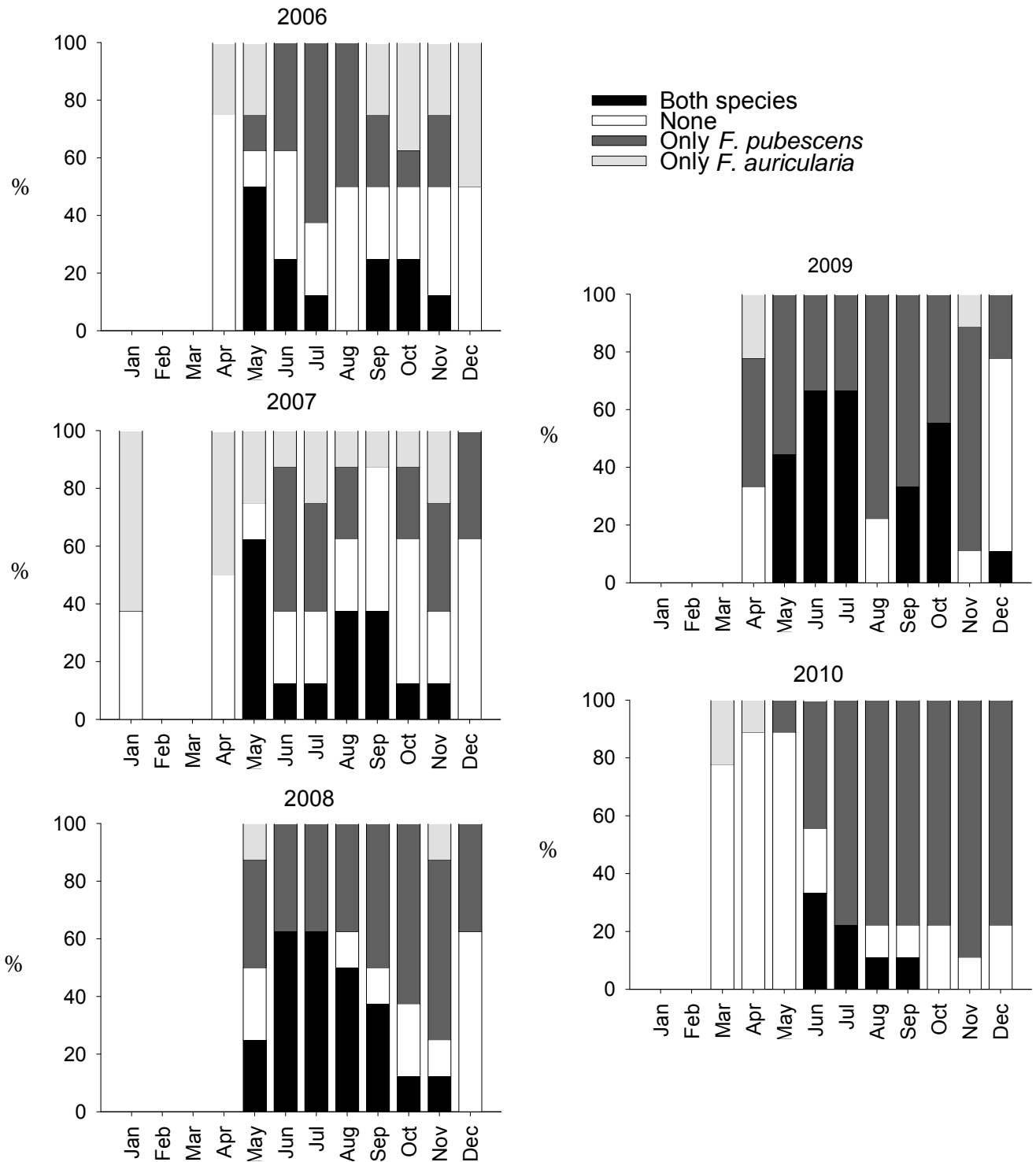| 2006 n=8		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DES
Forficula auricularia	♀	0	0	0	0.13 ± 0.13	0.50 ± 0.19	0.38 ± 0.26	0.13 ± 0.13	0	0.13 ± 0.13	0.38 ± 0.18	0.25 ± 0.16	0
	♂	0	0	0	0	0.88 ± 0.30	0.13 ± 0.13	0.13 ± 0.13	0	0.50 ± 0.27	0.38 ± 0.26	0	0
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0	0	0	0	0	0	0	0.13 ± 0.13	0
	N3	0	0	0	0	0.13 ± 0.13	0	0	0	0	0	0.13 ± 0.13	0.75 ± 0.31
	N4	0	0	0	0.13 ± 0.13	0	0	0	0	0	0	0	0
Forficula pubescens	♀	0	0	0	0	0	0.13 ± 0.13	0.88 ± 0.35	0.38 ± 0.26	0.25 ± 0.16	0.38 ± 0.26	0.25 ± 0.16	0
	♂	0	0	0	0	0	0.63 ± 0.26	0.88 ± 0.48	0.50 ± 0.19	0.38 ± 0.26	0.88 ± 0.52	0.25 ± 0.16	0
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0	0	0	0	0	0	0	0	0
	N3	0	0	0	0	0	0	0	0	0	0	0	0
	N4	0	0	0	0	0	0	0	0	0	0	0	0
	N5	0	0	0	0	1.00 ± 0.38	0.38 ± 0.26	0	0	0	0	0	0

2007 n=8		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DES
Forficula auricularia	♀	0	0	0	0.25 ± 0.25	1.00 ± 0.50	0.38 ± 0.26	0.25 ± 0.16	0.13 ± 0.13	0.13 ± 0.13	0.25 ± 0.16	0.25 ± 0.16	0
	♂	0	0	0	0.38 ± 0.18	0.50 ± 0.19	0.25 ± 0.25	0.50 ± 0.38	0.50 ± 0.27	0.63 ± 0.26	0	0.13 ± 0.13	0
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0.13 ± 0.13	0	0	0	0	0	0	0	0	0	0	0
	N3	0.25 ± 0.16	0	0	0	0.13 ± 0.13	0	0	0	0	0	0	0
	N4	0.38 ± 0.18	0	0	0.13 ± 0.13	1.25 ± 0.45	0	0	0	0	0	0	0
Forficula pubescens	♀	0	0	0	0	0	0.63 ± 0.18	1.00 ± 0.42	0.38 ± 0.18	0.13 ± 0.13	0.38 ± 0.26	0.25 ± 0.16	0.13 ± 0.13
	♂	0	0	0	0	0	0.13 ± 0.13	0.25 ± 0.25	0.88 ± 0.30	0.38 ± 0.26	0.25 ± 0.16	0.25 ± 0.16	0.25 ± 0.16
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0	0	0	0	0	0	0	0	0
	N3	0	0	0	0	0.13 ± 0.13	0	0	0	0	0	0	0
	N4	0	0	0	0	0.13 ± 0.13	0	0	0	0	0	0	0
	N5	0	0	0	0	1.13 ± 0.48	0	0	0	0	0	0	0

2008 n=8		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DES
Forficula auricularia	♀	0	0	0	0	0.13 ± 0.13	0.63 ± 0.18	0.75 ± 0.62	0.13 ± 0.13	0	0	0.13 ± 0.13	0
	♂	0	0	0	0	0.38 ± 0.26	0.25 ± 0.16	0.75 ± 0.37	0.38 ± 0.18	0.38 ± 0.18	0.13 ± 0.13	0.13 ± 0.13	0
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0	0	0	0	0	0	0	0	0
	N3	0	0	0	0	0	0	0	0	0	0	0	0
	N4	0	0	0	0	0.13 ± 0.13	0	0	0	0	0	0	0
Forficula pubescens	♀	0	0	0	0	0	1.25 ± 0.31	1.88 ± 0.64	2.00 ± 0.73	0.63 ± 0.26	1.88 ± 0.99	1.13 ± 0.35	0.50 ± 0.27
	♂	0	0	0	0	0	0.25 ± 0.16	1.75 ± 0.53	1.88 ± 0.69	1.13 ± 0.30	1.50 ± 0.46	1.00 ± 0.33	0.13 ± 0.13
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0	0.13 ± 0.13	0	0	0	0	0	0	0
	N3	0	0	0	0	0	0	0	0	0	0	0	0
	N4	0	0	0	0	0.38 ± 0.26	0.25 ± 0.16	0	0	0	0	0	0
	N5	0	0	0	0	0.50 ± 0.27	0.88 ± 0.23	0.13 ± 0.13	0	0	0	0	0

Table s3.1. Average number (Mean ± SE) of earwigs captured per canopy each month of the studied period (2006-2008). The number of tree canopies sampled each year is indicated in the upper left corner. Nymphs are classified according to its stage of development (first instars = N1, second instars = N2, and so on). Adult numbers are segregated by sex.

2009 n=9		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DES
Forficula auricularia	♀	0	0	0	0	0.56 ± 0.38	0.44 ± 0.24	1.44 ± 0.67	0	0.22 ± 0.22	0.11 ± 0.11	0.11 ± 0.11	0
	♂	0	0	0	0	0.67 ± 0.33	0.44 ± 0.18	0.22 ± 0.15	0	0.33 ± 0.24	0.44 ± 0.18	0	0
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0	0	0	0	0	0	0	0	0.11 ± 0.11
	N3	0	0	0	0	0	0	0	0	0	0	0	0
	N4	0	0	0	0.22 ± 0.15	0.11 ± 0.11	0	0	0	0	0	0	0
Forficula pubescens	♀	0	0	0	0.11 ± 0.11	0.22 ± 0.15	7.00 ± 1.20	11.56 ± 2.72	3.22 ± 1.44	8.89 ± 4.22	3.22 ± 0.62	0.89 ± 0.35	0.56 ± 0.29
	♂	0	0	0	0	0	5.78 ± 1.02	13.89 ± 4.76	4.44 ± 1.39	9.56 ± 3.87	4.56 ± 0.75	0.89 ± 0.42	0.11 ± 0.11
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0.33 ± 0.17	0.11 ± 0.11	0	0	0	0	0	0	0
	N3	0	0	0	0.11 ± 0.11	0.33 ± 0.17	0	0	0	0	0	0	0
	N4	0	0	0	0	2.67 ± 0.67	0.22 ± 0.15	0.11 ± 0.11	0	0	0	0	0
	N5	0	0	0	0	1.22 ± 0.49	12.11 ± 1.64	0	0	0	0	0	0

2010 n=9		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DES
Forficula auricularia	♀	0	0	0	0.11 ± 0.11	0	0.11 ± 0.11	0	0.11 ± 0.11	0	0	0	0
	♂	0	0	0	0	0	0.11 ± 0.11	0.22 ± 0.15	0.11 ± 0.11	0.11 ± 0.11	0	0	0
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0	0	0	0	0	0	0	0	0
	N3	0	0	0	0	0	0	0	0	0	0	0	0
	N4	0	0	0.22 ± 0.15	0	0	0.11 ± 0.11	0	0	0	0	0	0
Forficula pubescens	♀	0	0	0	0	0	0.67 ± 0.29	6.33 ± 1.78	4.22 ± 1.19	2.78 ± 0.89	1.78 ± 0.55	1.22 ± 0.36	0.44 ± 0.18
	♂	0	0	0	0	0	0.78 ± 0.28	4.89 ± 1.26	3.00 ± 0.75	3.33 ± 1.80	1.78 ± 0.49	1.11 ± 0.56	0.44 ± 0.19
	N1	0	0	0	0	0	0	0	0	0	0	0	0
	N2	0	0	0	0	0	0	0	0	0	0	0	0
	N3	0	0	0	0	0	0	0	0	0	0	0	0
	N4	0	0	0	0	0.11 ± 0.11	0.67 ± 0.67	0	0	0	0	0	0
	N5	0	0	0	0	0.11 ± 0.11	1.00 ± 0.41	0	0	0	0	0	0

Table s3.1. Average number (Mean ± SE) of earwigs captured per canopy each month of the studied period (2009-2010). The number of tree canopies sampled each year is indicated in the upper left corner. Nymphs are classified according to its stage of development (first instars = N1, second instars = N2, and so on). Adult numbers are segregated by sex.

Detecting aphid predation by earwigs in organic citrus orchards using molecular markers

Abstract

Aphids (Hemiptera: Aphidoidea) can damage citrus trees via direct damage to leaves and flowers or via the indirect transmission of viruses. Predators such as the European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae), may assist in keeping aphid populations under control in citrus orchards. Group-specific primers were developed to detect aphid DNA in earwigs, in order to determine earwig predation rates in aphids in Mediterranean organic citrus trees. These primers were designed in accordance with the alignment of comparable sequences of aphids and earwigs and they amplified a 224 bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) region. Following the consumption of three to five *Aphis spiraecola* Patch, aphid DNA was still detectable in 50% of earwigs one day after the ingestion. When predation was evaluated in the field, aphid DNA was detected in earwigs in May, June and July but not in April and August. The most interesting result is that of May, when aphid abundance was very low but 30% of the earwigs tested positive for aphid DNA. This finding suggests that earwigs are important aphid predators in citrus orchards, as they probably alter aphid dynamics as a result of early seasonal pressure on this pest.

Introduction

Aphids (Hemiptera: Aphidoidea) are phloem-feeder insects present in a quarter of the plant species in temperate regions (Dixon et al. 1987). Aphids can negatively affect trees via direct damage to leaves and flowers or via the indirect transmission of viruses (Ebeling 1959). Seven species of aphids have been found so far in the organic citrus orchard under study, the most abundant being *Aphis spiraecola* Patch and *Aphis gossypii* Glover (Piñol et al. 2009a). These two species are in fact well known for being responsible for the worst direct damage to citrus trees (Barbagallo et al. 2007; Urbaneja et al. 2008). The cotton aphid, *A. gossypii*, is also the most efficient vector of the Citrus tristeza virus (Closteroviridae; genus *Closterovirus*) in the Mediterranean basin (Belliure et al. 2008), a pathogen that has affected the citrus industry worldwide (Moreno et al. 2008).

In order to minimize aphid damage, their populations must be kept below an economic threshold (Irwin et al. 2007; Belliure et al. 2008). Natural enemies such as predators may help regulate aphid populations in citrus orchards. There are several key aphid predators, including ladybird beetles, anthocorid and mirid bugs, lacewings, hoverflies and earwigs (Dixon 1998; Solomon et al. 2000). In this study we focus on earwigs, in particular on the most common species, the European earwig *Forficula auricularia* L. (Dermaptera: Forficulidae). Even if earwigs have been reported to be active aphid predators in apple and citrus orchards (Mueller et al. 1988; Nicholas et al. 2005; Piñol et al. 2009a; Dib et al. 2010), their predation rates have never been measured.

Different methods have been used to identify the prey remains in predators' guts, including dissection, radio-isotope labelling, stable isotopes, electrophoresis, chromatography, monoclonal antibodies and DNA-based analysis (reviewed in Symondson 2002, and in Hardwood & Obrycki 2005). Nowadays, the DNA-based approach, using the polymerase chain reaction (PCR) amplification of the DNA of the prey remains, is the most common method (King et al. 2011). Several single and multiple-copy DNA regions from nuclear and mitochondrial genes have been targeted and sequenced in insects, and many group- and species-specific primers have already been designed (King et al. 2008; Kuusk & Agustí 2008) and used to study invertebrate prey-predator interactions in the field (Agustí et al. 2003a; Harper et al. 2005; Ma et al. 2005; Read et al. 2006; Hardwood et al. 2007; Juen & Traugott 2007; Zhang et al. 2007; Monzó et al. 2010; Moreno-Ripoll et al. 2012).

To correctly interpret DNA-based outcomes of predation, it is necessary to know the time during which prey remains can be detected in the guts of predators (Greenstone et al. 2007). Predators with long detection periods might appear to predate more than they actually do if the

data are not weighted according to the decay in detectability (Chen et al. 2000; Greenstone et al. 2007). The detectability half-life is a measure of the decay in detectability and is defined as the time needed to reduce the number of predators that test positive for prey DNA to 50% (Greenstone et al. 2007). The detectability half-life is used to standardize predation rates when different predator taxa are compared (King et al. 2008). Detectability half-lives have been obtained for many arthropod predators, such as spiders, beetles, heteropterans, neuropterans, mites and collembolans, but never for earwigs.

In short, our objectives were: (i) to develop group-specific PCR primers for aphids, capable of detecting the most common aphid species in citrus orchards, (ii) to measure the detectability half-life of aphid DNA in earwigs by laboratory feeding trials, and (iii) to track aphid predation rates by the European earwig under natural field conditions in Mediterranean organic citrus trees. To our knowledge, this is the first time DNA-based approaches have been used to study earwig predation in the field.

Materials and methods

Primer design and species specificity

The mitochondrial cytochrome *c* oxidase subunit I (COI) region was used for the design of a pair of aphid-specific primers. The following GenBank aphid and earwig sequences were used: EU701503 (*Aphis spiraecola*), EU701399 (*Aphis gossypii*), EU701935 (*Toxoptera aurantii* (Boyer de Fonscolombe)), EU701804 (*Myzus persicae* (Sulzer)), EU701313 (*Aphis craccivora* (Koch)), EU701729 (*Macrosiphum euphorbiae* (Thomas)), EU701935 (*Aulacorthum solani* (Kaltenabach)), EU701813 (*Nasonovia ribisnigri* (Mosley)) and AF015230 (*F. auricularia*). Sequences were aligned using CLUSTALW2 (www.ebi.ac.uk/Tools/msa/clustalw2; Larkin et al. 2007), while primers were designed as described in Agustí et al. (2003a), following the guidelines of Innis & Gelfand (1990) and Saiki (1990).

Primer specificity was tested by attempting to amplify DNA from several aphid species, as well as from earwigs and other potential prey, predators and parasitoids (Table 4.1). All seven aphid species present in the study site were tested, as well as the most common non-aphid species susceptible to be predated by earwigs. Three to five individuals from each species were tested, most of them collected at the study site (see below).

Group	P	Order	Family/Tribe	Species	Origin
Aphids	✓	Hemiptera	Aphidini	*Aphis spiraecola* Patch	La Selva
	✓	Hemiptera	Aphidini	*Aphis gossypii* Glover	La Selva
	✓	Hemiptera	Aphidini	*Toxoptera aurantii* (B. de F.)	La Selva
	✓	Hemiptera	Aphidini	*Aphis craccivora* (Koch)	La Selva
	✓	Hemiptera	Macrosiphini	*Myzus persicae* (Sulzer)	CRAG
	✓	Hemiptera	Macrosiphini	*Macrosiphum euphorbiae* (Thomas)	CCMA-CSIC
	✓	Hemiptera	Macrosiphini	*Aulacorthum solani* (Kaltenbach)	CCMA-CSIC
	✓	Hemiptera	Macrosiphini	*Hyperomyzus lactucae* (L.)	CCMA-CSIC
	x	Hemiptera	Macrosiphini	*Nasonovia ribisnigri* (Mosley)	Cabrils
Non-aphid	✓	Psocoptera	Trichopsocidae	*Trichopsocus clarus* (Banks)	La Selva
	✓	Psocoptera	Ectopsocidae	*Ectopsocus briggsi* McLachlan	La Selva
	✓	Hemiptera	Coccidae	*Ceroplastes sinensis* Del Guercio	La Selva
	✓	Hemiptera	Margarodidae	*Icerya purchasi* Maskell	La Selva
	✓	Hemiptera	Coccidae	*Saissetia oleae* (Olivier)	La Selva
	✓	Hemiptera	Aleyrodidae	*Aleurothrixus floccosus* (Maskell)	La Selva
	x	Hemiptera	Aleyrodidae	*Bemisia tabaci* (Gennadius)	IRTA
	✓	Lepidoptera	Gracillariidae	*Phyllocnistis citrella* Stainton	La Selva
	-	Collembola	Entomobryidae	*Entomobrya sp.*	Cabrils
	-	Thysanoptera	Thripidae	*Frankliniella occidentalis* (Pergande)	IRTA
Predators	✓	Dermaptera	Forficulidae	*Forficula auricularia* L.	La Selva
	✓	Coleoptera	Coccinellidae	*Adalia decempunctata* (L.)	La Selva
	✓	Araneae	Philodromidae	*Philodromus cespitum* (Walckenaer)	La Selva
	-	Araneae	Araneidae	Unknown species	Cabrils
	-	Diptera	Syrphidae	*Episyrphus balteatus* (De Geer)	Commercial 1
	x	Hemiptera	Anthocoridae	*Orius majusculus* (Reuter)	IRTA
Parasitoids	✓	Hymenoptera	Aphelinidae	*Aphelinus abdominalis* (Dalman)	Commercial 2
	x	Hymenoptera	Braconidae	*Aphidius colemani* Viereck	Commercial 2

Table 4.1. Aphids, non-aphid prey, predators and parasitoids tested for primer specificity. All aphids belong to the family Aphididae (tribe is indicated). The column "P" indicates presence (✓), absence (X), or unknown presence (-) of the species at the study site. The source of sampled individuals was: La Selva = Experimental citrus orchard, La Selva del Camp, Tarragona; CRAG = Laboratory colonies at the Center for Research in Agricultural Genomics, Barcelona; CCMA-CSIC = Laboratory colonies at the Center for Environmental Sciences, Madrid; Cabrils = Experimental lettuce fields, Cabrils, Barcelona; IRTA = Laboratory colonies at IRTA, Cabrils, Barcelona; Commercial 1 = Koppert Biological Systems S.L., Almería; Commercial 2 = Biobest Biological Systems S.L., Águilas.

DNA extraction and PCR analysis

DNA was extracted using the DNeasy Tissue Kit (QIAGEN, Hilden, Germany) according to the protocol for insects included in the manufacturer's instructions. The whole body was used for all insects except for earwigs, in which only the abdomen was used for DNA extraction, in keeping with reports that most of the digestive system of earwigs is located in their abdomen (Albouy & Caussanel 1990). Total DNA was eluted in 100 µl of AE buffer and stored at -20ºC. DNA amplifications were performed in 25 µl reaction volumes containing 1 µl of resuspended DNA, 0.2 mM of dNTPs (Promega Corporation, WI, USA), 2 mM of $MgCl_2$, 0.2 µM of each primer and 0.6 U *Taq* DNA polymerase (Invitrogen Corporation, CA, USA) in 10x manufacturer's buffer. Samples were amplified in a 2720 thermal cycler (Applied Biosystems, CA, USA) for 35 cycles at 94ºC for 20 s, 54ºC for 30 s and 72ºC for 45 s. An initial denaturation step was carried out at 94ºC for 2 min, and a final extension step was performed at 72ºC for 2 min. Target DNA and water were always included as positive and negative controls, respectively. PCR products were separated by electrophoresis in 1.5% agarose gels stained with ethidium bromide and visualized under UV light. Each extracted sample was tested up to three times and considered positive if aphid DNA was detected in one of them.

Feeding trials

Forficula auricularia feeding trials were performed to determine aphid detection decay rates in earwig gut. After 11 days of starvation at room temperature, earwigs collected at the study site (see below) were individually placed in plastic containers (5 cm diameter) with a humidity source (cotton soaked in water). They were allowed to consume five frozen *A. spiraecola* for a maximum of two hours. This was the aphid species most abundant at the study site (Piñol et al. 2009a). Only those earwigs that had been observed to feed on three to five aphids were immediately frozen (t=0) at -20ºC or maintained for 3, 6, 14, 24, 48 and 72 h at 25ºC ± 1ºC, 70% ± 10% RH and L16:D8 photoperiod and then frozen. Ten individuals were tested for each time period (males and females). The half-life of aphid DNA in earwigs (50% of earwigs testing positive) was obtained from an exponential function fitted between the percentage of aphid detection in earwigs and time. We estimated the function parameters by least squares using SigmaPlot 8.0 (SPSS Inc. Chicago, IL, USA).

Field analysis of predation

The field site is an organic citrus orchard located in La Selva del Camp (Tarragona, NE Spain; 41º 13' 7"N, 1º 8' 35"E). The orchard consisted of *ca*. 300 Clementine trees grafted on the hybrid rootstock Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb.). An artificial refuge was placed on two branches of fifteen trees. These refuges, similar to those

described by Suckling et al. (2006), consisted of plastic shell vials (7.6 cm length; 1.2 cm diameter) covered with garden hosing with caps on top to create darkness. The refuges were tied to branches with tape and were checked once a month (from April to August) for earwig presence. Earwigs were collected in the morning and preserved at -80ºC for subsequent molecular analysis. Twenty earwigs (10 males and 10 females) were tested every month using the designed primers, except in April, when only sixteen earwigs were obtained from the refuges (five males and eleven females). The aphid detection percentages obtained from males and females were compared using the Pearson chi-square test gathering all month's results in a unique analysis. The software used was SPSS 15.0 (SPSS Inc. Chicago, IL, USA).

The abundance of aphids in the canopy was calculated in nine different trees from the same orchard. These trees were randomly chosen at the beginning of the experiment and periodically sampled on the same day that earwigs were collected. Aphids were sampled using beating trays (three vigorous blows to the tree canopy in opposite directions), captured with entomological aspirators, and immediately preserved in 70% ethanol. The total number of aphids found in each sample was counted.

Results

Primers

A pair of aphid-specific primers was successfully designed from the COI region. Primer sequences were: 5'-ATTTGGTATTTGATCAGG-3' (AphF1) and 5'-CGTGGAAAAGATATATCTGGAC-3' (AphR3). They amplified a fragment of 224 bp for all the aphid species tested without amplifying the DNA of other potential prey species, such as two Psocoptera, five non-aphid Hemiptera, one Lepidoptera, one Collembola and one Thysanoptera (fig. 4.1). Predators such as earwigs, ladybird beetles, spiders, anthocorid bugs and hoverflies also tested negative, as did two aphid parasitoids (Hymenoptera: Aphelinidae, Braconidae). Even though throughout the manuscript we refer to aphid-specific primers, they have been only tested on species belonging to the Aphididae family.
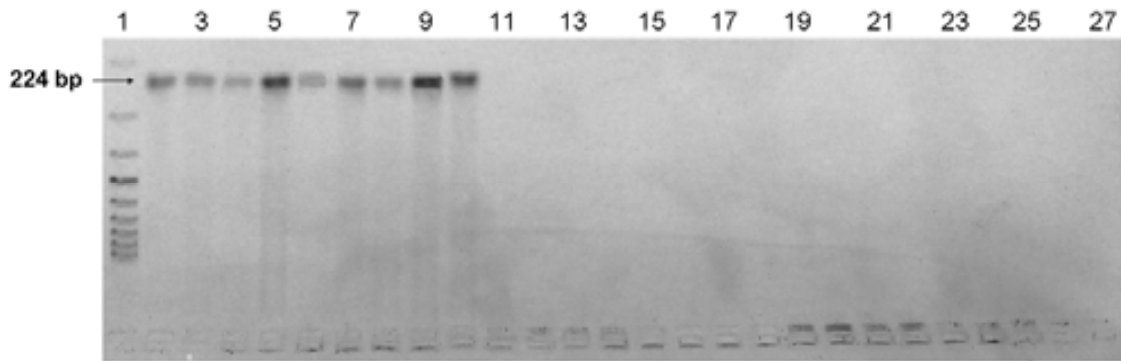
Fig. 4.1. Agarose gel electrophoresis of PCR-amplified DNA using the aphid-specific COI primers AphF1/AphR3 (224 bp). Lane 1, 100 bp molecular-size marker; lane 2, *Aphis spiraecola*; lane 3, *Aphis gossypii*; lane 4, *Toxoptera aurantii*; lane 5, *Myzus persicae*; lane 6, *Aphis craccivora*; lane 7, *Nasonovia ribisnigri*; lane 8, *Macrosiphum euphorbiae*; lane 9, *Aulacorthum solani*; lane 10, *Hyperomyzus lactucae*; lane 11, *Trichopsocus clarus*; lane 12, *Ectopsocus briggsi*; lane 13, *Ceroplastes sinensis*; lane 14, *Icerya purchasi*; lane 15, *Saissetia oleae*; lane 16, *Aleurothrixus floccosus*; lane 17, *Phyllocnistis citrella*; lane 18, *Bemisia tabaci*; lane 19, *Entomobrya* sp.; lane 20, *Frankliniella occidentalis*; lane 21, *Forficula auricularia*; lane 22, *Adalia decempunctata*; lane 23, *Philodromus cespitum*; lane 24, *Orius majusculus*; lane 25, *Episyrphus balteatus*; lane 26, Araneidae; lane 27, negative control.

Aphid DNA detection

Aphid DNA was detected in 70% of earwigs immediately after feeding (t=0). At t=3h and t=6h aphid detection increased to 100%, before dropping to 20% at 72h after ingestion (fig. 4.2). The low percentage of detection at t=0 (70%) was considered to be an artifact, as DNA was extracted only from earwig abdomens. The length of earwigs (males 14-21 mm; females 13-18 mm; Albouy & Caussanel 1990) makes it likely that some prey had not yet reached the abdomen immediately after feeding. Thus, this value (70% at t=0) was not used to draw the function of decay in detectability. The detection of aphid DNA in earwigs was better fitted to an exponential decay with a half-life of 23.8 hours.
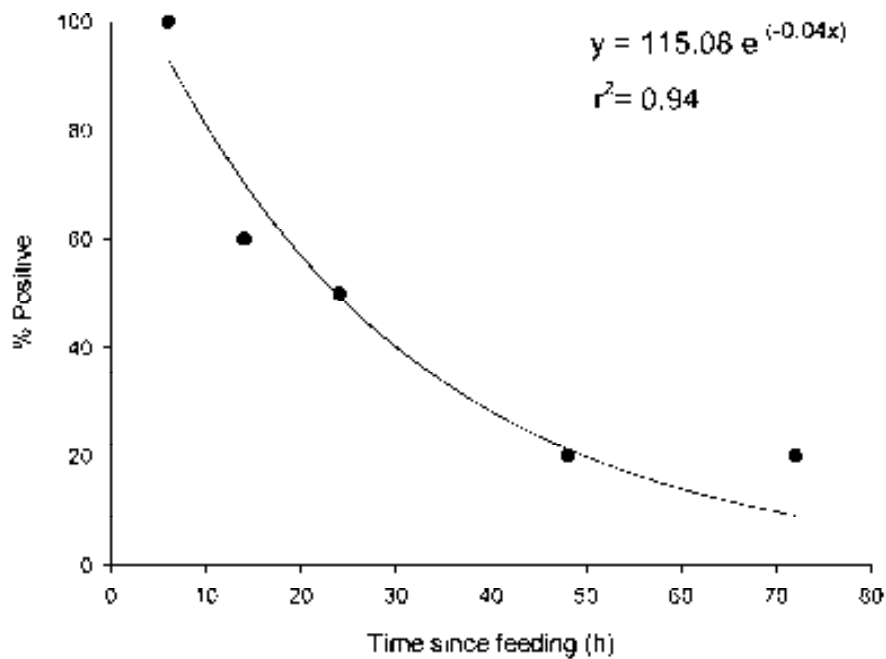
Fig. 4.2. Detection of *Aphis spiraecola* DNA in *Forficula auricularia* at different times after ingestion in a laboratory feeding trial performed at 25ºC.

Field analysis of predation

Aphids were scarce in the trees in April, May and August (Mean ± SE; 0.1 ± 0.1 aphids per sample) (fig. 4.3). June was, by far, the month with the highest population of aphids in canopies (203 ± 53 aphids per sample), followed by July, albeit with a much lower population (0.8 ± 0.3 aphids per sample). In April and August no earwig tested positive for aphid DNA presence. In the other months, earwigs contained aphid remains (30% in May, 50% in June and 35% in July). The aphid peak in the field occurred in June, and this was also the month with the highest percentage of earwigs screening positive for aphid DNA. No difference was found in the detection of aphid DNA between earwig males and females (n= 60; χ^2 = 0.64, *P =* 0.43).
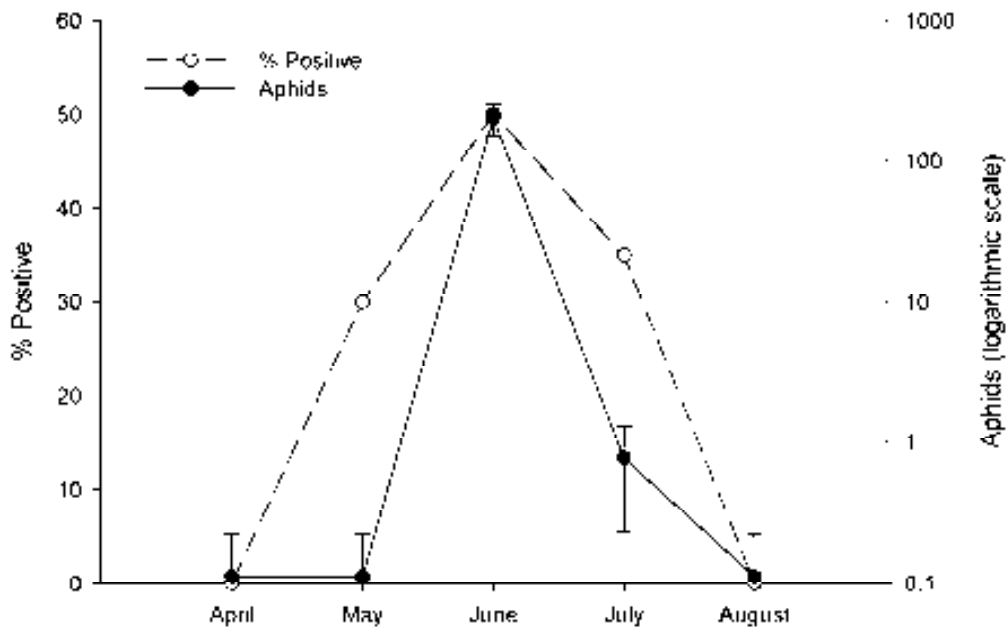
Fig. **4.3**. Percentage of *Forficula auricularia* screening positive by PCR using AphF1/AphR3 primers (dashed line) and mean abundance (± SE) of aphids captured per citrus canopy (solid line) in each tested month.

Discussion

The group-specific primers that we designed successfully amplified a 224 bp fragment of the COI region of all the aphid species tested, which belong to two different tribes of the Aphididae family. They also made it possible to detect aphid remains in earwigs. Multiple-copy DNA regions (such as COI) and short amplified sequences (100-300 bp) have previously proved suitable for primer design in gut-content analyses (Agustí et al. 2003a; King et al. 2008). The aphid-specific primers developed by Harper et al. (2005) were inadequate for our purposes as they detected earwig DNA. On the contrary, the primers that we designed are aphid-specific in the context of this study, as tested negative when earwigs were screened and also when non-aphid prey, parasitoids and predators other than earwigs were tested. The designed primers could probably be of interest to study antagonistic interactions not only in citrus crops, but also in other agroecosystems as many of the insect species tested here are common in several agroecosystems. For instance, *Nasonovia ribisnigri* is a widespread aphid pest in lettuce fields (Reinink & Dieleman 1993; Díaz et al. 2010) and the syrphid *Episyrphus balteatus* and the parasitoid *Aphidius colemani* Viereck are natural enemies of *Myzus persicae* on sweet-pepper plants (Pineda et al. 2007).

The detectability half-life of aphid remains in earwig guts was approximately one day. Spiders usually have longer detection periods (Agustí et al. 2003a; Ma et al. 2005; Sheppard et al. 2005; Greenstone et al. 2007; Hosseini et al. 2008; Monzó et al. 2010) whereas in other predators, such as beetles, heteropterans, neuropterans and mites, the detection periods are generally shorter (Chen et al. 2000; Agustí et al. 2003b; Ma et al. 2005; De León et al. 2006; Read et al. 2006; Juen & Traugott 2007; Hardwood et al. 2007; Zhang et al. 2007; Hosseini et al. 2008). However, there are exceptions to the above distinction between spiders and insects; i.e. the detectability half-life of aphids in lycosid spiders was much lower (t= 3.7h; Kuusk et al. 2008) than that of aphids in carabids (t=28.9h; Sheppard et al. 2005). In fact, there are some factors that affect the detectability of prey DNA in predators that should be considered before comparing half-lives, such as the feeding mode and the digestive physiology of each specific predator (Greenstone et al. 2007). Temperature also influences the detectability of prey DNA in the predator gut (Hoogendoorn & Heimpel 2001; Hosseini et al. 2008; von Berg et al. 2008). In this study, earwigs were maintained at 25 ºC, close to summer field conditions in the study area. Even if higher temperatures usually imply lower detection rates (von Berg et al. 2008) presumably as a result of an increased digestion rate (Hoogendoorn & Heimpel 2001), in the case of earwigs detection was still quite high at 25ºC.

Aphid DNA was found in field-collected earwigs in three out of the five months. In May, before the severe aphid attack started, 30% of the earwigs tested positive for aphid DNA, while in June, during the pest outbreak, detection rose to 50%. In July, when aphid populations were declining, 35% of earwigs still tested positive. This means that earwigs fed on aphids before, during and after the aphid outbreak. The 50% of positives found in June is a high value, as – although higher percentages in detectability in other prey-predator associations have been found (Ma et al. 2005; Hardwood et al. 2007; Zhang et al. 2007; Moreno-Ripoll et al. 2012) –, most studies usually report lower percentages, ranging from 0% to 40% (Agustí et al. 2003a; Harper et al. 2005; Read et al. 2006; Hardwood et al. 2007; Juen & Traugott 2007; Kuusk et al. 2008; Kuusk & Ekbom 2010; Monzó et al. 2010; Moreno-Ripoll et al. 2012). However, the predation on aphids in June, when aphid density was at its maximum, and in July, when the aphid population was already declining, probably had little effect on the overall aphid dynamics. What is really important for the dynamics of the aphid population is the 30% predation detected in May. Even though aphid outbreak still took place -probably because earwig abundance was below the threshold needed to eradicate aphid populations (Piñol et al. 2009b)-, 30% predation in May most probably reduced aphid outbreak extent. When aphid density is very low, predation can have a disproportionate effect on final aphid density, because the per capita effect of each predation event on the aphid population is higher (Piñol et al. 2009b). This would confirm the European earwig's important role as an aphid predator in citrus orchards as a consequence of its early seasonal pressure on this pest (Romeu-Dalmau et al. 2011).

To conclude, molecular techniques have been successfully used to track aphid predation by earwigs, providing a new tool for estimating the earwig's role as a biological control agent. We showed that earwigs are important predators of aphids in citrus orchards, particularly as a result of their early pressure on this pest. The designed primers might also be useful in agroecosystems other than citrus orchards.

Acknowledgements

Chapter 5

Friend or foe? The role of earwigs in a Mediterranean organic citrus orchard

Submitted to *Biological Control*;

Carla Romeu-Dalmau, Josep Piñol & Xavier Espadaler

Abstract

As earwigs (Insecta: Dermaptera) are considered both effective predators of aphids and pests in their own right in citrus orchards, the aim of the present study was to examine their relative role as pest versus predator. We conducted a two-year experiment of earwig exclusion from citrus canopies and compared aphid attack, flower survival and fruit yield in trees with earwigs (control trees) with those in trees without earwigs (banded trees). However, as not only earwigs but also all other crawling insects were excluded from the banded trees, we added a third group of trees (earwig trees) where crawling insects were excluded but earwigs were added to the canopy every 1-2 weeks. We hypothesized that if the same results were obtained in control and earwig trees, and both differed from those obtained in banded trees, earwigs would most probably be the cause of these differences. Overall, aphid attack in trees with earwigs was less severe than aphid attack in trees without earwigs; we also found that aphid density was negatively related to earwig abundance. Earwigs also negatively influenced flower survival but this effect was no longer observed once trees naturally abscised their own flowers and fruitlets. Finally, we did not find any difference in fruit yield between the treatments, or any relationship between earwig abundance and fruit production. Thus, as earwigs appeared to control aphid populations while not affecting fruit yield, we can conclude that earwigs are beneficial insects in this Mediterranean organic citrus orchard.

Introduction

Earwigs (Insecta: Dermaptera) are common in agro-ecosystems. As omnivores, they have been considered both natural enemies of pests and pests in their own right. As insectivores, earwigs have proven effective predators of aphids in apple, cherry and citrus trees (Mueller et al. 1988; Nicholas et al. 2005; Piñol et al. 2009a; Dib et al. 2010 & 2011; Stutz & Entling 2011). They are also key biocontrol agents of several non-aphid pests, such as the leafroller *Epiphyas postvittana* (Walker) in vineyards (Frank et al. 2007), the psyllid *Cacopsylla pyri* L. in pears (Debras et al. 2007; Höhn et al. 2007) or the fall armyworm *Spodoptera frugiperda* (Smith) in maize (Wyckhuys & O'Neil 2006). As herbivores, earwigs are perceived as pests since they may feed on leaves, flowers and soft fruits (Brindley 1918; Fulton 1924; McLeod & Chant 1952; Grafton-Cardwell et al. 2003). In citrus groves, some Californian growers claim that heavy infestations of earwigs may require treatment as they have observed earwigs feeding on plant material (Kallsen 2006).

Earwigs are essentially nocturnal; they forage at night and seek dry and cool places to hide during the day (Albouy & Caussanel 1990). In the studied citrus grove, two earwig species regularly climb to tree canopies searching for food and shelter: the European earwig *Forficula auricularia* L. and *Forficula pubescens* Gené. In this study we focus on the European earwig, *F. auricularia*. We considered only this species because we have previously shown that it appeared to regulate aphid populations while *F. pubescens* did not (Romeu-Dalmau et al. 2011).

In citrus canopies earwigs have been considered both as pests (Grafton-Cardwell et al. 2003; Kallsen 2006) and as beneficial insects (Piñol et al. 2009a). The aim of the present study was to examine earwigs' relative role as pest versus predator. To achieve this goal, we excluded earwigs from canopies and compared aphid attack, flower survival and fruit yield in trees with earwigs (control trees) with those in trees without earwigs (banded trees). However, to exclude earwigs from canopies we used a sticky barrier that also excluded other crawling insects, mainly ants (Piñol et al. 2009a). For this reason, we added a third group of trees, where earwigs were present but ants were not (earwig trees). The hypothesis was that if similar results were obtained in the control and earwig trees (both treatments with earwigs), and those results were different from those obtained in the banded trees (without earwigs), this would indicate that the differences were caused by earwigs.

Materials and methods

Study site and experimental setting

The study was conducted in a citrus plantation located in La Selva del Camp (Tarragona, NE Spain; 41° 13' 7"N, 1° 8' 35"E). The climate is Mediterranean, with a rainy spring and autumn and a dry winter and summer. The grove consisted of ca. 300 clementine trees grafted onto the hybrid rootstock Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb.). The plantation complies with all organic agriculture standards. The trees were watered when necessary.

The experiment was conducted on trees planted in 2001, originally grafted as "Marisol" clementines but re-grafted in 2006 to "Clemenules" clementines. The trees were set in four different rows, and each row was divided into two blocks. Each year, we applied a randomized block design by randomly assigning each tree within a block to one of the three treatments. In the control trees (n=15 in 2009 and n=8 in 2010), crawling insects (and thus earwigs) had free access to the canopy. In the banded trees (n=8), crawling insects were excluded using a polibutene-based sticky barrier (Rata Stop®, Frabo Adesivi, Milan, Italy) applied to an alimentary plastic sheet tightly attached over a padding cylinder in contact with the trunk (Samways & Tate 1985). In the earwig trees (n=8), crawling insects were excluded with the same sticky barrier as in the banded trees but earwigs were added to the canopy every 1-2 weeks. As the aim was to achieve the same number of earwigs in the earwig trees as in the control trees, the number of individuals in both was recorded beforehand (see below) to estimate the number of earwigs that needed to be added to the earwig trees. The added earwigs were European earwigs *Forficula auricularia*, and they were collected from non-experimental citrus trees on the same day that earwig abundance was recorded.

The treatments were established in March 2009 and in February 2010. Twice a month we checked the sticky barriers to guarantee that they were operative. Tall grasses and forbs were also periodically trimmed so that they would not act as bridges for crawling insects to reach the canopy.

Earwig abundance

An artificial refuge was placed on two branches of each sampled tree. These refuges, similar to those described by Suckling et al. (2006), consisted of plastic shell vials (7.6 cm length; 1.2 cm diameter) covered with garden hosing with caps on one side to create darkness. The refuges were tied to branches with tape and checked fortnightly (from April to October) for earwig

abundance.

Aphid attack

Aphid population in tree canopies was monitored every 1-2 weeks during the main aphid attack (May-June). Two circles of 0.125 m^2 were haphazardly selected on two opposite sides of each canopy (Hermoso de Mendoza et al. 2001; Piñol et al. 2009a). Within each circle, each tender leaf was classified into one of the following categories, according to aphid abundance: no aphids, 1-5, 6-25, 26-100 and more than 100 aphids per leaf. To calculate aphid density within 0.25 m^2 (considering both circles), the above-mentioned abundance ranges were considered as having 0, 2, 12, 50 and 250 aphids, respectively.

We did not distinguish between aphid species, but seven species of aphids have been found so far in the studied organic citrus orchard, the most abundant being *Aphis spiraecola* Patch and *Aphis gossypii* Glover (Piñol et al. 2009a).

Flower survival

Flower buds were marked with a wool thread tied to the flower peduncle on May 20th 2009 (40 flowers per tree) and on June 1st 2010 (50 flowers per tree). The number of marked flowers (subsequently fruitlets) still in the tree was periodically recorded until the end of August. Throughout the manuscript we refer to flower/fruitlet to indicate that even though we initially marked flowers, after a few weeks of marking they became fruitlets. Finally, as citrus trees naturally shed damaged and undamaged flowers and fruits (Stephenson 1981), the proportion of marked flowers/fruitlets still in the tree was analyzed on two different days, i.e. before and after the natural flower and fruitlet abscission occurred.

Fruit yield

The fruit was harvested by hand in December, and the total fruit yield per tree was recorded (kilograms).

Statistical analysis

In order to avoid the temporal pseudo-replication that results from the repeated monitoring of each tree (Crawley 2007), we used cumulative values (for earwig and aphid populations) or the values obtained on a specific day (for flower survival and fruit yield) as dependent variables to run the statistical tests. To obtain the cumulative values we plotted the abundance of earwigs or the density of aphids over time and measured the area below each resultant curve using

Sigmaplot 8.0 tools (SPSS Inc. Chicago, IL, USA). To analyze the effect of treatments on each variable studied, we used univariate permutational ANOVA's. For earwig abundance, aphid density and fruit yield, the raw data was square-root transformed, while for flower survival we used the arcsine transformation of the square root of each proportion. Treatment and block were considered as fixed and random factors, respectively. The Euclidean distance was used to calculate the dissimilarity between samples and pair-wise tests (corrected with the False Discovery Rate; Benjamini & Hochberg 1995) were conducted following significant differences between treatments. The software used was PRIMER v6 and PERMANOVA + (Anderson et al. 2008).

We performed additional analyses to relate earwig abundance (independent variable) with the other variables studied (dependent variables) in the control trees. Distance-based linear models (DISTLM) were used to examine each relationship. When Euclidean distances are used, and even though the P-values are obtained by permutation, DISTLM can be considered equivalent to traditional linear regressions (Anderson et al. 2008). For each studied relationship (i.e. earwig abundance vs aphid density, earwig abundance vs. flower survival and earwig abundance vs. fruit yield; for 2009 & 2010), the cumulative abundance of earwigs varied, as it was calculated up to the particular day in question. For instance, to study the relationship between earwigs and flower survival before abscission occurred, the cumulative earwig abundance was calculated up to the day considered prior to abscission. Furthermore, to study earwigs' role as an aphid predator we used earwig cumulative abundance and aphid cumulative density up to aphid peak, as from previous studies we know that only predation occurring before aphid population peaks is actually effective in controlling aphid outbreak (Piñol et al. 2009b). Whenever it was necessary, we also performed permutational ANCOVA's to combine data from both years in the same analysis and obtain more comprehensive results. In these ANCOVA analyses earwig cumulative abundance was considered a covariate and the year a random factor.

Results

Earwig abundance

Both earwig species, *Forficula auricularia* and *F. pubescens*, used the refuges. However, the European earwig was much more abundant in the refuges than *F. pubescens* (only 1% of the earwigs found were *F. pubescens*), and thus, throughout the paper, when we refer to earwigs we are mainly dealing with the European earwig, *F. auricularia*.

Earwig abundance differed significantly, depending on the treatment (Pseudo-$F_{2,21}$=167; P=0.0001 for 2009; Pseudo-$F_{2,14}$=59; P=0.0001 for 2010). Control trees had more earwigs than earwig trees, and both treatments had significantly more earwigs than banded trees (fig. 5.1).
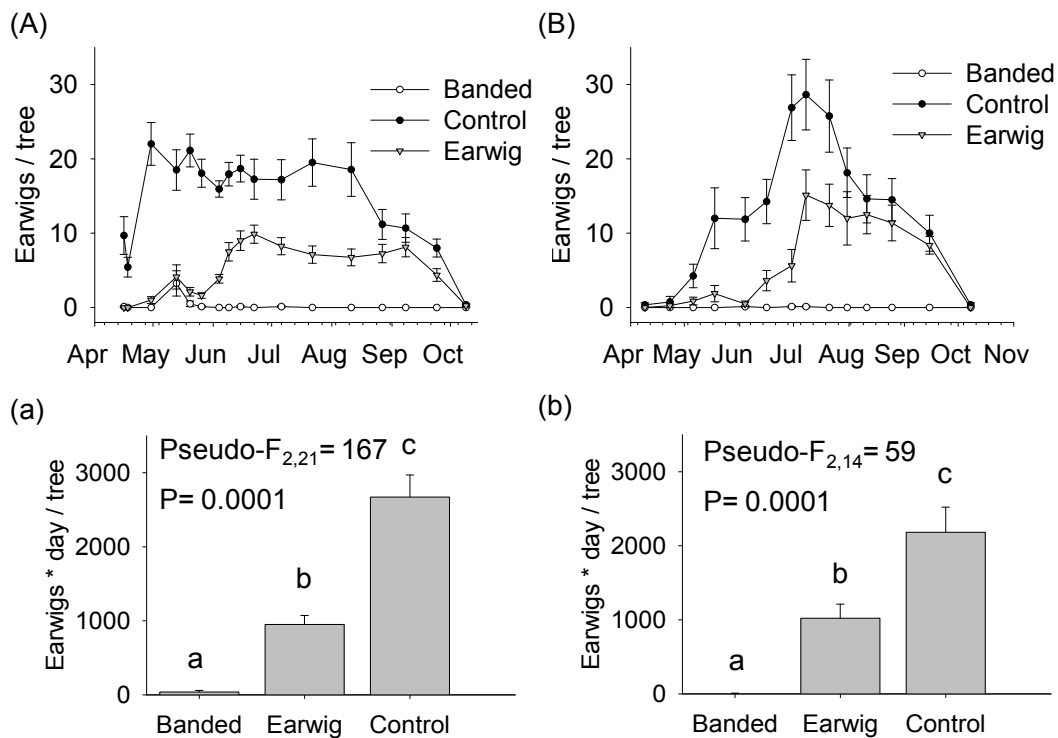

Fig. 5.1. Mean (± SE) abundance of earwigs found in the refuges per tree per sampling date; (A) 2009, (B) 2010. The mean cumulative earwig abundance (earwigs*day) per tree and per treatment for the whole period of study is represented in (a) for 2009 and in (b) for 2010. Each (a) and (b) diagram shows the Pseudo-F statistic resulting from the univariate permutational ANOVA and its statistical significance. Different letters indicate treatments with significantly different earwig cumulative abundances (Pair-wise tests corrected with the False Discovery Rate).

Earwig abundance from April to aphid peak (early-mid June) in 2009 was higher than in 2010 (Pseudo-$F_{1,36}$=8.1; P=0.007; Table 5.1). The same tendency was observed when earwig abundance was considered prior to the abscission of flowers (April to late June; Pseudo-$F_{1,36}$=6.0; P=0.017).

Treatment	Earwigs to aphid peak		Earwigs to abscission	
	2009	2010	2009	2010
Control	16.0 ± 1.0	7.1 ± 1.3	16.5 ± 0.8	9.9 ± 1.6
Earwig	1.8 ± 0.3	1.2 ± 0.4	3.9 ± 0.5	1.8 ± 0.5

Table 5.1. Mean (± SE) abundance of earwigs per tree in control and earwig trees. 'To aphid peak' means all sampling dates up to 3 June in 2009 and up to 16 June in 2010. 'To flower abscission' means all sampling dates up to 22 June in 2009 and up to 30 June in 2010.

Aphid attack

An aphid outbreak occurred in June of both years and each time it was significantly different between treatments (Pseudo-$F_{2,21}$=9.8; P=0.001 for 2009; Pseudo-$F_{2,14}$=3.8; P=0.046 for 2010; fig. 5.2). In 2009 aphid attack was significantly less severe in the control and earwig trees than in the banded trees. In 2010 the control trees also had a less severe aphid attack than the banded trees but these differences were only marginally significant after the pair-wise test was corrected.
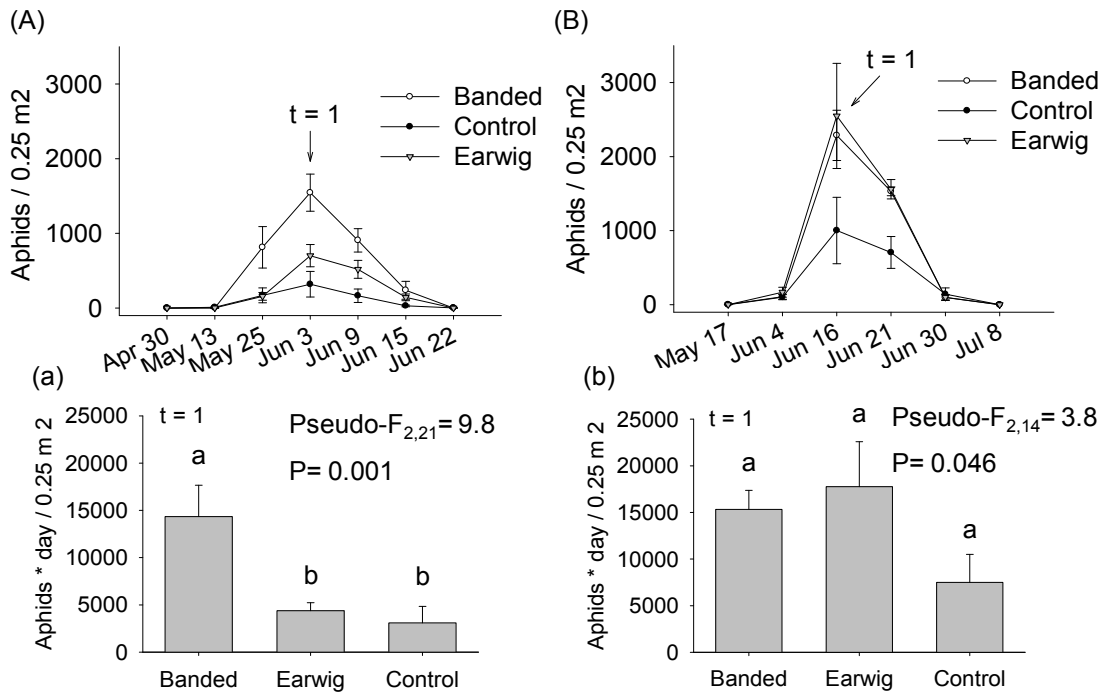
Fig. 5.2. Mean (± SE) density of aphids per 0.25 m² of the canopy in 2009 (A) and in 2010 (B). The mean cumulative aphid density to aphid peak (t=1) per tree and per treatment is represented below for 2009 (a) and for 2010 (b). Diagrams (a) and (b) report the Pseudo-F statistic resulting from the univariate permutational ANOVA and its statistical significance. Different letters indicate treatments with significantly different aphid cumulative densities (Pair-wise tests corrected with the False Discovery Rate).

Aphid attack was more severe in 2010 than in 2009 in both the control and earwig trees (Pseudo-$F_{1,36}$=14.9; P=0.0004; Table 5.2), while the aphid attack in the banded trees was very similar in both years (Pseudo-$F_{1,14}$=0.16; P=0.69).

Treatment	Aphids / 0.25 m²	
	2009	**2010**
Control	166 ± 67	368 ± 171
Earwig	286 ± 80	907 ± 332
Banded	787 ± 177	799 ± 244

Table 5.2. Mean (± SE) abundance of aphids per tree (0.25 m²) up to aphid peak (3 June in 2009 and 16 June in 2010) per treatment.

There was a negative relationship between cumulative earwig and aphid abundance in the control trees. This relationship was statistically significant in 2010 (Pseudo-F= 7.7, P=0.032; fig. 5.3b) and marginally significant in 2009 (Pseudo-F= 4.1, P=0.068; fig. 5.3a). Over the two years (ANCOVA), the dependence of aphid density on earwig abundance was statistically significant (Pseudo-$F_{1,20}$=14.8; P=0.003).
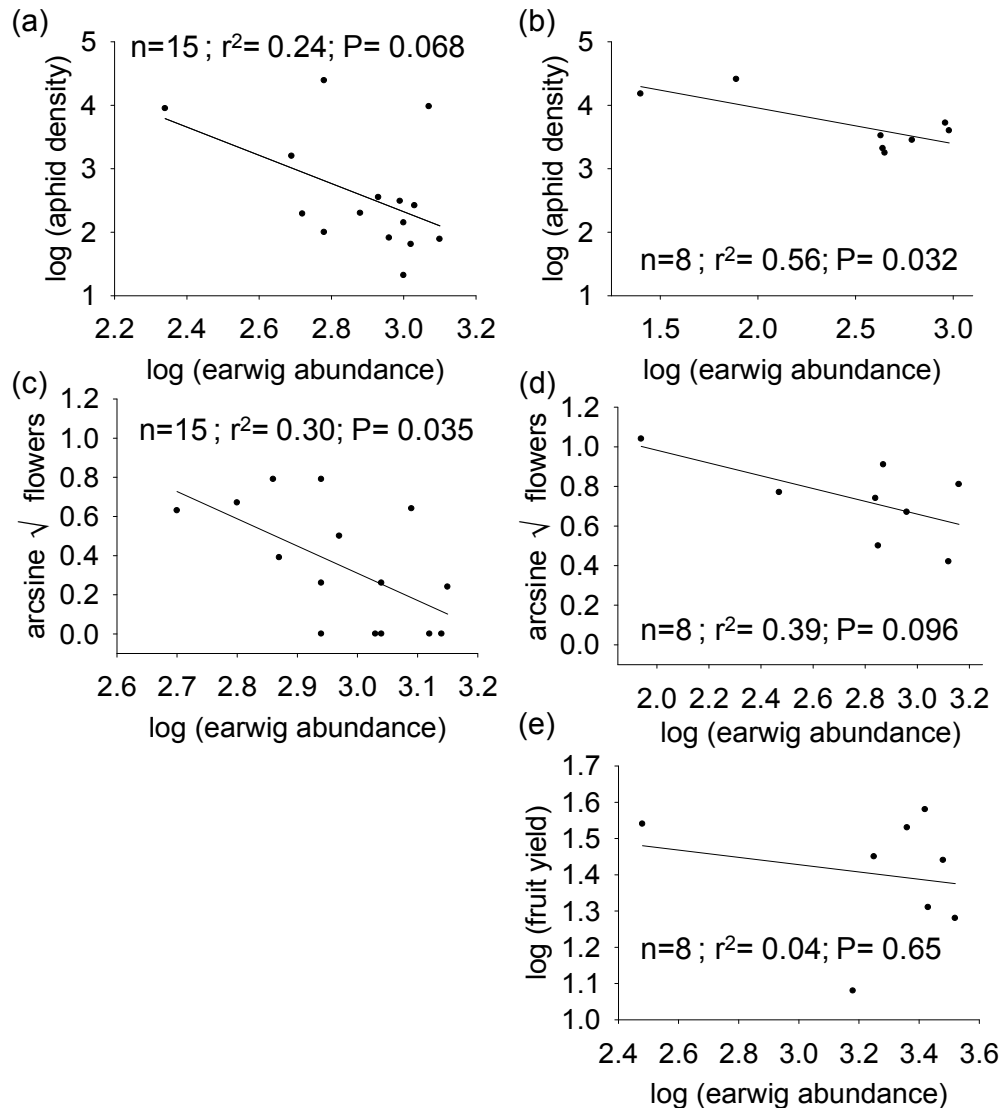

Fig. 5.3. Simple linear regressions illustrating the relationship between earwig cumulative abundance (independent variable) and the other variables of study (dependent variables) in control trees. Diagrams (a) and (b) show earwig cumulative abundance vs. aphid cumulative density to aphid peak per tree in 2009 and 2010, respectively. Diagrams (c) and (d) show earwigs vs. flower survival per tree before the abscission occurred in 2009 and 2010, respectively. Diagram (e) shows the relationship between earwigs and fruit yield in 2010. For each diagram, the total number of samples, the coefficient of determination and its statistical significance obtained from DISTLM are given.

Flower survival

In 2009 there were significant differences in flower survival between treatments before the flower/fruitlet abscission (Pseudo-$F_{2,21}$=10.7; P=0.0009). The post-hoc analysis revealed that fewer flowers survived in the control trees than in the other treatments (fig. 5.4A and 5.4a). After the abscission occurred in late June, the differences between treatments disappeared (Pseudo-$F_{2,21}$=0.53; P=0.60). In 2010, no significant differences between treatments were found either before or after the natural drop occurred (Pseudo-$F_{2,14}$=0.13; P=0.88 before; Pseudo-$F_{2,14}$=0.37; P=0.69 after; fig. 5.4B and 5.4b). It is worth mentioning that the percentage of marked flowers/fruitlets still in the tree after the natural drop was very low, regardless of the treatment (Mean ± SE; 1.4 ± 0.6 % in 2009 and 2.9 ± 0.6 % in 2010).
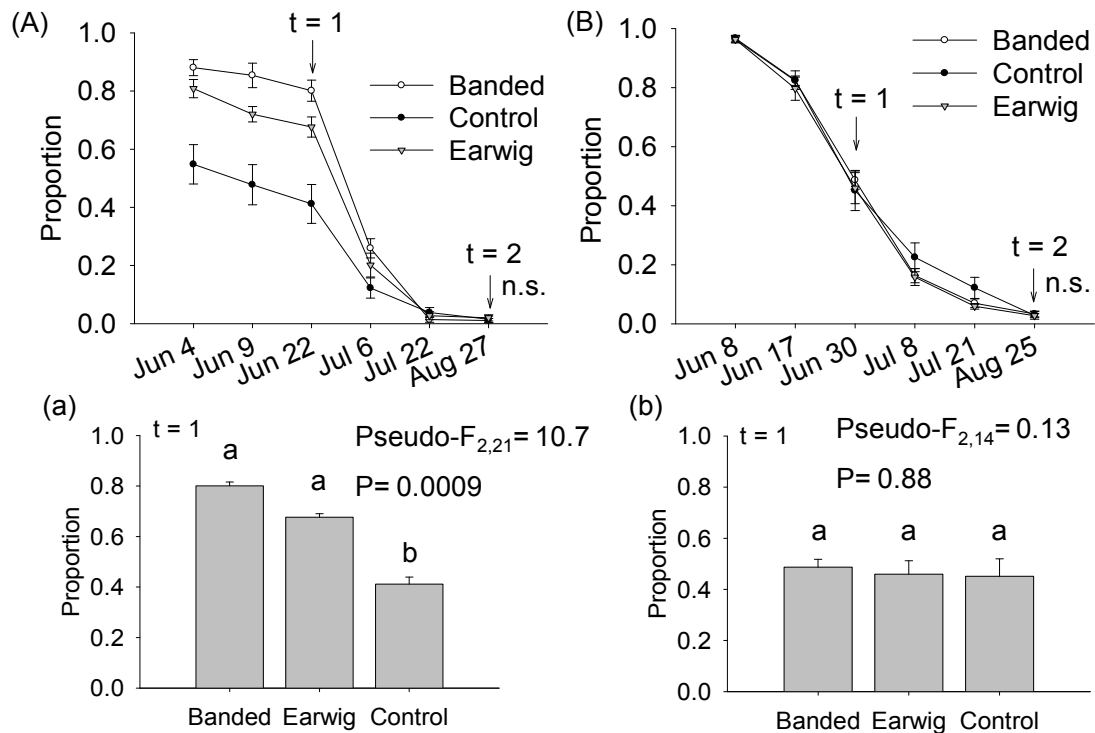

Fig. 5.4. Mean (± SE) proportion of marked flowers/fruitlets still in the tree in 2009 (A) and in 2010 (B). Diagrams (a) and (b) represent the mean proportion of flower survival per tree and per treatment on 22/06/2009 and 30/06/2010, respectively, i.e. before the abscission occurred (t=1). Diagrams (a) and (b) show the Pseudo-F statistic resulting from the univariate permutational ANOVA and its statistical significance. Different letters indicate treatments with significantly different flower survival (Pair-wise tests corrected with the False Discovery Rate). 't=2' indicates the sampling date used to run the post-abscission statistical tests. 'n.s' means that no significant differences were found in flower survival between treatments on that date.

There was a negative relationship between the cumulative abundance of earwigs and flower survival before the natural drop occurred in the control trees. This relationship was statistically significant in 2009 (Pseudo-F= 5.6, P=0.035; fig. 5.3c), and marginally significant in 2010 (Pseudo-F=3.9, P= 0.096; fig 5.3d). Over the two years (ANCOVA), earwig abundance as a covariate of flower survival before the natural drop was marginally significant (Pseudo-$F_{1,20}$=4.0; P=0.059). After the abscission, the relationship between earwigs and flower survival was no longer observed (Pseudo-F=1.99, P=0.20 for 2009; Pseudo-F=0.29, P=0.75, for 2010).

Fruit yield

As the fruit yield in 2009 was extremely low, regardless of the treatment (Mean ± SE; 1.1 ± 0.4 kg per tree) – probably as a consequence of inadequate pruning –, we discarded the 2009 yield and it was no longer considered in the study. In 2010 the mean production increased up to 22.9 ± 1.9 kg/tree. There were no differences in fruit yield between the treatments in 2010 (Pseudo-$F_{2,14}$ =1.2; P=0.34; fig. 5.5) and there was a non-significant relationship between earwig abundance and fruit yield in the control trees (Pseudo-F=0.26, P=0.65; fig. 5.3e).
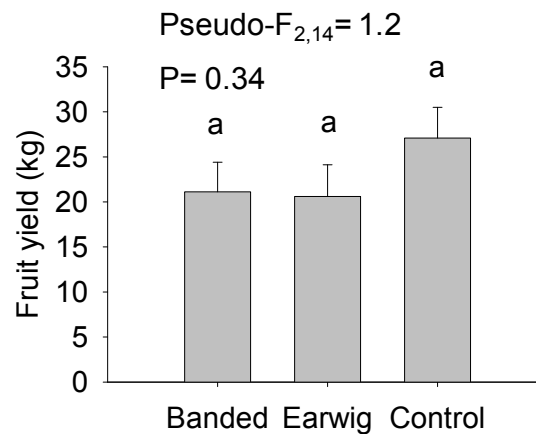

Fig. 5.5. Mean (± SE) fruit yield per tree and per treatment. The Pseudo-F statistic resulting from the univariate permutational ANOVA and its statistical significance are given. Identical letters indicate treatments with equivalent fruit yield (Pair-wise tests corrected with the False Discovery Rate).

Discussion

The experimental setting was partially successful as even though the control and earwig trees had earwigs while banded trees did not, earwig abundance in the earwig trees was not as high

as in the control trees. In future experiments more individuals should be added to earwig trees to achieve greater earwig abundance, comparable to those in the control trees. Earwig abundance also varied from year to year: i.e., from April to June – when earwigs mainly interacted with aphids and flowers – it was significantly higher in 2009 than in 2010. These differences in earwig abundance between treatments and years clearly influenced the results and thus need to be considered in the following interpretation.

Earwigs significantly affected aphid populations. Aphid attack in trees with earwigs was generally less severe than in trees without earwigs. Moreover, in 2009 – when earwig abundance was higher than in 2010 – both the control and earwig trees had significantly less aphid attack than in 2010. Finally, we found that aphid density was related to earwig abundance. Thus, our data strongly suggest that earwigs are important biocontrol agents of aphids in citrus trees, as reported before in the same orchard (Piñol et al. 2009a; Romeu-Dalmau et al. 2011). Earwigs have also been proven to be key predators of aphids in other agro-ecosystems, such as apple orchards, where various studies have shown that the greater the abundance of earwigs, the lesser the extent of the aphid attack (Mueller et al. 1988; Nicholas et al. 2005; Dib et al. 2010).

Earwigs appeared to negatively influence flower survival. In 2009 flowers survived less in control trees than in the other treatments and there was a significant negative relationship between earwig abundance and flower survival. In 2010 no differences in flower survival were observed between treatments, although a marginally significant negative relationship was found between earwig abundance and flower survival. As earwig abundance was much lower in 2010 than in 2009, this factor probably caused these contrasting results between years. This would imply that the effect of earwigs on flower survival has a threshold below which damage to flowers from earwigs is not detectable. Nevertheless, after the natural drop, very few (1-3%) marked fruitlets were still in the tree and earwig damage to citrus flowers was no longer observed. These percentages of flower and fruit survival after abscission, although low, are higher than those previously published by Stephenson (1981), who observed that only 0.2-1% of the citrus flowers matured to fruits. Finally, we did not observe any effect of treatments on fruit yield or any relationship between earwig abundance and fruit yield, which concurs with the abovementioned results of no differences in post-abscission flower survival between treatments.

As in this study, other works that have measured earwigs' relative role as pest versus predator in other agro-ecosystems also found their role as a natural enemy more important than their role as an herbivore. For instance, Boukary et al. (1997) concluded – after analyzing the gut content of more than 500 individuals – that the role of *Forficula senegalensis* Audinet-Serville as a pest in millet should be reconsidered, as they observed that arthropods were an important part of its diet while plants were not. Carroll & Hoyt (1984) also concluded that earwigs were

beneficial insects in apple orchards as, even though they chew on leaves, they also control aphid populations without causing any damage to fruit yield.

Conclusions

In accordance with previous results obtained in the same orchard, we have shown here that earwigs are important aphid predators. What is new in this study is that we have also shown a negative effect of earwigs on flower survival. These two results taken together would imply a dual role for earwigs in the studied citrus orchard, as both friend (reduction of aphid attack) and foe (reduction of flower survival). This negative effect of earwigs on flower survival was, however, overcome by the abscission of flowers and fruitlets and the final fruit production was not affected by earwig abundance. We can thus conclude that earwigs are beneficial insects in this Mediterranean organic citrus orchard as they reduced aphid attack but did not affect fruit yield.

Acknowledgements

Earwigs: pests or beneficials in California citrus orchards?

This chapter was published in a farmer's journal. Thus its style differs from that in the other chapters.

A supplementary section was added at the end of this chapter to give further information on the methodology used and on the statistical outputs.

Growers have noticed an increase in earwig abundance in California citrus orchards in recent years. This is probably due to a decline in the use of broad spectrum insecticides for pests such as California red scale and citrus thrips. The two earwig species most commonly found in California citrus orchards are the European earwig, *Forficula auricularia* L. and the ring-legged earwig *Euborellia annulipes* (Gené) (fig. 6.1). The ring-legged earwig is wingless, smaller in size, darker, and with shorter forceps (pincers) compared to the European earwig. Since the ring-legged species is rarely found on trees, it is not damaging to citrus and so it can be ignored. The European earwig can be found both on the ground and in the tree and has been observed to damage leaves and fruit and so it is of most interest to citrus growers. Thus, the European earwig was the species targeted in our study of the role of earwigs in California citrus.

Fig. 6.1. Adults (female and male) of the two earwig species most commonly found in California citrus orchards, the European earwig *Forficula auricularia* L. and the ring-legged *Euborellia annulipes* (Gené).

General information about the European earwig

Earwigs are nocturnal insects, they forage at night and during the daytime they seek dry and cool places to hide in such as rolled-up leaves, tree wraps, and trunk or soil crevices. Earwig females create nests in the soil in which they deposit their eggs. The females guard their eggs and when the eggs hatch the females feed the early instars in their subterranean nests. From the second nymphal stage onwards, European earwig nymphs gradually leave the nest and begin the free-foraging phase on the soil surface and in trees until they enter the soil again to reproduce as adults.

In the spring of 2011, European earwigs deposited many eggs in the soil and they readily hatched into nymphs. Based on the literature, we expected to see another period of egg laying in the fall. There was some egg laying, however, it was very small compared to the spring and few of the eggs hatched.

Earwigs are omnivorous; they feed on both plant and animal material. As an insectivore, the European earwig is considered a key biocontrol agent of important pests. It has been proven to regulate pest populations in orchards such as the woolly apple aphid *Eriosoma lanigerum* (Hausmann) in apple trees, the leafroller *Epiphyas postvittana* (Walker) in apple orchards and vineyards, and the pear psylla *Cacopsylla pyri* L. in pears. In fact, it is known that earwigs can consume higher amounts of aphids than other common predators such as ladybugs and green lacewings. Earwigs also feed on scale insects, mites, Collembola and fungi. As an herbivore, the European earwig can at times be a springtime pest as growers have observed them feeding on citrus leaves and fruit (fig. 6.2). Thus, it is not a simple matter to decide what role earwigs play in citrus orchards; are they pests or beneficials?

Fig. 6.2. Earwig potential damage to fruit. Damage to fruit may occur just after petal fall when the fruit is small.

The impact of earwigs on citrus leaves flush

To begin to understand the role of European earwigs, we ran several experiments in the field and the greenhouse to determine how much leaf flush the earwigs might consume.

Field leaf feeding experiment

We utilized tango-mandarin trees that were field planted one year earlier at the University of California Lindcove Research and Extension Center. Ten trees, called *earwig* trees, had foam wraps around the trunks. Earwigs could hide in these wraps and freely access the canopy. Ten trees, called *non-earwig* trees, had the tree foam wraps removed and earwigs were excluded using a sticky barrier (Stikem Special) applied to plastic wrap (Saran) tightly wound over a cylinder of batting in contact with the trunk (fig. 6.3). This system excluded earwigs but not other flying insects. Only ants could also be excluded with this system, but they were barely present in this plot. Ten flushes per tree were marked before earwig damage started and then sampled weekly. Damage was defined as chewed marks in the edge or middle of the leaf (fig. 6.4). We rated the percentage of leaf surface area lost (0%, 1-25%, 26-50%, 51%-75% or over 76%) per leaf. The treatment was applied on 13 April 2011 and weekly sampling continued until 27 June.

Fig. 6.3. One-year old tango-mandarin trees used in the field experiment. In *earwig* trees, earwigs had free access to the canopies; in *non-earwig* trees, earwigs were excluded using a sticky barrier applied to plastic wrap wound tightly over a batting cylinder in contact with the trunk.

During the first flush (from April to the beginning of May), leaves in *earwig* trees lost an average of 6.9 ± 1.6 % of their leaf surface area (fig. 6.4), while leaves in *non-earwig* trees lost an average of 0.8 ± 0.2 % leaf surface area. The average number of earwigs found per sampled day per tree was 3 in the *earwig* trees (both nymphs and adults present) and zero in

the *non-earwig* trees. Thus, earwigs did feed on and cause significant damage to leaves during the first flush; however we do not know if this level of damage affected the long-term growth of the trees. We plan to conduct additional studies with varying levels of earwig densities to determine the threshold of leaf damage that results in reduced growth and/or yield of young trees.

Fig. 6.4. Earwig damage to one-year old tango-mandarin leaves (May 6th).

Significant leaf damage was not observed in the second flush (middle of May until June) in either the *earwig* or *non-earwig* trees (fig. 6.5). The average number of earwigs found during this second flush per sampled day per tree was one in the *earwig* trees (only adults) and again zero in the *non-earwig* trees. Thus earwigs were still present during the second flush although in a much lower abundance.

Fig. 6.5. Second flush of the two-year old tango-mandarin (June 27th), undamaged by earwigs.

Greenhouse leaf feeding experiments

We conducted a set of experiments during July under greenhouse conditions (heating below 60 °F and cooling above 75 °F, shade all the time), with 1-year-old Valencia or Rough Lemon seedlings. We placed ten field-collected adult earwigs (5 males and 5 females) inside each cage (fig. 6.6) with one or two food sources for two weeks. The food source was (i) only a citrus seedling, to monitor leaf damage, or (ii) a citrus seedling and a California red scale infested lemon, to study earwig preferences between animal and plant material. We completed eight replicates for each food source.

In the first treatment (i), there was 0.2 ± 0.1% of surface area lost per leaf. In the second treatment (ii), there was 1.1 ± 0.2 % of surface area lost per leaf and earwigs ate 47 ± 9 % of the scales (fig. 6.7). In both experiments, earwigs did almost no damage to leaves, a result that contrasts with the results found in the field for the first flush, when there was 6.9% of surface lost per leaf. We need to conduct more tests, however, we believe that either the earwigs are changing their feeding habits as the season progresses or the nymphs and adults have different feeding preferences. During the first flush of the field trees many fourth instar nymphs were present while the earwigs in second flush of the field trees and those used in the greenhouse experiments were only adults. Nymphs seem to attack leaves much more than adults and adults seem to prefer to predate on California red scale. Since only adults are found in summer and they don't seem to feed on foliage very much at that time of year, our results suggest that control of European earwigs is not necessary in the summer.

Fig. 6.6. Greenhouse experiments. Twenty citrus trees were individually placed inside cages, each one with 10 earwigs (5 females and 5 males). Additionally, 10 of the cages also received a California red scale-infested lemon.

Fig. 6.7. Earwigs fed on California red scale infesting the lemon, leaving behind white areas indicating where the scales were completely consumed.

Pesticide trials

During 2011 we began laboratory and field studies to determine what types of insecticides would effectively control earwigs and so reduce the damage they cause to leaves and fruit in field situations during spring. We screened a wide variety of insecticides using a petri dish method. We soaked filter paper in the petri dish with a field rate of insecticide mixed in 100 gpa water and added individual earwigs to the dishes (fig. 6.8) for a total of 10 dishes per insecticide. We screened an array of registered insecticides (Table 6.1) including fairly broad spectrum insecticides such as organophosphate, carbamate, pyrethroid and neonicotinoid insecticides and also fairly selective insecticides such as Delegate, Success, Agri-Mek, Altacor and various insect growth regulators. We considered an earwig dead when it was not able to walk. As expected, we did not see any negative effect of the insect growth regulators on adult earwigs because they are not molting. We found that a very limited number of insecticide groups were fully effective in killing adult female earwigs. Lorsban, Sevin, Seduce, Leverage and Baythroid were effective in causing 100% kill by day 14. We found that some pyrethroids would initially 'knock down' or temporarily paralyze the earwigs, but earwigs would sometimes recover and we would see lower mortality at 14 days compared to 2 days.

Fig. 6.8. Petri dish bioassay for testing the effects of insecticides on earwigs. The filter paper is soaked in insecticide and the leaf disk is provided as food for the earwig.

Chemical group	Formulation	chemical	Rate per acre	% mortality at 2 days	% mortality at 14 days
Organophosphate	Lorsban Advanced	Chlorpyrifos	1 qt	100	100
Carbamate	Sevin XLR Plus	Carbaryl	5 qts	50	100
Pyrethroid	Baythroid XLR	Beta cyfluthrin	6.4 fl oz	60	100
Pyrethroid	Mustang	Zeta cypermethrin	4.3 fl oz	50	20
Pyrethroid	Danitol	Fenpropathrin	21 1/3 fl oz	80	60
Neonicotinoid	Assail 30 SG	Acetamiprid	6 oz	0	20
Neonicotinoid	Admire Pro	Imidacloprid	7 fl oz	0	10
Neonicotinoid	Actara	Thiamethoxam	5.5 oz	0	40
Mixture of pyrethroid and neonicotinoid	Leverage 2.7	Cyfluthrin and imidacloprid	11.6 fl oz	30	100
Spinosyns	Delegate WG	Spinetoram	6 oz	0	0
Spinosyns	Success	Spinosad	10 fl oz	0	20
	Seduce bait*		.16 gm/dish	90	100
Avermectins	Agri-Mek 0.7 SC	Abamectin	3.5 fl oz	0	0
Insect growth regulator	Esteem 0.86 EC	Pyriproxyfen	16 fl oz	0	0
Insect growth regulator	Micromite 80 WGS	Diflubenzuron	6.25 oz	0	0
Insect growth regulator	Applaud 70 DF	Buprofezin	46 oz	0	0
Tetronic acid derivative	Movento 240 SC	Spirotetramat	10 fl oz	0	0
Diamides	Altacor WDG	Chlorantraniliprole	4 oz	0	10

Table 6.1. Effects of various broad spectrum (orange highlight) and soft (green highlight) insecticides on survival of adult female earwigs. Rates of insecticides mixed in 100 gpa water volume. *Seduce applied as bait and fed on by earwigs.

Several important points about the pesticide test results should be highlighted. First, only Lorsban Advanced was able to quickly kill the earwigs (full control within 2 days). Secondly, most of the highly effective insecticides are very broad spectrum and so are difficult to integrate with natural enemies needed for other pests. Finally, Seduce was very effective in killing earwigs when it was provided as a bait that the earwigs fed on (rather than treated filter

paper). Seduce is an organically approved product that is very soft on natural enemies, thus it has great potential for use by citrus growers. We plan to repeat testing of all of these insecticides using nymphs to see if that stage is more or less susceptible to the insecticides.

In the field, the grower applied treatments of Sevin XLR and Baythroid XL (fig. 6.9) using a speed sprayer and 100 gpa on 15 April 2011 to the foliage of a 7-yr-old block of Rush navel oranges in Porterville, CA. The plots were 5 rows by 16 trees and replicated 3 times. We sampled the center 5 trees in each plot by shaking the foliage onto a beating sheet on two sides of the trees. In addition, on 25 April Seduce bait was applied to the ground of 3 plots. The foliar treatments successfully suppressed earwigs for about 3 weeks and then the adults were able to return to the trees. The Seduce treatment was ineffective in reducing earwigs. This was probably due to the fact that at the time of year the study was conducted (May), earwigs were predominantly in the trees and not foraging on the ground. This product may be more successful in the fall when earwigs are likely to be building nests in the ground. We plan to study Seduce treatment timing further. We surveyed the trees and did not find any fruit damage in either treated or untreated trees.
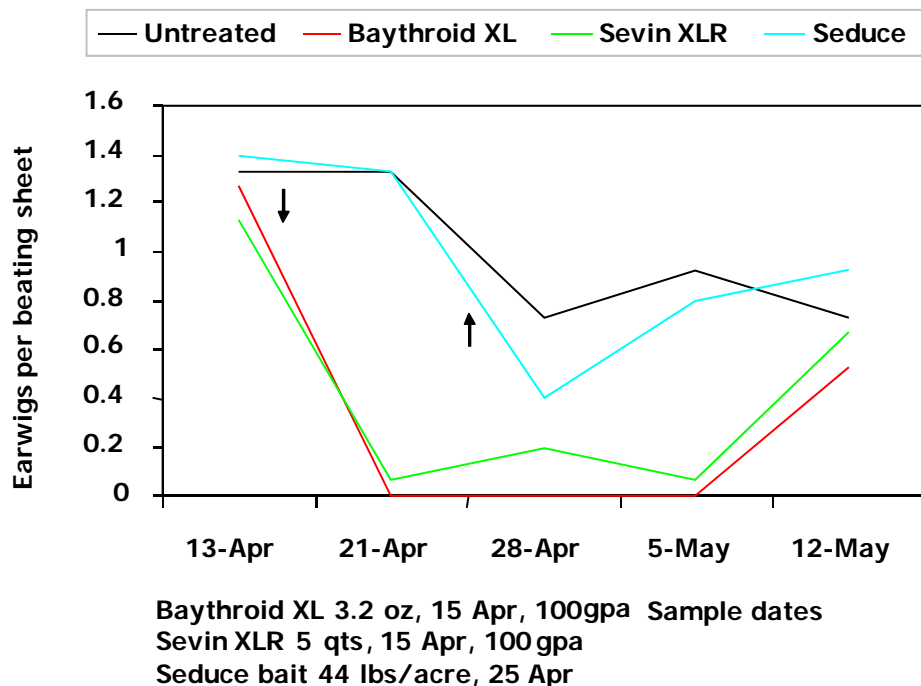

Fig. 6.9. Field Trial to determine the impact of various insecticides on earwig population. The Sevin XLR and Baythroid XL were applied on 15 April and the Seduce was applied on 25 April.

What we have learned so far

(i)   There are two species of earwigs in California citrus orchards. The European earwig is the one that growers should learn to identify, because it is the only one that climbs into the trees.

(ii)   Earwigs (and especially nymphs) may damage citrus first flush in spring. We did not observe fruit damage in our experiments, but others have observed fruit damage occurring at petal fall.

(iii)   Earwigs do not behave uniformly throughout the year. Even if they damage leaves during the first flush, they do not damage flush in the subsequent spring and summer flushes. This may be a seasonal change in diet or activity in the trees, or a difference between nymph and adult feeding habits.

(iv)   Earwigs are also predators of pests. In fact, in greenhouse conditions adults preferred feeding on California red scale rather than on leaves.

(v)   With one exception (Seduce bait), earwig adults are difficult to kill with anything but very broad spectrum insecticides such as organophosphate, carbamate and pyrethroid insecticides.

(vi)   Seduce bait is soft on natural enemies and very effective in the laboratory, but did not work in a spring field trial indicating that timing of application to attract ground feeding earwigs may be critical for successful control of earwigs.

Earwig Management Tactics

We have, in a very short period of time, learned some information about earwigs that will improve their management. In young nonbearing trees protecting growth is most important. The best management tactic to protect spring flush from earwig damage is to either remove the tree wraps (to limit earwig refuges) or spray broad spectrum organophosphate, carbamate or pyrethroid pesticides into the wraps and/or on the foliage. After the first spring flush, the earwigs become adults, they are found less frequently in the trees and they don't seem to damage leaves. In summer, the wraps could be replaced to provide protection against sunburn and to reduce suckering.

In mature orchards, leaf flush damage is of little concern because the tree can tolerate heavy damage and maintain production. In mature trees, earwigs will only be a problem if they attack the fruit at petal fall. We did not observe earwig damage to the fruit in our field study, but we know that it occurs in some orchards as growers and PCAs have observed it. We did observe feeding by adult earwigs on California red scales, indicating the adults can be significant beneficial predators. Therefore, the role of the European earwig in mature trees is not easy to

define: pest or beneficial? When earwigs are found to be attacking fruit, a management tactic would be to apply a full rate of pyrethroid or organophosphate with the petal fall citrus thrips-katydid treatment to protect the new fruit. We will continue our studies of Seduce earwig bait and other soft insecticides to develop a softer, more integrated approach to earwig management for mature orchards with chronic earwig problems.

Take home message

Are earwigs pests or beneficials? At this point we would describe them as both – depending on the time of year and the situation. Earwigs can be pests of flush in young trees in the spring when they build up in wraps. They can also be pests of mature trees if they feed on new fruit at petal fall. However, they can also be natural enemies of citrus pests such as California red scale and we did not find them damaging citrus flush or fruit in the summer. We will continue our studies to determine thresholds for treatment and management methods to help tip the balance towards European earwig as a beneficial insect.

Supplementary material

In this section, non-published information related to the methodology used and to the statistical outputs is presented.

Field leaf feeding experiment

The field experiment was conducted in a citrus plantation located at University of California Lindcove Research and Extension Center (Exeter, California; 36° 22' 23″ N, 119° 03' 45″ O). The experiment was conducted on tango-mandarin trees (*Citrus reticulata* Blanco var. 'Tango') planted in 2010. Trees were located in two different rows, and each row was divided into five blocks. We applied a randomized block design by randomly assigning each tree within a block to one of the two treatments (earwig and non-earwig). Treatments were established in April 2011.

To analyze the effect treatments had on leaves, we performed univariate permutational ANOVA's. We used the arcsine transformation of the square root of the proportion of leaf surface lost. Treatment and block were considered as fixed and random factors, respectively. The Euclidean distance was used to calculate the dissimilarity among samples and the software used was PRIMER v6 and PERMANOVA + (Anderson et al. 2008). We also performed additional analyses to relate earwig cumulative abundance (independent variable; sum of earwigs per tree) with leaf surface lost per tree in earwig trees. Distance-based linear models (DISTLM) - equivalent to simple linear regressions (Anderson et al. 2008) - were used to examine this relationship.

Leaves in non-earwig trees lost significantly less surface than leaves in earwig trees (0.8 ± 0.2 and 6.9 ± 1.6 %, respectively; Pseudo-$F_{1,9}$=19.4; P=0.003). However, the relationship between earwig abundance and leaf surface lost per tree in earwig trees was not statistically significant (Pseudo-F= 1.04; r^2= 0.12; P= 0.33).

During the second flush (middle of May until June) there was not any damage to any of the leaves either in earwig or non-earwig trees.

Greenhouse experiment

The greenhouse experiment was conducted at the UC Kearney Agricultural Research and Extension Centre facilities (Parlier, California; 36° 36' 2″ N, 119° 30' 39″ O). Earwigs (*Forficula auricularia* L.) were obtained from an almond orchard close to the station, as earwigs normally used the wraps that covered almond trunks as shelters. The experiment was conducted on 1-

year-old Valencia (*Citrus sinensis* (Linnaeus) var. 'Valencia'; treatment-1) or Rough Lemon (*Citrus jambhiri* Lush; treatment-2) seedlings and lasted two weeks. In treatment-1 (n=7[1]) earwig only food source was the plant material, while in treatment-2 (n=8) earwig food source was the plant material and a California red scale (CRS) infested lemon. Due to the limited number of earwigs found in the field, we placed fewer earwigs in treatment-1 (n=6[2]; 3 males and 3 females) than in treatment-2 (n=10; 5 males and 5 females). Lemons were infested with CRS three weeks before the experiment took place, and the number of CRS's per lemon was counted before and after the experiment. To study CRS mortality in natural conditions, we also performed a control treatment (n=10) where 10 seedlings were placed with a CRS infested lemon without earwigs for two weeks.

To analyze the effect treatments had on leaves and on CRS we performed univariate permutational ANOVA's. We used the arcsine transformation of the square root of CRS survival or of leaf surface lost. To standardize, leaf surface lost was divided by the number of earwigs added into each tree, because as stated above treatment-2 had more earwigs than treatment-1. Treatment was considered as a fixed factor, and as in the above mentioned analyses, the Euclidean distance was used to calculate the dissimilarity among samples.

Leaves in treatment-1 lost significantly less surface than leaves in treatment-2 (0.03 ± 0.02 and 0.1 ± 0.02 % respectively; Pseudo-$F_{1,13}$=12.5; P=0.009). This could indicate that earwigs preferred Rough Lemon variety than Valencia. Nevertheless, both percentages of surface lost were really low, and they were significantly lower than the 6.9 ± 1.6 % of surface lost per leaf in earwig trees in the field experiment (Pseudo-$F_{2,22}$=23.6; P=0.0001).

In treatment-2 -where a CRS infested lemon was also placed inside the cage with earwigs- 47 ± 9% of the scales disappeared while in controls (without earwigs) there was only a 2.7 ± 0.9% CRS mortality. These differences were statistically significant (Pseudo-$F_{1,16}$=34.9; P=0.0001) and indicated that earwigs predated on CRS.

Pesticide trials

Fig. s6.1 is fig. 6.9 but with the SE. Please note that the names of the pesticides are trade names. Thus, as shown in Table 6.1, Baythroid XL are cyfluthrins, Seduce is a spinosad bate and Sevin XLR is a suspension of carbaryl insecticide.

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[1] See below

[2] In the published manuscript we stated that we completed 8 replicates and used 10 earwigs per treatment to make the reading simpler.
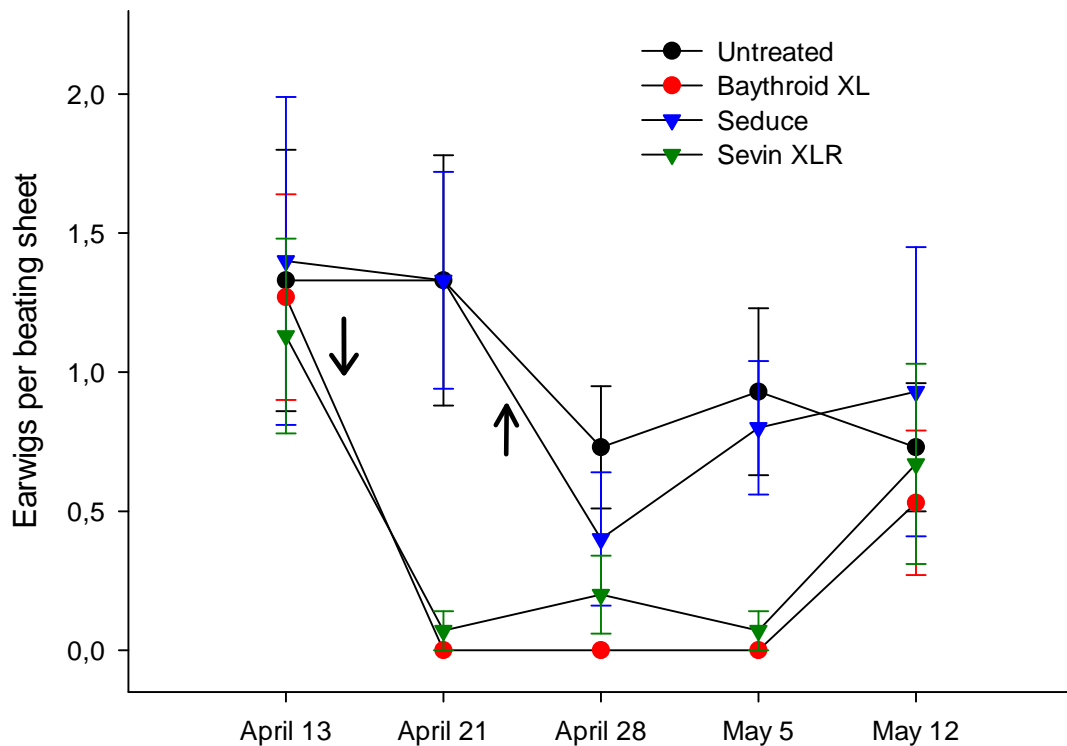
Fig. s6.1. Mean abundance of earwigs (± SE) found per beating sheet.

To analyze the effect each treatment (untreated; Baythroid XL; Sevin XLR; Seduce) had on earwig survival, we performed univariate permutational ANOVA's at each sampled day. Earwig abundance was log-transformed. Treatment was considered as a fixed factor. The Euclidean distance was used to calculate the dissimilarity among samples and pair-wise tests (corrected with the False Discovery Rate; Benjamini and Hochberg, 1995) were conducted following significant differences between treatments.

There were significant differences in earwig abundance between treatments on April 21 (Pseudo-$F_{3,56}$=10.8; P=0.0001), April 28 (Pseudo-$F_{3,56}$=4.3; P=0.007) and May 5 (Pseudo-$F_{3,56}$=7.2; P=0.0002). The post-hoc analyses revealed that on April 21 and May 5, Baythroid-treated trees and Sevin-treated trees had similar earwig abundances, significantly lower than those in Seduce-treated and untreated trees (both with similar earwig abundances). On April 28, Baythroid-treated trees had significantly less earwigs than untreated trees but no more significant differences were found between treatments. On the remaining dates, April 13 and May 12, no significant differences were found between treatments (Pseudo-$F_{3,56}$=0.0; P=0.99 and Pseudo-$F_{3,56}$=0.3; P=0.82, respectively).

Chapter 7

General discussion

"Increasing knowledge and
appreciation of the values
of invertebrates is [...] crucial" New, 2005

Earwigs and ants in Mediterranean organic citrus canopies

In the Mediterranean organic citrus orchard of this study, earwigs and ants have important roles (Piñol et al. 2009a & 2012). Their roles tend to be opposite when plant-sucking insects such as aphids are studied, i.e. ants are aphid mutualists (Way 1963; Stadler & Dixon 2005) and earwigs are aphid predators (Mueller et al. 1988; Nicholas et al. 2005; Dib et al. 2010). As earwigs and ants were concurrently excluded from canopies when common exclusion methods of crawling insects were used (Piñol et al. 2009a, 2010 & 2012), there was the subsequent question of which of them was actually responsible for the observed effects. Therefore, a method was designed to differentially exclude ants and earwigs from citrus canopies (Chapter 2). This method allowed free access of small and medium-sized ants to the canopy, but excluded earwigs. Although this method was finally not applied in this thesis, it should be useful in other agroecosystems where ants and earwigs co-occur and have important roles from an ecological point of view. This method is also useful to differentially exclude ants from tree canopies based on ant body size. In fact, in terrestrial communities, ants are the most frequently excluded insect, and thus this method might provide an opportunity to study the effect of individual or groups of ant species in comparison to other ant species, or to the entire ant community. As ants have had full consideration in previous studies (Piñol et al. 2009a, 2010 & 2012), in this thesis earwigs were chosen as the key studied insect, and another method was finally used which allowed the presence of earwigs but not of ants in canopies (Chapter 5).

Most studies on the European earwig have been conducted in USA (Washington and Oregon), Oceania (New Zealand and Australia) and North-Central Europe (Belgium, UK and the Netherlands), and in apple and pear orchards, but very little is known of the European earwig in Mediterranean citrus trees. In this particular agroecosystem, *Forficula auricularia* L. had two reproductive periods per year (Chapter 3), such as in other temperate climates (Fulton 1924; Crumb et al. 1941; Lamb 1974; Vancassel & Foraste 1980; Wirth et al. 1998; Burnip et al. 2002). However, the second brood occurred much later - in November and December - than in colder temperate regions (August; Crumb et al. 1941, Washington, USA; Behura 1956, Edinburg, UK; Lamb & Wellington 1975, Vancouver, Canada). Moreover, the European earwig was active in citrus canopies during a longer period (April to December) than in colder regions (May to October; Crumb et al. 1941; Behura 1956; Gobin et al. 2008; Moerkens et al. 2009; Moerkens et al. 2011). These differences between the Mediterranean study site and colder regions were expected, because as poikilotherms, the biological cycle of earwigs is dependent on temperature (Crumb et al. 1941; Behura 1956; Helsen et al. 1998; Moerkens et al. 2011). The most interesting result of this is that the European earwig's potential role as biocontrol agent of pests and/or a pest in its own right is likely to be higher in the Mediterranean due to this longer active period.

This thesis also makes a contribution to the knowledge of the earwig species *Forficula pubescens* Gené, which was found active in Mediterranean organic citrus canopies from May to December and had one reproductive period per year.

Finally, it was detected that *F. auricularia* and *F. pubescens* co-occurred in time and in space in citrus tree canopies without being in association or having any negative effect on each other. It was also observed that the abundance of both species significantly changed over a five-year period. As a similar significant interannual variation in the abundance of other insects has already been described in the citrus canopy arthropod community (Piñol et al. 2012), it appears that short-term studies can be misleading and that long-term monitoring should be conducted whenever possible.

Earwigs as predators in citrus canopies

The European earwig is an important aphid predator in citrus canopies (Cañellas et al. 2005). A negative relationship between aphid and earwig abundance was observed, suggesting a top-down control of aphids by earwigs (Chapter 3 & 5). This control was most likely a consequence of European earwig early seasonal pressure on aphids - i.e. 30 % predation detected in May, when the abundance of aphids was still extremely low (Chapter 4) -, which would have had a disproportionate effect on the final aphid density (Piñol et al. 2009b). However, even though earwigs exercised this early pressure on aphids, aphid outbreak still took place (see Figure 5.2, Chapter 5). This is because the effect of earwig density on the cumulative density of aphids is not linear, but has a threshold below which the density of aphids is high, and above which the density of aphids is very low (Piñol et al. 2009b). Thus, the European earwig appears to regulate aphid populations by reducing aphid outbreak, but if earwig density is not high enough, aphid outbreak still takes place.

The co-occurring earwig *F. pubescens*, climbed one month later to canopies (in May, instead of April as the European earwig) and apparently did not regulate aphid populations (Chapter 3). However, we know that this species is an aphid predator because preliminary visual gut-content analyses of *F. pubescens* digestive tubes showed aphid remains (unpublished observations). In addition, other studies considered this earwig species as an active aphid predator in apple and pear orchards (Debras et al. 2007; Dib et al. 2010).

Early predation of biocontrol agents such as coleopterans and heteropterans has already been proved to be important for the regulation of aphid populations in cereal (Ekbom et al. 1992), apple (Brown 2010), alfalfa (Pons et al. 2009) and soybean fields (van den Berg et al. 1997). Early predation is also crucial to control populations of the brown citrus aphid (BCA), *Toxoptera*

citricida (Kirkaldy), the most efficient vector of the Citrus tristeza virus (Michaud & Browning 1999; Urbaneja et al. 2008). As only alate aphids tend to move from tree to tree, mature colonies – which export alates – are the ones that need to be controlled to stop BCA colonization of new orchards. Early seasonal predation can significantly reduce the growth of BCA populations with the consequential reduction of alates (Michaud & Browning 1999). An integrated management program for BCA (and in consequence for Citrus Tristeza) should conserve aphid natural enemies, because they are likely the greatest contributor to BCA suppression (Michaud 1999).

Besides being aphid predators, both earwig species (*F. pubescens* and *F. auricularia*) are potential predators of other citrus pests as they co-occur with many of them, both in time and space (Chapter 3). Although it remains unknown if earwigs predate on other key citrus pests shown in Figure 3.4, different studies indicate that earwigs are important natural enemies of insects such as lepidopterans and non-aphids homopterans (McLeod & Chant 1952; Jauset et al. 2005; Suckling et al. 2006; Wyckhuys & O'Neil 2006; Xiushan et al. 2006; Debras et al. 2007; Frank et al. 2007; Höhn et al. 2007). In addition, in Chapter 6 it was observed that the European earwig predated on the California red scale *Aonidiella aurantii* (Maskell), a key non-aphid homopteran pest in citrus orchards (Urbaneja et al. 2008).

Earwigs as pests in citrus canopies

The European earwig can also be a pest in its own right in citrus orchards, as earwigs may feed on flowers, leaves and fruits. On one hand, it was observed in this thesis that earwig damage to flowers occurred, but this effect was overcome during the natural abscission of fruitlets, and the final fruit yield was not affected (Chapter 5). On the other hand, earwig damage to leaves and fruits may be important in some occasions. While in mature orchards leaf flush damage is of little concern because the tree can tolerate heavy leaf damage and maintain production, in young non-bearing trees earwig damage to spring flush (such as observed in Chapter 6) may affect the long-term growth of trees. Earwigs may also perform direct aesthetical damage to fruit by scarring the fruit rind, what considerably downgrades fruit value in conventional markets (Grafton-Cardwell et al. 2003). In such situations where earwigs might cause problems, and if broad spectrum insecticides are to be avoided to achieve a more integrated approach to pest management, soft insecticides such as spinosyns might be useful (Table 6.1.; consistent with Cisneros et al. 2002; Shaw & Wallis 2010).

The role of the other earwig species present in the organic citrus canopies (*F. pubescens*) as a pest was not studied in this thesis. However, this species is most likely not a pest in the studied agroecosystem as even though fewer flowers survived in control trees (with both *F. pubescens*

and *F. auricularia*) than in banded trees, the final fruit yield was not affected by any treatment (Chapter 5).

Earwigs in citrus canopies: friend or foe?

Earwigs have the capacity to feed both on animal and plant material, a characteristic shared with many natural enemies, mainly predators, from most insect orders, i.e. Heteroptera, Thysanoptera, Neuroptera, Coleoptera, Diptera and Hymenoptera (Albajes et al. 2006). There are both negative and positive costs of omnivory. For instance, omnivore insects may damage crops, but simultaneously this plant-feeding behavior of predators can also be positive from a biocontrol point of view, as it allows such predators to survive in the agroecosystem when prey is unavailable (Eubanks & Denno 1999), and to establish on the crop before pest populations buildup (Coll & Guershon 2002; Naranjo & Gibson 1996). The ability of omnivores to exploit various resources benefits them nutritionally and reduces interspecific competition between omnivores, but it also implies that they predate on non-target prey and/or on other predators (Coll & Guershon 2002).

In order to be able to consider omnivore insects as biocontrol agents of pests, it is necessary to measure their contribution to pest management while determining their potential damage to crops (Alomar 2002). The European earwig was proven to be a beneficial insect in a Mediterranean organic citrus orchard, as it reduced aphid attack but did not affect the final fruit yield (Chapter 5). In addition, when adult earwigs were offered both plant and animal material in greenhouse conditions, they preferred to feed on California red scale rather than on leaves (Chapter 6). Thus, in both cases, the earwig role as an insectivore appeared to be more important than earwig role as an herbivore, what concurred with other studies in millet (Boukary et al. 1997) and in apple orchards (Carroll & Hoyt 1984). However, earwigs cannot be considered beneficial insects in orchards as a rule, because the status of an omnivore as a pest or as a predator can vary depending on several factors such as environmental conditions, insect stage, prey abundance, and the susceptibility of the particular crop (Alomar 2002; Albajes et al. 2006). For instance, Arnó et al. (2010) observed that when a generalist zoophytophagous predator such as the mirid *Nesidiocoris tenuis* (Reuter) is carried into extreme conditions of prey shortage, it feeds significantly more on the host plant and it becomes a pest. This might also be the case of earwigs in some situations, such as when too many chemicals are added and not many insects are left behind but earwigs, which are difficult to kill with most insecticides except for very broad spectrum pesticides (as seen in Chapter 6 and also by Epstein et al. 2000; Nicholas & Thwaite 2003; Maher et al. 2006; Colvin & Cranshaw 2009). Thus, when earwigs are present in such situations (i.e. the California orchard; Chapter 6), they may feed on

plant material more than they do in other situations where there are food alternatives (i.e. the Mediterranean organic citrus orchard).

To conclude, even though it is still not clear if organic farming can meet the future needs, as there are both positive (Mäder et al. 2002; Pimentel et al. 2005; Perfecto & Vandermeer 2008) and negative reports (Trewavas 2001; Green et al. 2005) on this topic, some principals of agroecology, such as biological control of pests, are starting to be deployed even in conventional agriculture –i.e. IPM programs - as their benefits are well-recognized (Macilwain 2004; New 2005). Earwigs can be important biocontrol agents as pests, but as many factors influence the behavior of omnivores and their diet choice, the earwig's relative role as pest versus predator needs to be measured in each particular agroecosystem before using earwigs in biological control programs. Anyhow, as Hunter (2009) stated, most omnivores tend to be our *friends* in agroecosystems. Earwigs appear to be among them.

Future perspectives

Future research of earwigs in citrus orchards should focus on earwig damage to young trees flushes, and on earwig's potential for aesthetic damage to fruits. In Chapter 6 we observed that earwigs damaged an average of 6.9 ± 1.6 % of leaf surface during the spring flush of young trees in conventional orchards, and this may influence the tree development, but it remains unknown to which extent. Likewise, Grafton-Cardwell et al. (2003) stated that earwigs damage citrus fruit by scarring the rind, but to date no studies have been conducted that measure earwig potential aesthetic damage to citrus fruit.

Chapter 6 preliminary results suggested that earwigs do not behave uniformly throughout the year; i.e. they damaged leaves during the spring flush but they did not damage the leaves of subsequent spring and summer flushes. It was argued that these results might be caused by seasonal changes in earwig diet or activity in canopies, or by differences between nymph and adult feeding habits. Anyhow, if earwigs do not behave uniformly throughout the year, this implies that the earwig's relative role as pest versus predator does not only depend on the particular agroecosystem under study, but also on the time of the year.

Additional future research should consider the whole citrus grove when studying earwig role, i.e. including ground-dwelling earwigs. In the citrus orchard under study, three earwig species are regularly found in the ground: *Euborellia moesta* (Gené), *Euborellia annulipes* (Dohrn), and *Nala lividipes* (Dufour) (unpublished observations). Earwigs in the ground are usually considered pest predators (Horton et al. 2003; Urbaneja et al. 2006; Frank et al. 2007; Simon et al. 2007; Farinós et al. 2008), and thus earwig role as biocontrol agent of pests in citrus orchards would be more important if ground-dwelling earwigs were considered.

Finally, stable isotope analyses might provide more detailed information on earwig trophic level and consequently might help to better understand the earwig's relative role as insectivore versus herbivore in orchards. Moreover, as five earwig species were found in the studied Mediterranean citrus orchard, it would be interesting to compare the trophic level of each. This tool could be also used to track eventual changes of earwig diet throughout the year.

Conclusions

i) The European earwig has a longer active period in the Mediterranean than in colder regions (Chapter 3).

ii) *Forficula auricularia* and *F. pubescens* co-occur in time and space in citrus canopies without having any positive or negative effect on each other (Chapter 3).

iii) The European earwig, *F. auricularia*, significantly affects aphid populations in the studied Mediterranean organic citrus canopies, as a consequence of their early predation on this pest (Chapters 3, 4 & 5).

iv) The other species present in the organic citrus canopies under study, *F. pubescens*, apparently does not regulate aphid populations, most likely due to its late appearance in canopies. However, similar to the European earwig, it co-occurs with many citrus pests and as a generalist predator, most likely it feeds on citrus pests (Chapter 3).

v) The European earwig negatively influences flower survival. However, after the natural drop of fruitlets occurs, earwig damage to citrus flowers is no longer observed, and the final fruit production is not affected by earwig abundance. Thus, the European earwig is a beneficial insect in the studied Mediterranean organic citrus canopies as it reduces aphid attack but does not affect fruit yield (Chapter 5).

vi) Adult earwigs prefer to predate on California red scale rather than on citrus leaves in greenhouse conditions (Chapter 6).

vii) Earwigs are difficult to kill with anything but very broad spectrum insecticides (Chapter 6). This implies that, in some situations, earwigs are present when not many insects are, resulting in extreme conditions of food shortage for earwigs, forcing them towards increased herbivory.

viii) In this thesis earwigs appeared to be important biocontrol agents of pests in an organic citrus orchard, but it was also observed that in some occasions, earwigs might act as pests. The critical issue is to measure earwig contribution to pest management while defining earwig damage to crops. Earwigs, as omnivores, can have a dual role in citrus agroecosystems, but this fact should not undervalue earwig contribution to biological control.

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