

Universitat de Lleida

## Enhancing biological control in apple orchards

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# Enhancing biological control in apple orchards

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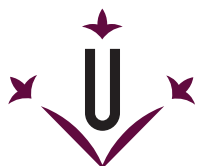
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Juny 2014



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“Tot sembla impossible fins que es fa”

Nelson Mandela

Als meus pares i al Joan.

A la Núria.



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## Resum

Depredadors, parasitoides i entomopatògens són els pilars de les estratègies actuals de control integrat de plagues, els quals poden contribuir a una agricultura més eficient i sostenible. Conèixer la seva presència i fenologia és molt important per tal d'afavorir els enemics naturals més eficaços contra cada plaga en concret. L'objectiu general d'aquesta tesi és conèixer la presència de les tisoretetes en les parcel·les de pomera mediterrànies, la seva fenologia i avaluar les infraestructures ecològiques que podrien millorar el control biològic mitjançant la promoció de fauna auxiliar. Es va avaluar la interacció del pugó llanut *Eriosoma lanigerum* Hausmann amb els enemics naturals i les variables climàtiques.

La tisoreta comuna *Forficula auricularia* Linnaeus i *Forficula pubescens* Gené (Dermaptera : Forficulidae ) van ser les espècies més abundants en les parcel·les mediterrànies. Aquestes dues espècies es poden trobar durant tot l'any, coexistint en els arbres. Donat el seu llarg període d'activitat, poden tenir un paper decisiu com a depredadors. L'ús de refugis impregnats amb la feromona d'agregació de les tisoretetes pot ser una eina útil per tal d'afavorir-les com a depredadors en plantacions de fruita de llavor però també per capturar-les en finques de fruita de pinyol. Es va observar que un refugi impregnat per 0,2 individus/cm<sup>2</sup> durant una setmana tenia una capacitat d'atracció de tisoretetes durant 5 setmanes amb un abast de 50 cm.

Es va avaluar la compatibilitat entre la tisoreta i nematodes entomopatògens (NEPs). *Steinernema carpocapsae* va ser l'únic NEPs capaç de matar-la. No obstant, la tisoreta pot detectar la presència de *S. carpocapsae* i per tant evitar els refugis tractats amb nematodes. També es va observar una activitat dissuassòria en larves de carpocapsa *Cydia pomonella* L. (Lepidoptera: Tortricidae) mortes per NEPs, reduint l'alimentació de la tisoreta sobre cadàvers que contenien nematodes al seu interior, suggerint una compatibilitat entre la tisoreta i NEPs.

La presència de tisoretetes i aranyes (Araneae) es va observar all llarg de tot l'any, però tan sols les tisoretetes van contribuir a reduir les infestacions de pugó llanut. La migració dels primers estadis ninfals de pugó llanut amunt i avall del tronc es dona des de la primavera fins a la tardor; les re-infestacions en les regions mediterrànies s'originen tant a partir de colònies aèries com subterrànies. D'aquesta manera, el control d'aquest pugó cal que estigui dirigit tant cap a les colònies aèries com a les arrels. El parasitoide *Aphelinus mali* Haldeman va aparèixer quan les infestacions aèries ja estaven desenvolupades, sent capaç de controlar el pugó llanut tant sols quan el nivell d'infestació era baix.

L'ús d'infraestructures ecològiques pot augmentar el control biològic de plagues, proporcionant un entorn més favorable i aliments i refugis alternatius als enemics naturals. *Anacyclus clavatus* Desf., *Dorycnium pentaphyllum* Scop., *Erucastrum nasturtiifolium* Poiret, *Euphorbia serrata* L., *Hedysarum confertum* Desf., *Papaver rhoeas* L., *Trifolium pratense* L. a la primavera, i *Atriplex* sp., *Dittrichia viscosa*



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L., *Medicago sativa* L., *Moricandia arvensis* L., *Salsola kali* L., *Sorghum halepense* (L.) Pers., *Suaeda spicata* Willd. i *Verbena* sp. a la tardor es van observar com flora autòctona útil per proporcionar refugi i aliment a les aranyes. Quaranta-tres espècies d'arbres i arbustos es van mostrejar per avaluar la seva idoneïtat per a ser utilitzats en tanques vegetals per afavorir la fauna auxiliar. *Viburnum tinus* L., *Euonymus japonicus* L. fil. i *Pistacia lentiscus* L. van mostrar resultats prometedors per a augmentar la riquesa i abundància d'enemics naturals.

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## Resumen

Depredadores, parasitoides y entomopatógenos son los pilares de las estrategias actuales de control integrado de plagas, los cuales pueden contribuir a una agricultura más eficiente y sostenible. Conocer su presencia y fenología es muy importante para favorecer los enemigos naturales más eficaces contra cada plaga en concreto. El objetivo general de esta tesis es conocer la presencia de las tijeretas en las parcelas mediterráneas de manzano, su fenología y evaluar las infraestructuras ecológicas que podrían mejorar el control biológico mediante la promoción de fauna auxiliar. Se evaluó la interacción del pulgón lanígero *Eriosoma lanigerum* Hausmann con los enemigos naturales y variables climáticas.

La tijereta común *Forficula auricularia* Linnaeus y *Forficula pubescens* Gené (Dermaptera: Forficulidae) fueron las especies más abundantes en las parcelas mediterráneas. Estas dos especies se pueden encontrar durante todo el año, coexistiendo en los árboles. Dado su largo periodo de actividad, pueden tener un papel decisivo como depredadores. El uso de refugios impregnados con la feromona de agregación de las tijeretas puede ser una herramienta útil para favorecerla como depredador en plantaciones de frutales de pepita pero también para capturarlas en frutales de hueso. Se observó que un refugio impregnado por 0,2 individuos/cm<sup>2</sup> durante una semana tenía una capacidad de atracción de tijeretas durante 5 semanas con un alcance de 50 cm.

Se evaluó la compatibilidad entre la tijereta y nematodos entomopatógenos (NEPs). *Steinernema carpocapsae* fue el único NEPs capaz de matarla. No obstante, la tijereta puede detectar la presencia de *S. carpocapsae* y evitar refugios tratados con nematodos. También se observó una actividad disuasoria en larvas de carpocapsa *Cydia pomonella* L. (Lepidoptera: Tortricidae) muertas por NEPs, reduciendo la alimentación de la tijereta sobre cadáveres que contenían nematodos en su interior, sugiriendo una compatibilidad entre la tijereta y NEPs.

La presencia de tijeretas y arañas (Araneae) se observó durante todo el año, aunque sólo las tijeretas contribuyeron a reducir las infestaciones de pulgón lanígero. La migración de los primeros estadios ninfales de pulgón lanígero arriba y abajo del tronco se da desde la primavera hasta el otoño; las re-infestaciones en las regiones mediterráneas se pueden originar tanto a partir de las colonias subterráneas como de las aéreas. Por tanto, el control de este pulgón debe dirigirse tanto a las colonias aéreas como a las raíces. El parasitoide *Aphelinus mali* Haldeman apareció cuando las infestaciones aéreas ya estaban desarrolladas, siendo capaz de controlar el pulgón lanígero sólo cuando el nivel de infestación era bajo.

El uso de infraestructuras ecológicas puede aumentar el control biológico de plagas, proporcionando un entorno más favorable y alimentos y refugios alternativos a los enemigos naturales. *Anacyclus clavatus* Desf., *Dorycnium pentaphyllum* Scop., *Erucastrum nasturtiifolium* Poiret, *Euphorbia serrata* L., *Hedysarum confertum* Desf., *Papaver rhoeas* L., *Trifolium pratense* L. en

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primavera, y *Atriplex* sp., *Dittrichia viscosa* L., *Medicago sativa* L., *Moricandia arvensis* L., *Salsola kali* L., *Sorghum halepense* (L.) Pers., *Suaeda spicata* Willd. y *Verbena* sp. en otoño se observaron como flora autóctona útil para proporcionar refugio y alimento a las arañas. Cuarenta y tres especies de árboles y arbustos fueron muestreados para evaluar su utilidad en setos vegetales para favorecer la fauna auxiliar. *Viburnum tinus* L., *Euonymus japonicus* L. fil. y *Pistacia lentiscus* L. mostraron resultados prometedores para aumentar la riqueza y abundancia de enemigos naturales.

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## Abstract

Predators, parasitoids and entomopathogens are cornerstones of the current integrated pest management strategies, which can lead to more efficient and sustainable agriculture. Knowledge on their presence and phenology is crucial when promoting the most efficient natural enemies against each target pest. The general aim of this thesis was to know the presence of earwigs within Mediterranean apple orchards, their phenology, and to assess native ecological infrastructures that would improve the biological control through enhancement of beneficials. Interaction of woolly apple aphid *Eriosoma lanigerum* Hausmann with natural enemies and climate variables was assessed.

The European earwig *Forficula auricularia* Linnaeus and *Forficula pubescens* Gené (Dermaptera: Forficulidae) were the most abundant species within Mediterranean apple orchards. These two species can be found throughout the year and co-occurred in canopies. Given their long activity period, they may play a crucial role as biocontrol predators. Impregnated shelters may be useful to promote earwigs in orchards devoted to pip fruit and also to capture them in those used for stone fruit production. A shelter impregnated by 0.2 individuals/cm<sup>2</sup> over one week was observed to attract earwigs during 5 weeks with a range of 50 cm.

Compatibility between European earwig and entomopathogenic nematodes (EPN) was evaluated. *Steinernema carpocapsae* was the only tested EPN capable of killing the European earwig. However, the European earwig can detect the presence of *S. carpocapsae* and therefore avoid nematode-treated shelters. An earwig deterrent activity in EPN-killed codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) larvae that reduces the foraging of European earwig on insect cadavers containing nematodes was also observed, suggesting compatibility between the European earwig and EPNs.

European earwigs and spiders (Araneae) were found throughout the year, but only earwigs contributed to reduce woolly apple aphid infestations. Woolly apple aphid crawlers migrate upward and downward from spring to autumn; winter temperatures did not kill the canopy colonies, and both canopy and root colonies are the source of reinfestations in Mediterranean areas. Thus the control of this aphid in such environments should focus on roots and canopy. *Aphelinus mali* Haldeman appeared when aerial infestations were already developed, and it was able to control woolly apple aphid when the level of infestation was low.

The use of ecological infrastructures may increase the biological control of pests, providing a more favorable environment and additional food and shelter for natural enemies. *Anacyclus clavatus* Desf., *Dorycnium pentaphyllum* Scop., *Erucastrum nasturtifolium* Poiret, *Euphorbia serrata* L., *Hedysarum confertum* Desf., *Papaver rhoeas* L., *Trifolium pratense* L. in spring, and *Atriplex* sp., *Dittrichia viscosa* L., *Medicago sativa* L., *Moricandia arvensis* L., *Salsola kali* L., *Sorghum halepense* (L.) Pers., *Suaeda spicata* Willd. and *Verbena* sp. in fall were observed as native

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flora useful to provide shelter and food for spiders. Forty-three species of trees and shrubs were sampled to evaluate their suitability to be used in hedgerows to enhance beneficials. *Viburnum tinus* L., *Euonymus japonicus* L. fil. and *Pistacia lentiscus* L. showed promising results in order to enhance abundance and richness of natural enemies.

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## **Chapter I. General introduction**







## 1. Towards sustainable agriculture

To satisfy a societal demand for environmentally friendly systems and healthy fruits, there is a challenge to keep the pests and diseases below economic thresholds reducing the pesticide use (Simon et al. 2009). Protection and promotion of biodiversity by the lower-input approaches of integrated pest management, organic agriculture and no-till or conservation agriculture may be a key issue to meet both ecological and agronomic purposes (FAO 2002; Simon et al. 2009). However, due to the expansion and intensification of production, the loss of biodiversity will remain serious over the next 30 years, even in the developed countries where nature is highly protected (FAO 2002).

Pest management strategies include preventive approaches that may stand from the plant resistance and cultural methods to the enhancement of natural control; whereas remedial approaches encompass the inundative releases of natural enemies, biotechnical methods and the application of pesticides (Hill et al. 1999; Kogan 1998; Zehnder et al. 2007). In many agro-ecosystems predators, parasitoids and pathogens are the cornerstone of integrated pest management strategies (Kogan 1998), and in recent decades biological control has been gaining importance, being currently one of the preferred techniques to control mainly indirect pests, due to its performance criteria such as economic, ecological and social (Jacas and Urbaneja 2008).

Natural control (biological) takes place without human intervention and occurs in the world within 89.5 million km<sup>2</sup> of all terrestrial ecosystems. Moreover, most of the potential agricultural pests (95% of 100,000 phytophagous arthropod species) are also under natural control, so the rest of the control methods used today are aimed at the remaining 5,000 species of arthropod pests. It is estimated that this biocontrol ecosystem service reaches a minimum annual value of US\$ 400 billion per year (Van Lenteren 2008).

To buy and release beneficials is not always a practical method, and in some cases can even deteriorate the natural control by eroding the genetic diversity of the agro-ecosystems (Rissler and Mellon 1996). Therefore, the best biological control method relies on enhancing the naturally occurring predators and parasitoids (conservation biological control) (Helyer et al. 2003; Pywell et al. 2005).

Location, plant material, spacing, irrigation, training system, soil management, etc., are decisions made when designing the orchard that can not be easily modified later, and all of them will influence phytophagous and natural enemies at the long run. In addition, the fruit ecosystem provides a much more predictable resources for phytophagous than for natural enemies; more phytophagous are associated permanently in comparison with the lower number of resident natural enemies (Avilla et al. 2008).

According to Helyer et al. (2003), predators can be classified within two groups: residents and colonists. Residents tend to be polyphagous and are present throughout the year, for instance earwigs and spiders. These predators are well established and can predate on pests early in the season. The other group of predators is the colonists, which are highly mobile and can occur in extremely great numbers. These predators tend to be attracted to the orchards when there is high prey presence, for instance pest outbreaks. Predators of the families Anthocoridae, Miridae, Nabidae, Coccinellidae, Syrphidae and Chrysopidae might be considered as colonists.

Success of biological control relies on promoting those predators that are more effective for each target pest. Therefore, knowledge on the presence and phenology of natural enemies in each area, and their prey and host preference may be crucial when trying to improve the biological control of pests (Alomar and Albajes 2005; Avilla et al. 2008; Jones et al. 2012; Sigsgaard 2005; Symondson et al. 2002; Zehnder et al. 2007).

Although spiders tend to be in the orchards as residents, their role as predators has been underestimated (Samu et al. 1999; Sunderland and Greenstone 1999; Toft 1999). Spiders are high insect consumers, and due to a wide variety of lifestyles and foraging strategies, they may play an important function in stabilizing or regulating insect populations (Chiri 1989; Nyffeler and Sunderland 2003). The results of different investigations conducted in apple orchards demonstrated that spiders are important natural enemies of aphids, mites and lepidopterans (Boreau de Roince et al. 2013; Mansour et al. 1980; McCaffrey and Horsburgh 1980; Wyss et al. 1995).

Pesticide use in agro-ecosystems has decreased the populations of residents as spiders, affecting their ability to control pest species (Riechert and Lockley 1984; Young and Edwards 1990). Moreover, there is a dearth of knowledge on the potential role of spiders from Southern Europe and Mediterranean climates (Nyffeler and Sunderland 2003).

Regarding other residents, in Central-North Europe, low presence of European earwig *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae) has been related to woolly apple aphid *Eriosoma lanigerum* Hausmann (Hemiptera: Aphididae) outbreaks (Helsen and Simonse 2006; Helsen et al. 2007; Mols 1996; Mueller et al. 1988; Stap et al. 1987).

The European earwig is an omnivorous insect worldwide distributed, which plays an important role as biocontrol agent (Albouy and Caussanel 1990; Carroll and Hoyt 1984; Helsen and Simonse 2006; Suckling et al. 2006). Considering their importance in biological control, European earwig has been cited as a predator of codling moth *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) (Glenn 1977; Jones et al. 2012; Sauphanor et al. 2012), apple leaf-curling midge *Dasineura mali* Kieffer (Diptera: Cecidomyiidae) (He et al. 2008), diaspidid scale insects (Hill et al. 2005; Logan et al. 2007), pear psylla *Cacopsylla pyri* Linnaeus (Hemiptera:

Psyllidae) (Höhn et al. 2007; Lenfant et al. 1994; Sauphanor et al. 1994), the leafroller *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) (Frank et al. 2007; Suckling et al. 2006) and aphids (Hemiptera: Aphididae) such as woolly apple aphid (Asante 1995; Mueller et al. 1988; Nicholas et al. 2005), rosy apple aphid *Dysaphis plantaginea* Passerini (Brown and Mathews 2007; Dib et al. 2010) and green apple aphid *Aphis pomi* DeGeer (Hagley and Allen 1990). Due to its key role as biocontrol agent, phenology of European earwig has been broadly studied (Burnip et al. 2002; Crumb et al. 1941; Fulton 1924; Gobin et al. 2008; Helsen et al. 1998; Kocarek 1998; Lamb 1975; Lamb and Wellington 1975; Lamb 1976a; Moerkens et al. 2009; Phillips 1981; Romeu-Dalmau et al. 2011; Suckling et al. 2006), but very little is known in Mediterranean apple orchards.

On the other hand, woolly apple aphid has recently become a more prevalent and important pest (Beers et al. 2010; Warner 2006), associating its increase with disruption of biological control and changes in pesticide programs (Gontijo et al. 2012). While earwigs, spiders, ladybird beetles, lacewings, hoverflies and the parasitoid *Aphelinus mali* Hald (Hymenoptera: Aphelinidae) are reported abroad as woolly apple aphid natural enemies (Asante 1995; Asante 1997; Gontijo et al. 2012; Monteiro et al. 2004; Mueller et al. 1988; Nicholas et al. 2005; Short and Bergh 2004), few data are available for the Mediterranean area.

Environment manipulation and ecological infrastructures such as hedgerows, flower strips and cover crops may provide additional resources to beneficials. Access to shelter and overwintering habitats, alternative preys/hosts and appropriate microclimates will bring more favorable conditions to natural enemies, enhancing their survival, fecundity, longevity and behavior, which will improve the biocontrol efficacy (Barberi et al. 2010; Boller et al. 2004; Jonsson et al. 2008; Landis et al. 2000; Nicholls and Altieri 2012).

Beneficials are estimated to provide US agriculture with US\$ 8 billion worth of pollination and pest control each year (Isaacs et al. 2009), and by the promotion of biodiversity within crops and landscapes, this value could be increased (Bianchi et al. 2006; Boller et al. 2004; Landis et al. 2000).

Many authors have reported promotion of natural enemies through implementation of ecological infrastructures. For instance, several of these studies have used flower strips of annual plants such as *Phacelia tanacetifolia* Bentham and *Lobularia maritima* (L.) Desv. (Ambrosino et al. 2006; Baggen et al. 1999; Gontijo et al. 2013; Hickman and Wratten 1996; Holland and Thomas 1996; Long et al. 1998; Lövei et al. 1992; Nicholls and Altieri 2012; Pontin et al. 2006). The use of perennial plants as hedgerows has also been reported by many authors to increase and improve performance of natural enemies (Bianchi et al. 2006; Macfadyen et al. 2011; Morandin et al. 2011; Navntoft et al. 2009; Pisani Gareau and Shennan 2010; Ricci et al. 2011; Varchola and Dunn 2001).

There are many species of plants that have been evaluated abroad for the promotion of beneficials; however, in their selection, promotion of pests, diseases

and other aspects such as the regional climate must be considered (Baggen et al. 1999; Boller et al. 2004). In addition by supporting the biodiversity of the native fauna and being more adapted to local environment, native plants might be as useful as the non-natives that are widely cited in references to promote natural enemies (Danne et al. 2010; Fiedler and Landis 2007; Isaacs et al. 2009).

## **2. Aims and outline of the thesis**

Use of predators, parasitoids and entomopathogens can lead to more efficient and sustainable pest management programs. However, the interaction between all these agents may play an important role. Knowledge on presence and abundance, phenology and habitats of the most efficient biocontrol agents within each area may be the key to improve biological control of pests. The general aim of this thesis was to know the presence of earwigs within Mediterranean apple orchards, their phenology, and to assess native ecological infrastructures that would improve the biological control through enhancement of beneficials. The interaction of natural enemies with woolly apple aphid will provide more knowledge to evaluate the role that biological control may play to control this pest.

The specific objectives of the thesis were:

- To know the earwig species present in Mediterranean apple orchards, their phenology and the role that they may have as biocontrol agents (Chapter II).
- To know if pheromone-impregnated shelters might be useful to promote and/or control earwigs due to their aggregation behavior (Chapter III).
- To assess the compatibility of the European earwig with entomopathogenic nematodes (EPNs) (Chapter IV).
- To know the behavior and population dynamics of the woolly apple aphid (WAA) in relation to climatic variables and natural enemies (Chapter V).
- To know the spider diversity and identify common plants of the Mediterranean area which are more suitable for them (Chapter VI).
- To evaluate the suitability of native and naturalized trees and shrubs to be included in hedgerows to enhance beneficials (Chapter VII).

**Chapter II. Phenology and interspecific association of *Forficula auricularia* and *Forficula pubescens* in apple orchards**



## **Abstract**

The European earwig *Forficula auricularia* L. (Dermaptera: Forficulidae) has been widely studied as a key predator of pests in colder regions, but their phenology and behavior may differ in warmer areas such as the Mediterranean. Therefore, in these regions, in order to promote earwigs but also to optimize their control in crops where they are pests, more information is needed. The phenology, aggregation, and interspecific association of *F. auricularia* and *Forficula pubescens* Gené were assessed in Mediterranean apple orchards. Suitability of day degree models elaborated for temperate regions were evaluated to predict the phenology of the European earwig in a Mediterranean climate. *F. auricularia* and *F. pubescens* co-occurred in canopies without apparent competition. This study provides useful data about the weekly phenology of earwigs throughout the year that can be used to improve the promotion of this insect in pip fruit orchards or to control them in stone fruit crops

**Keywords:** Biological control, Dermaptera, earwig, Forficulidae, Mediterranean, pest.

## 1. Introduction

The European earwig, *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae), is an important predator of pear psylla *Cacopsylla pyri* Linnaeus (Hemiptera: Psyllidae) (Höhn et al. 2007; Lenfant et al. 1994; Sauphanor et al. 1994), codling moth *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) (Glenn 1977; Jones et al. 2012; Sauphanor et al. 2012), apple leaf-curling midge *Dasineura mali* Kieffer (Diptera: Cecidomyiidae) (He et al. 2008), diaspidid scale insects (Hill et al. 2005; Logan et al. 2007), the leafroller *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) (Frank et al. 2007; Suckling et al. 2006) and aphids (Hemiptera: Aphididae) such as woolly apple aphid (WAA) *Eriosoma lanigerum* Hausmann (Asante 1995; Mueller et al. 1988; Nicholas et al. 2005), rosy apple aphid (RAA) *Dysaphis plantaginea* Passerini (Brown and Mathews 2007; Dib et al. 2010) and green apple aphid *Aphis pomi* DeGeer (Carroll and Hoyt 1984; Hagley and Allen 1990). However, due to their omnivorous diet, European earwigs can cause economic damage to some crops (Albouy and Caussanel 1990; Grafton-Cardwell et al. 2003; Huth et al. 2011; Kuthe 1996). In addition, their frass can negatively influence the aroma and flavor of some wines (Burdet et al. 2013).

Another earwig species, *Forficula pubescens* Gené, has been observed to prey on pear psyllids (Debras et al. 2007) and RAA (Dib et al. 2010). There are few studies of the phenology of *Forficula pubescens* (Herter 1964; Romeu-Dalmau et al. 2011). Most studies have been conducted on *F. auricularia* in Central-Northern Europe (Gobin et al. 2008; Helsen et al. 1998; Kocarek 1998; Moerkens et al. 2009; Phillips 1981), New Zealand (Burnip et al. 2002; Suckling et al. 2006), and North America (Crumb et al. 1941; Fulton 1924; Lamb 1975; Lamb and Wellington 1975; Lamb 1976a); however, little is known about this insects in Mediterranean apple orchards, where they may also play an important role as predators in pip fruit and citrus orchards but become pests in stone fruit orchards and vineyards.

The common European earwig is classified into two distinct populations on the basis of their reproductive strategy. Single-brood populations (SBPs) are characterized by prolonged maternal care by the female, whereas in double-brood populations (DBPs) maternal care is shorter, and immediately after the first molt the female abandon their young and start a second nest (Lamb 1976b; Vancassel and Quris 1994). SBPs lay eggs in autumn (November to December), while for DBPs, which have two reproductive cycles per year, this process occurs in winter (January to February) and late spring-summer (June to July) (Helsen et al. 1998; Kocarek 1998; Lamb and Wellington 1975; Moerkens et al. 2009; Phillips 1981; Vancassel and Quris 1994). When the nesting phase ends, N2 and N3 nymph instars from the DBPs and SBPs, respectively, start the free-foraging phase on the ground (Helsen et al. 1998; Lamb 1975; Moerkens et al. 2009). The arboreal phase starts once earwigs from the DBP and SBP molt to N3 and N4 nymph instar respectively. At this point, they forage and shelter on trees, where they later molt



into adults and remain until autumn (Helsen et al. 1998; Lamb and Wellington 1975; Moerkens et al. 2009).

European earwig forage at night and seek shelter during the day (Albouy and Caussanel 1990; Helsen et al. 1998). Given that these insects are important biocontrol agents, their promotion through the use of additional shelters has been assessed in apple, pear, and kiwifruit orchards (Gobin et al. 2006; Logan et al. 2011; Solomon et al. 1999). As earwigs have a univoltine life cycle, any disruption on their cycle one year can have long-lasting repercussions on their populations (Gobin et al. 2006; Peusens and Gobin 2008; Peusens et al. 2010). To minimize negative effects on vulnerable life stages of earwigs, the prediction of their phenology will contribute to determining the precise timing for spray applications and soil tillage, thereby improving orchard management (Belien et al. 2012; Belien et al. 2013; Moerkens et al. 2012; Peusens et al. 2010). For instance, commonly pesticides sprayed in orchards, such as chlorpyrifos, deltamethrin, indoxacarb and spinosad, have been reported by several authors to have lethal effects on European earwig (Fountain et al. 2013; Peusens and Gobin 2008; Peusens et al. 2010; Vogt et al. 2010). Software applications and prediction models have been developed to optimize orchard management techniques geared to promoting European earwig (Belien et al. 2012; Belien et al. 2013; Helsen et al. 1998; Moerkens et al. 2011). However, these studies have been conducted in colder regions. Earwig phenology and behavior may differ in warmer areas such as the Mediterranean. Therefore, in these regions, in order to promote earwigs but also to optimize their control in crops where they are pests, more information is needed.

The objective of this study was to assess the phenology, aggregation, and interspecific association of *F. auricularia* and *F. pubescens* in Mediterranean apple orchards. Suitability of day degree models elaborated for temperate regions were evaluated to predict the phenology of the European earwig in a Mediterranean climate.

## 2. Materials and Methods

### 2.1. Phenology

Trials were conducted in four apple orchards under organic management located in Catalonia (NE Spain): Les Borges Blanques (BB) (41°30'23.06''N; 0°51'05.93''E), Mollerussa (MO) (41°36'51.13''N; 0°52'22.75''E), Ivars d'Urgell (IU) (41°41'06.19''N; 0°58'06.09''E), and Miralcamp (MI) (41°36'31.89''N; 0°52'24.62''E). The climate is semi-arid Mediterranean, with a mean annual rainfall of 350 mm.

BB was an experimental orchard of 'Fuji Kiku 8' apple grafted onto M9, planted in 2003, and trained to a central leader with spacing of 4 x 1.4 m. MO was a commercial orchard of 'Golden Smoothee' apple grafted onto M9, planted in 1985, and trained to a double-axis system with spacing of 4 x 1.2 m. IU was a

commercial orchard of ‘Golden Smoothie’ apple grafted onto M9, planted in 1993, and trained to a central leader with spacing of 4 x 1.1 m. MI was a commercial orchard of ‘Golden Smoothie’ apple grafted onto M9, planted in 2000, and trained to a central leader with spacing 4 x 1.2 m.

To follow earwig phenology, BB was sampled for 4 years (2010-2013), MO and IU for 3 (2011-2013), and MI for 2 (2012-2013). For each orchard from 2010 onwards, 10 shelters were set on the second scaffold limb of various trees (tree shelters). From 2012 onwards, 10 additional shelters were tied at the base of 10 supplementary trees in each orchard (ground shelters). Shelters were prepared by rolling a piece of corrugated cardboard to obtain cylinders (12 cm height x 9 cm diameter), which were protected from rain and adverse conditions by a PVC tube (15 cm height x 9.5 cm diameter). Similar shelters have been used in studies of European earwigs elsewhere (Burnip et al. 2002; Gobin et al. 2006; He et al. 2008; Helsen et al. 1998; Logan et al. 2007; Moerkens et al. 2009; Phillips 1981; Solomon et al. 1999). Every week throughout the year, species, number, phenological stage, and sex of adult earwigs for each shelter were recorded, and earwigs were then released at the base of the assessed tree. Presence of wings was used to distinguish between *F. auricularia* and *F. pubescens* adults. Cerci dimorphism was used to distinguish sex, and size and number of antennal segments and the apparent wing buds on the 3<sup>rd</sup> segment of the thorax to distinguish nymph stages (Albouy and Caussanel 1990).

## 2.2. Evaluation of the day degree models

The European earwig phenological day degree model designed by Moerkens et al. (2011) was tested in our region. Daily minimum and maximum temperatures required to run the model were obtained from the closest automatic weather station of the Meteorological Service of Catalonia (*Meteocat, Departament de Territori i Sostenibilitat, Generalitat de Catalunya*). For BB, data were from the Castellans station 8.5 km away, for IU from the Castellnou de Seana station 3 km away and for MO and MI from the Mollerussa station 0.5 km and 1 km away respectively. From 2011 onwards, daily soil temperatures at a depth of 5 cm were also available from the Mollerussa station, which is 12 km from BB and 10 km from IU. The model was checked for 2012-2013 based on the dates of first appearance and peak of the developmental stage observed in the field. For BB and IU, the model was run with soil data from MO.

The sum in day-degrees (DD) up to the first and maximum number of N3, N4 nymph instars and adults were calculated for each orchard and year and compared with those reported by Helsen et al. (1998). The minimum and maximum temperatures from each weather station were used to calculate the effective temperature for each orchard and year. The effective temperature sum in DD was calculated through the sine wave approximation (Rabbinge 1976), using a lower threshold of 6°C and taking 1<sup>st</sup> January as the biofix. These parameters were chosen according to Helsen et al. (1998).

### 2.3. Data analysis

Data from April to July —when more earwigs were recorded— were used to compare abundance among years within orchards. Replicates were the weekly mean abundance of the 10 canopy shelters. *F. auricularia* data were log-transformed and ANOVA assumptions (normality and homoscedasticity) were confirmed before analysis. Means were compared at the  $P = 0.05$  level, and a Tukey HSD test was used to separate means. Due to heterogeneity of variance, *F. pubescens* data were analyzed by Welch's test.

To compare abundance between earwig species, data from April to July in canopy shelters were used. Replicates were the weekly mean abundance of the 10 shelters, and in this case abundances were compared between both species within orchards by Welch's test.

Data from June and July —when more adults were recorded on canopy shelters— were used to calculate and analyze the sex ratio for *F. auricularia* and *F. pubescens* within orchards. Data were log-transformed and analyzed by a nonparametric Wilcoxon test. Homogeneity of variance was also confirmed before each analysis.

Aggregation in shelters was evaluated by fitting data to Taylor's power law (Taylor 1961):

$$S^2 = a \cdot m^b \quad (1)$$

Where  $S^2$  is the variance,  $m$  is the sample mean,  $a$  is a sampling factor and  $b$  indicates whether the population distribution is regular ( $b < 1$ ), random ( $b = 1$ ) or aggregated ( $b > 1$ ).

For *F. auricularia*, the weekly mean data of the 10 shelters from June to July from all the years were used, while for *F. pubescens* the data used were from IU 2011-2012 and MI 2012. Equation (1) was log-log transformed to estimate  $a$  and  $b$ .

To evaluate the interspecific association between *F. auricularia* and *F. pubescens*, data from IU 2011-2012 and MI 2012 were used. Tree and ground shelters were assigned to one of the following categories based on insect presence: (a) both earwig species; (b) only *F. auricularia*; (c) only *F. pubescens*; and (d) without earwigs. For each month, the number of shelters within each category was used to calculate the interspecific association coefficient ( $Cas$ ) following Yule's formula:

$$Cas = \frac{(ad-bc)}{(ad+bc)} \quad (2)$$

$Cas$  varies from -1 to +1. A negative value shows competition, zero no interaction, and a positive value an association between species (Legendre and Legendre 1984; Sauphanor and Sureau 1993).

Data were analyzed using the JMP statistical software package (Version 9; SAS Institute Inc., Cary, North Carolina).

### 3. Results

#### 3.1. Phenology

In addition to *F. auricularia* and *F. pubescens*, three other earwig species were found: *Labidura riparia* Pallas, *Nala lividipes* Dufour and *Euborellia moesta* Gené. *F. auricularia* and *F. pubescens* were detected in both tree and ground shelters (Table sII-1), whereas *L. riparia*, *N. lividipes* and *E. moesta* were found occasionally and only in ground shelters.

*F. auricularia* was very common in all the orchards during the study period, whereas *F. pubescens*, although observed in all the orchards, was not captured all the years (Table sII-1 and Figure II-1). Higher numbers of *F. auricularia* than *F. pubescens* were observed in all the orchards (Figure II-2). The abundance of *F. auricularia* did not change along the years in BB, IU or MI, whereas the population increased in MO over the years (ANOVA - Tukey HSD:  $F = 19.75$ ;  $df = 2,48$ ;  $P = 0.0001$ ) (Figure II-1). In contrast, the abundance of *F. pubescens* decreased in IU (Welch's test:  $F = 35.44$ ;  $df = 2,20$ ;  $P < 0.0001$ ) and MI (Welch's test:  $F = 9.49$ ;  $df = 1,19$ ;  $P = 0.006$ ) (Figure II-1).

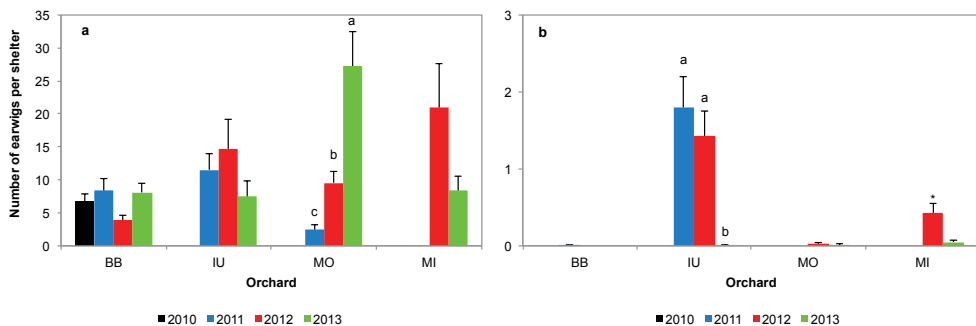


Figure II-1. Abundance of *Forficula auricularia* (a) and *Forficula pubescens* (b) (mean  $\pm$  SE) from April to July per year in BB, IU, MO and MI. Column bars marked with the same letter or without asterisk indicate no significant differences among years within each orchard according to the Tukey HSD or Welch's tests ( $P > 0.05$ ). Note that y-axis scales are different.

*F. auricularia* was found throughout the year (Figure II-3a-b and Table sII-1). From January to June, N2, N3 and N4 instars were found in ground shelters. At the end of January the population peaked with an average of 3 N3 instar individuals (Figure II-3b). The presence of the N4 instar rose from mid-March to the end of May, after which time no more N4 were observed in ground shelters (Figure II-3b). The presence of the N2 instar was intermittent during winter and early spring, and more regular from May to June; however, the population peak was observed in November, with an average close to 3 individuals per ground shelter (Figure II-3b).

Adults were found in ground shelters from May to November, but their abundance was lower than that of nymphs (Figure II-3b).

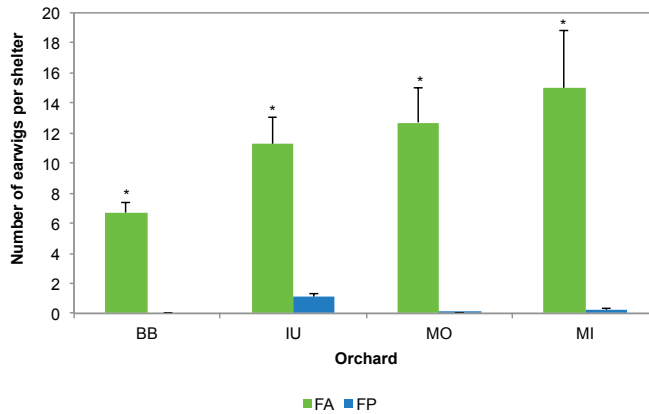


Figure II-2. Number of *Forficula auricularia* and *Forficula pubescens* (mean  $\pm$  SE) per orchard. Column bars marked with an asterisk indicate significant differences among earwig species within orchards according to Welch's test ( $P < 0.05$ ).

In contrast, adults were most abundant in the tree shelters (Figure II-3a). Adults were captured from April to November, but higher presence was observed from mid-May to the beginning of July, with a peak of 23 individuals per shelter (Figure II-3a). N4 was the most abundant instar found in tree shelters from the end of March to mid-May, with a population peak of 14 individuals per shelter in mid-May (Figure II-3a). The N3 instar was also observed in tree shelters one month after the N4 was found. The abundance of N3 was much lower, with an average of 3 individuals per shelter (Figure II-3a).

Regarding *F. pubescens*, adults were found in ground shelters from mid-February to April, and after that N2, N3, N4 and N5 instars were successively observed either in ground or in tree shelters until July (Figure II-3c-d). The N2 instar of *F. pubescens* was more common in ground shelters, while it was barely observed in tree shelters. In contrast, the N1 instar was not found in tree or ground shelters (Figure II-3c-d). Adults of *F. pubescens* were observed from March to April and from June to December in canopies, with a maximum of 2 individuals per shelter (Figure II-3c).

Capture rates dropped for both earwig species during molting into adults (Figure II-3). No significant differences were observed between number of male and female individuals ( $P > 0.05$ , Wilcoxon test).

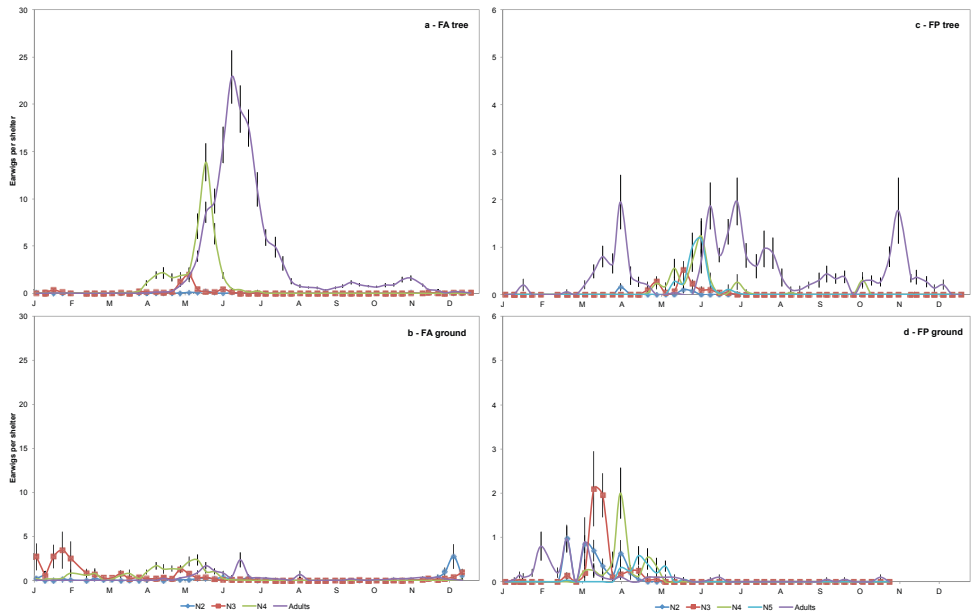


Figure II-3. Weekly mean earwig individuals per tree and ground shelters for *Forficula auricularia* (a-FA and b-FA) and *Forficula pubescens* (c-FP and d-FP) throughout the year for nymph stages (N2, N3, N4 and N5) and adults. Note that y-axis scales are different. FA figures were calculated with data from all the orchards and years, whereas FP figures were calculated on the basis of IU 2011-2012 and MI 2012.

### 3.2. Aggregation behavior and interspecific association

The relationship between the variance and the mean was studied by Taylor’s law. The distribution of *F. auricularia* in shelters was observed to be aggregated, as the *b* coefficient was higher than 1 in all the orchards (Table II-1). On the other hand, for *F. pubescens*, the *b* coefficient was higher than 1 in IU, also indicating an aggregated distribution. In contrast, in MI this distribution could not be confirmed (Table II-1).

Table II-1. Taylor’s parameters for each orchard and species; *b* indicates when the population in shelters was regular ( $b < 1$ ), random ( $b = 1$ ) or aggregated ( $b > 1$ ).

Species	Orchard	n	b	SE	t ratio	Prob>t	CI 95%	R <sup>2</sup>
<i>Forficula auricularia</i>	BB	33	<b>1.43</b>	0.06	22.52	<0.0001	1.30 1.56	0.94
	IU	25	<b>1.73</b>	0.07	25.96	<0.0001	1.59 1.87	0.97
	MO	25	<b>1.48</b>	0.06	23.22	<0.0001	1.35 1.61	0.96
	MI	17	<b>1.73</b>	0.08	22.56	<0.0001	1.57 1.90	0.97
<i>Forficula pubescens</i>	IU	16	<b>1.24</b>	0.17	7.48	<0.0001	0.88 1.60	0.80
	MI	7	<b>0.92</b>	0.48	1.94	0.1103	-0.30 2.14	0.43

*F. auricularia* and *F. pubescens* showed mainly a positive association (Figure II-4). A few negative values were observed occasionally (Figure II-4).

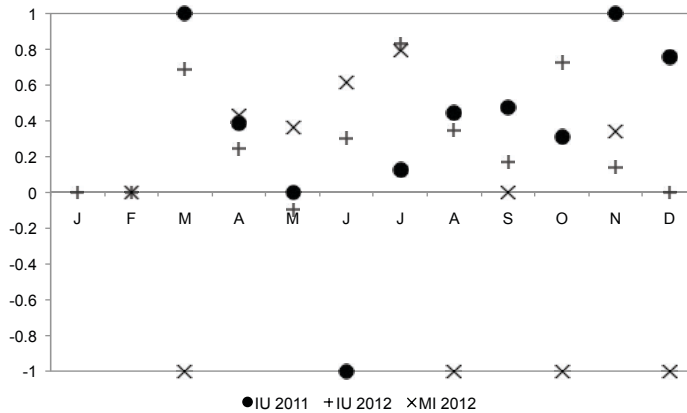


Figure II-4. Monthly interspecific association coefficients between *F. auricularia* and *F. pubescens* for IU and MI orchards 2011-2012. A negative value shows active competition, zero no interaction, and a positive value an association between species.

### 3.3. Evaluation of the day degree models

No matches among observed and estimated dates were found for any of the developmental stages detected in tree or in ground shelters running the model of Moerkens (Table II-2). Regarding the model of Helsen, the N3 nymph instar was observed to appear at 215 DD; however, large differences between orchards were found (Table II-3). Although smaller differences were observed for the N4 instar (264 DD) and adult stage (250 DD), there were no matches between observed and estimated dates (Table II-3). We found only some coincidences when predicting the maximum number of N4 (613 DD) and adult individuals (1035 DD), with a range from 0 to 29 days between observed and estimated data (Table II-3).

Table II-2. Estimated appearance dates for the first and maximum number of individuals of each European earwig developmental stage according to the day degree model (Model) and observations (Tree and Ground).

1st individual																
Orchard	Year	N1			N2			N3			N4			Adult		
		Model	Tree	Ground	Model	Tree	Ground	Model	Tree	Ground	Model	Tree	Ground	Model	Tree	Ground
MO	2012	24-Mar	-	10-Dec	10-Apr	3-Jan	5-Mar	27-Apr	5-Mar	27-Mar	10-May	27-Mar	27-Mar	24-May	5-Mar	16-Apr
MO	2013	21-Feb	-	21-Jun	12-Mar	14-Jan	2-Jan	1-Apr	21-Jan	2-Jan	21-Apr	7-Feb	2-Jan	17-May	31-Jan	2-Jan
BB	2012	24-Mar	-	5-Dec	10-Apr	2-Jan	15-Nov	27-Apr	17-Jan	28-Feb	10-May	20-Mar	6-Mar	23-May	17-Apr	24-Apr
BB	2013	21-Feb	-	3-May	13-Mar	22-Apr	20-Feb	30-Mar	3-Jan	3-Jan	17-Apr	3-Jan	3-Jan	10-May	13-Mar	14-Feb
IU	2012	24-Mar	-	-	10-Apr	10-Apr	10-Apr	28-Apr	25-Apr	10-Apr	11-May	2-Apr	3-May	25-May	15-Mar	10-Apr
IU	2013	21-Feb	3-May	3-May	12-Mar	3-May	4-Apr	31-Mar	4-Mar	3-May	17-Apr	21-Mar	3-May	12-May	21-Jan	4-Apr
MI	2012	24-Mar	-	-	10-Apr	21-May	21-May	27-Apr	16-Apr	16-Apr	10-May	10-Apr	10-Apr	24-May	27-Mar	19-Mar
MI	2013	21-Feb	21-Jun	23-May	12-Mar	31-May	26-Mar	1-Apr	22-Apr	3-May	21-Apr	9-May	9-May	17-May	21-Jan	31-Jan

max																
Orchard	Year	N1			N2			N3			N4			Adult		
		Model	Tree	Ground	Model	Tree	Ground	Model	Tree	Ground	Model	Tree	Ground	Model	Tree	Ground
MO	2012	24-Mar	-	10-Dec	10-Apr	27-Nov	10-Dec	27-Apr	21-May	17-Dec	10-May	15-May	15-May	24-May	21-May	6-Aug
MO	2013	21-Feb	-	21-Jun	12-Mar	6-Nov	8-Jan	1-Apr	3-May	31-Jan	21-Apr	23-May	21-Feb	17-May	13-Jun	21-Jun
BB	2012	24-Mar	-	5-Dec	10-Apr	17-Jan	11-Dec	27-Apr	24-Jan	18-Dec	10-May	11-Apr	11-Apr	23-May	22-May	22-May
BB	2013	21-Feb	-	3-May	13-Mar	20-Nov	12-Apr	30-Mar	26-Mar	21-Jan	17-Apr	12-Apr	12-Apr	10-May	28-Jun	31-May
IU	2012	24-Mar	-	-	10-Apr	24-May	10-Apr	28-Apr	3-May	3-May	11-May	24-May	7-May	25-May	13-Jun	16-May
IU	2013	21-Feb	3-May	3-May	12-Mar	31-May	5-Jun	31-Mar	9-May	9-May	17-Apr	31-May	31-May	12-May	13-Jun	31-May
MI	2012	24-Mar	-	-	10-Apr	21-May	21-May	27-Apr	30-Apr	21-May	10-May	21-May	7-May	24-May	11-Jun	4-Jun
MI	2013	21-Feb	21-Jun	13-Jun	12-Mar	31-May	28-Jun	1-Apr	6-Jun	28-Jun	21-Apr	31-May	13-May	17-May	28-Jun	28-Jun

## Phenology of *F. auricularia* & *F. pubescens*

Table II-3. Observed and accumulated degree days (DD > 6°C, from 1 January on) for first and maximum number of European earwig individuals for each developmental stage found in tree canopies.

1st N3					Max N3				
Orchard	Year	Date	DD > 6°C (1 Jan)	Observed - Estimated	Orchard	Year	Date	DD > 6°C (1 Jan)	Observed - Estimated
MO	2011	5-Dec	3473	270	MO	2011	12-Dec	3486	236
MO	2012	5-Mar	126	-5	MO	2012	21-May	721	31
MO	2013	21-Jan	27	-49	MO	2013	3-May	491	13
BB	2010	23-Mar	199	13	BB	2010	31-Mar	250	-20
BB	2011	22-Mar	235	12	BB	2011	5-Apr	350	-15
BB	2012	17-Jan	34	-53	BB	2012	24-Jan	47	-87
BB	2013	3-Jan	3	-67	BB	2013	26-Mar	251	-25
IU	2011	13-Apr	397	34	IU	2011	4-May	582	14
IU	2012	25-Apr	433	46	IU	2012	3-May	502	13
IU	2013	4-Mar	124	-6	IU	2013	9-May	573	19
MI	2012	16-Apr	370	37	MI	2012	30-Apr	480	10
MI	2013	22-Apr	424	43	MI	2013	6-Jun	807	47
Average		10-Mar			Average		20-Apr		
Average (Mean ± SE)			215.49 ± 50.30		Average (Mean ± SE)			459.48 ± 66.70	
1st N4					Max N4				
Orchard	Year	Date	DD > 6°C (1 Jan)	Observed - Estimated	Orchard	Year	Date	DD > 6°C (1 Jan)	Observed - Estimated
MO	2011	11-Apr	391	19	MO	2011	9-May	659	-1
MO	2012	27-Mar	243	4	MO	2012	14-May	647	4
MO	2013	7-Feb	63	-45	MO	2013	23-May	669	13
BB	2010	31-Mar	250	8	BB	2010	26-Apr	434	-14
BB	2011	5-Apr	350	13	BB	2011	19-Apr	492	-21
BB	2012	20-Mar	230	-3	BB	2012	11-Apr	380	-29
BB	2013	3-Jan	3	-80	BB	2013	12-Apr	370	-28
IU	2011	30-Mar	250	7	IU	2011	18-May	749	8
IU	2012	2-Apr	293	10	IU	2012	24-May	761	14
IU	2013	21-Mar	203	-2	IU	2013	31-May	749	21
MI	2012	10-Apr	340	18	MI	2012	21-May	721	11
MI	2013	9-May	559	47	MI	2013	31-May	732	21
Average		22-Mar			Average		9-May		
Average (Mean ± SE)			264.47 ± 41.88		Average (Mean ± SE)			613.42 ± 43.57	
1st Adult					Max Adult				
Orchard	Year	Date	DD > 6°C (1 Jan)	Observed - Estimated	Orchard	Year	Date	DD > 6°C (1 Jan)	Observed - Estimated
MO	2011	2-May	576	46	MO	2011	14-Jun	1129	1
MO	2012	5-Mar	126	-12	MO	2012	21-May	721	-23
MO	2013	31-Jan	47	-46	MO	2013	13-Jun	904	0
BB	2010	26-Apr	434	40	BB	2010	22-Jun	1115	9
BB	2011	19-Apr	492	33	BB	2011	14-Jun	1177	1
BB	2012	17-Apr	413	31	BB	2012	22-May	789	-22
BB	2013	13-Mar	188	-4	BB	2013	28-Jun	1174	15
IU	2011	30-Mar	250	13	IU	2011	22-Jun	1240	9
IU	2012	15-Mar	174	-2	IU	2012	13-Jun	1078	0
IU	2013	21-Jan	27	-56	IU	2013	13-Jun	916	0
MI	2012	27-Mar	243	10	MI	2012	11-Jun	1055	-2
MI	2013	21-Jan	27	-56	MI	2013	28-Jun	1117	15
Average		16-Mar			Average		13-Jun		
Average (Mean ± SE)			249.73 ± 54.41		Average (Mean ± SE)			1034.54 ± 47.40	

## 4. Discussion

The occasional presence of *L. riparia*, *E. moesta*, and *N. lividipes* may be explained by their low aggregation coefficient and, in some cases, solitary behavior (Albouy and Caussanel 1990; Sauphanor and Sureau 1993). The observation that these species were found only in ground shelters is consistent with their low appearance in literature as biocontrol agents in fruit orchards, as those surveys addressed mainly tree canopies. *L. riparia*, *N. lividipes*, and *E. moesta* have been described as important biocontrol agents in cereal and cotton crops (Albouy and Caussanel 1990; Shepard et al. 1973). As ground dwelling, these species might play a role in predateding pests with developmental stages on the ground, such as WAA, codling moth, and Mediterranean fruit fly (*Ceratitis capitata* Wiedemann; Diptera: Tephritidae); however, this need to be further tested.



The distribution of *F. pubescens* in field shelters was not clearly aggregated. In contrast, Sauphanor and Sureau (1993) observed high gregariousness in laboratory trials. On the other hand, the aggregation behavior of *F. auricularia* that we observed is similar to that reported by those authors. These differences may be due to the fact that *F. pubescens* was not abundant in field shelters, thus the opportunity to aggregate was lower than in lab trials, where more individuals per shelter were present. This observation agrees with Taylor et al. (1978), who reported that in the majority of species the degree of aggregation is density dependent. In both species, we found that the presence of males and females was similar, with a sex ratio of 1:1, coinciding with the observations made by Romeu-Dalmau et al. (2011) in citrus orchards.

While in our study the average number of *F. auricularia* was higher than *F. pubescens*, in citrus orchards the opposite was observed (Romeu-Dalmau et al. 2011). However, as different sampling methods were used in each study, it is difficult to draw conclusions about the relative abundance of the two species. In general terms, abundance of *F. auricularia* among years within orchards did not change, and only in one orchard an increase was detected, while the abundance of *F. pubescens* decreased. Moerkens et al. (2009) reported large variations in population density among orchards and years for *F. auricularia*. Winter temperatures and soil tillage can have an important influence on earwig abundance within orchards (Moerkens et al. 2012). In addition, SBPs have been reported to be more susceptible to cold temperatures than DBPs (Moerkens et al. 2012). Therefore, depending on the population type prevailing in each orchard, distinct population fluctuations might be observed.

*F. auricularia* and *F. pubescens* showed a tendency to associate positively. The few negative values that we observed appeared only in months when they were barely found in the shelters. Coinciding with our results, Sauphanor and Sureau (1993) also observed a positive association, estimating a coefficient value of 0.75. High association values were observed when more earwigs were found in the shelters, thus resembling the conditions tested by Sauphanor and Sureau (1993) in laboratory trials. Even in the field, Debras et al. (2007) reported the absence of competition between *F. auricularia* and *F. pubescens*. We can assume that when both earwig species are found in high numbers in the shelters, no competition between them occurs. This may be linked to high availability of food or to the different diet preferences of each species, which prevent interspecific competition. Sauphanor and Sureau (1993) suggested that the aggregation pheromone of the most abundant species can act as a kairomone for other species of the genus.

Concerning earwig phenology, individuals were found throughout the year in apple orchards. The mature stages of *F. auricularia* were observed mainly from May to November in tree shelters and immature ones from October to June in ground shelters. Most published studies were based on tree sampling, reporting the presence of *F. auricularia* individuals from May to October, with a May-June peak for N3 and N4 instars, and the abundance of adults in July (Gobin et al. 2008;

Helsen et al. 1998; Lamb and Wellington 1975; Moerkens et al. 2009; Moerkens et al. 2011; Phillips 1981). Romeu-Dalmau et al. (2011) also observed a longer active period in Mediterranean citrus orchards, which coincides with our results. The decrease in tree shelter captures during the summer months may be explained by the increased availability of natural shelters during this period. For instance, Helsen et al. (1998) observed that when the size of apples increases, many earwigs are found in fruit clusters, thus reflecting the availability of alternative shelters in the tree canopy, and Moerkens et al. (2009) reported an increase in adults in the shelters right after the harvest of pears.

In our study, N2, N3 and N4 instars were not found in a consecutive order along different months of the year in tree or in ground shelters. These findings may indicate the coexistence of single brood and double brood strategies, as observed by Helsen et al. (1998), Gobin et al. (2008) and Moerkens et al. (2009) in pip fruit orchards in Central-Northern Europe. Although low temperatures can be considered a crucial determinant of earwig mortality (Moerkens et al. 2012), in Mediterranean orchards nymphs were also found during winter, thereby indicating that earwig development in these conditions does not stop, as nymphs also hibernate. Due to these differences in phenology, abundance and population dynamic predictions through the day degree models will not be appropriate in Mediterranean orchards.

Adult individuals of *F. pubescens* were observed year-round —except in May in tree shelters— and nymph instars were detected from April to June in ground as well as in tree shelters. However, Romeu-Dalmau et al. (2011) observed individuals only from May to December, this could be attributed not only to the sampling methodology but also to the crop suitability, for example in shelter and diet. Phillips (1981) proposed that diet affects earwig development rate, as they develop faster on a mixed diet with aphids; and Mols (personal communication in Helsen et al. (1998)) also supported the relevance of diet, noting that the body size of earwigs reared in captivity is often lower than that of those collected in the field.

Occurrence of the different nymph instar stages of *F. pubescens* in apple orchards is not reported in bibliography to our knowledge. The N1 instar was never observed, as this stage is very short and the nymphs probably remained in the nest with the female (Albouy and Caussanel 1990). We found the N2 instar mainly in ground shelters from April to mid-May. After this time, the successive instars were also detected in tree and ground shelters. We found nymph instars only from April to July, thus indicating a single reproductive period per year, similar observations were made by Romeu-Dalmau et al. (2011).

For both earwig species, after the peak numbers of N4 instars, a population crash during molting into adults was observed. Moerkens et al. (2009) proposed that this decrease was caused by competition for limited resources, such as hiding places and food, when the population increases; but also to an increase of cannibalism and intraguild predation, as insects are very vulnerable during molting.

In addition to the object of the study, individuals of *E. moesta*, *N. lividipes*, and *L. riparia* were also found in Mediterranean apple orchards, but only on the ground. *F. auricularia* and *F. pubescens* are the most abundant species and are present throughout the year. In Mediterranean apple orchards, nymphs also hibernate. Therefore in Mediterranean areas *F. auricularia* does not have a synchronized cycle. *F. auricularia* and *F. pubescens* co-occur in canopies and may play an important role as biocontrol agents in pip fruit orchards as a result of their long activity period. This long period also explains their damage in peaches, nectarines, apricots and cherries. New day degree models better fitted to Mediterranean conditions are required in order to improve the protection of earwigs in pip fruit canopies and to control them in stone fruit orchards and vineyards. This study provides useful data about the weekly phenology of earwigs throughout the year that can be used to develop new phenological models for Mediterranean areas.

## 5. Supplementary material

Table sII-1. Monthly average (Mean ± SE) of earwigs found in tree and ground traps in each orchard (BB, IU, MO and MI) and year (2010-2013). Nymph instar (N1, N2, N3, N4 and N5) is shown for each earwig species (*F. auricularia* and *F. pubescens*), and adults are segregated by sex.

Orchard	2010 Tree trap	J	F	M	A	M	J	J	A	S	O	N	D	
BB	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	
	<i>Forficula auricularia</i>	N3	0.65 ± 0.30	0.30 ± 0.0	0	0.16 ± 0.0	0.03 ± 0.0	0	0	0	0	0	0	0
		N4	0.50 ± 0.21	6.10 ± 0.0	1.30 ± 0.0	0.23 ± 0.0	0	0	0	0	0	0	0	0
	Male	0	0.03 ± 0.0	1.74 ± 0.0	5.65 ± 0.0	0.81 ± 0.0	0.06 ± 0.0	0.1 ± 0.0	0.08 ± 0.0	0.02 ± 0.0	0	0	0	
	Female	0	0.03 ± 0.0	2.70 ± 0.0	7.90 ± 0.0	1.35 ± 0.0	0.11 ± 0.0	0.33 ± 0.0	0.15 ± 0.0	0.13 ± 0.0	0.11 ± 0.0	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	
	<i>Forficula pubescens</i>	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	0	0	0	0	0	0	0	0
		Male	0	0	0	0	0	0	0	0	0	0	0	0
		Female	0	0	0	0	0	0	0	0	0	0	0	0
	BB	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	
<i>Forficula auricularia</i>		N3	0	0.03 ± 0.0	0	0	0	0	0	0	0	0.03 ± 0.0	0.13 ± 0.0	
		N4	0	0.05 ± 0.0	0.13 ± 0.0	0.04 ± 0.0	0	0	0	0	0	0.05 ± 0.0	0.23 ± 0.0	
Male		0	1.80 ± 0.0	0.88 ± 0.0	0.08 ± 0.0	0	0	0	0	0	0	0	0	
Female		0	0	0.01 ± 0.0	4.68 ± 0.0	6.55 ± 0.0	0.55 ± 0.0	0.28 ± 0.0	0.28 ± 0.0	0.12 ± 0.0	0.05 ± 0.0	0	0	
Female		0	0	0.28 ± 0.0	7.10 ± 0.0	8.79 ± 0.0	1.50 ± 0.0	1.06 ± 0.0	1.33 ± 0.0	0.36 ± 0.0	0.43 ± 0.0	0.08 ± 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	
<i>Forficula pubescens</i>		N3	0	0	0	0	0.03 ± 0.03	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	0	0	0	0	0	0	0	0
		Male	0	0.05 ± 0.03	0	0	0	0	0	0	0	0	0	0
		Female	0	0.05 ± 0.03	0	0	0	0	0	0	0	0	0	0
IU	N1	0	0	0.03 ± 0.03	0	0	0	0	0	0	0	0	0	
	N2	0	0	0.05 ± 0.05	1.05 ± 0.25	0	0	0	0	0	0	0	0	
	<i>Forficula auricularia</i>	N3	0	1.93 ± 0.73	5.53 ± 2.16	0.09 ± 0.05	0	0	0	0	0	0	0	
		N4	0.02 ± 0.02	0.13 ± 0.08	12.03 ± 2.44	0.76 ± 0.19	0.03 ± 0.03	0	0	0	0	0	0	
	Male	0	0	0.38 ± 0.14	6.89 ± 1.04	1.00 ± 0.28	0.4 ± 0.12	0.53 ± 0.16	0.36 ± 0.08	0.10 ± 0.06	0.36 ± 0.17			
	Female	0.04 ± 0.03	0.08 ± 0.04	0.85 ± 0.22	11.78 ± 1.79	2.27 ± 0.55	0.56 ± 0.15	0.60 ± 0.12	0.36 ± 0.08	0.45 ± 0.14	0.08 ± 0.04			
	N1	0	0	0	0	0	0	0	0	0	0	0	0	
	N2	0	0	0	0.08 ± 0.06	0.04 ± 0.03	0	0	0	0	0	0	0	
	<i>Forficula pubescens</i>	N3	0	0	0.38 ± 0.15	0.26 ± 0.08	0	0	0	0	0	0	0	0
		N4	0	0	0	1.28 ± 0.26	0.24 ± 0.12	0.02 ± 0.02	0	0.18 ± 0.11	0	0	0	
		N5	0	0	0	0	0	0	0	0	0	0	0	0
		Male	0.78 ± 0.16	0.23 ± 0.10	0	0.2 ± 0.06	1.11 ± 0.30	0.31 ± 0.12	0.50 ± 0.11	0.40 ± 0.11	0.73 ± 0.25	0.03 ± 0.03		
		Female	0.04 ± 0.04	0.63 ± 0.14	0	1.2 ± 0.34	1.32 ± 0.19	0.60 ± 0.15	0.48 ± 0.12	0.30 ± 0.09	1.20 ± 0.31	0.23 ± 0.09		
	MO	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	0.03 ± 0.03	
<i>Forficula auricularia</i>		N3	0	0	0	0	0	0	0	0	0	0	0	0.13 ± 0.06
		N4	0	0	0.03 ± 0.03	0.18 ± 0.11	0	0	0	0	0	0	0	0
Male		0	0	0	1.20 ± 0.36	2.28 ± 0.72	0.23 ± 0.08	0.16 ± 0.09	0.13 ± 0.06	0.12 ± 0.07	0.03 ± 0.03			
Female		0	0	0	1.82 ± 0.44	3.18 ± 1.05	0.53 ± 0.19	0.18 ± 0.11	0.28 ± 0.16	0.10 ± 0.06	0.20 ± 0.11	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	
<i>Forficula pubescens</i>		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	0	0	0	0	0	0	0	0
		Male	0	0	0	0	0	0	0	0	0	0	0	0
		Female	0	0	0	0	0	0	0	0	0	0	0	0

# Chapter II

Orchard	2012 Tree trap	J	F	M	A	M	J	J	A	S	O	N	D	
BB	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0	0
		N2	0.13 ± 0.0	0	0	0	0	0	0	0	0	0	0	0
		N3	0.52 ± 0.0	0	0.08 ± 0.0	0.11 ± 0.0	0.02 ± 0.0	0	0	0	0	0	0	0
		N4	0	0	0.64 ± 0.0	6.84 ± 0.0	0.56 ± 0.0	0.03 ± 0.0	0	0	0	0	0	0.03 ± 0.0
	Male	N1	0	0	0	0.05 ± 0.0	1.44 ± 0.0	1.55 ± 0.0	0.22 ± 0.0	0.16 ± 0.0	0.07 ± 0.0	0.02 ± 0.0	0.03 ± 0.0	0.07 ± 0.0
		N2	0	0	0	0.18 ± 0.0	1.98 ± 0.0	2.75 ± 0.0	0.32 ± 0.0	0.1 ± 0.0	0.20 ± 0.0	0.04 ± 0.0	0.50 ± 0.0	0.03 ± 0.0
	Female	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
	<i>Forficula pubescens</i>	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	0	0	0	0	0	0	0	0
		Female	0	0	0	0	0	0	0	0	0	0	0	0
IU	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0	
		N2	0	0	0	0.03 ± 0.03	0.02 ± 0.02	0	0	0	0	0	0	0
		N3	0	0	0	0.13 ± 0.09	0.98 ± 0.25	0.49 ± 0.17	0	0	0	0	0	0
		N4	0	0	0	0.11 ± 0.05	18.79 ± 3.37	0.67 ± 0.22	0	0	0	0	0	0
	Male	N1	0	0	0	0.03 ± 0.03	2.0 ± 0.5	8.31 ± 1.84	1.68 ± 0.41	0.06 ± 0.03	0.4 ± 0.13	0.18 ± 0.07	0.08 ± 0.06	0
		N2	0	0	0.08 ± 0.05	0.26 ± 0.10	2.56 ± 0.59	13.23 ± 2.95	2.38 ± 0.55	0.14 ± 0.06	0.4 ± 0.15	0.42 ± 0.14	0.26 ± 0.10	0
	Female	N1	0	0	0	0	0	0	0	0	0	0	0	0
		N2	0	0	0	0.16 ± 0.09	0	0	0	0	0	0	0	0
	<i>Forficula pubescens</i>	N1	0	0	0	0	0.25 ± 0.09	0.03 ± 0.03	0	0	0	0	0	0
		N2	0	0	0	0	0.48 ± 0.11	0.21 ± 0.11	0	0	0	0	0	0
		N3	0	0	0	0	0.17 ± 0.06	1.46 ± 0.33	0.10 ± 0.08	0	0	0	0	0
		N4	0.04 ± 0.03	0.04 ± 0.04	0.3 ± 0.1	0.32 ± 0.13	0.02 ± 0.02	0.21 ± 0.07	0.50 ± 0.14	0.06 ± 0.04	0.03 ± 0.03	0.04 ± 0.03	0.05 ± 0.04	0
		Male	0	0	0.11 ± 0.06	1.5 ± 0.5	0.02 ± 0.02	0.36 ± 0.16	0.55 ± 0.18	0.10 ± 0.04	0.08 ± 0.04	0.10 ± 0.05	0.23 ± 0.08	0
		Female	0	0	0	0	0	0	0	0	0	0	0	0
MO	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0	
		N2	0.04 ± 0.03	0	0	0	0	0	0	0	0	0.02 ± 0.02	0.08 ± 0.06	0
		N3	0	0	0.03 ± 0.03	0	0.13 ± 0.06	0	0	0	0	0	0.03 ± 0.03	0
		N4	0	0	0.03 ± 0.03	1.29 ± 0.37	6.73 ± 2.61	0.4 ± 0.18	0.02 ± 0.02	0	0	0	0	0
	Male	N1	0	0	0	0.27 ± 0.11	4.48 ± 1.14	4.5 ± 1.0	3.28 ± 0.87	0.26 ± 0.07	0.63 ± 0.26	0.66 ± 0.23	0.36 ± 0.15	0
		N2	0	0	0.03 ± 0.03	0.17 ± 0.08	6.05 ± 1.39	7.98 ± 1.47	5.2 ± 1.3	0.30 ± 0.09	1.57 ± 0.49	0.94 ± 0.24	1.44 ± 0.30	0
	Female	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
	<i>Forficula pubescens</i>	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	0.03 ± 0.03	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	0	0	0	0	0	0	0	0
		Female	0	0	0	0	0	0.02 ± 0.02	0.02 ± 0.02	0	0	0	0	0
MI	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0	
		N2	0	0	0	0	0.03 ± 0.03	0	0	0	0	0	0	0
		N3	0	0	0	0.20 ± 0.11	0.13 ± 0.05	0.10 ± 0.05	0	0	0	0.02 ± 0.02	0	0
		N4	0	0	0	0.80 ± 0.31	14.21 ± 3.81	0.48 ± 0.13	0	0	0	0	0	0.04 ± 0.04
	Male	N1	0	0	0.06 ± 0.03	5.13 ± 1.10	24.05 ± 3.47	0.51 ± 0.12	0.10 ± 0.05	0.20 ± 0.11	0.50 ± 0.15	0.54 ± 0.16	0	
		N2	0	0.05 ± 0.04	0.10 ± 0.07	7.41 ± 1.66	38.45 ± 4.69	1.49 ± 0.34	0.38 ± 0.12	0.23 ± 0.09	1.24 ± 0.33	1.05 ± 0.25	0.07 ± 0.05	
	Female	N1	0	0	0	0	0	0	0	0	0	0	0	
		N2	0	0	0	0	0	0	0	0	0	0	0	
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0	
		N2	0	0	0	0	0	0	0	0	0	0	0	
		N3	0	0	0	0.05 ± 0.04	0.03 ± 0.03	0	0	0	0	0	0	0
		N4	0	0	0	0.31 ± 0.14	0	0	0	0	0	0	0	0
		N5	0	0	0	0.18 ± 0.07	0.33 ± 0.09	0	0	0	0	0	0	0
		Female	0	0	0	0	0.35 ± 0.08	0.11 ± 0.05	0	0	0	0	0	0
Female	0	0	0.03 ± 0.03	0	0	0.38 ± 0.09	0.13 ± 0.06	0	0	0.02 ± 0.02	0.03 ± 0.03	0		

# Phenology of *F. auricularia* & *F. pubescens*

Orchard	2013 Tree trap	J	F	M	A	M	J	J	A	S	O	N	D		
BB	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0		
		N2	0	0	0	0.04 ± 0.0	0	0	0	0	0	0	0	0.07 ± 0.0	
		N3	0.08 ± 0.0	0.03 ± 0.0	0.42 ± 0.0	0.15 ± 0.0	0.02 ± 0.0	0	0	0	0	0	0	0.13 ± 0.0	
		N4	0.04 ± 0.0	0.08 ± 0.0	0.29 ± 0.0	3.27 ± 0.0	1.49 ± 0.0	0.10 ± 0.0	0	0	0	0	0	0	
		Male	0	0	0	0.08 ± 0.0	2.35 ± 0.0	8.28 ± 0.0	2.03 ± 0.0	0.16 ± 0.0	0.3 ± 0.0	0.12 ± 0.0	0	0	
		Female	0	0	0.05 ± 0.0	0.08 ± 0.0	3.31 ± 0.0	6.79 ± 0.0	3.71 ± 0.0	0.51 ± 0.0	0.43 ± 0.0	0.26 ± 0.0	0.13 ± 0.0	0	
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0		
		N2	0	0	0	0	0	0	0	0	0	0	0		
		N3	0	0	0	0	0	0	0	0	0	0	0		
		N4	0	0	0	0	0	0	0	0	0	0	0		
		N5	0	0	0	0	0	0	0	0	0	0	0		
		Female	0	0	0	0	0	0	0	0	0	0	0	0.03 ± 0.03	
	IU	<i>Forficula auricularia</i>	N1	0	0	0	0	0.02 ± 0.02	0	0	0	0	0	0	
			N2	0	0	0	0	0.29 ± 0.08	0.05 ± 0.04	0	0	0	0	0	
N3			0	0	0.05 ± 0.04	0	0.67 ± 0.21	0.22 ± 0.10	0.03 ± 0.03	0	0	0	0		
N4			0	0	0.03 ± 0.03	0	9.64 ± 1.97	1.97 ± 0.74	0.03 ± 0.03	0	0	0	0		
Male			0.04 ± 0.03	0	0.03 ± 0.03	0	0.44 ± 0.18	4.73 ± 0.84	0.38 ± 0.09	0.06 ± 0.03	0.13 ± 0.09	0.08 ± 0.04	0	0	
Female			0	0.03 ± 0.03	0.05 ± 0.04	0	1.04 ± 0.43	7.24 ± 1.28	0.53 ± 0.13	0.04 ± 0.03	0.20 ± 0.07	0.35 ± 0.10	0.2 ± 0.1	0	
<i>Forficula pubescens</i>		N1	0	0	0	0	0	0	0	0	0	0	0		
		N2	0	0	0	0	0	0	0	0	0	0	0		
		N3	0	0	0	0	0	0	0	0	0	0	0		
		N4	0	0	0	0	0	0	0	0	0	0	0		
		N5	0	0	0	0	0	0	0	0	0	0	0		
		Female	0	0.03 ± 0.03	0.14 ± 0.06	0	0	0	0	0.02 ± 0.02	0	0	0	0	
MO		<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0	
			N2	0.02 ± 0.02	0	0	0.03 ± 0.03	0	0.06 ± 0.06	0	0	0	0	0	0.23 ± 0.12
	N3		0.04 ± 0.04	0.03 ± 0.03	0.10 ± 0.05	0.27 ± 0.2	0.94 ± 0.24	0.28 ± 0.25	0	0	0	0	0	0.20 ± 0.09	
	N4		0	0.03 ± 0.03	0.28 ± 0.14	1.60 ± 0.66	9.32 ± 1.53	3.50 ± 0.84	0.62 ± 0.39	0	0	0	0	0	
	Male		0.02 ± 0.02	0	0	0	5.11 ± 1.47	23.97 ± 3.89	15.38 ± 2.76	1.15 ± 0.27	0.54 ± 0.13	1.42 ± 0.23	0.9 ± 0.32	0	
	Female		0	0	0	0.37 ± 0.3	6.91 ± 1.52	17.47 ± 3.65	18.03 ± 3.08	1.83 ± 0.41	1.5 ± 0.53	2.42 ± 0.48	2.07 ± 0.55	0	
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0		
		N2	0	0	0	0	0	0	0	0	0	0	0		
		N3	0	0	0	0	0	0	0	0	0	0	0		
		N4	0	0	0	0	0	0	0	0	0	0	0		
		N5	0	0	0	0	0	0	0	0	0	0	0		
		Female	0	0	0	0	0	0	0	0	0	0	0	0	
	MI	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0.03 ± 0.03	0	0				
			N2	0	0	0	0	0.06 ± 0.04	0.10 ± 0.05	0.03 ± 0.03	0				
N3			0	0	0	0.04 ± 0.04	0.18 ± 0.08	0.90 ± 0.64	0.05 ± 0.04	0					
N4			0	0	0	0	6.62 ± 2.32	1.41 ± 0.50	0	0					
Male			0	0	0	0.04 ± 0.04	0.4 ± 0.15	6.15 ± 1.44	3.67 ± 0.85	1.10 ± 0.66					
Female			0.02 ± 0.02	0	0	0	0.6 ± 0.21	6.87 ± 1.69	4.41 ± 1.10	1.30 ± 0.62					
<i>Forficula pubescens</i>		N1	0	0	0	0	0	0	0	0					
		N2	0	0	0	0	0	0	0	0					
		N3	0	0	0	0	0	0	0	0					
		N4	0	0	0	0	0	0	0	0					
		N5	0	0	0	0	0	0	0	0					
		Female	0	0	0	0	0	0.03 ± 0.03	0.05 ± 0.05	0					
Female		0	0	0	0	0	0.03 ± 0.03	0.10 ± 0.06	0						

# Chapter II

Orchard	2012 Ground trap	J	F	M	A	M	J	J	A	S	O	N	D
BB	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0.67 ± 0.40
		N2	0	0	0	0	0	0	0	0	0	0.08 ± 0.04	0.23 ± 0.17
		N3	0.03 ± 0.03	0.18 ± 0.07	0.18 ± 0.06	0.06 ± 0.03	0	0	0	0	0	0	0.20 ± 0.11
		N4	0	0.10 ± 0.06	1.63 ± 0.48	0.30 ± 0.15	0	0	0	0	0	0	0.07 ± 0.07
		Male	0	0	0.03 ± 0.03	0.40 ± 0.11	0.08 ± 0.04	0	0.02 ± 0.02	0	0.04 ± 0.03	0	0.07 ± 0.05
		Female	0	0	0	0.28 ± 0.11	0.10 ± 0.06	0	0.04 ± 0.04	0.1 ± 0.06	0.08 ± 0.05	0.15 ± 0.07	0.07 ± 0.07
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0
		N2	0	0	0	0	0	0	0	0	0	0	0
		N3	0	0	0	0	0	0	0	0	0	0	0
		N4	0	0	0	0	0	0	0	0	0	0	0
		Male	0	0	0	0	0	0	0	0	0	0	0
		Female	0	0	0	0	0	0	0	0	0	0	0
IU	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0
		N2	0	0	0.03 ± 0.03	0.04 ± 0.03	0	0	0	0	0	0	0
		N3	0	0	0.25 ± 0.1	0.71 ± 0.27	0	0	0	0	0	0	0
		N4	0	0	0	0.78 ± 0.21	0.08 ± 0.06	0	0	0	0	0	0
		Male	0	0	0	0.02 ± 0.02	0.05 ± 0.04	0.05 ± 0.03	0.08 ± 0.05	0	0	0	0
		Female	0	0	0.03 ± 0.03	0.06 ± 0.03	0	0.03 ± 0.03	0.04 ± 0.03	0	0.02 ± 0.02	0	0
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0
		N2	0	0	0.93 ± 0.25	0.63 ± 0.16	0.03 ± 0.03	0	0	0	0	0	0
		N3	0	0	0.18 ± 0.08	1.39 ± 0.41	0.14 ± 0.07	0	0	0	0	0	0
		N4	0	0	0.13 ± 0.13	0.69 ± 0.17	0.35 ± 0.12	0	0	0	0	0	0
		N5	0	0	0.03 ± 0.03	0.04 ± 0.03	0.27 ± 0.07	0	0	0	0	0	0
		Female	0.03 ± 0.03	0.40 ± 0.11	1.05 ± 0.33	0.12 ± 0.07	0	0	0.04 ± 0.03	0	0.02 ± 0.02	0	0.03 ± 0.03
MO	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0.10 ± 0.06
		N2	0	0.06 ± 0.08	0.06 ± 0.03	0.03 ± 0.03	0	0	0	0	0.02 ± 0.02	0.60 ± 0.27	5.40 ± 1.86
		N3	0	0.08 ± 0.04	0.12 ± 0.05	0.44 ± 0.23	0.03 ± 0.03	0	0	0	0	0.28 ± 0.12	1.87 ± 0.54
		N4	0	0.08 ± 0.06	1.16 ± 0.39	3.15 ± 1.36	0.10 ± 0.06	0	0	0	0	0	0.13 ± 0.08
		Male	0	0	0.12 ± 0.05	1.0 ± 0.4	0.13 ± 0.08	0	0.18 ± 0.16	0	0.22 ± 0.09	0.05 ± 0.03	0
		Female	0	0	0.12 ± 0.05	1.12 ± 0.29	0.28 ± 0.09	0.12 ± 0.05	0.46 ± 0.30	0.07 ± 0.05	0.10 ± 0.04	1.23 ± 0.24	0.23 ± 0.09
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0
		N2	0	0	0	0	0	0	0	0	0	0	0
		N3	0	0	0	0.38 ± 0.18	0	0	0	0	0	0	0
		N4	0	0	0	0.38 ± 0.17	0	0.02 ± 0.02	0	0	0	0	0
		N5	0	0	0	0	0	0	0	0	0	0	0
		Female	0	0	0.02 ± 0.02	0	0	0	0	0	0	0	0
MI	<i>Forficula auricularia</i>	N1	0	0	0	0	0	0	0	0	0	0	0
		N2	0	0	0	0.05 ± 0.03	0	0	0	0	0	0	0
		N3	0	0	0.26 ± 0.07	0.3 ± 0.1	0.03 ± 0.03	0	0	0	0	0	0
		N4	0	0	0.72 ± 0.21	2.88 ± 0.99	0.05 ± 0.03	0	0	0	0	0	0.03 ± 0.03
		Male	0	0.03 ± 0.03	0.04 ± 0.03	0.43 ± 0.19	0.80 ± 0.23	0.08 ± 0.04	0	0.08 ± 0.04	0	0.03 ± 0.03	0
		Female	0	0.03 ± 0.03	0.06 ± 0.03	0.55 ± 0.16	2.75 ± 0.58	0.34 ± 0.10	0.03 ± 0.03	0	0	0.08 ± 0.06	0.07 ± 0.05
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0
		N2	0	0	0.24 ± 0.08	0.15 ± 0.08	0	0	0	0	0	0	0
		N3	0	0	0.16 ± 0.06	0.40 ± 0.17	0.03 ± 0.03	0	0	0	0	0	0
		N4	0	0	0.02 ± 0.02	0.65 ± 0.28	0.18 ± 0.09	0	0	0	0	0	0
		N5	0	0	0	0.23 ± 0.10	0.48 ± 0.12	0.02 ± 0.02	0	0	0	0	0
		Female	0	0.05 ± 0.03	0	0	0.08 ± 0.04	0	0	0	0	0.03 ± 0.03	0
Female	0	0	0.02 ± 0.02	0	0.08 ± 0.04	0.06 ± 0.03	0.03 ± 0.03	0	0	0	0.03 ± 0.03		

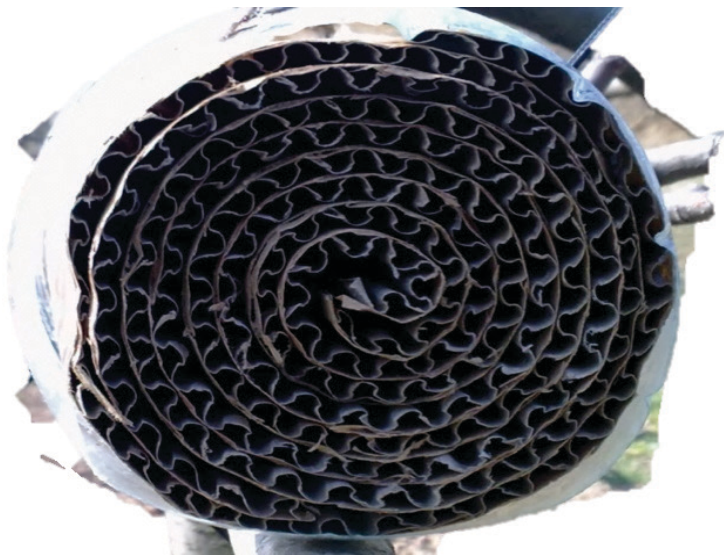
# Phenology of *F. auricularia* & *F. pubescens*

Orchard	2013 Ground trap	J	F	M	A	M	J	J	A	S	O	N	D	
BB	<i>Forficula auricularia</i>	N1	0	0	0	0	0.02 ± 0.02	0	0	0	0	0	0	
		N2	0	0.03 ± 0.03	0.03 ± 0.03	0.47 ± 0.34	0	0	0	0	0	0	0.10 ± 0.08	
		N3	0.74 ± 0.31	0.23 ± 0.15	0.30 ± 0.12	0.20 ± 0.11	0	0	0	0	0	0	0.45 ± 0.31	
		N4	0.22 ± 0.08	0.28 ± 0.14	0.45 ± 0.16	2.20 ± 0.49	0.62 ± 0.16	0	0	0	0	0	0	0
	Male	Female	0	0	0	0	0.98 ± 0.23	0.20 ± 0.08	0.07 ± 0.05	0.05 ± 0.03	0	0.12 ± 0.05	0	0
		Female	0	0.03 ± 0.03	0	0	1.02 ± 0.24	0.33 ± 0.13	0.07 ± 0.05	0	0.13 ± 0.1	0.04 ± 0.03	0.07 ± 0.05	0
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0	
		N2	0	0	0	0	0	0	0	0	0	0	0	
		N3	0	0	0	0	0	0	0	0	0	0	0	
		N4	0	0	0	0	0	0	0	0	0	0	0	
		N5	0	0	0	0	0	0	0	0	0	0	0	
		Male	Female	0	0	0	0	0	0	0	0	0	0	0
	IU	<i>Forficula auricularia</i>	N1	0	0	0	0	0.20 ± 0.09	0.10 ± 0.06	0	0	0	0	0
			N2	0	0	0	0.07 ± 0.07	0.66 ± 0.15	0.63 ± 0.31	0	0	0	0	0
N3			0	0	0	0	0.48 ± 0.18	0.05 ± 0.03	0	0	0	0	0	
N4			0	0	0	0	1.84 ± 0.48	0.25 ± 0.12	0	0	0	0	0	
Male		Female	0	0	0	0	0.05 ± 0.05	0.08 ± 0.04	0	0	0	0	0	
		Female	0	0	0	0.07 ± 0.05	0.14 ± 0.06	0.15 ± 0.06	0	0	0	0	0	
<i>Forficula pubescens</i>		N1	0	0	0	0	0	0	0	0	0	0	0	
		N2	0	0	0	0	0	0	0	0	0	0	0	
		N3	0	0	0	0	0	0	0	0	0	0	0	
		N4	0	0	0	0	0	0	0	0	0	0	0	
		N5	0	0	0	0	0	0	0	0	0	0	0	
		Male	Female	0	0.15 ± 0.11	0.09 ± 0.05	0	0	0	0	0	0	0	0
MO		<i>Forficula auricularia</i>	N1	0	0	0	0	0	0.03 ± 0.03	0	0	0	0.02 ± 0.02	0.10 ± 0.06
			N2	0.82 ± 0.40	0.50 ± 0.36	0	0.57 ± 0.26	0.38 ± 0.17	0.03 ± 0.03	0.15 ± 0.15	0	0	0.02 ± 0.02	0.41 ± 0.14
	N3		7.02 ± 2.17	5.35 ± 2.09	2.73 ± 0.85	0.87 ± 0.23	2.52 ± 0.71	0.16 ± 0.09	0.04 ± 0.04	0	0.07 ± 0.07	0	0.10 ± 0.08	
	N4		0.44 ± 0.16	3.10 ± 1.23	2.88 ± 1.01	5.40 ± 1.29	3.56 ± 0.66	0.16 ± 0.07	0.04 ± 0.04	0	0	0	0	
	Male	Female	0	0.03 ± 0.03	0	0	0.85 ± 0.28	1.11 ± 0.68	0.27 ± 0.13	0.08 ± 0.05	0.03 ± 0.03	0.14 ± 0.05	0.21 ± 0.14	
		Female	0.04 ± 0.03	0	0.03 ± 0.03	0	0.83 ± 0.24	1.87 ± 0.99	0.23 ± 0.12	0.10 ± 0.04	0.20 ± 0.07	0.18 ± 0.07	0.24 ± 0.11	
	<i>Forficula pubescens</i>	N1	0	0	0	0	0	0	0	0	0	0	0	
		N2	0	0	0	0	0	0	0	0	0	0	0	
		N3	0	0	0	0	0	0	0	0	0	0	0	
		N4	0	0	0	0	0	0	0	0	0	0	0	
		N5	0	0	0	0	0	0	0.04 ± 0.04	0	0	0	0	
		Male	Female	0	0	0	0	0	0	0	0	0	0	
	MI	<i>Forficula auricularia</i>	N1	0	0	0	0	0.04 ± 0.04	0.23 ± 0.10	0	0	0	0	
			N2	0	0	0.03 ± 0.03	0	0.27 ± 0.09	1.18 ± 0.30	0.06 ± 0.04	0	0	0	
N3			0	0	0	0	0.08 ± 0.04	0.38 ± 0.25	0.09 ± 0.06	0	0	0		
N4			0	0	0	0	0.58 ± 0.20	0.13 ± 0.07	0	0	0	0		
Male		Female	0	0	0	0	0	0.15 ± 0.08	0.32 ± 0.11	0	0	0		
		Female	0.02 ± 0.02	0	0	0	0.06 ± 0.04	0.20 ± 0.09	0.18 ± 0.08	0	0	0		
<i>Forficula pubescens</i>		N1	0	0	0	0	0	0	0	0	0	0		
		N2	0	0	0	0	0	0	0	0	0	0		
		N3	0	0	0	0	0	0	0	0	0	0		
		N4	0	0	0	0	0	0	0	0	0	0		
		N5	0	0	0	0	0	0	0	0	0	0		
		Male	Female	0	0	0	0	0	0.05 ± 0.03	0.18 ± 0.07	0	0	0	





# **Chapter III. Aggregation behavior in the European earwig: response to impregnated shelters**



## **Abstract**

The European earwig *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae) is a key predator of pests in pip fruit orchards; however, this insect can also cause economic damage in stone fruit crops. Pheromone-impregnated shelters may be useful to promote earwigs in orchards devoted to pip fruit and also to capture them in those used for stone fruit production. By using corrugated cardboard traps in four orchards during two years, we observed the aggregation behavior of European earwig in canopies. Under laboratory conditions, a corrugated cardboard shelter impregnated by 0.2 individuals/cm<sup>2</sup> over one week attracted earwigs for 5 weeks within a range of 50 cm. Future field work should examine the potential of impregnated shelters to promote earwigs in pip fruit orchards and to remove them from stone fruit ones.

**Keywords:** Aggregation behavior, biological control, *Forficula auricularia*, pest, predator.

## 1. Introduction

The European earwig, *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae), is an important predator in pip fruit (Asante 1995; He et al. 2008; Lenfant et al. 1994; Nicholas et al. 2005), kiwifruit (Hill et al. 2005) and citrus (Piñol et al. 2009; Piñol et al. 2010) orchards. However, given its omnivorous regime, this insect can damage shoots, leaves, flowers and fruits (Pollini 2010), becoming a pest of stone fruit crops (Albouy and Caussanel 1990; Cranshaw 2000; Flint 2012; Grafton-Cardwell et al. 2003; Kuthe 1996) and vineyards, where in addition to its direct damage on berries, its frass can negatively influence the aroma and flavor of some wines (Burdet et al. 2013; Huth et al. 2011). The incidence and severity of earwig outbreaks has recently increased in peaches (*Prunus persica* (L.) Batsch var. *persica*), nectarines (*Prunus persica* (L.) Batsch var. *nectarine* (Aiton) Maxim. and *Prunus persica* (L.) Batsch var. *nucipersica* (Borkh.) Schneider), apricots (*Prunus armeniaca* L.) and cherries (*Prunus avium* L.), reaching in some cases 10-15% of damage in Mediterranean areas (Asteggiano and Vittone 2013; Pollini 2010; Saladini et al. 2012; Servei de Sanitat Vegetal 2013). Therefore, earwig management practices should be adopted in accordance with the fruit crop. To control them in conventional production, growers are looking for effective pesticides, whereas in organic production they are looking for alternative strategies such as mass trapping and exclusion by setting glue around the base of trunks (Alston and Tebeau 2011; Saladini et al. 2012).

The European earwig is a thigmotactic insect that shelters during the day and forages at night (Albouy and Caussanel 1990; Burnip et al. 2002). It is usually found in clusters across the orchard, taking refuge in shelters previously occupied by earwigs (Sauphanor and Sureau 1993). In laboratory experiments, this insect has been observed to aggregate, which is postulated to be elicited by a pheromone (Evans and Longépé 1996; Hehar et al. 2008; Sauphanor 1992; Sauphanor and Sureau 1993; Walker et al. 1993). Gregarious behavior confers protection against predators, increases mate encounters, and enhances juvenile growth and development (Antony et al. 1985; Fuchs et al. 1985; Sauphanor and Sureau 1993; Walker et al. 1993).

Laboratory experiments revealed that females, males, and nymphs produce and respond to an airborne aggregation pheromone; however, its source and composition are still under debate (Evans and Longépé 1996; Hehar et al. 2008; Sauphanor 1992; Walker et al. 1993). Sauphanor (1992) suggested that the pheromone was segregated on tibial glands, while Walker et al. (1993) associated it with fecal excreta and cuticular lipids. Evans and Longépé (1996) reported that leg extracts were not active and pointed to the body cuticle as the source of the pheromone, whereas Hehar et al. (2008) observed that neither fresh frass extracts nor body washes elicited significant responses. Although the source and composition of the pheromone remains unclear, Hehar et al. (2008) proposed that this chemical cue is perceived by olfaction rather than by contact chemoreception,

and Evans and Longép  (1996) had already determined that it was detectable by the antennae.

Evans and Long p  (1996), Sauphanor and Sureau (1993) and Hehar et al. (2008) observed that filter papers, cardboard shelters, and paper-towel disks previously in contact with European earwig individuals elicited aggregation behavior. In this regard, the use of corrugated cardboard shelters in pear orchards has been reported to increase populations of European earwig which results in a reduction of the densities of pear psylla *Cacopsylla pyri* L. (Hemiptera: Psyllidae) (Solomon et al. 1999). Suckling et al. (2006) suggested that high populations of earwigs may have significant contribution to biological control, suppressing several pests species below economic threshold. This has been found in pests such as woolly apple aphid (WAA) *Eriosoma lanigerum* Hausmann (Asante 1995; Mueller et al. 1988; Nicholas et al. 2005), and green apple aphid *Aphis pomi* DeGeer (both Hemiptera: Aphididae) (Carroll and Hoyt 1984; Hagley and Allen 1990), apple leaf-curling midge *Dasineura mali* Kieffer (Diptera: Cecidomyiidae) (He et al. 2008) and diaspidid scale insects (Hill et al. 2005; Logan et al. 2007).

While the pheromone emitted by earwigs is not commercially available, impregnated shelters may be useful to promote earwigs in orchards devoted to pip fruit, where growers have tried, with little success up to now, to enhance earwig populations (Moerkens et al. 2009). Impregnated shelters might be also useful to capture individuals in orchards used for stone fruit production. However, such applications are hindered because there is no method to ensure long-term impregnation of shelters for this purpose.

Here we evaluated the aggregation behavior of the European earwig in field conditions; determined in the laboratory the number of earwigs required to impregnate a shelter, the duration of such impregnation, and the distance at which the insect can respond to the pheromonal signal emitted by these shelters.

## 2. Materials and Methods

### 2.1. Aggregation behavior in field conditions

The trials were performed in the following four apple orchards located in Catalonia (NE Spain): Les Borges Blanques (41°30'23.06''N; 0°51'05.93''E), Mollerussa (41°36'51.13''N; 0°52'22.75''E), Ivars d'Urgell (41°41'06.19''N; 0°58'06.09''E), and Miralcamp (41°36'31.89''N; 0°52'24.62''E). All orchards were under organic management. To evaluate earwig aggregation behavior, 10 cardboard traps per orchard were set up in the canopy of trees (one trap per tree). For this purpose, a piece of corrugated cardboard was rolled into a cylinder (12 cm height x 9 cm diameter) and inserted into a PVC tube (15 cm height x 9.5 cm diameter) to protect it from rain and adverse conditions. Similar traps have been used in studies of European earwigs elsewhere (Burnip et al. 2002; Gobin et al. 2006; He et al. 2008; Helsen et al. 1998; Logan et al. 2007; Moerkens et al. 2009; Phillips 1981;

Solomon et al. 1999). Every week from March to September in 2012 and 2013, we recorded the number and phenological stage of *F. auricularia* in each trap. As two earwig species were found, absence of wings in *Forficula pubescens* Gené was used to distinguish adults from those of *F. auricularia*; while to distinguish the nymphs we took into account the size, colour and setae type of the cerci (Albouy and Caussanel 1990). The number of antennal segments and presence of wing buds on the 3<sup>rd</sup> segment of the thorax were used to distinguish nymph stages (Albouy and Caussanel 1990). After identification and enumeration, insects were released at the base of the assessed tree.

### 2.2. Aggregation pheromone trials

The European earwigs used in the experiments were collected with cardboard traps from Les Borges Blanques and Ivars d'Urgell orchards in 2011. They were fed *ad libitum* on a semi-artificial diet (Eizaguirre and Albajes 1992) and kept in colonies always under a 16:8 h light/dark cycle at  $25 \pm 3$  °C and  $75 \pm 5\%$  RH.

### 2.3. Shelter impregnation by the aggregation pheromone

The shelters used in the experiments were prepared by rolling a piece of corrugated cardboard into cylinders (5.5 cm height x 3 cm diameter). Earwigs were confined with the cardboard cylinders in plastic containers (14 x 10 x 20 cm).

To determine the minimum number of earwigs needed to impregnate shelters, we performed tests with 10, 20 and 40 individuals (with equal number of males and females). The gender of earwigs was determined by dimorphism of the cerci (Albouy and Caussanel 1990). Each group of earwigs (pheromone group, PG) was placed in a plastic container, together with a shelter, and 2 g of semi-artificial diet during one week. One week later, the earwigs and food were removed and the shelter was considered 'impregnated'.

To evaluate the attraction of pheromone-impregnated shelters, 10 earwigs (5 males and 5 females) were used (evaluation group, EG). At 3.00 p.m. on the day before the assessment, the EG was put in plastic containers with a semi-artificial diet until 8.00 a.m. on the following day (day of assessment). The EG was used in a choice test the day of assessment: This experiment consisted of placing an impregnated pheromone shelter (P) and a non-impregnated shelter (C) at the opposite ends of a rectangular plastic container (30 x 20 x 10 cm), releasing the EG at its center.

To prevent any effect of orientation, the relative position of shelters was reversed for each replication. Seven hours later, still during the photophase, the number and the gender of earwigs in each shelter were recorded. The impregnated shelters were kept individually in plastic containers without earwigs until they were used again in the next test to evaluate duration of the effect. The first test was always performed the day after the impregnation week. The time between tests was 1 week in shelters impregnated by 10 or 20 earwigs. For 40 individuals, there were 3 weeks between the first and the second test; from this on, tests were performed

fortnightly. Tests were carried out until no effect was detected for 2 consecutive tests. Before and after each evaluation, containers were cleaned with 99% ethyl alcohol. Earwigs belonging to the EG and PG were randomly obtained from laboratory colonies. We performed four replicates for each treatment.

### 2.4. Range of pheromone perception

Following the same method described in section 2.2.1, new shelters impregnated with pheromone by 40 European earwigs over one week were used in this experiment. To evaluate the range of pheromone attraction, a P shelter and a C shelter were placed at opposite ends of a plastic channel (250 cm long x 13.5 cm diameter). The channel was set up in a room with no air current. An EG was released at an equal distance from each shelter. The number and the gender of earwigs in each shelter were recorded 15 minutes after their release. The earwigs were released at four distances from the shelters: 10, 25, 50 and 100 cm. Before and after each evaluation, the plastic channel was cleaned with 99% ethyl alcohol. Earwigs belonging to the EG were randomly taken from the laboratory colonies and the relative position of shelters was reversed for each replication. We performed four replicates of each treatment.

### 2.5. Data analysis

Comparisons between nymph and adult densities were made for each year using a mixed procedure as statement repeated measures (week) with a first order autoregressive covariance structure. Orchard was considered as a random factor. Densities were transformed ( $\sqrt{(x+3/8)}$ ) before analysis to normalize their distribution.

For each year, the aggregation index ( $I_A$ ) in traps for adults and nymphs was calculated using the variance/mean ratio. The sampling unit for variance and mean determination was the weekly average of the ten traps per orchard. Each orchard was considered a replication. The  $I_A$  departure from a ratio equal to one was tested by a Chi-square test (Southwood and Henderson 2000).

To evaluate the attraction of pheromone-impregnated shelters, its duration and range, the number of individuals between shelters was compared to a random response by a Chi-square test. The number of males and females within the impregnated shelters was also compared to a random response by a Chi-square test to assess differences in attraction regarding gender of earwigs. Earwigs that occasionally were found out were not included in the analysis. The loss of attraction of the shelters impregnated by 40 individuals along the time was calculated by a regression.

Data were analyzed using the SAS (Version 9.2; SAS institute Inc., Cary, North Carolina) and the JMP statistical software package (Version 9; SAS Institute Inc., Cary, North Carolina).

### 3. Results

#### 3.1. Aggregation behavior in field conditions

European earwig nymphs in field traps were observed from April to the beginning of June, whereas adults were mainly found from June to August (Figure III-1a-b). Nymph density peaked at the end of May, with an average of  $23.78 \pm 4.89$  (mean  $\pm$  SE) individuals per trap in 2012 and  $14.68 \pm 3.18$  individuals per trap in 2013 (Figure III-1a-b). The number of adults per trap had one peak ( $37.53 \pm 7.1$ ) in June 2012 and two similar peaks in June-July 2013 ( $23.34 \pm 4.65$  and  $21.41 \pm 5.38$ ) (Figure III-1a-b). From April to mid May, density of nymphs was significantly higher than density of adults, while from then on the reverse was true (Figure III-1a-b). Regarding the aggregation in traps, in both years, the nymph and adult values of  $I_A$  were significantly  $>1$  from April to August ( $P < 0.05$ ) (Figure III-1c-d), indicating an aggregated behavior (Figure III-1c-d). Numerical values of  $I_A$  followed a similar pattern than the density, with greater values coinciding with higher densities (Figure III-1).

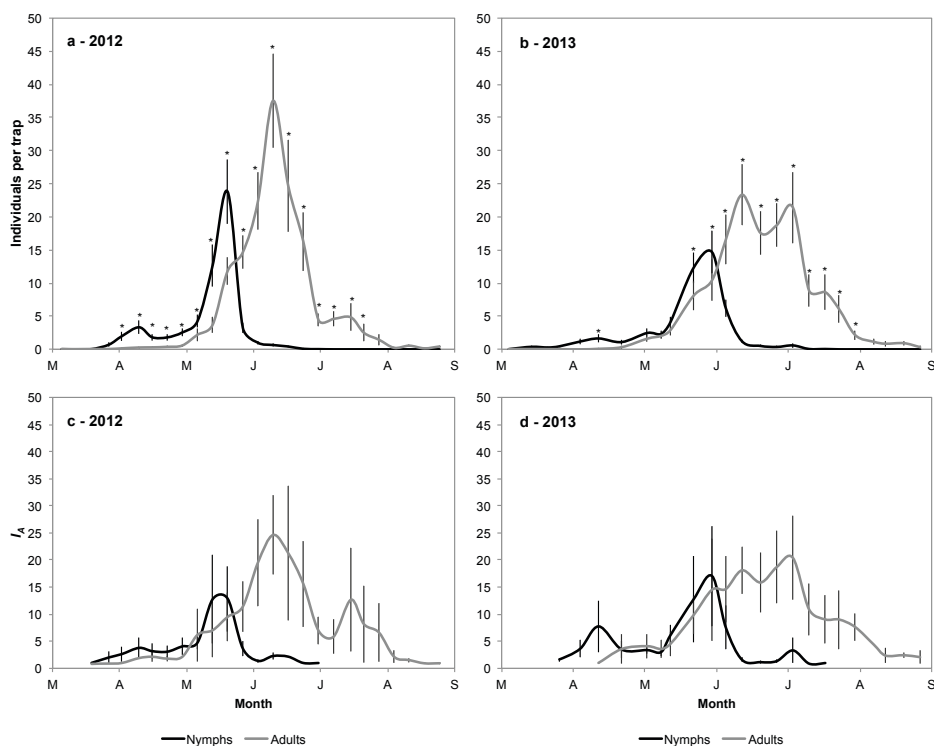


Figure III-1. European earwig densities (a-b) and aggregation index ( $I_A = s^2/\bar{x}$ ) (c-d) (mean  $\pm$  SE) of nymphs and adults in 2012 and 2013. Significant differences in densities between adults and nymphs are marked with an asterisk ( $P < 0.05$ ). A Chi-square test indicated that all  $I_A$  values were significantly higher than one ( $P < 0.05$ ).



## 3.2. Aggregation pheromone

### 3.2.1. Shelter impregnation by the aggregation pheromone

To impregnate a shelter, 10 earwigs (10i) were not enough to induce an attractive response even the day after the impregnation (w0) (Table III-1). When 20 individuals were used for this purpose, the pheromone effect was detected the day after impregnation (20i w0) and one week later (20i w1) (Table III-1). Finally, the more long-lasting effect was observed in shelters impregnated by 40 earwigs, where significant responses were recorded even 5 weeks after impregnation (40i w5) (Table III-1). There was a significant regression between the number of individuals (Y) choosing the shelter impregnated by 40 individuals and the time (x) since the shelter had been impregnated ( $Y = 7.85 - 0.23x$ ;  $F = 49.71$ ;  $df = 1,3$ ;  $P = 0.0059$ ;  $R^2 = 0.94$ ).

No significant differences ( $P > 0.05$ ) in behavior were observed between males and females (data not shown).

Table III-1. Number of European earwig individuals (i) (mean  $\pm$  SE) found inside pheromone-impregnated shelter (P) and non-impregnated shelter (C) for each treatment depending on the individuals that impregnated the shelter (10i, 20i and 40i) and the number of weeks post-impregnation that the shelter was evaluated (w0, w1, w2, w3, w5, w7 and w9).

Treatment	Individuals (mean $\pm$ SE)		df	$\chi^2$	Prob > Chisq
	P Shelter	C Shelter			
10i w0	4.8 $\pm$ 0.5	4.8 $\pm$ 0.8	1	0.00	1.000
10i w1	6.0 $\pm$ 1.1	3.8 $\pm$ 0.9	1	2.08	0.150
20i w0	7.0 $\pm$ 1.5	2.0 $\pm$ 1.1	1	11.11	0.001
20i w1	7.2 $\pm$ 1.8	2.8 $\pm$ 1.8	1	8.10	0.004
20i w2	5.8 $\pm$ 0.9	4.3 $\pm$ 0.9	1	0.90	0.343
20i w3	4.8 $\pm$ 1.9	5.2 $\pm$ 1.9	1	0.10	0.752
40i w0	8.0 $\pm$ 0.4	1.8 $\pm$ 0.5	1	16.02	< 0.001
40i w3	7.0 $\pm$ 0.7	3.0 $\pm$ 0.7	1	6.40	0.011
40i w5	6.8 $\pm$ 0.8	3.0 $\pm$ 0.6	1	5.77	0.016
40i w7	6.0 $\pm$ 1.2	3.5 $\pm$ 1.0	1	2.63	0.105
40i w9	6.0 $\pm$ 0.4	3.5 $\pm$ 0.5	1	2.63	0.105

### 3.2.2. Range of pheromone perception

The attraction of impregnated shelters was detected up to 50 cm (Table III-2). No differences between P and C shelters were observed when the distance from the EG was 100 cm (Table III-2). No significant differences ( $P > 0.05$ ) were observed between males and females (data not shown).

Table III-2. Number of European earwigs (mean  $\pm$  SE) found inside each pheromone-impregnated shelter (P) and non-impregnated shelter (C) for each distance.

Treatment	Individuals (mean $\pm$ SE)		df	$\chi^2$	Prob > Chisq
	P Shelter	C Shelter			
10 cm	7.0 $\pm$ 0.4	3.0 $\pm$ 0.4	1	6.4	0.0114
25 cm	7.0 $\pm$ 0.9	2.5 $\pm$ 1.0	1	8.52	0.0035
50 cm	7.3 $\pm$ 1.1	2.8 $\pm$ 1.1	1	8.10	0.0044
100 cm	5.8 $\pm$ 0.9	4.0 $\pm$ 0.9	1	1.26	0.2623

## 4. Discussion

### 4.1. Aggregation behavior

Earwigs in tree traps were observed from April to August, with higher densities between May and June. Similar results were observed by Romeu-Dalmau et al. (2011) under Mediterranean climates, while in colder areas of Central-Northern Europe, they tend to appear later on the season, with density peaks in June-July (Gobin et al. 2008; Helsen et al. 1998; Moerkens et al. 2009; Moerkens et al. 2011). These differences between warmer and colder areas may be explained because earwigs are highly temperature-dependent (Crumb et al. 1941; Helsen et al. 1998; Moerkens et al. 2011), and thus their phenology and behavior may differ. According to our field results, *F. auricularia* showed an aggregated behavior. Similar behavior was also observed by Sauphanor and Sureau (1993) in laboratory trials. This behavior, which brings individuals together, has also been described in other Dermaptera (Albouy and Caussanel 1990) and in insects such as *Blattella germanica* (L.) (Dictyoptera: Blattellidae) (Ishii and Kuwahara 1968), *Acheta domesticus* (L.) (Orthoptera: Gryllidae) (McFarlane et al. 1983), *Thermobia domestica* (Packard) (Tremblay and Gries 2003), *Lepisma saccharina* (L.), and *Ctenolepisma longicaudata* (Escherich) (Thysanura: Lepismatidae) (Woodbury and Gries 2007). The aggregation index of *F. auricularia* observed in field conditions were higher than those reported by Sauphanor and Sureau (1993) in laboratory trials. For instance, densities of 25 and 35 individuals per trap were

related to  $I_A$  values around 15 and 25 for nymph and adults, respectively; whereas, Sauphanor and Sureau (1993) observed  $I_A$  values around 4 for both adults and nymphs at densities of 40 individuals. Also, while in laboratory trials no differences were observed between mature and immature stages (Sauphanor and Sureau 1993), in our field conditions adults of *F. auricularia* aggregated more than nymphs. Adults spent most of their time in tree canopies, where traps were placed, whereas nymph instars spent part of their time on the ground. The aggregated behavior followed the density pattern, suggesting that the degree of aggregation is density dependent. This observation agrees with Taylor et al. (1978), who reported that in the majority of species the degree of aggregation changes with the population density.

### 4.2. Aggregation pheromone

We observed that at least 20 European earwigs were required to impregnate a shelter that elicits aggregation behavior; however, this impregnation lasted only one week. As 10 earwigs over one week were insufficient to impregnate the shelters, additional impregnations by the EG were discarded as they were only in the shelter for a maximum of 7 hours at every test. The effect of shelter impregnation by 40 individuals over one week persisted longer, and aggregation behavior was still observed after 5 weeks. Other authors reported aggregation behavior with more European earwigs but shorter impregnation times (Evans and Longép  1996; Hehar et al. 2008; Sauphanor and Sureau 1993). If we compare impregnation by surface unit ( $\text{cm}^2$ ) and day to homogenize data with these authors, in our study, 0.016 individuals/ $\text{cm}^2 \cdot \text{day}$  (20 individuals) caused impregnation lasting for 1 week while for 0.032 individuals/ $\text{cm}^2 \cdot \text{day}$  (40 individuals) the effect lasted up to 5 weeks. In contrast, Sauphanor and Sureau (1993) used 0.98 individuals/ $\text{cm}^2 \cdot \text{day}$ , Evans and Longép  (1996) 0.64 individuals/ $\text{cm}^2 \cdot \text{day}$ , and Hehar et al. (2008) 0.09 individuals/ $\text{cm}^2 \cdot \text{day}$ . Nevertheless, those authors did not evaluate the duration of the impregnation. Our results provide information about the precise number of individuals per area required to achieve lasting impregnation of shelters. The regression analysis indicated a reduction of the number of individuals within the impregnated shelter (by 40 individuals) of only 0.23 per week. The attraction lasting obtained will be enough to be effective in the field, as shelters will be re-impregnated by the earwigs attracted during the first weeks. In addition, Sauphanor and Sureau (1993) found that earwigs of others species of the genus *Forficula* (*F. pubescens* and *F. decipiens* Gen ), and *Euborellia moesta* Gen  were also attracted to shelters impregnated by *F. auricularia*. Although the beneficial or damaging effect of these species is not well known, the pheromone-impregnated shelters might be used for similar purposes than for *F. auricularia*. However, further research is needed in order to evaluate the role of these earwig species and their response to *F. auricularia*-impregnated shelters.

A minimum of 0.2 individuals/ $\text{cm}^2$  of *F. auricularia* during one week is needed to impregnate a shelter in such a way as to achieve a long-lasting effect on *F.*

*auricularia*. Shelters impregnated with the aggregation pheromone could be used to attract European earwigs to initial focus of pests that tend to highly aggregate in orchards such as woolly apple aphid (Asante et al. 1993), thus boosting biological control. Impregnated shelters may provide a useful tool for fruit production, on the one hand such shelters could contribute to improving biological control in pip fruit orchards, while on the other hand they could serve to capture and remove earwigs from stone fruit orchards. Further research is needed to determine whether using more individuals and/or during more time to impregnate shelters would increase the range of perception, as the obtained range of 50 cm may be a limiting aspect. After that, field work will be necessary to assess their practical use.



**Chapter IV. Compatibility between  
*Forficula auricularia* and  
entomopathogenic nematodes to be used  
in pome fruit pest management**



## **Abstract**

Use of predators, parasitoids and entomopathogens as biocontrol agents in pome fruit production can lead to more efficient and sustainable pest management programs. The European earwig (*Forficula auricularia* Linnaeus [Dermaptera: Forficulidae]) is a major predator of key pests in pome fruit orchards, and entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are obligate parasites of a large number of insect species. Therefore, the interaction between earwigs and EPNs can play an important role in pest management programs. Susceptibility of the European earwig to *Steinernema carpocapsae*, *Steinernema feltiae* (Steinernematidae) and *Heterorhabditis bacteriophora* (Heterorhabditidae) was evaluated. *S. carpocapsae* was the only tested EPN capable of killing the European earwig. However, the European earwig can detect the presence of *S. carpocapsae* and therefore avoid nematode-treated shelters. An earwig deterrent activity in EPN-killed codling moth larvae that reduces the foraging of European earwig on insect cadavers containing nematodes and allows nematodes to complete their life cycle was also assessed with the three species of nematodes. These findings suggest a positive compatibility between the European earwig and EPNs.

**Keywords:** Avoidance, biological control, European earwig, evasion, deterrent activity, *Steinernema carpocapsae*.

## 1. Introduction

The European earwig, *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae), is a major predator of key pome fruit orchard pests such as the woolly apple aphid *Eriosoma lanigerum* Hausmann (Hemiptera: Aphididae) (Asante 1995; Helsen et al. 2007; Mueller et al. 1988; Nicholas et al. 2005), the rosy apple aphid *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) (Brown and Mathews 2007; Dib et al. 2010), the pear psylla *Cacopsylla pyri* Linnaeus (Hemiptera: Psyllidae) (Höhn et al. 2007; Lenfant et al. 1994; Sauphanor et al. 1994) and the codling moth *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) (Glenn 1977; Jones et al. 2012; Sauphanor et al. 2012). Actions to enhance European earwig as biocontrol agent of orchard pests in several fruit species have been taken in several countries; for instance, the use of corrugated cardboard shelters has been proposed on kiwifruit (Logan et al. 2011), apple (Gobin et al. 2006) and apple and pear (Solomon et al. 1999).

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are obligate parasites of a large number of insect species (Kaya and Gaugler 1993) that have great potential as biological control agents of insect pests (Grewal et al. 2005). The codling moth, one of the most serious apple pests, has been shown to be highly susceptible in the laboratory to different isolates of steinernematid and heterorhabditid nematodes (78%-100 % mortality) (De Waal et al. 2011). In field trials nematode applications proved to be effective (mortality >50%) against diapausing codling moth larvae (De Waal et al. 2011).

As both biocontrol agents could be applied simultaneously within a pest management program, the compatibility of these agents must be ascertained before they are used together.

EPNs are usually applied in inundative biological control programs (Parkman and Smart 1996). Once applied, they can interact with non-target arthropods or even parasitize alternate hosts, and thus recycle and persist longer in the habitat (Georgis et al. 1991; Hodson et al. 2011; Kaya 1990). However, only a few negative effects of EPNs on natural enemies of pests used as biological control agents have been observed. Hymenoptera parasitoids such as Braconidae (Everard et al. 2009; Head et al. 2003; Mbata and Shapiro-Ilan 2010), Ichneumonidae (Lacey et al. 2003) and Eulophidae (Head et al. 2003; Sher et al. 2000) have been shown to be potential hosts of EPNs. Limited information is available about the susceptibility of insect predators to EPNs. Powell and Webster (2004) showed that applications of *S. carpocapsae* resulted in significant mortality of an aphid predator, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae). Hodson et al. (2011) determined the susceptibility of the European earwig *F. auricularia* to the nematode *S. carpocapsae*, suggesting that this earwig may be a potential host for this nematode. Some non-target insects and also some target insects have developed behavioral, morphological and physiological barriers to avoid nematode infection



(Sicard et al. 2004). The first step to avoid nematode infection is based on behavioral defenses such as avoidance of areas contaminated with nematodes (Ennis et al. 2010; Vincent and Bertram 2010) and grooming to eliminate nematodes attached to the insect cuticle (Gaugler et al. 1994). The next step relies on the morphology of orifices (mouth, spiracles and anus) and the structure of the cuticle which can restrict the entry of nematodes into the insect (Ishibashi and Kondo 1990). Finally, once nematodes are in the insect hemolymph, the immune system of the insect can avoid the nematode infection (Castillo et al. 2011).

An opposite interaction between EPNs and non-target insects (predators and scavengers) may occur. Predation of nematode-killed insects may interrupt the life cycle of EPNs by aborting the production of infective juveniles (Kaya et al. 1998). To avoid this negative interaction, some EPN species can be protected from being eaten during their reproduction and development in the insect cadavers by one or more chemical compounds produced by the symbiotic bacteria that deter scavengers (the scavenger deterrent factor) (Gulcu et al. 2012). This deterrent effect has been confirmed in scavengers such as ants (Baur et al. 1998; Zhou et al. 2002) and crickets and wasps (Gulcu et al. 2012) but nothing is known about this effect in the omnivorous *F. auricularia*.

Therefore, since the interaction between EPNs and predators such as earwigs can play an important role in orchard pest management programs, our general aim was to check the compatibility between the European earwig and EPNs. The specific aims of this study were (i) to evaluate the susceptibility of the European earwig to three species of EPNs (*S. feltiae*, *S. carpocapsae* and *H. bacteriophora*), (ii) to test the hypothesis that the European earwig responds to the presence of EPNs by avoiding treated shelters, and (iii) to determine whether there is a deterrent activity that reduces the foraging of the European earwig on the insect cadavers containing nematodes.

## 2. Materials and Methods

### 2.1. Earwig and nematode source

European earwigs used for the experiments were natural populations collected with cardboard traps from organic apple orchards of the fruit tree-growing area of Lleida (Catalonia, NE Spain). They were fed *ad libitum* on a semi-artificial diet (Eizaguirre and Albajes 1992) and kept in colonies at  $25 \pm 3$  °C,  $75 \pm 5\%$  RH and a 16:8 light dark cycle. According to the forceps length and body weight described by Forslund (2003), the males used in the experiments belonged to the same brachylabic morphotype.

Three Spanish native species of nematodes were used in the study: *Heterorhabditis bacteriophora* (strain F11), *Steinernema carpocapsae* (strain B14) and *Steinernema feltiae* (strain D114). Nematodes were cultured at 25 °C in last instar larvae of *Galleria melonella* (L.) (Lepidoptera: Pyralidae) according to the method

of Woodring and Kaya (1998). Infective juveniles (IJs) that emerged from cadavers were recovered using White traps (Kaya and Stock 1997) and stored in tap water at 7 °C for no longer than 2 weeks prior to the experiments. Before application, the viability of the IJs was checked by observation of movement under a stereomicroscope. All experiments were conducted twice using different batches of nematodes and insects, with an equal number of mature European earwig males and females.

### 2.2. Experiment 1 - Susceptibility

European earwigs were placed individually in 5-cm-diameter Petri dishes lined with two moistened filter paper discs and exposed to a dose of 980 IJs (50 IJs/cm<sup>2</sup>). Nematodes were applied in sterile tap water to the filter paper, then dishes were sealed with Parafilm<sup>®</sup> and kept in a climate chamber at 23 ± 2 °C in the dark. For each treatment, 10 earwigs were exposed individually. The control treatment received only sterile tap water. Death of earwigs was recorded for 17 days, every 12 h during the first 4 days and every 24 h thereafter. Twenty-four hours after death, the earwigs were dissected. Only earwigs with nematodes inside were recorded as dead due to nematodes.

### 2.3. Experiment 2 - EPN avoidance

To check the compatibility between the European earwig and EPNs, earwig avoidance of a shelter contaminated with *S. carpocapsae* (the only EPN species that was virulent in the susceptibility experiment) was evaluated. The experimental units for the tests were plastic containers (5.25 cm radius x 5 cm height) into which two different shelters and an earwig were introduced. The shelters used in these experiments were prepared by rolling a piece of 9 cm x 4 cm corrugated cardboard to obtain cylinders (4 cm height x 1.5 cm diameter). The treated shelters (N) were submerged for 5 seconds in an *S. carpocapsae* solution of 4000 IJ/ml and the control shelters (C) were submerged in water without IJs.

In each container one shelter (n 1) was introduced with one earwig and 2 g of diet. About two hours later, when the insect had entered the shelter, a second shelter (n 2) was introduced into the same container (Figure IV-1). Three different tests were carried out: C-C, in which both shelters were untreated ones, to test whether there was an exploratory behavior or fidelity to the first shelter used; N-C, in which first a shelter with nematodes was introduced and later a control shelter; and C-N, in which the first shelter was a control and the second contained nematodes. Every morning for 3 consecutive days the shelter chosen by each European earwig was recorded. For the N-C and C-N tests, each day that the earwigs were found in each shelter was counted as one time unit to calculate the proportional exposure time. On the third day, the shelters were removed and washed in water to check for live nematodes. Earwigs were kept individually in 5-cm-diameter Petri dishes lined with two moistened filter paper discs in a climate chamber at 25± 3 °C in the dark for two weeks more to test mortality due to nematodes. For each test (C-C, N-C, C-

N) 18 individuals were divided into three replications, and the experiment was conducted twice.

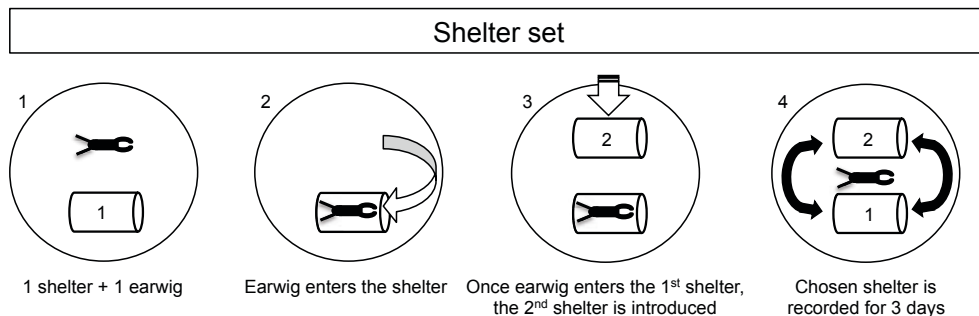


Figure IV-1. Set up of the EPN-avoidance experiment.

## 2.4. Experiment 3 - Deterrent activity

The scavenger behavior of the European earwig on insect cadavers containing EPNs was evaluated with last instar larvae of codling moth exposed to the three EPN species tested (*S. carpocapsae*, *S. feltiae* and *H. bacteriophora*) in 9-cm-diameter Petri dishes lined with moistened filter paper discs. In each dish 20 larvae were exposed to 1000 IJs, sealed with Parafilm<sup>®</sup> and kept in a climate chamber at  $25 \pm 3$  °C in the dark. To ensure bacteria development and prevent IJs from emerging from cadavers, 3-day-old cadavers were used. Freeze-killed codling moth larvae were used as a control to compare the foraging of European earwig on the insect cadavers with and without nematodes. A choice test was carried out with one nematode-killed larva and one freeze-killed larva. The two larvae were placed on a piece of  $2.5 \times 4$  cm<sup>2</sup> corrugated cardboard and offered in a 5-cm-diameter Petri dish to one earwig. Earwigs used for each treatment had been starved for 7 days. The Petri dishes were sealed with Parafilm<sup>®</sup> and kept at  $25 \pm 3$  °C,  $75 \pm 5\%$  RH and a 16:8 light dark cycle. After 48 h, predation of cadavers was visually evaluated and recorded. For each treatment (*S. carpocapsae*, *S. feltiae* and *H. bacteriophora*) 18 individuals were divided into three replications, and the experiment was conducted twice.

## 2.5. Data analysis

To evaluate earwig susceptibility, a chi-square test of independence was used to compare mortality frequencies between nematode species. To evaluate EPN avoidance, the percentage of individuals per shelter and the proportional exposure time for each individual were used. Percentage of codling moth predation by earwigs was used to evaluate the deterrent activity. All the percentages were arcsine transformed before the analysis and analyzed by one-way ANOVA. Means were compared at the  $P = 0.05$  level, and a Tukey HSD test was used to separate means. Since the experiments were all conducted twice and no significant differences were observed between them, the results are the pooled data of both,

using all the replicates of both experiments together for the statistical analysis. All data were analyzed using the JMP statistical software package (Version 9; SAS Institute Inc., Cary, North Carolina).

### 3. Results

#### 3.1. Experiment 1 - Susceptibility

Differences in virulence between nematode species were observed. *Steinernema carpocapsae* was the only nematode species that was virulent against earwigs (50% mortality) and was significantly different to the control (chi-square = 13.333, 1 d.f.,  $P = 0.0003$ ,  $N = 20$ ) (Table IV-1). We observed that 80% of the mortality due to EPNs occurs within the first 3 days after treatment, and new mortalities can also occur up to 17 days after treatment (Figure IV-2). From 17 days on, no new deaths were observed (data not shown). No significant differences were observed between males and females regarding mortality ( $P > 0.05$ , chi-square). No significant differences were observed between mortality caused by *S. feltiae* (0%), *H. bacteriophora* (5%) and the control (0%) ( $P > 0.05$ , chi-square).

Table IV-1. Mortality of the European earwig after 17 days of exposure to *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* at 50 IJs/cm<sup>2</sup>.

Treatment %	Mortality, mean $\pm$ SEM	Prob>ChiSq
Control	0	
<i>S. carpocapsae</i> (B14)	50 $\pm$ 13.7	0.0003
<i>S. feltiae</i> (D114)	0	ns
<i>H. bacteriophora</i> (F11)	5 $\pm$ 5.0	ns

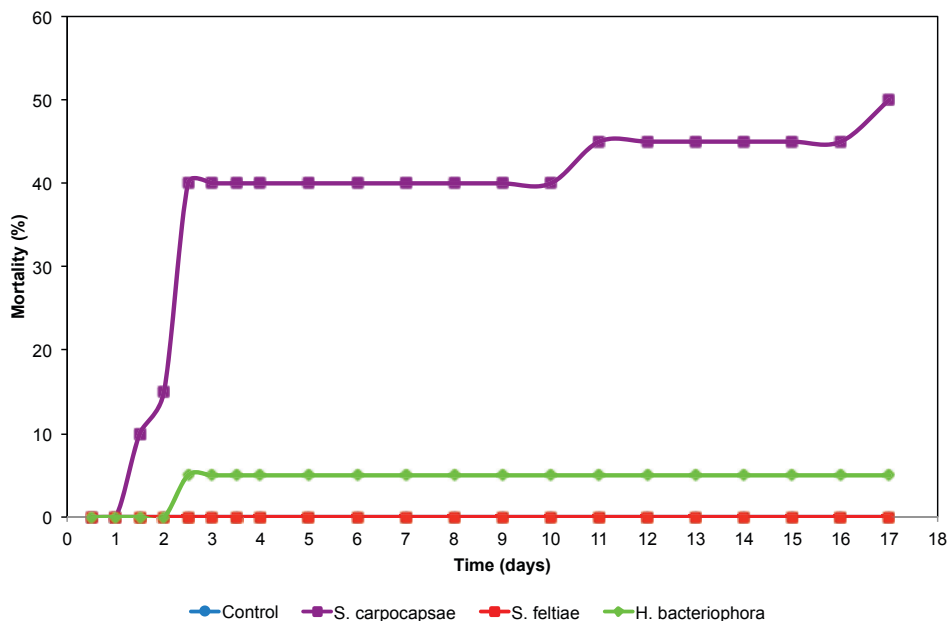


Figure IV-2. Evolution over days of European earwig mortality due to *S. carpocapsae*, *S. feltiae*, *H. bacteriophora* and control. Mean mortality (%). For the *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* treatments, the mortality due to EPNs is only represented when infective juveniles were found inside earwig individuals.

### 3.2. Experiment 2 – EPN avoidance

In the test in which two control shelters without nematodes were used (C-C test), the same percentage of earwigs was observed in both shelters on all three days (Figure IV-3A).

In the test in which the shelter with nematodes was introduced first and later the shelter without nematodes (N-C test), only on the first day after the earwigs entered the nematode-treated shelter were there significantly fewer earwigs in the nematode-treated shelter (F-value = 106.48; d.f. = 1,10;  $P < 0.0001$ , N = 36) (Figure IV-3B). In the test in which earwigs were not previously in contact with nematodes because the shelter without nematodes was introduced first and the treated shelter later (C-N test), there were significantly fewer earwigs in the nematode-treated shelters at day 1 (F-value = 20.00; d.f. = 1,10;  $P = 0.0012$ , N = 36), day 2 (F-value = 14.30; d.f. = 1,10;  $P = 0.0036$ , N = 36) and day 3 (F-value = 5.95; d.f. = 1,10;  $P = 0.0349$ , N = 34) (Figure IV-3C). No significant differences ( $P > 0.05$ , ANOVA) were observed between males and females regarding the chosen shelter. At the end of the N-C and C-N tests, live nematodes were recovered from both shelters.

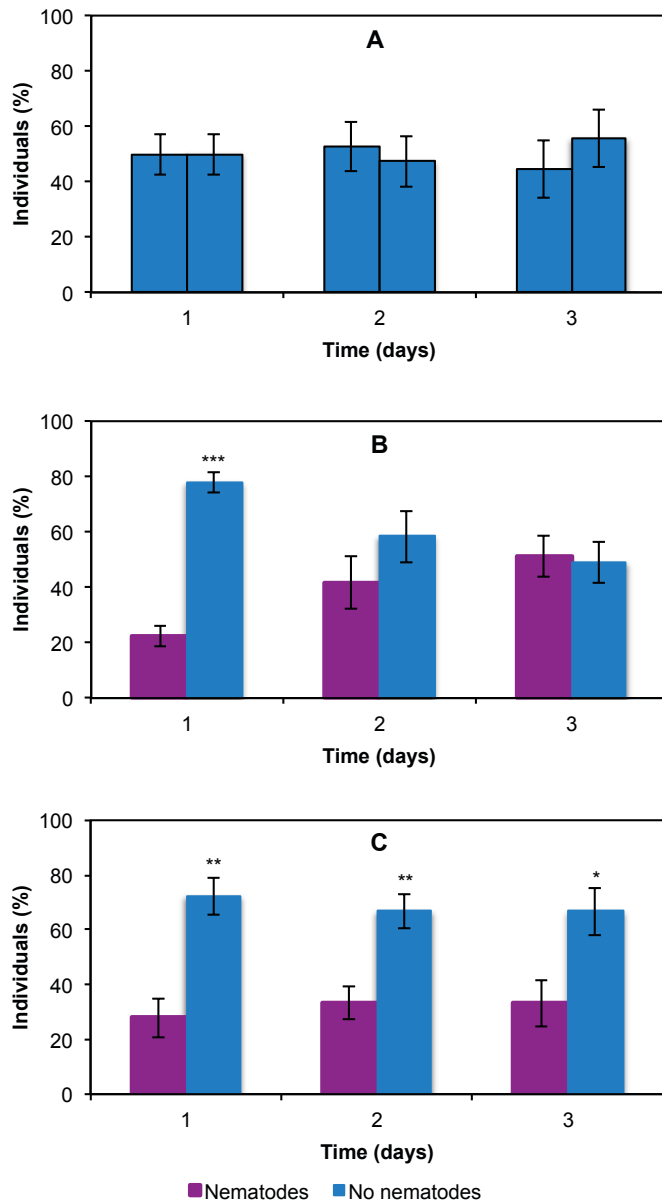


Figure IV-3. Percentage of European earwig individuals per shelter (mean and SEM) over 3 days. A (C-C): first one control shelter was introduced and later another control shelter. B (N-C): first a shelter with nematodes was introduced and later a control shelter. C (C-N): first a control shelter was introduced and later a shelter with nematodes. Significant treatment effects per day are marked with asterisks (\*\*\*) =  $P < 0.001$ , \*\* =  $P < 0.01$ , \* =  $P < 0.05$ ).

At the end of the N-C test 42% of the individuals were dead, while at the end of the C-N test only 31% were dead. No mortality was observed in the C-C test. Earwigs that survived had significantly less proportional exposure time (36%) to nematode shelters than those that died (F-value = 24.70; d.f. = 1,90;  $P < 0.0001$ , N = 46).

### 3.3. Experiment 3 - Deterrent activity

All EPN species tested were able to reduce the foraging of the European earwig on the insect cadavers containing nematodes. Between 44% and 69% of earwigs preyed on freeze-killed larvae and only between 3% and 6% on nematode-killed larvae (Figure IV-4). Differences in earwig predation on nematode-killed larvae and freeze-killed larvae were observed for *S. carpocapsae* (F-value = 53.43; d.f. = 1,10;  $P < 0.0001$ , N = 36), *S. feltiae* (F-value = 70.13; d.f. = 1,10;  $P < 0.0001$ , N = 36) and *H. bacteriophora* (F-value = 22.49; d.f. = 1,10;  $P = 0.0008$ , N = 36) (Figure IV-4). There were no significant differences ( $P > 0.05$ , ANOVA) between treatments (nematode species) or between male and female earwigs in each treatment ( $P > 0.05$ , ANOVA).

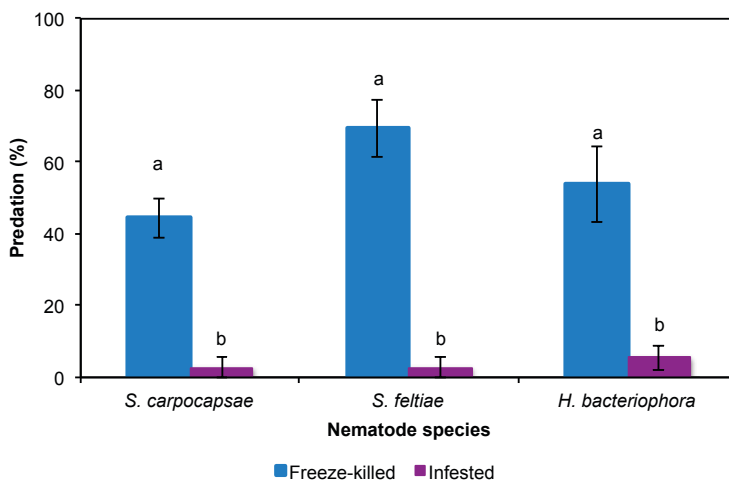


Figure IV-4. Percentage of predation by European earwig of freeze-killed or nematode-killed codling moth larvae (cadavers). Mean predation (%) and SEM. Columns marked with the same letter are not significantly different ( $P > 0.05$ ); N=36 per treatment.

## 4. Discussion

European earwig was not affected by *H. bacteriophora* and *S. feltiae* at a dose of 50 IJs/cm<sup>2</sup> under laboratory conditions. Grewal et al. (1993) also found no mortality of European earwig caused by *Steinernema scapterisci* (Nguyen and Smart) at a higher concentration (102 IJs/cm<sup>2</sup>). Georgis et al. (1991) found that at a dose of 20 IJs/cm<sup>2</sup> immature and adult stages of the earwig *Labidura riparia* Pallas

were refractory to *H. bacteriophora* and *S. carpocapsae* infection, and no mortality was observed 4 days after treatment. On the other hand, our results showed that in a filter paper assay, 50 IJs/cm<sup>2</sup> of *S. carpocapsae* kills up to 50% of the European earwig population, either males or females, under laboratory conditions. This mortality is lower than that reported by Hodson et al. (2011), which at 25 IJs/cm<sup>2</sup> was around 42.7% after 24 h of exposure and 84.3% after 48 h. However, the higher susceptibility reported by Hodson et al. (2011) was observed for females and macrolabic males, whereas for brachylabic males it was significantly lower (60%) and more similar to the susceptibility observed in our experiment. These differences may be due to different pathogenicity among EPNs strains or to the more favorable conditions for EPNs provided by the sand in their experiments instead of the filter paper in ours: providing greater moisture and allowing the earwigs to dwell in the sand, thus favoring the contact with the nematodes. In addition, earwig mortality due to EPNs tends to occur within the first 3 days after the application, as shown by Hodson et al. (2011) and thereafter it is difficult to get more infestations.

Regarding the selection of shelters, our data showed an exploratory behavior of the earwigs as a similar number of insects chose one or the other when two shelters without nematodes were offered. However, when the earwigs entered first a nematode-treated shelter and were later offered a shelter without nematodes they preferred to shelter within the untreated one than the treated one. The nuisances caused by nematodes in treated shelters makes earwigs abandon them, but this behavior is only observed on the first day after the introduction of the clean shelter. After that, there were no differences between treatments, probably because individuals carried nematodes on their bodies and did not feel comfortable in either of the shelters, which both contained nematodes at the end. The nuisance that nematodes can cause to insects has been reported by Gaugler et al. (1994). These authors showed how insect's grooming behavior might be used to eliminate nematodes attached to the insect cuticle. Grooming behavior has been observed in earwigs when they are in contact with nematodes (Hodson et al. 2011) and in some other insects such as the ants *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Drees et al. 1992), the termites *Zootermopsis angusticollis* Hagen, *Coptotermes formosanus* Shiraki and *Coptotermes vastator* Light (Isoptera: Rhinotermitidae) (Mankowski et al. 2005; Wilson-Rich et al. 2007) and the pine weevil *Hylobius abietis* Linnaeus (Coleoptera: Curculionidae) (Ennis et al. 2010).

Grooming and avoidance of contaminated areas are the most common behavioral defenses employed by animals against parasites (Ennis et al. 2010). In the present study we also detected avoidance of nematode-treated shelters by earwigs. When European earwigs were inside an untreated shelter and were offered a nematode-treated shelter, they explored the treated shelter but it seemed as if they detected the presence of nematodes and avoided them. Since the earwigs that died in this experiment had spent more time within nematode-treated shelters than those that survived, this finding supports the hypothesis that earwig avoidance of shelters



with presence of nematodes reduced their risk of mortality. This type of nematode avoidance has also been observed by other authors in insects such as the cockroach *Blattella germanica* Linnaeus (Blattodea: Blattellidae) (Appel et al. 1993), the Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae) (Gaugler et al. 1994; Schroeder et al. 1993) and the pine weevil (Ennis et al. 2010).

Although earwigs are major predators of insects, Baur et al. (1998) mention them as scavengers. The significant preference of earwigs to predate freeze-killed insects instead of nematode-killed insects observed in our study confirms the presence of an earwig deterrent activity produced by the nematodes that reduces the foraging of the European earwig on insect cadavers with EPNs. This deterrent effect has previously been reported as a “scavenger deterrent factor” in other insects such as the ants *Linepithema humile* Mayr and *Lepisiota frauenfeldi* Mayr (Hymenoptera: Formicidae) (Baur et al. 1998; Gulcu et al. 2012), the beetles *Pterostichus melanarius* Illiger (Coleoptera: Carabidae) (Foltan and Puza 2009), the crickets *Gryllus bimaculatus* DeGeer (Orthoptera: Gryllidae), the vespid wasps *Vespa orientalis* Linnaeus and *Paravespula* sp. (Hymenoptera: Vespidae) and the calliphorid flies *Chrysomya albiceps* Wiedemann (Diptera: Calliphoridae) (Gulcu et al. 2012). Baur et al. (1998) attributed the production of this scavenger deterrent factor to the symbiotic bacteria of the EPNs. Gulcu et al. (2012) suggested that the bacteria *Xenorhabdus bovienii* (associated with *S. feltiae*) produced a concentration of scavenger deterrent factor that was different to or higher than produced by than *X. nematophila* (associated with *S. carpocapsae*). Furthermore, Baur et al. (1998) reported that ants scavenged significantly more steinernematid-killed insects (60%-85%) than heterorhabditid-killed insects (10%-20%), suggesting that *Photorhabdus luminescens* (associated with *H. bacteriophora*) has a greater deterrent activity than *Xenorhabdus* species. In the present study we observed no significant differences in the scavenger behavior of earwigs between steinernematid-killed insects and heterorhabditid-killed insects, probably because European earwigs are not specialist scavengers: after starving for 7 days more than 30% of the earwigs did not attack even the freeze-killed larvae.

We can conclude that the use of *S. feltiae* and *H. bacteriophora* for pest control does not pose a threat to the European earwig population. Although *S. carpocapsae* can kill the European earwig under laboratory conditions, under field conditions the European earwig seemed to detect the presence of *S. carpocapsae*, differentiating between areas with and without nematodes, as has been shown in the experiment of EPN avoidance. This avoidance behavior can be beneficial to earwigs, reducing risk of the lethal effect that EPNs may have on them. Hodson et al. (2012) after the treatment with *S. carpocapsae*, observed a small reduction in the catches of European earwig in pistachio orchards and attributed this reduction to lethal effects. According to our results, this could also be due to avoidance of the treated area. On the other hand, earwigs will not interrupt the EPN cycle due to the presence of a deterrent activity of nematode-killed cadavers that has been observed in mature earwigs for the first time in this study.

## Compatibility between European earwig & EPNs

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Although these experiments were conducted on mature stages, Hodson et al. (2011) reported that the body size significantly increases the mortality by EPNs in earwigs, so we would expect nymph stages to be less susceptible than mature ones. The interaction under field conditions should be checked, but our data provide evidence that using EPNs to control codling moth can be compatible with promoting the predator *F. auricularia* in pome fruit orchards.



**Chapter V. Woolly apple aphid *Eriosoma lanigerum* Hausmann ecology and its relationship with climatic variables and natural enemies in the Mediterranean areas**



## **Abstract**

A multi-lateral approach that includes both biotic and climatic data was developed to detect the main variables that affect the ecology and population dynamics of woolly apple aphid *Eriosoma lanigerum* (Hausmann) (WAA). Crawlers migrated up and down the trunk from spring to autumn and horizontal migration through the canopy was observed from May to August. Winter temperatures did not kill the canopy colonies, and both canopy and root colonies are the source of reinfestations in Mediterranean areas. Thus, control measures should simultaneously address roots and canopy. European earwigs *Forficula auricularia* (Linnaeus) were found to reduce the survival of overwintering canopy colonies up to June, allowing their later control by the parasitoid *Aphelinus mali* (Haldeman) from summer to fall. Preliminary models to predict canopy infestations were developed.

**Keywords:** *Aphelinus mali*, crawler, European earwig, *Forficula auricularia*, multivariate analysis, winter survival.

## 1. Introduction

Woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), is a worldwide pest of apple *Malus domestica* (Borkhausen). It is native of North America, where the American elm *Ulmus americana* (Linnaeus) (Urticales: Ulmaceae) is the primary host and apple the secondary one; in the absence of the primary host it develops on apple throughout the year.

The biology of WAA has been widely studied in the United States (Beers et al. 2007; Beers et al. 2010; Brown and Schmitt 1994; Hoyt and Madsen 1960; Walker 1985; Walker et al. 1988), New Zealand (Alspach and Bus 1999; Sandanayaka and Bus 2005), Australia (Asante et al. 1993; Asante 1994; Asante 1999) and South Africa (Damavandian and Pringle 2007; Heunis and Pringle 2006; Pringle and Heunis 2001; Pringle and Heunis 2008). However, little information is available in Europe (Evenhuis 1958; Theobald 1921), especially in Mediterranean areas.

This aphid colonizes roots and sites on the trunk and branches that have been previously injured, but can also colonize undamaged current year shoots (Asante et al. 1993; Asante 1994; Beers et al. 2010; Brown et al. 1991; Childs 1929; Pringle and Heunis 2001; Weber and Brown 1988). WAA is distributed irregularly across the orchard, gathering on given trees or along isolated rows (Asante et al. 1993). The principal dispersion method between trees involves first instar nymphs (crawlers), which are transported by orchard management practices, migration or wind (Nel 1983; Schoene and Underhill 1935; Walker 1985).

Several studies have linked canopy infestations with the upward movement of crawlers from the roots, suggesting that the root colonies are the constant source of canopy infestations (Heunis and Pringle 2006; Nel 1983; Theobald 1921). This can be especially important in areas where canopy colonies are highly affected by low winter temperatures (Walker 1985), but the role that these cold temperatures may have on canopy colonies in Mediterranean areas has not been checked.

The increase in WAA outbreaks appears to be associated with changes in pesticide programs and the disruption of biological control (Gontijo et al. 2012). Information on the efficacy of WAA parasitoid *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) to control arboreal populations is contradictory. Therefore, while in warmer regions, such as Brazil, no chemical control is necessary due to high parasitism rates (Monteiro et al. 2004), under cool climatic conditions *A. mali* is not effective in preventing economic damage (Asante and Danthanarayana 1992; Heunis and Pringle 2006). Predators such as ladybird beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), hoverflies (Diptera: Syrphidae), earwigs (Dermaptera: Forficulidae) and spiders (Araneae) are reported to be WAA predators; of these, earwigs are cited as the most important (Asante 1995; Asante 1997; Gontijo et al. 2012; Mueller et al. 1988; Nicholas et al. 2005; Short and Bergh 2004). However, few data are available on the efficacy of earwigs to control WAA in the Mediterranean area.

Aims of this study were to know the ecology of WAA in Mediterranean areas, the winter survival of the canopy colonies and the role that natural enemies may play in such areas. Climatic conditions are important to explain arthropod development rates and activity, but very little is known about WAA population dynamics as affected by climatic variables. Therefore, in order to improve WAA control, a multi-lateral approach that includes both biotic and climatic data was developed to detect the main variables that affect their ecology and population dynamics.

## 2. Materials and Methods

### 2.1. Study orchards

Trials were performed in three apple orchards located in Catalonia (NE Spain): les Borges Blanques (BB) (41°30'23.06''N; 0°51'05.93''E), Mollerussa (MO) (41°36'51.13''N; 0°52'22.75''E), and Ivars d'Urgell (IU) (41°41'06.19''N; 0°58'06.09''E). The climate is semi-arid Mediterranean, with a mean annual rainfall of 350 mm. All the orchards had major infestations of WAA and were under organic management. The orchards were treated with pesticides as follows: Azadiracthin, maximum twice a year around the end of March-April to control rosy apple aphid (*Dysaphis plantaginea* (Passerini), Hemiptera: Aphididae), before WAA aerial infestations initiate their development; granulosis virus in April and May against codling moth (*Cydia pomonella* Linnaeus, Lepidoptera: Tortricidae); and lime sulphur from April to May to control apple scab (*Venturia inaequalis* Cooke). In addition, to control codling moth, Spinosad was applied twice to IU in June and July 2012.

BB was an experimental orchard of 'Fuji Kiku 8' apple grafted onto M9, planted in 2003, and trained to a central leader with a spacing of 4 x 1.4 m. MO was a commercial orchard of 'Golden Smoothee' apple grafted onto M9, planted in 1985, and trained to a double-axis system with a spacing of 4 x 1.2 m. IU was a commercial orchard of 'Golden Smoothee' apple grafted onto M9, planted in 1993, and trained to a central leader with a spacing of 4 x 1.1 m. BB and MO were drip-irrigated, whereas IU was flood-irrigated.

Hourly climatic variables such as maximum temperature (T<sub>max</sub>, °C), minimum temperature (T<sub>min</sub>, °C), number of hours above or below several temperature thresholds (h>20 °C, h> 25 °C, h<10 °C and h<7 °C), minimum relative humidity (rh min%), solar radiation (Sun, W/m<sup>2</sup>), rainfall (Rain, mm), and wind speed (Wind, m/s), were obtained from the closest automatic weather station of the Meteorological Service of Catalonia (*Meteocat, Departament de Territori i Sostenibilitat, Generalitat de Catalunya*). For BB, data were obtained from the Castellidans station 8.5 km away, for IU from the Castellnou de Seana station 3 km away and for MO from the Mollerussa station 0.5 km away.

## 2.2. Crawler movement

To assess crawler movement from root and aerial colonies, 50 trees with WAA infestations were selected in each orchard. BB was sampled for three years (2010-2012), while MO and IU were sampled for two (2011-2012).

Upward (from root colonies) and downward (from aerial colonies) crawler movement was evaluated weekly in 20 trees over the whole year. Of these trees, 10 were consistently included in the evaluation while the other 10 rotated every week, being repeated every four weeks in order to minimize interference with WAA phenology.

For each tree, two 2.5-cm-wide adhesive tapes (Tesa Tape S.A.; Argenton, Spain) placed 3 cm apart were wrapped around the trunk above the graft union. A thin bead (1.5-cm-wide) of insect trapping medium (Tree Tanglefoot; the Tanglefoot Company, Grand Rapids, MI) was centered along each tape. Aphids moving up from the root colonies were trapped on the lower tape while those moving down from the canopy were trapped on the higher one. Tapes were replaced weekly throughout the year, and WAA number on each tape was visually estimated by a qualitative index of six categories. This index was developed through a geometrical scale ( $a_n = a \cdot r^{n-1}$ ) where  $r=3$ ,  $a=4$  and  $n$  is from 2 to 7 (Table V-1). The use of this scale allowed us to adopt the same index category regardless of trunk diameter. For data analysis, categories were transformed to the mean aphid number of each interval (Table V-1).

Table V-1. Interval and mean number of aphids for each category according to the qualitative index.

Category	Number of aphids	Mean
1	0-12	6
2	13-36	25
3	37-108	73
4	109-324	217
5	325-972	649
6	973-2916	1945

In addition, the numbers of *A. mali* and the most abundant predators, such as spiders, earwigs, and velvet mites (Trombidiformes: Trombidiidae), trapped on each tape were recorded as an indicator of presence. Given that earwigs are considered the most important predator of WAA and we were unsure whether the tapes would trap them, their number was also assessed by means of shelters. For this purpose, we set up 10 earwig shelters on the second scaffold limb of 10 different trees randomly selected within the infested ones in each orchard. The shelters were prepared by rolling a piece of corrugated cardboard into a cylinder (12 cm height x 9 cm diameter), which was protected from rain and adverse conditions by a PVC tube (15 cm height x 9.5 cm diameter). Similar traps have been used in studies of European earwigs elsewhere (Burnip et al. 2002; Gobin et al. 2006; He et al. 2008; Helsen et al. 1998; Logan et al. 2007; Moerkens et al. 2009; Phillips 1981; Solomon et al. 1999). Every week throughout the year, we



counted the number of earwigs per shelter. After counts, the insects were released at the base of the assessed tree.

Horizontal movement between trees through the canopy (C) was assessed fortnightly from May to December 2012. In each orchard, 10 of the trees used to assess the crawler movement were included. Five of these were permanently taped while the other five were those taped every four weeks. One glue tape (described above) per tree was wrapped around the base of a random branch that was in contact with branches of a neighbor tree. The tapes were removed one week later and aphids were individually counted under a stereomicroscope.

### 2.3. WAA aerial infestation and parasitism

This study was carried out from May through December for two years (2011-2012). To assess the canopy infestation, 20 trees per orchard were used. Ten trees with permanent trunk tapes used to evaluate crawler movement (section above) were included, together with another 10 that were WAA-infested and had never been trunk-taped. For each tree, five shoots were randomly selected. Every two weeks, the total length of the shoot and the length occupied by WAA were measured to calculate the percentage of the aerial infestation (AI). The percentage of infested shoots (IS) was evaluated at the same time. Also, the percentage of the length of each colony parasitized by *A. mali* (parasitism) was assessed visually using a qualitative scale (<10%, 10-50%, 51-90% and >90%). The mean value for each category was used to represent and analyze parasitism. The same 20 WAA-infested trees monitored in each orchard were used during the two years of evaluation.

### 2.4. Winter survival of WAA aerial colonies

This study was carried out in the BB orchard in 2012. At the beginning of February, the coldest month in our area, 75 shoots that had had similar levels of WAA infestations the previous summer were selected. Of these, 25 were covered with a cloth bag to exclude natural enemies and WAA recolonization, 25 were glue-taped (trapping medium) at the base to prevent WAA recolonization, and the other 25 were used as controls. The glue was checked regularly to ensure its effectiveness. At the end of June, when aerial colonies reach their maximum development, AI was evaluated. The air temperature inside and outside the cloth bag was recorded by data loggers (Testo 177-T4; Testo AG; Lenzkirch, Germany) over three weeks in February. For this purpose, five control shoots and five shoots covered by a cloth bag were randomly selected, and a temperature sensor was placed on each one.

### 2.5. Data analysis

The annual cumulative number of aphids captured moving up and down was analyzed per year within orchards by one-way ANOVA; data were log-

transformed and ANOVA assumptions (normality and homoscedasticity) were confirmed before analysis. Tukey HSD tests were used to compare means. The number of aphids captured on trees that were permanently taped and trees that were included in the evaluation every four weeks was log-transformed and analyzed by a non-parametric Wilcoxon test. To evaluate AI at the end of the winter survival trial, data were tested for significance by a non-parametric Kruskal-Wallis test, and the Steel-Dwass method was used to separate treatments. These nonparametric tests were used because the ANOVA assumptions were violated. Temperature inside and outside the shoot bags was analyzed by one-way ANOVA. Data were analyzed using the JMP statistical software package (Version 9; SAS Institute Inc., Cary, North Carolina).

Multivariate projection methods were applied to simultaneously analyze biotic and abiotic variables. For this purpose, we used the following variables for each orchard and year: the weekly number of aphids captured on the bands (Up, Down and C), the accumulated number of aphids captured each week (Up ac and Down ac), the AI, IS, the mean values of the classes of parasitism, the number of earwigs and *A. mali* individuals captured on the bands (EarwC and MaliC, respectively), and the number of earwigs present in shelters (EarwP). For every week that crawler movement and AI were evaluated, a mean value of each climatic variable was calculated, with the exception of rainfall, for which accumulated rain was used. All the variables were analyzed in the same matrix.

We performed a PCA and a regression model by PLS for one-dependent variable (PLS-1) and two dependent variables (PLS-2). Regression procedures by means of PLS-1 methods were carried out to predict the Up and Up ac variables, whereas the AI and IS variables were studied together by means of a PLS-2 technique. According to their contribution to explain the overall variance in the PCA and to the easiness to evaluate them, the X-variables used to construct the PLS-1 were: MaliC, Parasitism, Tmax, Tmin, Wind, Sun, Up, EarwP, rh min%, Rain,  $h < 7\text{ }^{\circ}\text{C}$  and  $h < 10\text{ }^{\circ}\text{C}$ . To construct the PLS-2, the X-variables used were: AI, IS, Parasitism, Up ac, Tmax, Tmin, Wind, Sun, EarwP, rh min%,  $h < 7\text{ }^{\circ}\text{C}$  and  $h < 10\text{ }^{\circ}\text{C}$ . Before analysis, all the data were centered and standardized by dividing each variable by its standard deviation. Both the PCA and PLS models were validated using the full cross-validation method. All these multivariate models were performed using The Unscrambler software (Version 7.6; Camo Process AS, Oslo, Norway).

### 3. Results and Discussion

#### 3.1. WAA ecology in Mediterranean areas

For all the orchards and years, no differences were observed between trees that were taped every four weeks and those taped continuously (data not shown). Therefore, data were pooled for the analysis.

Crawler movement was recorded almost year-round in all the orchards, although with very low numbers of crawlers catches from fall to early spring (Figure V-1). Peak captures were observed from May to June, and in some years and orchards there seemed to be 2 annual peaks (Figure V-1), probably due to fluctuation of the maximum temperatures in summer. These relations are addressed more in detail in the multi-lateral approach analysis. The up:down ratio of accumulated crawlers was highly variable even in the same orchard (Figure V-1 and Table V-2). We observed ratios from 1:1 (IU both years) to 11:1 (BB 2012) (Figure V-1 and Table V-2).

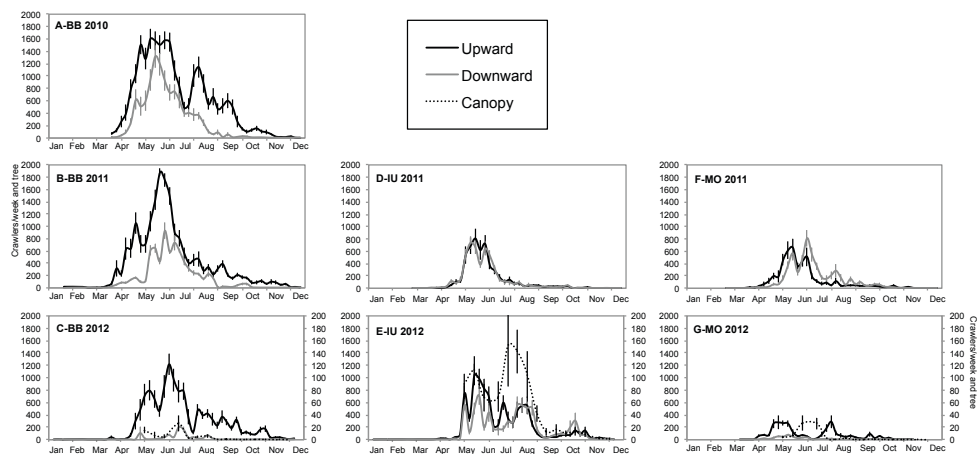


Figure V-1. Number of woolly apple aphid crawlers captured per tree per week (mean  $\pm$  SEM) throughout the year. Note that crawlers through the canopy are referred to the secondary axis and are present only in 2012.

Although it is difficult to extrapolate the results of three orchards to the whole area, some common aspects can be highlighted. For example, the captures on the trunk tapes, which show the pattern of upward and downward crawler movement occurred consistently from mid-April to November with a plateau around May-June, while the movement across the canopy was higher from May to August (Figure V-1). The maximum number of aphids captured per tree over one week (1,800 upward captures) occurred in BB in 2011 (Figure V-1B). Analogous results, using similar sampling methods, were found by Beers et al. (2010) in Washington, where crawler movement started in May but diminished considerably after July, and the migration pattern resembled a peak rather than a plateau, with a maximum of 1,500 upward crawlers per tree per week. In California, Hoyt and Madsen (1960) observed year-round crawler movement and, despite increasing in May and June, the highest level was observed in July and August, declining from September onwards. A year-round migration pattern with peaks in late spring and from late summer to autumn was also reported by Asante (1994) in Australia and by Heunis and Pringle (2006) in South Africa, with the greatest movement occurring from October to December (equivalent to April-June in the Northern hemisphere).

## WAA ecology & relationship with climatic variables & NE

Table V-2. Number (Mean  $\pm$  SEM) of annual cumulative woolly apple aphid crawlers per orchard on the lower (Up) and upper (Down) bands. Column values followed by different letters or asterisk indicate significant differences within orchards, as determined by the Tukey HSD test ( $P < 0.05$ ).

Orchard/year	Up accumulated	Down accumulated
<b>BB</b>		
2010	23,684 $\pm$ 2,257a	10,885 $\pm$ 1,417a
2011	18,867 $\pm$ 1,055a	7,380 $\pm$ 553a
2012	12,646 $\pm$ 1,094b	1,097 $\pm$ 146b
d.f.	2,57	2,57
F value	7.63	51.33
Prob>F	0.0012	<0.0001
<b>IU</b>		
2011	5,375 $\pm$ 910	5,145 $\pm$ 795
2012	9,377 $\pm$ 1,433*	7,228 $\pm$ 1,492
d.f.	1,38	1,38
F value	7.88	1.12
Prob>F	0.0078	ns
<b>MO</b>		
2011	4,897 $\pm$ 704*	5,504 $\pm$ 807*
2012	2,814 $\pm$ 373	656 $\pm$ 44
d.f.	1,38	1,38
F value	5.72	126.25
Prob>F	0.0218	<0.0001

Regarding the captures of crawlers moving through the canopy, the highest captures were in June, coinciding with the peak of downward movement, and immediately after the peak of upward movement was recorded (Figure V-1C). The captures of aphids moving through the canopy seemed to follow the same pattern as the captures of the downward crawlers (Figure V-1). Asante et al. (1993) observed that at low infestations the aphid is confined to the trunk and large branches, but disperses to establish colonies on twigs or new lateral growths during peak populations. Taking into account only the movement of crawlers, we cannot find a consistent relationship between canopy and root colonies. The same observation was made by Beers et al. (2010) in Washington. Therefore, to detect the main driving variables that explain the dynamics of WAA, a multi-lateral approach that includes both biotic and climatic data would be more appropriate than trying to separate the contribution of each individual factor.

### 3.2. Winter survival and role of natural enemies

In our study, low winter temperatures did not kill aerial colonies of WAA. High AI rates were observed on shoots on which recolonization by crawlers and access of natural enemies were prevented by cloth bags (Table V-3). Shoot temperature was only 0.7 °C higher in bag-covered shoots than in control ones ( $F = 23.8011$ ; d.f. = 1,10606;  $P < 0.0001$ ), and as no differences in AI were observed between shoots without bags (glue and control) and those with bags containing earwigs, bag protection against cold was discarded. Therefore, the effect of subterranean WAA populations on AI is expected to be less significant than in areas where aerial colonies are killed or reduced, for instance in central Washington, where Walker

(1985) observed high mortality in winter; or in South Africa, where Heunis and Pringle (2006) stated that aerial infestations originate every year from the roots.

Table V-3. AI (percentage of shoot length occupied by woolly apple aphid, mean ± SEM) at the end of June 2012 for the BB orchard in the winter survival trial. Values followed by different letters indicate significant differences, as determined by the Kruskal-Wallis test and Steel-Dwass method ( $P < 0.05$ ).

Treatment	Aerial infestation (%)
Bag (N=9)	59.2 ± 8.5a
Bag with earwigs (N=16)	10.0 ± 4.0b
Glue (N=25)	5.9 ± 2.0b
Control (N=25)	2.7 ± 0.8b
d.f.	3
Chi-Square	25.89
Prob>ChiSq	<0.0001

The less isolated the shoots, the less AI was found. This observation could be attributed to the difficulty encountered by predators to reach them. Earwigs had entered some of the bags used to assess winter survival (16 of the initial 25) through small holes, probably made by the insects themselves. AI was close to 60% on bag-isolated shoots (the remaining nine) and reached only 10% on shoots with earwigs (Table V-3). The glue at the base of some shoots prevented crawler recolonization, but it was not enough to impede the movement of earwigs. Thus, earwig exclusion on shoots with glue was also discarded. This makes earwigs good candidates as natural enemies of WAA, and the temporal coincidence with the maximum crawler movement (Table V-4) reinforces this observation. The capacity of earwigs to control WAA populations (Helsen et al. 2007; Mueller et al. 1988; Nicholas et al. 2005; Stap et al. 1987), and their promotion through the use of additional shelters in orchards (Gobin et al. 2006; Logan et al. 2011; Solomon et al. 1999) has been reported. Moreover, Noppert (1987) and Philips (1981) estimated a minimum of seven earwigs per tree to control WAA in apple orchards.

Table V-4. Crawlers (up, down and canopy), *A. mali* and predators (earwigs, spiders and velvet mites) trapped on the glue tapes and the AI (mean monthly percentage of the total year data from all the orchards in 2011-2012). Higher presence is shown by darker cells. Note that parasitism is represented by the mean recorded parasitism (%) for each month of all the years and of all three orchards.

	J	F	M	A	M	J	J	A	S	O	N	D
<b>Up</b>	0	0	1	4	27	32	13	11	5	4	2	1
<b>Down</b>	0	0	1	3	21	34	16	16	3	5	1	0
<b>Canopy</b>	Not evaluated				24	10	29	30	3	3	1	0
<b>AI</b>	Not evaluated				6	19	20	22	12	6	10	5
<b>Parasitism</b>	Not evaluated				5	7	49	58	74	73	86	81
<b><i>A. mali</i></b>	0	0	12	5	2	9	16	20	9	18	8	1
<b>Earwigs</b>	1	0	2	9	34	31	11	2	1	1	3	5
<b>Spiders</b>	4	3	9	7	19	12	12	7	4	8	6	9
<b>Velvet mites</b>	1	3	10	6	1	1	10	16	11	24	12	5

< 5%

5% - < 25%

> 25%

Individuals of the WAA parasitoid *A. mali* were detected on the tapes from March to December, but parasitism on the canopy was recorded mainly from July to December (Table V-4 and Figure V-2). It was observed that when AI was less than 10% in May, no outbreaks occurred later, and that when AI was higher early in the season the parasitism reached 80% already in June but did not maintain infestation under low levels (Figure V-2). These findings reinforce the importance of promoting earwigs early in the season to maintain low levels of AI until the levels of parasitism by *A. mali* takes over from summer onwards.

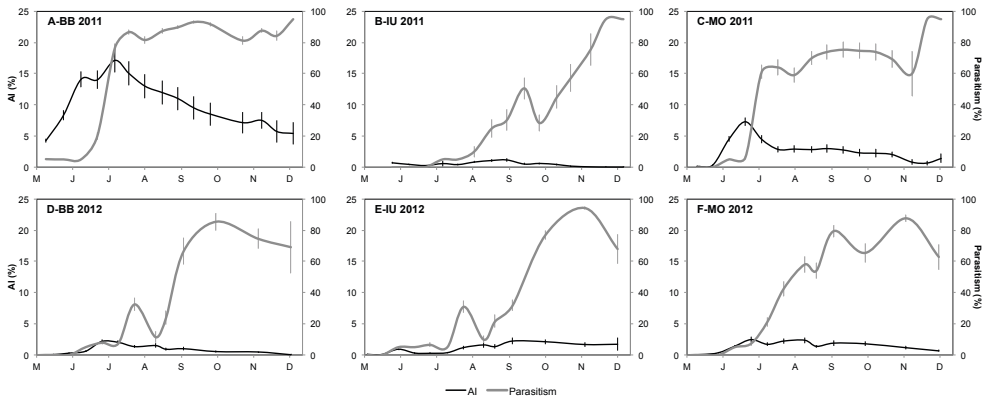


Figure V-2. Woolly apple aphid aerial infestation (AI) and parasitism (mean  $\pm$  SEM) for each orchard and year.

Other WAA predators as spiders and velvet mites were trapped from March to December (Table V-4), and due to this extended appearance they could be considered candidates as predators of crawlers. While few data can be found regarding velvet mites and predation on aphids (Helyer et al. 2003; Marko et al. 2008; Sundic and Pajovic 2012), several authors have proposed spiders for biological control purposes (Harwood et al. 2004; Sunderland and Samu 2000; Symondson et al. 2002; Thorbek et al. 2004). Using diagnostic polymerase chain reaction, Boreau de Roince et al. (2013) observed the importance of spiders in the early control of green apple aphid (*Aphis pomi* Linnaeus, Hemiptera: Aphididae) and rosy apple aphid in orchards. Furthermore, Wyss et al. (1995) in Switzerland, reported a significant reduction of rosy apple aphid density when weed strips were sown to provide food and refuge to spiders. As proposed by Nicholas (2005) and Gontijo (2011), the biological control of WAA can be achieved in orchards where natural enemies are not disrupted.

### 3.3. A multi-lateral approach to the role of biotic and climatic data on the ecology of WAA

Data from April to September, the period during which WAA population dynamics mainly occurred, were used to construct a PCA. Although the complexity of the data determined nine principal components (PC) to explain 90% of the variance,

the first two PCs were able to explained 57% (39% PC1, 18% PC2) of the overall variance (Figure V-3). The most important variables for the definition of the first PC were minimum and maximum temperatures ( $T_{min}$ ,  $T_{max}$ ) and the number of hours above or below several temperature thresholds ( $h < 10^{\circ}\text{C}$ ,  $h < 7^{\circ}\text{C}$ ,  $h > 20^{\circ}\text{C}$ ,  $h > 25^{\circ}\text{C}$ ) (Figure V-3), suggesting that these climatic variables may have an important contribution to the WAA ecology. By the use of the diagram of scores, we observed that the variables defined in the direction of maximum information of the data (first PC) were clearly related to the week number of the year (data not shown). The second PC was determined by weekly crawler movement, such as that through the canopy (C), and upward (Up) and downward (Down) displacement, and by the presence of earwigs either on the glue tapes (EarwC) or in the shelters (EarwP) (Figure V-3). Therefore, as seen before, earwigs may really have a role in the number of crawlers moving up, down and through the canopy. The percentage of infested shoots (IS) and the percentage of the shoot length occupied by WAA (AI) were highly correlated, and both variables were important in the definition of the first and the second PCs (Figure V-3). Therefore, as both variables are highly correlated, IS might be used instead of AI to evaluate the level of WAA infestation, as it was much easier to obtain.

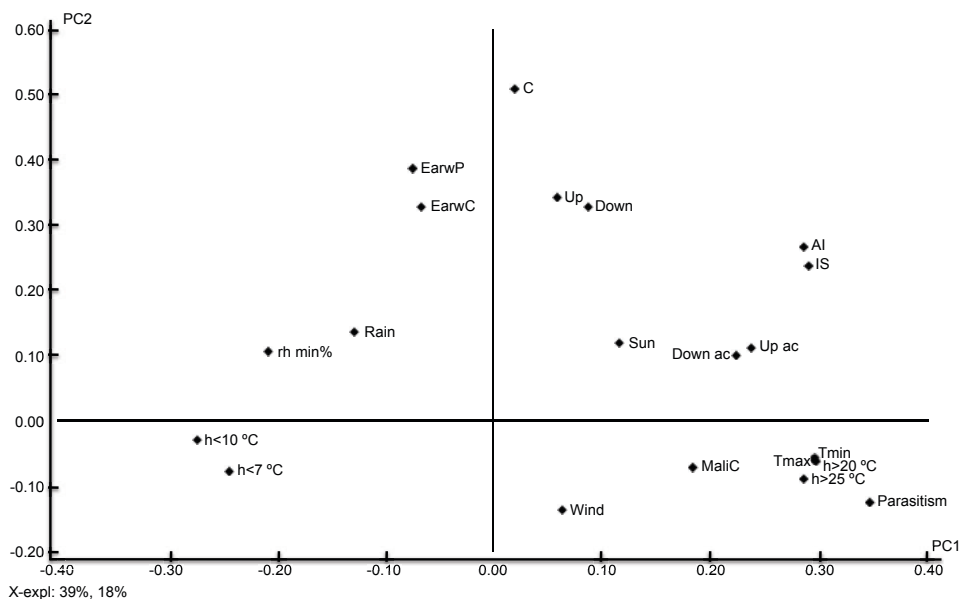


Figure V-3. Variable loadings represented in the plane defined by the first two principal components. Variables are: the weekly number of aphids captured on the bands (Up, Down and Canopy (C)), the accumulated number of aphids captured each week (Up ac and Down ac), the percentage of aerial infestation (AI), the percentage of infested shoots (IS), the mean values of the classes of parasitism, the number of earwigs and *A. mali* individuals captured on the bands (EarwC and MaliC, respectively), and the number of earwigs present in shelters (EarwP), climatic variables such as maximum temperature ( $T_{max}$ ,  $^{\circ}\text{C}$ ), minimum temperature ( $T_{min}$ ,  $^{\circ}\text{C}$ ), number of hours above or below temperature thresholds ( $h > 20^{\circ}\text{C}$ ,  $h > 25^{\circ}\text{C}$ ,  $h < 10^{\circ}\text{C}$  and  $h < 7^{\circ}\text{C}$ ), minimum relative humidity (rh min%), solar radiation (Sun,  $\text{W}/\text{m}^2$ ), rainfall (Rain, mm), and wind speed (Wind, m/s).



Crawler upward (Up) and downward (Down) migration per week were highly correlated, and the movement through the canopy (C) was one of the variables that contributed most to the overall variance and did not seem to have a close relationship with the AI or IS (Figure V-3). These observations are consistent with the hypothesis mentioned above, that there is not a close relationship between canopy and root colonies. Peak captures of crawlers moving through the canopy (C) were observed in the warmer months of the year (Figure V-1); however, with the multi-lateral approach we cannot confirm a clear correlation of canopy movement with the temperatures, and it may be more related to other variables not yet detected. The variables Up ac and Down ac had a high negative correlation with the number of hours below 10 °C ( $h < 10$ ) and 7 °C ( $h < 7$ ) (Figure V-3), suggesting that crawlers moving up and down the trunk will be more important when temperatures are higher than 10 °C. Hoyt and Madsen (1960) also reported that temperatures below 10 °C inhibited crawler movement in laboratory conditions. Asante et al. (1991) observed that temperatures above 25 °C were detrimental regarding optimal fecundity and survival rates, but no information related to crawler movement was provided.

We found no clear relation between rainfall (Rain) and relative humidity (rh min %) with crawler movement (Up, Down, C), AI or IS (Figure V-3). A negative influence of rainfall on crawler migration was observed by Hoyt and Madsen (1960), Bhardwaj (1995), and Heunis and Pringle (2006). The lack of correlation that we observed may be explained because in the conditions of our study, maximum crawler captures were observed during the driest weeks of the year, when rainfall was rare, more similar to the conditions in which Beers et al. (2010) performed their study in Washington.

The strong correlation observed between EarwP and EarwC suggests that glue tapes are a practical and efficient means by which to estimate the presence of earwigs in the orchard, without the need for special shelters. The number of *A. mali* trapped on the tapes (MaliC) appeared to be negatively correlated with rainfall (Rain) and not correlated with parasitism. Many *A. mali* were found on the tapes at the beginning of spring. This observation could be attributed to these insects emerging from overwintering mummies. The positive correlation found between parasitism and temperatures above 20 °C (Figure V-3), is consistent with the high rates of parasitism observed by Monteiro et al. (2004) in the warmer climate of Brazil. In addition, these observations also are supported by the high parasitism from July onwards (Figure V-2), coinciding with the highest temperatures of the year.

Spiders and velvet mites were ruled out as main variables of the PCA as they had a null contribution to the overall explained variance. Wind and solar radiation (Sun) did not make an important contribution to the overall variance as well (Figure V-3), with similar conditions among orchards, the study area was not especially windy, and the solar radiation was not limiting. In contrast, Hoyt and Madsen (1960) suggested the relevance of solar radiation on daily crawler migration, as



they observed the greatest movement in late afternoon and very little during darkness; however, in our analysis this daily dynamic was not observed as we recorded weekly captures.

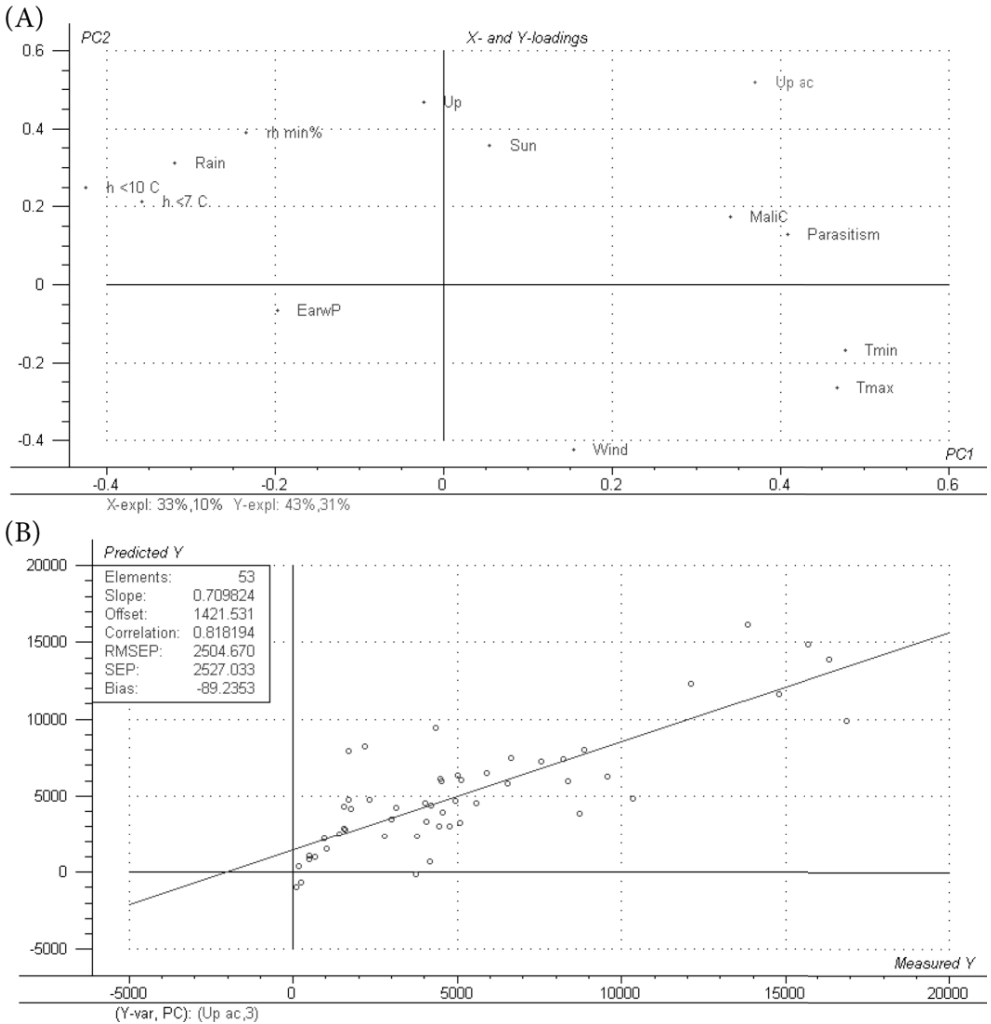


Figure V-4. Up ac PLS-1: X and Y loadings represented in the plane defined by the two first PLS factors (A) and predicted vs. measured diagram for the regression model of Up ac on the 12 variables analyzed (B). Variables are: the weekly number of aphids captured on the upper band (Up), the accumulated number of aphids captured each week on the upper band (Up ac), the mean values of the classes of parasitism, the number of *A. mali* individuals captured on the bands (MaliC), the number of earwigs present in shelters (EarwP), climatic variables such as maximum temperature (Tmax, °C), minimum temperature (Tmin, °C), number of hours below temperature thresholds (h < 10 °C and h < 7 °C), minimum relative humidity (rh min%), solar radiation (Sun, W/m<sup>2</sup>), rainfall (Rain, mm), and wind speed (Wind, m/s).

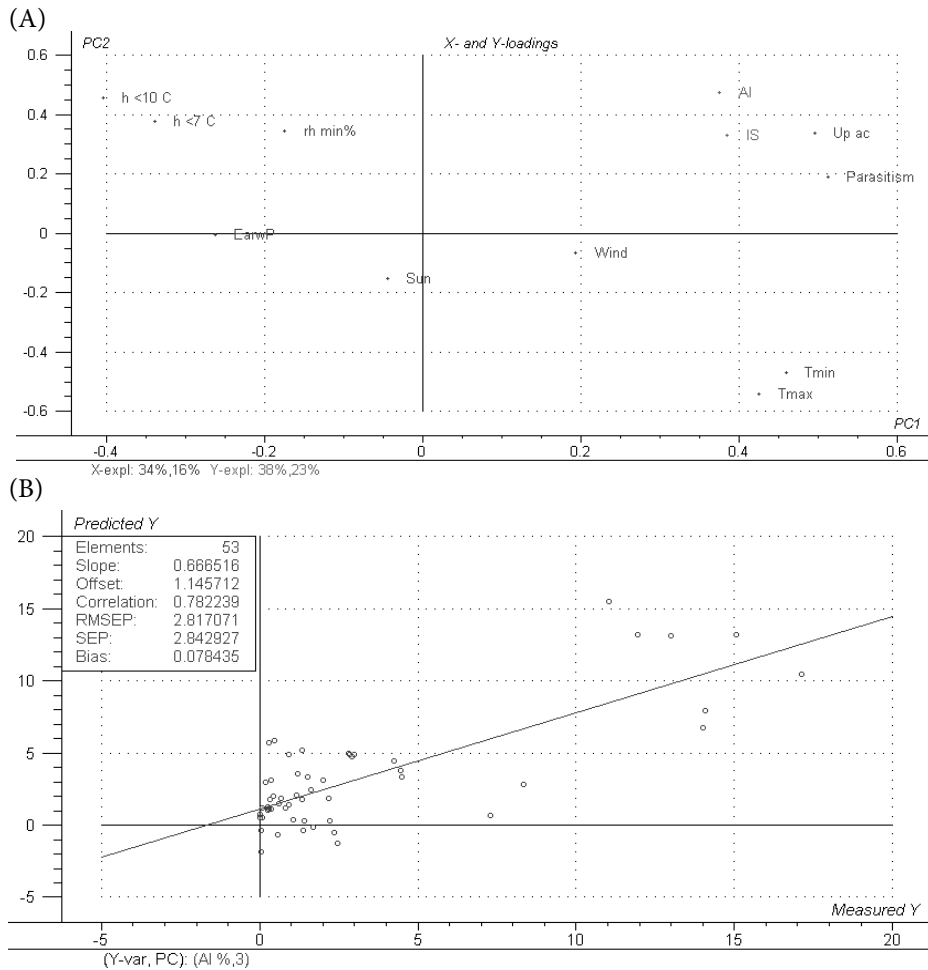


Figure V-5. AI and IS PLS-2: X and Y loadings represented in the plane defined by the two first PLS-factors (A) and predicted vs. measured diagram for the regression model of AI-IS on the 10 variables analyzed (B). Variables are: the accumulated number of aphids captured on the upper band each week (Up ac), the percentage of aerial infestation (AI), the percentage of infested shoots (IS), the mean values of the classes of parasitism, the number of earwigs present in shelters (EarwP), climatic variables such as maximum temperature (Tmax, °C), minimum temperature (Tmin, °C), number of hours below temperature thresholds (h<10 °C and h<7 °C), minimum relative humidity (rh min%), solar radiation (Sun, W/m<sup>2</sup>), rainfall (Rain, mm), and wind speed (Wind, m/s).

Regarding the PLS-1 to predict Up, the first two PLS factors explained 52% of the variance of the X-variables and only 26% of the information concerning the Up with a Root Mean Square Error of Prediction (RMSEP) value of 372.94 (data not shown) within a 0-2,000 data rank. With these results, the model was considered not to be accurate enough to predict Up. On the other hand, in the PLS-1 method used to predict Up ac, 43% of the information contained in the X-variables explained 74% of the Y information (Figure V-4A). The latter model showed a

coefficient of determination of 0.82 between predictions and reference values, and a RMSEP value of 2,504.67 (data rank 0-20,000) to predict the Up ac between April and October. These values suggest that a reliable model can be constructed to predict the accumulated number of crawlers and that variables in addition to Up, such as MaliC, Parasitism, EarwP, Wind, Tmin, Tmax, Sun, rh min, Rain, h<10 and h<7 have to be taken into account (Figure V-4). To reduce the unexplained variance (26%) additional variables not evaluated in this study that could have a direct effect on WAA or through an effect on natural enemies should also be included in the model.

In the PLS-2 procedure used to jointly analyze AI and IS, the first two PLS factors explained 51% of the variance of the X-variables and 61% of the Y information (Figure V-5A). The model obtained had a coefficient of determination of 0.78 between predictions and reference values and an RMSEP value of 2.82 (Figure V-5B) within a data rank of 0-20. These results were similar to those obtained from the PLS-1 to predict Up ac. The same considerations regarding the way to improve this model would also be suitable in this case. The role of earwigs can be highlighted in both PLS-1 and PLS-2 models, with a negative correlation with the canopy infestations and the number of crawlers cumulated over the year (Figure V-4A and Figure V-5A).

To our knowledge, this is the first study aimed at modeling canopy infestations and crawler movement. Climatic variables were used in multivariate techniques (via principal component analysis (PCA)) by Howling et al. (1993) to predict the first appearance dates of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and by Semeao et al. (2012) to predict the natural mortality of *Triozoida limbata* (Enderlein) (Hemiptera: Psyllidae). For WAA, only linear models based on temperature (Asante et al. 1991) or on developmental times (Bodenheimer 1947; Bonnemaison 1965; Evenhuis 1958) have been reported.

## 4. Conclusions

The aim of this study was to provide knowledge to improve WAA management in Mediterranean areas. We conclude that both canopy and root colonies are the source of reinfestations in Mediterranean areas, as crawlers migrated upward and downward throughout the year and winter temperatures did not kill the aerial colonies. Therefore, measures of control must be addressed as well on roots as on the canopy.

Earwigs were found to reduce the survival of overwintering canopy colonies up to June. Predation of such colonies by earwigs in early spring is important to maintain them under low levels, allowing their later control by the parasitoid from summer to fall, and this role was also highlighted in the predictive models. Therefore, it is important to promote or at least not to disrupt neither earwigs nor *A. mali* in order to improve natural control of WAA.

To improve the accuracy of the models in the prediction of canopy infestations, other variables that could affect WAA and/or natural enemies must be included. Further research is needed to determine an infestation threshold in spring to evaluate whether the natural control would be enough or if additional measures must be applied.



## **Chapter VI. Diversity and abundance of spiders in the flora of the fruit area around Lleida (NE Spain)**



**Abstract:** The identification of flora that is useful to provide shelter and food for spiders thereby increasing the biological control of pests was studied in the fruit tree region of Lleida (Spain). The study was carried out in different areas, according to the presence of fruit tree orchards and edapho-climatic conditions. Herbaceous plants were sampled by an insect suction sampler. All the individuals captured in each sample were identified at family level. The main plants hosting spiders during spring were *Anacyclus clavatus* (Desf.), *Dorycnium pentaphyllum* (Scop.), *Erucastrum nasturtifolium* (Poiret), *Euphorbia serrata* (L.), *Hedysarum confertum* (Desf.), *Papaver rhoeas* (L.) and *Trifolium pratense* (L.). For the autumn period, most important species were *Atriplex* sp., *Dittrichia viscosa* (L.), *Medicago sativa* (L.), *Moricandia arvensis* (L.), *Salsola kali* (L.), *Sorghum halepense* (L.), *Suaeda spicata* (Willd.) and *Verbena* sp. The spiders' families more abundant on the plants were Thomisidae, Linyphiidae and Oxyopidae.

**Key words:** Spider, biodiversity, biological control.

## **Chapter VII. Enhancing beneficials through hedgerow design**





## **Abstract**

Ecological infrastructures can be used to increase the biological control of pests by providing a more favorable environment and additional food and shelters for natural enemies. However, such infrastructures should not share pests or diseases with the crop; thus, selective management of these infrastructures is decisive to improve conservation biological control. The use of native plants, which are more adapted to local environment and soil conditions, may contribute to the success of ecological infrastructures. Here we assessed the flowering period of 43 species of trees and shrubs and the beneficials associated with the same. We identified 6,752 arthropods, of which 1,582 were natural enemies. Our findings highlight that the inclusion of certain trees and shrubs in hedgerows could strengthen ecological infrastructures for the purpose of biological control. As a first approach, we evaluated 8 species of trees and shrubs in an experimental trial. Of these, *Viburnum tinus* L., *Euonymus japonicus* L. fil., and *Pistacia lentiscus* L. showed potential to enhance the abundance and richness of natural enemies.

**Keywords:** Biodiversity, biological control, ecological infrastructures, flowering period, natural enemies, predator.

## **Chapter VIII.      General discussion**





## 1. Earwigs in Mediterranean apple orchards

Five species of earwigs have been found in Mediterranean apple orchards. While *L. riparia*, *E. moesta*, and *N. lividipes* were mainly found on the ground, *F. pubescens* and *F. auricularia* were observed as well on the ground as up on the canopy (Chapter II). Presence of *F. auricularia* and *F. pubescens* has been reported in apple orchards of Central-North Europe (Debras et al. 2007; Gobin et al. 2008; Helsen et al. 1998; Kocarek 1998; Moerkens et al. 2009; Phillips 1981), North America (Crumb et al. 1941; Fulton 1924; Lamb 1975; Lamb and Wellington 1975; Lamb 1976a) and New Zealand (Burnip et al. 2002; Suckling et al. 2006). Regarding Mediterranean orchards, *F. auricularia*, *F. pubescens*, *L. riparia*, *E. moesta*, *N. lividipes* and *Euborellia annulipes* Dohrn have been also observed by Romeu-Dalmau et al. (2011) in citrus canopies. The last four earwig species are barely cited in the bibliography, probably because they are only found on the ground, and the main studies have been addressed to the canopy. In addition, these ground-dwelling earwigs have significantly lower abundance than those from the genus *Forficula*, thus, due to these low numbers of individuals, it is more difficult to study their phenology.

The phenology that we observed for *F. auricularia* and *F. pubescens* was similar to the reported by Romeu-Dalmau et al. (2011) in citrus orchards and different from the reported in other areas of Central-North Europe (Gobin et al. 2008; Helsen et al. 1998; Moerkens et al. 2009; Phillips 1981). We observed that *F. auricularia* nymphs hibernate here, being present from October to June, whereas in colder areas of Northern Europe only adults hibernate. For *F. pubescens* we observed nymphs from April to June, but this cannot be compared to colder areas, as there is a lack of such data. Regarding mature stages, they were observed all year round for both species. This longer activity period, compared with colder areas (Gobin et al. 2008; Helsen et al. 1998; Lamb and Wellington 1975; Moerkens et al. 2009; Moerkens et al. 2011; Phillips 1981) may affect their potential as biocontrol agents, as earwigs in Mediterranean orchards will be able to predate on pests before outbreaks occur. However, this longer period may also have negative effects in other crops, for instance, in peaches, nectarines, apricots and cherries.

Aggregation of earwigs in field conditions was assessed. We observed that *F. auricularia* and *F. pubescens* did not have a random distribution across the orchards. Furthermore, the aggregation that we observed in field conditions was even higher than the reported by Sauphanor and Sureau (1993) in laboratory trials, and in addition, both species were observed sharing the same shelters. In our trials we observed that earwigs were attracted to shelters that were pheromone-impregnated by 0.2 individuals/cm<sup>2</sup> at distances up to 50 cm. A study made by Sauphanor and Sureau (1993) reported that individuals of the genus *Forficula* were attracted to shelters impregnated by *F. auricularia*; thus, we may assume that shelters impregnated by this species may also serve to attract *F. pubescens* and *F. decipiens* individuals. The use of shelters has been proposed in kiwifruit, apple and

pear orchards to enhance the biocontrol role of earwigs (Gobin et al. 2006; Logan et al. 2007; Solomon et al. 1999). Attraction and promotion through shelters might be improved by the use of pheromone-impregnated shelters. Therefore, these findings would improve biological control in pip fruit orchards, but in other hand could also serve to capture and remove earwigs from stone fruit orchards. However, further research is needed to increase the perception range in order to make these impregnated-shelters a useful tool.

*Forficula auricularia* had an important role in the control of overwintering aerial colonies of woolly apple aphid (Chapter V). Success of biological control may be increased when different biocontrol agents interact simultaneously within the same pest management program. However, some entomopathogens may also have negative effects on natural enemies (Everard et al. 2009; Head et al. 2003; Lacey et al. 2003). Compatibility of entomopathogenic nematodes and earwigs was evaluated in Chapter IV. *S. feltiae* and *H. bacteriophora* did not have negative effects on European earwig, whereas *S. carpocapsae* was able to kill 50 % of individuals in laboratory trials. We observed that European earwig was able to recognize nematode-treated shelters, thus, we assume that this avoidance behavior observed in laboratory trials may also guarantee compatibility of earwig with *S. carpocapsae* in field conditions, where more shelters and chances to scape from nematodes would be available. In addition, the presence of a deterrent activity of nematode-killed cadavers will allow nematodes to complete their life cycle and so, increase their biocontrol efficacy.

## **2. Woolly apple aphid and its interaction with climatic variables and natural enemies**

Ecology of woolly apple aphid and its relation with biotic and abiotic factors throughout the year has been assessed (Chapter V). We developed a visual index to count the crawlers trapped on the glue tapes. The use of glue tapes has been already used by some authors to assess the crawler movement (Beers et al. 2010; Bhardwaj et al. 1995; Heunis and Pringle 2006; Hoyt and Madsen 1960; Walker 1985); however, in those studies they counted the number of aphids under a binocular microscope. The use of the visual index that we elaborated reduces the assessing time when studying the crawler movement and ensures a sufficient accuracy.

The woolly apple aphid crawler movement up and down the trunks was similar in timing to the observed by other authors in USA, South Africa and Australia (Asante 1994; Beers et al. 2010; Heunis and Pringle 2006). However, the main difference that we observed in Mediterranean orchards is that the winter temperatures did not kill the aerial colonies, thus, reinfestations were not exclusively initiated from the root populations. Therefore, control of woolly apple aphid in milder climates should focus on roots and canopy populations. Also, the role that earwigs can have to maintain low levels of aerial infestations indicate that

promotion of such predator must be also ensured in Mediterranean orchards to guarantee a successful control of the pest. These findings coincide with the observed by other authors (Helsen et al. 2007; Mueller et al. 1988; Nicholas et al. 2005; Noppert et al. 1987; Phillips 1981; Stap et al. 1987). On the other hand, the contribution of spiders to control woolly apple aphid was not clear. In contrast, Boreau de Roince et al. (2013) in France and Wyss et al. (1995) in Switzerland, suggested the importance of spiders in reducing the green and rosy apple aphid populations, respectively. Presence of root colonies that are hardly accessible for spiders, and a walking dispersion, make woolly apple aphid less susceptible to spiders than rosy and green apple aphids, which disperse flying.

*A. mali* was observed to have an important role late on the season, being more effective in years with dry and warm springs, and only when the initial levels were low. These findings coincide with the observed by Asante and Danthanarayana (1992) in Australia and Heunis and Pringle (2006) in South Africa, and are in contrast with the observed by Monteiro et al. (2004) in Brazil. The temperature and rainfall ranges may explain these differences, as the Mediterranean climate is more similar to the study area of Australia and South Africa than to Brazil.

### 3. Biodiversity management to improve biological control

Information attained in the first chapters (II-III-IV) will bring us knowledge about the reasons and how we should promote a resident predator as the European earwig. However, biological control of pests will be more successful if multiple agents act simultaneously. In Chapter VI and VII we report the potential of the native flora to attract beneficials. By providing a more favorable environment and additional food and shelters to natural enemies, the use of these ecological infrastructures would increase the biological control of pests.

Thomisidae, Linyphiidae and Oxyopidae were the families of spiders more abundant in our area (Chapter VI). Implementation of flower strips or cover crops with mixtures of *Anacyclus clavatus* Desf., *Dorycnium pentaphyllum* Scop., *Erucastrum nasturtiifolium* Poiret, *Euphorbia serrata* L., *Hedysarum confertum* Desf., *Papaver rhoeas* L., *Trifolium pratense* L. in spring and *Atriplex* sp., *Dittrichia viscosa* L., *Medicago sativa* L., *Moricandia arvensis* L., *Salsola kali* L., *Sorghum halepense* (L.) Pers., *Suaeda spicata* Willd. and *Verbena* sp. in fall, would increase presence of spiders within orchards.

On the other hand, high numbers of colonists were observed to be associated with trees and shrubs (Chapter VII). The most abundant families were Coccinellidae, Anthocoridae, Chrysopidae and Aeolothripidae for predators, while Chalcididae, Encyrtidae and Eulophidae were the most abundant parasitoids. Several authors have suggested that these natural enemies play an important role as biocontrol agents (Andreev et al. 2006; Feraru and Mustata 2006; Helyer et al. 2003; Lind et

al. 2003; Mols and Boers 2001; Ribes et al. 2004; Wyss 1995; Wyss et al. 1995; Wyss 1999).

Comparing the same tree and shrubs species, more beneficials were observed during the survey than in the experimental trial. We believe that these differences may be attributed to the fact that trees and shrubs from the survey were older and bigger, thus, beneficials were already established and had more resources to take advantage. These hypothesis coincides with the observations made by Olson and Andow (2008), Bezemer et al. (2010), Bryant et al. (2013) and Blaauw and Isaacs (2012). In addition, evergreen species such as *E. japonicus*, *P. lentiscus*, and *V. tinus* had high abundance of natural enemies associated with them. The increase of richness and evenness, more than abundance itself, will enhance the performance of natural enemies by adding stability to the ecosystem (Cardinale et al. 2004; Colfer and Rosenheim 2001; Landis et al. 2000; Macfadyen et al. 2011; Stiling and Cornelissen 2005).

### 4. Future research

Future work should take into account the predatory role that ground-dwelling earwigs may play. Most of the ground-dwelling species are considered predators (Albouy and Caussanel 1990; Frank et al. 2007; Horton et al. 2003; Shepard et al. 1973), and their interaction with other species as competition for resources must be assessed. In addition, aggregation within species and attraction by pheromone-impregnated shelters should also be assessed. An increase of the range at which the impregnated shelters are perceived will facilitate their use as a valuable tool when promoting biological control, but also to be used as mass trapping in stone fruit orchards.

A phenological day degree model for Mediterranean populations would be a useful tool to manage orchards in order to promote earwigs and avoid harmful effects on them. Moerkens et al. (2011) and Belien et al. (2013) elaborated an earwig phenological model for colder regions, however, it does not work under Mediterranean climates, thus new models need to be elaborated.

Native plant species have been selected for their potential to attract and harbor beneficials. This is crucial for functional biodiversity, but this is only the first step to improve the biological control through biodiversity management. Additional future research must determine whether the abundance and diversity increase of natural enemies on the ecological infrastructures has direct effect on the biological control of the crop pests. Furthermore, management of these infrastructures may drive natural enemies to pests, but so far all these techniques need more research before they might be used in commercial orchards.

Besides the reported associations of spiders with plants, in successive years, the same and more native plants have been sampled to assess their attraction potential to beneficials. Data are still being processed, up to now 30,000 arthropods have been identified at family level and in the upcoming months more knowledge

regarding native flora will increase the list of candidate plants that may be used as ecological infrastructures, not only hedgerows, but also flower strips and cover crops.

## 5. Conclusions

- i. *Forficula auricularia*, *Forficula pubescens*, *Euborellia moesta*, *Nala lividipes*, and *Labidura riparia* are present in Mediterranean apple orchards. The former two species are the most abundant and are present throughout the year, thus they can contribute as biocontrol agents to maintain some pests under low levels. The last three species are ground-dwelling and their role as biocontrol agents is unclear (Chapter II).
- ii. The current phenological models of *F. auricularia* do not fit under Mediterranean climates because nymphs do also hibernate. One brood and two broods populations co-occur. (Chapter II).
- iii. Impregnated shelters may provide a useful tool for fruit management purposes (Chapter III).
- iv. Using entomopathogenic nematodes can be compatible with promoting the European earwig within the same pest management program (Chapter IV).
- v. The control of woolly apple aphid in Mediterranean areas should focus on roots and canopy as aerial colonies survive during winter (Chapter V).
- vi. Predation of canopy colonies by earwigs in early spring is important to maintain woolly apple aphid under low levels, allowing their control by *A. mali* from summer to fall (Chapter V).
- vii. The most abundant families of spiders were Linyphiidae, Thomisidae and Oxyiopidae; as they have different hunting strategies, their promotion can improve biocontrol of several kinds of pests.
- viii. Mixtures of *Anacyclus clavatus* Desf., *Dorycnium pentaphyllum* Scop., *Erucastrum nasturtiifolium* Poiret, *Euphorbia serrata* L., *Hedysarum confertum* Desf., *Papaver rhoeas* L., *Trifolium pratense* L. can promote spiders in spring; whereas mixtures of *Atriplex* sp., *Dittrichia viscosa* L., *Medicago sativa* L., *Moricandia arvensis* L., *Salsola kali* L., *Sorghum halepense* (L.) Pers., *Suaeda spicata* Willd. and *Verbena* sp. can be used in fall for the same purpose (Chapter VI).
- ix. Richness and abundance of natural enemies may be increased by the implementation of *V. tinus*, *E. japonicus* and *P. lentiscus* in hedgerows (Chapter VII).





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*If all insects on Earth disappeared, within 50 years all life on Earth would end. If all human beings disappeared from the Earth, within 50 years all forms of life would flourish.*

Jonas Salk