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Natural enemies for the conservation biological control of *Myzus persicae* in Mediterranean peach orchards

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La conservación es un estado de harmonía entre el hombre y la tierra.
Aldo Leopold.

To my parents and my brother,
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To Sergio,
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Abstract

Peach tree is the third most produced fruit species in the world. Within Europe, Spain is a leading producer and accounts for more than 30% of total European production. Most of Spanish peach orchards are concentrated in Catalonia, northeastern Spain. Peach tree is the primary host of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), which is one of the most damaging pests of the crop. To date, management of this aphid has mainly based on insecticides, but the risk they pose to the human health and the environment, requires a reduction in their usage. Biological control could be a good tool to improve the control of the pest in peach crops, because aphids have a wide array of natural enemies that could contribute to the management of this pest. Among the different biological control strategies that have been used against aphids, conservation of already existing natural enemies in the agroecosystem seems the more promising for fruit tree orchards. Therefore, the present thesis was undertaken to determine the possibility of implementing conservation biological control against *M. persicae* in peach and nectarine orchards in the Mediterranean area.

Conservation biological control relies on preserving the natural enemies present in a particular area, and in the implementation of strategies to enhance their populations in the agroecosystem. Therefore, the identification of the key natural enemies involved in *M. persicae* control has to be the first step to start a conservation biological control program. The inclusion of floral resources close to the orchards might help to enhance the biological control by providing natural enemies with nectar and pollen as food sources, thereby, contributing to boost their populations at the key moment for an effectively control of the pest. The first two chapters of this thesis are dedicated to identify the key predators and parasitoids of *M. persicae* in the Segrià area (Catalonia) and to evaluate, in field conditions, the potential contribution of insectary plants to enhance aphid natural enemy populations. Sentinel plants, small peach plants *adhoc* infested with *M. persicae*, were used to recruit the natural enemies associated to this aphid, and molecular and morphological methods were used to identify the most abundant species. Then, the attraction for the key natural enemies of sown flower patches including the following selected species *Achillea millefolium* L. (Compositae),

Lobularia maritima L. (Brassicaceae)*, Moricandia arvensis* L. (Brassicaceae), and *Sinapis alba* L. (Brassicaceae) was evaluated. Results revealed that the most abundant natural enemies of *M. persicae* were parasitoids. Although, there was a wide array of species, *Aphidius matricariae* Haliday (Hymenoptera: Braconidae: Aphidiinae) was by far the most prevalent. Ten different species of hyperparasitoids were obtained from *M. persicae* parasitized aphids. Nevertheless, the absence of hyperparasitoids early in the season, when *M. persicae* populations build up, suggest that biological control of this aphid would not be negatively impacted by the presence of hyperparasitoids. Regarding predators, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) were the most prevalent, followed by *Orius majusculus* Reuter (Hemiptera: Anthocoridae). Field experiments conducted during this thesis revealed that sown flower patches were highly attractive to hoverflies and parasitoids, and none of these plants was a reservoir of damaging aphids for peach crops. Among the four species tested in the experiment, *L. maritima* was a suitable resource for these natural enemies. However, and in spite of being attracted to the flowering plants in the sown patches, the proximity of these patches did not increase the abundance of natural enemies of *M. persicae* in the sentinel plants, probably due to the size of the orchards.

To effectively contribute to natural enemy fitness and therefore to biological control of *M. persicae*, flowering insectary plants have to provide to parasitoids and predators profitable food in terms of both quality and accessibility. Based on this, the third chapter of this thesis focused on assess whether the insectary plant *L. maritima* is a potential food source for the parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae: Aphidiinae) and the predator *A. aphidimyza.* With this aim, laboratory experiments were conducted to investigate if both natural enemies were attracted to blooming and non-blooming *L. maritima*. There were also tested the effects of flower availability on the longevity and reproductive potential of *A. ervi* and *A. aphidimyza*. Results of the olfactory bioassays showed that volatiles produced by *L. maritima*, with and without flowers, attracted both natural enemies. However, the attractiveness to the flowers was disrupted when compared with peach shoots infested with *M. persicae*. Although *A. aphydimiza* benefited from feeding on a sugar rich diet, availability of *L. maritima* flowers did not improved neither the longevity nor the reproduction of this predator, since the floral morphology of this species prevented females to feed on the nectar. Nevertheless, *L. maritima* flowers increased the longevity of *A. ervi* females when aphids were not present in the arenas. Thus, provision of other sugar resources, such as flowers with exposed nectaries and extra floral nectar may also be an option to improve the biological control of *M. persicae.*

The high diversity of natural enemies that attack *M. persicae* in the area of study, increases the probability of intraguild predation because these natural enemies may not only compete for the same food resource but also feed on each other. Due to the development of parasitoid larva within the aphid, predators might also eat juveniles of the parasitoids while preying on the host. Besides, the presence of natural enemies in an aphid colony can also produce behavioral changes in aphids. The last chapter of this thesis had the aim to evaluate the interactions among predators and parasitoids, and how these interactions may influence the biological control of *M. persicae.* With laboratory essays, the contribution of *E. balteatus, A. aphidimyza* and *O. majusculus* to the control of *M. persicae* was determined. The changes in the aphid's behavior mediated by the aphid cornicle exudate and the interaction of these predators with the parasitoid *A. matricariae* were also evaluated. Results showed that the most voracious predator was *E. balteatus*, followed by *A. aphidimyza.* In the experiments conducted during this thesis, *O. majusculus* did not reduce pest population. The cornicle exudate, produced by *M. persicae*, triggers fecundity compensation, that is to say a rise in the aphid offspring production. Although, *A. aphidimyza* and *O. majusculus* preferred to kill non-parasitized aphids, both predators were able to kill parasitized but still not mummified *M. persicae* individuals. Their preference to kill non-parasitized aphids, predicts that the coexistence of parasitoids and predators would not be negative for the biological control of *M. persicae.*

The results of this thesis, suggest that the conservation biological control can be a useful tool to improve *M. persicae* control in peach and nectarine crops.

Resumen

El melocotero y la nectarina pertenecen a la especie *Prunus persica* (L.) Batsch y es el tercer cultivo frutal con mayor producción a nivel mundial. En Europa, España es uno de los mayores países productores representando más del 30% del total de la producción. La mayoría de las plantaciones de melocotoneros y nectarinas están localizados en Cataluña, al noreste de España. *Prunus persica* es el huésped primario de *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), comúnmente conocido como el pulgón verde del melocotonero, que es una de las plagas más dañinas para el cultivo. Hasta la fecha el control de este pulgón se ha basado en la utilización de insecticidas pero, debido al riesgo que representan tanto para la salud humana como para el medio ambiente, se requiere reducir el uso de estos productos. El control biológico puede ser una buena herramienta para mejorar el control de la plaga en este cultivo debido a que los pulgones tienen un amplio rango de enemigos naturales que podrían contribuir a su control. Dentro de las diferentes estrategias de control biológico que se han utilizado contra los pulgones, la conservación de los enemigos naturales ya existentes en el agroecosistema parece ser la más prometedora para los cultivos frutales. Con estos antecedentes, la presente tesis se llevó a cabo para determinar la posibilidad de implementar el control biológico por conservación contra *M. persicae* en cultivos de melocotón y nectarina en el área del Mediterráneo.

El control biológico por conservación, se basa en preservar a los enemigos naturales presentes en la zona e implementar estrategias para mejorar sus poblaciones. Por lo tanto, la identificación de los enemigos naturales clave involucrados en el control de *M. persicae,* debe ser el primer paso para comenzar un programa de control biológico por conservación. La inclusión de recursos florales cerca del cultivo, podría ayudar a mejorar el control al ofrecer a los enemigos naturales recursos alimenticios como néctar y polen, contribuyendo así a aumentar sus poblaciones en el momento adecuado para controlar eficazmente la plaga. Los dos primeros capítulos de esta tesis, están dedicados a identificar los depredadores y parasitoides clave de *M. persicae* en la comarca del Segrià (Cataluña), y a evaluar, en condiciones de campo, el potencial de ciertas plantas insectarias para mejorar sus poblaciones. Para recolectar a los enemigos naturales de *M. persicae*, se utilizaron plantas centinela, es decir plantones de melocotonero

expresamente infestados con *M. persicae,* y para identificar a las especies más abundantes se utilizaron métodos moleculares y morfológicos. Posteriormente se evaluó la atracción de los enemigos naturales hacia manchas florales que incluían las siguientes especies: *Achillea millefolium* L. (Compositae)*, Lobularia maritima* L. (Brassicaceae)*, Moricandia arvensis* L. (Brassicaceae) y *Sinapis alba* L. (Brassicaceae). Los resultados mostraron que los enemigos naturales más abundantes de *M. persicae* fueron parasitoides. A pesar de que existió un amplio rango de especies, *Aphidius matricariae* Haliday (Hymenoptera: Braconidae: Aphidiinae) fue con mucho la más abundante. De los pulgones parasitados se identificaron, así mismo, diez especies de hiperparasitoides. Sin embargo, la ausencia de hiperparasitoides a principio de la temporada, cuando las poblaciones de *M. persicae* son elevadas, sugiere que el control biológico de este pulgón no se vería afectado negativamente. En cuanto a los depredadores, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) y *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) fueron los más abundantes, seguidos de *Orius majusculus* Reuter (Hemiptera: Anthocoridae). Los experimentos de campo realizados en esta tesis, mostraron que la presencia de manchas florales fue atractiva para los sírfidos y para los parasitoides y ninguna de las plantas evaluadas fue reservorio de pulgones dañinos para los cultivos de melocotón y nectarina. Entre las cuatro plantas insectarias evaluadas, *L. marítima* fue uno de los mejores para los enemigos naturales. Sin embargo, la proximidad de las plantas insectarias al cultivo no incrementó la abundancia de las poblaciones de enemigos naturales en las plantas centinela, probablemente a causa del tamaño de las parcelas.

Para poder contribuir de manera positiva al estado físico (*fitness* en inglés) de los enemigos naturales, y por lo tanto al control biológico de *M. persicae*, las flores de las plantas insectarias deben proporcionar a los parasitoides y depredadores un alimento que puedan aprovechar, tanto en términos de calidad como de accesibilidad. En base a ello, el tercer capítulo de esta tesis se centró en evaluar si la planta insectaria *L. marítima* es una fuente de alimento adecuada para el parasitoide *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae: Aphidiinae) y el depredador *A. aphidimyza.* Con este objetivo, se realizaron experimentos de laboratorio para investigar si ambos enemigos naturales eran atraídos hacia *L. marítima* en presencia y ausencia de flores. También se evaluaron los efectos de las flores en cuanto a la longevidad y el potencial reproductivo de estos insectos. Los resultados de los experimentos llevados a cabo en el olfactómetro mostraron que los compuestos volátiles producidos por *L. maritima*, con y sin flores, atraían a ambos enemigos naturales. Sin embargo, esta atracción se vio afectada cuando se comparó la *L. marítima* con brotes de melocotón infestados con *M. persicae*. Aunque *A. aphidimyza* se benefició de una alimentación rica en azúcar, la disponibilidad de *L. marítima* no mejoró ni la longevidad ni la reproducción del depredador, ya que la morfología floral de esta especie impidió que las hembras se alimentaran del néctar. En cambio, las flores de *L. maritima* incrementaron la longevidad de las hembras de *A. ervi* en ausencia de pulgones. Por lo tanto, la provisión de otros recursos azucarados, como nectarios extraflorales o flores con nectarios expuestos, pueden ser un recurso alimenticio viable para los enemigos naturales y por lo tanto ayudarían a mejorar el control biológico de *M. persicae*.

Debido a la gran diversidad de enemigos naturales que atacan a *M. persicae* en el área de estudio, existe una alta probabilidad de que ocurra depredación intragremial porque estos enemigos naturales no solo compiten por el mismo recurso alimenticio, sino que también pueden depredarse entre sí. Además, dado que el desarrollo de las larvas de los parasitoides ocurre dentro del pulgón, los depredadores pueden comérselos al alimentarse del pulgón. Por otro lado, la presencia de enemigos naturales en una colonia de pulgones también puede producirles cambios en su comportamiento. El último capítulo de esta tesis tuvo como objetivo evaluar las interacciones entre depredadores y parasitoides, y estudiar cómo estas interacciones pueden influir en el control biológico de *M. persicae*. En ensayos de laboratorio, se determinó la contribución de *E. balteatus, A. aphidimyza* y *O. majusculus* al control de *M. persicae*. También se evaluaron los cambios en el comportamiento del pulgón asociados por las secreciones de los cornículos o sifones, y la interacción de estos depredadores con el parasitoide *A. matricariae*. Los resultados mostraron que el depredador más voraz fue *E. balteatus*, seguido de *A. aphidimyza*. *Orius majusculus* no redujo la población de *M. persicae*. La secreción de los cornículos de *M. persicae* desencadenó una compensación de la fecundidad, es decir, un aumento de la descendencia. Aunque, *A. aphidimyza* y *O. majusculus* prefirieron matar los pulgones no parasitados, ambos depredadores fueron capaces de matar a los individuos de *M. persicae* parasitados pero aún no momificados. Su preferencia por matar pulgones no parasitados indica que la coexistencia de parasitoides y depredadores no sería negativa para el control biológico de *M. persicae*.

Los resultados de esta tesis, sugieren que el control biológico por conservación puede ser una herramienta útil para mejorar el control de *M. persicae* en cultivos de melocotón y nectarina.

Resum

Els préssecs i les nectarines Prunus persicae (L.) Batsch és el tercer conreu fruiter amb més producció a escala mundial. Dins d'Europa, Espanya és un dels majors productors amb el 30% del total de la producció europea. La majoria de camps de presseguer i nectarina estan localitzats a Catalunya, al nord-est d'Espanya. Prunus persicae és l'hoste primari del pugó verd del préssec, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) que és una de les plagues més nocives per al cultiu. Fins a la data actual, el control d'aquest pugó s'ha basat en l'ús d'insecticides, però el risc que representen tant per a la salut humana com per al medi ambient, fa necessari reduir-ne l'ús. El control biològic pot ser una bona eina per millorar el control d'aquesta plaga en els camps de cultiu, ja que els pugons tenen un ampli rang d'enemics naturals que podrien contribuir al seu control. Dins de les diferents estratègies de control biològic que s'han utilitzat contra els pugons, la conservació dels enemics naturals ja existents en l'agroecosistema sembla ser la més prometedora pels cultius de fruiters. Amb aquests antecedents, la present tesi es va dur a terme amb l'objectiu de determinar la possibilitat d'implementar el control biològic per conservació contra *M. persicae* en cultius de préssec i nectarina en l'àrea del Mediterrani.

El control biològic per conservació es basa en preservar als enemics naturals presents a la zona i en la implementació d'estratègies per millorar les seves poblacions en l'agroecosistema. Per tant, la identificació dels enemics naturals clau involucrats en el control de *M. persicae* ha de ser el primer pas per a començar un programa de control biològic per conservació. La implementació de recursos florals prop dels camps, podria ajudar a millorar el control, ja que ofereixen als enemics naturals recursos alimentaris com nèctar i pol·len, contribuint així a augmentar les seves poblacions en el moment adient per a controlar eficaçment la plaga. Els dos primers capítols d'aquesta tesi, estan dedicats a identificar els depredadors i parasitoides clau de *M. persicae* en la comarca del Segrià (Catalunya), així com a avaluar, en condicions de camp, el potencial de certes plantes insectàries per incrementar les poblacions d'enemics naturals. Per a determinar els enemics naturals de *M. persicae* es van utilitzar plantes sentinella, plançons de presseguer expressament infestats amb *M. persicae*. Per identificar les espècies més abundants es van utilitzar mètodes moleculars i morfològics. Posteriorment es va

avaluar l'atracció dels enemics naturals clau cap a marges florals que incloïen les següents espècies: *Achillea millefolium* L. (Compositae), *Lobularia maritima* L. (Brassicaceae), *Moricandia arvensis* L. (Brassicaceae) i *Sinapis alba* L. (Brassicaceae). Els resultats van mostrar que els enemics naturals predominants de *M. persicae* eren els parasitoides. Tot i l'ampli rang d'espècies, *Aphidius matricariae* Haliday (Hymenoptera: Braconidae: Aphidiinae) va ser amb diferència la més abundant. Dels pugons parasitats es van identificar també deu espècies d'hiperparasitoides. Amb tot, l'absència d'hiperparasitoides a principi de la temporada, quan les poblacions de *M. persicae* són elevades, suggereix que el control biològic d'aquest pugó no es veuria afectat negativament. Pel que fa als depredadors, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) i *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) van ser els més abundants, seguits de *Orius majusculus* Reuter (Hemiptera: Anthocoridae). Els experiments de camp realitzats en aquesta tesi, mostren que la presència de taques florals va ser atractiva per als sírfids i per als parasitoides, i cap de les plantes avaluades va ser reservori de pugons nocius per al cultiu. Entre les quatre plantes insectari avaluades, *L. marítima* va ser un bon recurs per als enemics naturals. No obstant això, la proximitat de les plantes insectàries no va incrementar l'abundància de les poblacions d'enemics naturals en les plantes sentinella, probablement degut a la mida de les parcel·les.

Per a poder contribuir de manera positiva al estat físic (*fitness* en anglès) dels enemics naturals, i per tant al control biològic de *M. persicae*, les flors de les plantes insectàries han de proporcionar als parasitoides i depredadors aliment que puguin aprofitar tant pel que fa a la qualitat com a l'accessibilitat. Basat en l'anterior, el tercer capítol d'aquesta tesi es va centrar en avaluar si la planta insectària *L. marítima* és una font d'aliment adequada per al parasitoide *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae: Aphidiinae) i el depredador *A. aphidimyza*. Amb aquest objectiu, es van realitzar experiments de laboratori per investigar si tots dos enemics naturals eren atrets cap a *L. marítima* en presència i absència de flors. També es van avaluar els efectes de les flors pel que fa a la longevitat i el potencial reproductiu. Els resultats dels experiments duts a terme en l'olfactòmetre van mostrar que els compostos volàtils produïts per *L. maritima*, amb i sense flors, atreien tots dos enemics naturals. Amb tot, aquesta atracció es veia afectada quan *L. marítima* es van comparar amb brots de presseguer infestats amb *M. persicae*. Tot i que *A. aphidimyza* es va beneficiar d'una alimentació rica en sucre, la disponibilitat de *L. maritima* no va millorar ni la longevitat ni la reproducció d’aquest depredador, ja que la morfologia floral d'aquesta espècie va impedir que les femelles s'alimentessin del nèctar. D'altra banda, la disponibilitat de flors de *L. maritima* va incrementar la longevitat de les femelles d’ *A. ervi* en absència de pugons. Per tant, la provisió d'altres recursos rics en sucre, com nectaris extraflorals i flors amb nectaris exposats poden ser una opció per millorar el control biològic de *M. persicae*.

A causa de la gran diversitat d'enemics naturals que ataquen a *M. persicae* en l'àrea d'estudi, existeix una alta probabilitat que hi hagi depredació intragremial, ja que aquests enemics naturals no només competeixen pel mateix recurs alimentari, sinó que també poden depredar-se entre ells. A més, degut al desenvolupament de les larves dels parasitoides dins del pugó, els depredadors se’ls poden menjar en alimentar-se de pugó. D’altra banda, la presència d'enemics naturals en una colònia de pugons també pot produir-los-hi canvis de comportament. L'últim capítol d'aquesta tesi va tenir com a objectiu avaluar les interaccions entre depredadors i parasitoides, i com aquestes interaccions poden influir en el control biològic de *M. persicae*. Es va determinar la contribució d’*E. balteatus, A. aphidimyza* i *O. majusculus* al control de *M. persicae*. També es van avaluar els canvis en el comportament del pugó derivats de el líquid secretat per les cornícules o sifons i la interacció d'aquests depredadors amb el parasitoide *A. matricariae*. Els resultats van mostrar que el depredador més voraç va ser *E. balteatus*, seguit d’ *A. aphidimyza*. *Orius majusculus* no va reduir la població de *M. persicae*. El líquid secretat per les cornícules de *M. persicae* va desencadenar una compensació de fecunditat, és a dir, un augment en la descendència. Tot i que, *A. aphidimyza* i *O. majusculus* van preferir matar pugons no parasitats, els dos depredadors van ser capaços de matar als individus de *M. persicae* parasitats que encara no estaven momificats. La seva preferència per matar pugons no parasitats suggereix que la coexistència de parasitoides i depredadors no seria negativa pel control biològic de *M. persicae*.

Els resultats d'aquesta tesi, suggereixen que el control biològic per conservació, pot ser una eina útil per millorar el control de *M. persicae* en cultius de préssec i nectarina.

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1 GENERAL INTRODUCTION

GENERAL INTRODUCTION

Peach taxonomy and origin

The peach is a deciduous tree that belongs to the family Rosaceae, subfamily Prunoideae, genus Prunus, subgenus Amygdalus, and species *persica* (L.) Batsch. It is native to China. Commercial peaches are usually divided by botanists into different varieties: yellow and white peaches, freestone, clingstone, doughnut peaches (var. platycarpa L.H. Bailey) and nectarine (var. nucipersica (Borkh) Schneider). In addition to the peach tree, other *Prunus* species are also of economic importance, such as the apricot (*P. armeniaca* L.), almond (*P. dulcis* Mill), cherry (*P. avium* L.), and plum (*P. domestica* L. and *P. salicina* L.).

Peach production in the World, Europe and Spain

Peach tree is the third most produced fruit species in the world after apples and pears. According to the Food and Agriculture Organization of the United Nations (FAO), in 2017, around 1.5 million hectares (Mha) of *P. persica* were cultivated worldwide with a production of almost 25 million tons (Mt). China is the world's leading producer, exceeding 50% of both; area and yield, followed by Europe and North America (Figure 1). Within Europe, in 2017 Spain was the main peach producer with more than 30% of the world's total production.

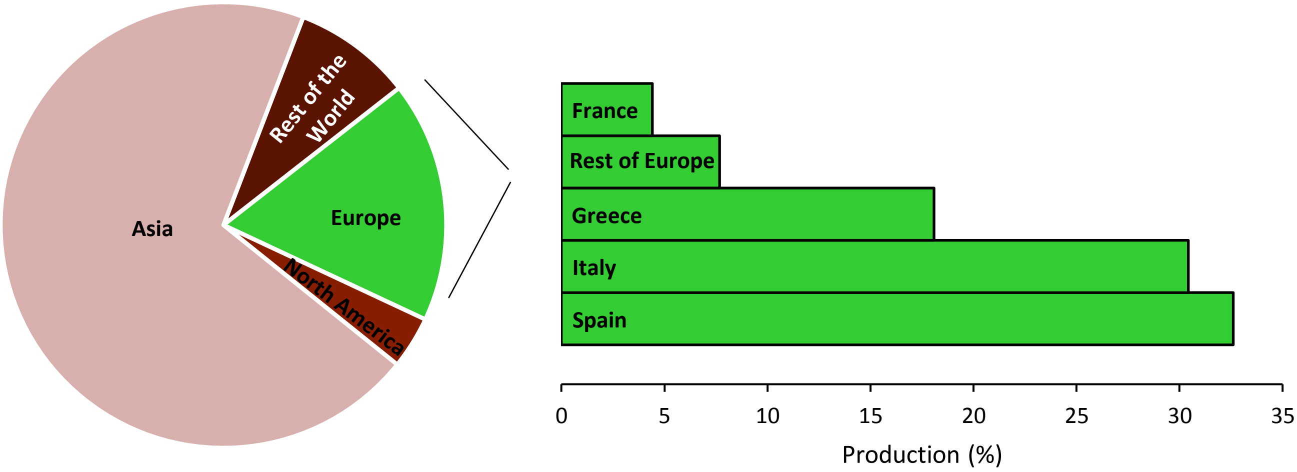

Figure 1. Distribution (%) of peach production in tones during 2017 (Source: FAOSTAT 2017).

The peach production in Spain has increased over the years not only due to an increase in the plantation area, but also because of the modernization of the Spanish peach industry. Peach orchards are mainly drip irrigated. They have adopted new efficient training systems, improved cultivars as well as implemented more efficient rootstocks (Llacer et al. 2009). Spain is the biggest exporter of stone fruit in Europe, with a share of around 50% (mainly peaches and nectarines). The main countries of destination are the United Kingdom, Germany and France (CBI, 2017).

According to the Ministerio de Agricultura, Pesca y Alimentación (MAPA) in 2017, the main peach producing regions in Spain were Catalonia, Aragon, Murcia, Extremadura and Andalusia (Figure 2). All of them share a dry and hot climate that are very suitable for peach production.

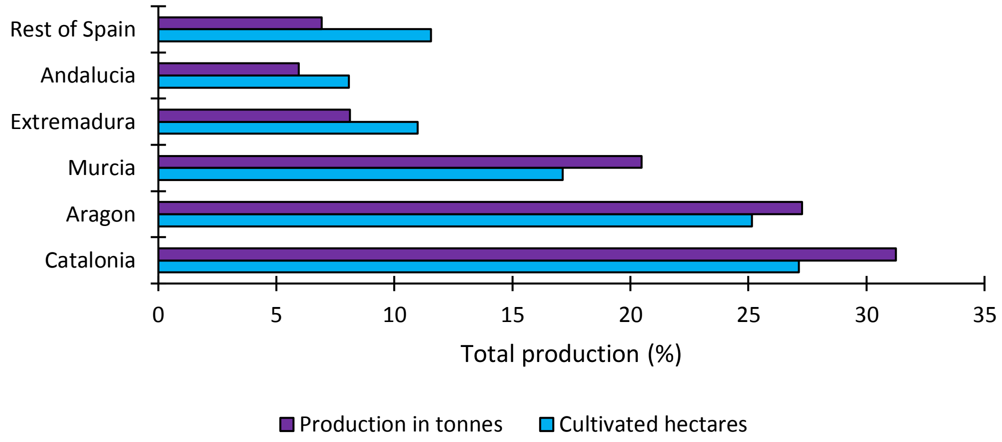

Figure 2. Main peach production Spanish regions (cultivated ha and tonnes) (Source: MAPA 2017).

In Catalonia, located in the North East of Spain, the area of Segrià (Figure 3) is the leading producer, exceeding 80% in terms of area and yield (16 thousand hectares and 450 thousand tonnes; DARP 2018). The production of peaches is predominant, with 65% of the total, followed by nectarines, with 33%, and doughnut peach, with 2% (Llácer et al., 2009).

Figure 3. Location of Segrià area in Catalonia and in Spain.

Main peach pests other than aphids, and strategies used on their control

According to a survey conducted in 2015 among the pest advisors working on fruit orchards in the Segrià area, besides of aphids, the most harmful pests of peach and nectarines include two species of Lepidoptera *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae) and the oriental fruit moth, *Grapholita molesta* Busck (Lepidoptera: Tortricidae); the fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae); the thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae); and the leafhopper *Asymmetrasca decedens* (Paoli) (Hemiptera: Cicadellidae) (Gabarra, Arnó, Riudavets and Aparicio, unpublished).

Among Lepidoptera, *A. lineatella* and *G. molesta* seriously affect peach trees (Rothschild and Vickers 1991; Damos and Savopoulou-Soultani 2006). *Anarsia lineatella* mostly attacks peach and almond. Other susceptible crops include apricot, nectarine, plum, prune, cherry, apple and persimmon trees (Ponomarenko 1990; Damos and Savopoulou-Soultani 2008). The larva causes severe damage by feeding on young twigs and fruits. As fruit matures, it becomes highly susceptible to attack; damage is most likely to occur from color break to harvest (Summers 1955; Curtis 1983). *Grapholita molesta* damages developing shoots and fruits. Larvae tunnel in tender twigs causing their dieback. However, the most severe damage occurs on fruit when the young larvae enter the fruit and bore to the center, feeding around the pit. After reaching maturity, they exit from the fruit and pupate.

The Mediterranean fruit fly (medfly) is a highly adaptive polyphagous tropical fruit fly (Papadopoulos et al., 1996), which attacks several plant species (Weems 1981; Liquido et al., 1991). Larvae feed upon the pulp of host fruits, sometimes tunneling through it, which allow the entry of secondary pathogens, which destroy the fruit (Bergsten et al., 1999). Peach pulp becomes soft, eventually acquiring an almost liquid consistency (Ros, 1988).

Frankliniella occidentalis is the principal thrips pest species in peach orchards in Spain (Lacasa 1993; Gonzalez et al., 1994) and in other Mediterranean areas such as Lleida (Torá et al., 2010; Teulon et al., 2018) and it is considered a serious pest especially in nectarines. They can be found in flowers and fruits, where most of the damage is shown. Feeding by thrips results in and unaesthetic 'silvering', that is more apparent when the fruit ripens downgrading its quality.

The leafhopper *A. decedens* (Paoli) is the predominant species affecting peach trees. Damage to plants is caused by feeding. Leaves suffer discoloration, deformation, leaf curling, or necrosis from the apex to the basis of the leaves, usually known as hopper burn (Alvarado et al., 1994; Jacas et al., 2000; Torres et al., 1998). Additionally, it has been demonstrated that this species can also transmit phytoplasms to plums and apricots (Pastore et al., 2004).

Avilla et al. (2008) reviewed the main strategies used in Integrated Pest Management (IPM) programs implemented in Catalonia. According to these authors, control techniques based on the semiochemicals are on use for a number of pests. Mating disruption is the main tool to control the Lepidoptera *A. lineatella* and *G. molesta*. This technique has shown to be effective even at high initial populations (Barnes and Blomefield 1997; Cardé et al., 1977; Gentry et al., 1980; Audemard et al., 1992; Minks et al., 1992; Rice et al., 1992). Control strategy for medfly is based in the use of mass trapping with very good results (Howse et al., 1998; Batllori et al., 2008). For thrips, alternative methods based in the detection of olfactory cues are under development but are not yet implemented (Teulon et al., 2018) and the control relies mostly in the use of insecticides, same as the control of *A. decedens.* Additionally, some insecticides are still needed to control *A. lineatella*, *G. molesta* and *C. capitata* because under certain circumstances, mating disruption and mass trapping do not provide complete control of these pests

(Avilla et al., 2008). As a consequence, the repetitive use of pesticides over years have facilitate the development of insecticide resistance to a number of compounds in lepidopteran pests (Kanga et al., 2003; Jones et al., 2011), medfly (Arouri et al., 2015; Magaña et al., 2007) and thrips (Bielza 2008; Espinosa et al., 2002, Herron et al., 2005).

***Myzus persicae* and its control**

The green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphidiidae) was ranked as the most harmful species to peach and nectarine trees in the survey conducted in the Segrià area in 2015 (Gabarra, Arnó, Riudavets and Aparicio, unpublished). Although to our knowledge there has been no formal evaluation of yield loss due to this aphid at any production area, it is considered as one of the most injurious species worldwide (Blackman and Eastop, 2000; Dedryver et al., 2010). *Hyalopterus* genus is also of economic importance in peach crops. Both cause important damage to the host such as leaf twisting, pitting and discoloring fruits (Fig. 4), and also are important vectors of the plum pox virus or Sharka (Isac et al., 1998; Katis et al., 2007; Barbagallo et al., 2017).

Figure 4. Peach shoot infested with *M. persicae* and damage to a nectarine fruit

The life cycle of M. persicae is shown in figure 5. *Prunus* spp. trees are the primary host of the aphids *M. persicae* and *Hyalopterus* spp. (van Emden 1969; Dixon 1987; Hardie 2017). They are necessary for aphid survival since they overwinter as eggs in peach trees. The eggs measure about 0.6 mm long and 0.3 mm wide, and they are initially yellow/green, but turn black. In spring, after egg hatching, the nymph feed on flowers, young foliage, and stems. Nymphs give rise to sequential generations of apterous morphs. Females reproduce parthenogenetically and give birth to young nymphs. Development often takes 10 to 12 days for a complete sexual generation. After several generations, winged dispersants move to summer hosts. As aphid densities increase or plant condition deteriorates, winged forms are again produced to disperse. The dispersants typically produce about 20 offspring, which are always wingless. This cycle is repeated throughout the period of favorable weather. In favorable climates, parthenogenetic reproduction continues through the year. In cold climates, adults return to *Prunus* spp. in the autumn. Females arrive first and give birth to wingless egg-laying forms (oviparae). Males are attracted to oviparae (egg-producing females) by a pheromone. The oviparous female (pink in color) deposits between four to 13 eggs, usually near of *Prunus* spp. buds.

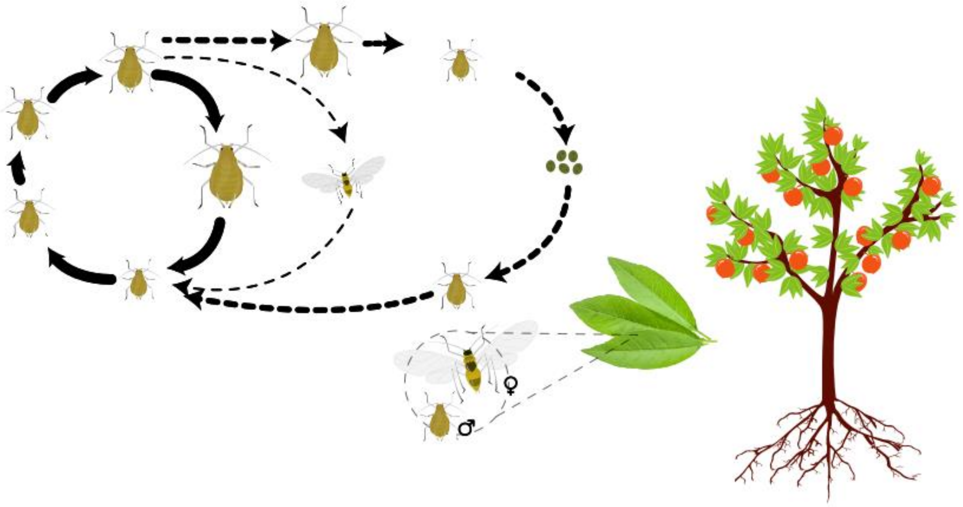

Figure 5. *Myzus persicae* life cycle

Although different aphid management tools, such as host-plant resistance, cultural and biological control (van Emden and Harrington 2017) have been studied, traditionally the most used tool for the control of aphids in peach trees have been insecticides (Barbagallo et al., 2017). Active ingredients used in aphid control have evolved with time from organophosphates and carbamates to pyretroids and neonicotinoinds, to move more recently to novel molecules with a friendlier environmental profile (Dewar and Denholm 2017). This over-reliance on pesticides has triggered the development of widespread and multiple forms of resistance to several insecticides such as pyrethroids, neonicotinoids, organophosphates and carbamates that often make chemicals ineffective (Foster et al., 2007). The long list of resistance by *M. persicae* to active ingredients (more than 80), makes it one of the most widely and strongly resistant species worldwide (APRD 2019). In the study area, resistance to neonicotinoids by *M. persicae* has also been detected, which has implications for aphid control in peach trees (Slater et al., 2012).

In parallel with these technical problems with the use of insecticides, a growing social concern to limit the risk associated with insecticides has developed in European and other developed countries. In this scenario biological control of aphids might be a useful tool to reduce the number of insecticide sprays in the peach orchards. Many natural enemies have been recorded feeding on aphids, mainly lady beetles (Coleoptera: Coccinellidae), hoverflies (Diptera: Syrphidae), lacewings (Neuroptera: mainly Chrysopidae), predatory midges (Diptera: Cecidomyiidae), predatory bugs (Hemiptera: Anthocoridae), parasitic wasps (Hymenoptera: Braconidae), and entomopathogenic fungi, mainly Entomophthorales (Barbagallo et al., 2017). Parasitoids are the most important control agents of aphids (Starý, 1973; Hagvar and Hofsvang, 1991; Völkl et al., 2007). The efficacy of parasitoids for *M. persiace* control in *Prunus* spp. trees has been demonstrated in several papers (e.g. Starý 1976; Kavallieratos and Tomanović 2001; Tomanović and Kavallieratos 2002; Kavallieratos et al., 2004, 2005, 2006; Tomanović et al., 2003, 2006).

Conservation Biological Control

Conservation Biological control (CBC) is a strategy aiming to protect and enhance the natural enemy population to contribute to the biological control of pests in the agroecosystem (Eilenberg et al., 2001; Begg et al., 2017). Limiting the use of insecticides is the first step to protect natural enemies present in the orchards, as was shown to occur in peach (Penvern et al., 2010). In addition, the provision of favorable habitats and resources, especially during non-crop periods, will contribute to increase diversity and abundance of predators and parasitoids. This will help to counterbalance the effects of agriculture intensification, tillage, short-blooming period of many crop plants, use of insecticides, and several other disturbances associated to modern cropping systems (e.g. Fiedler et al., 2008; Walton and Isaacs 2011; Begg et al., 2017).

A common practice in the CBC, is the implementation of ecological infrastructures such as ecological paths or strips, which typically consist of selected non-crop plants that provide food sources and overwintering shelters for local natural enemies, and also can protect them from pesticides (Landis et al., 2000; Tscharntke et al., 2007). It has been shown that the use of some perennial flowering plant species provides resources for an extended period of time (Fiedler and Landis, 2007), and they could enable natural enemies to remain near the field even when prey densities are low (Olson et al., 2005).

Although there are numerous papers reporting on the high diversity of natural enemies that are found to attack *M. persicae* (e.g. Brodeur et al., 2017), there is a consensus that these natural enemies do not provide an adequate aphid control in peach orchards (Avilla et al., 2008; Barbagallo et al., 2017). However, Dedryver et al., (2010) suggested that CBC are the best alternative for biological control of aphids in open field crops, which is the case of fruit orchards. There are some works exploring the possibilities of enhancing natural enemies by using habitat management in fruit crops. In Chinese peach orchards, Wan et al. (2014 a, b) demonstrated that a ground cover of *Trifolium repens* L. enhanced the diversity of generalist predators in trees canopies and decreased aphids and *G. molesta* incidence. Other studies have shown that ecological infrastructures in peach orchards could enhance diversity and abundance of natural enemies and may contribute to the control of aphids in citrus trees (Gomez-Marco et al., 2015) and apple trees (Simon et al., 2010; Rodríguez-Gasol et al., 2019).

The great diversity of aphid natural enemies increases the chances for Intraguild Predation (IGP) to exist. The IGP occurs when a number of natural enemies may compete for the same resource (extraguild prey) and additionally may feed on each other (intraguild predator over intraguild prey) (Wratten and Powell 1991; Rosenheim et al., 1995; Polis and Holt 1997; Dixon 1998; Lucas et al., 1998; Lee and Kang 2004; Lucas 2005). IGP can be unidirectional when one of the guild’s members is always the prey (IG-prey) and the other member always the predator (IG-predators), or mutual, when the interaction goes in both directions, the predator becoming the prey and vice-versa (Lucas 2005; van Emden and Harrintong 2017). In the case of parasitoids, the IGP is coincidental since predators kill the parasitoids when feeding on the aphid (Polis et al., 1989). IGP constitute a primary mortality factor for the most vulnerable stages of some aphidophagous species (Lucas 2005). Since in the field, most aphid colonies are exploited simultaneously by several aphidophagous species, they constitute ideal systems for intraguild interactions.

Despite the importance of peach crops in Europe, there are few studies aimed to study the potential of biological control in aphid management in this crop (Remaudière and Leclant 1971, Penvern et al., 2010, Aparicio et al., 2019). Therefore, the possibilities of implementing successful biological control programs in peach orchards remains unknown.

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OBJECTIVES

The main objective of this thesis was to evaluate the possibility of implementing conservation biological control against the aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) in peach and nectarine orchards in the Mediterranean area. To achieve this, four specific objectives were set:

Objective 1. Identify the key predators of *M. persicae*, and evaluate in field conditions, the potential contribution of insectary plants located in the vicinity of peach orchards to enhance populations of natural enemies of aphids (Chapter 1).

Objective 2. Identify the most abundant hymenopteran parasitoids and hyperparasitoids species attacking *M. persicae* in peach trees (Chapter 2).

Objective 3. Determine to what extent the flowers of the insectary plant *Lobularia maritima* can be used by *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) and *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), two natural enemies of *M. persicae*, as a food source that contribute to improve their fitness (Chapter 3).

Objective 4. Assess the interactions among three predators and one parasitoid species of *M. persicae* and their effects on aphid population growth. (Chapter 4).

CHAPTER 1

Can floral resources enhance the abundance of natural enemies of *Myzus persicae* in peach orchards?

17.1.1 Introduction

Peach and nectarine (*Prunus persica* L. Batsch) are important crops in Europe, which is the second worldwide producer after China. Spain is a leader producer in Europe with 30% of the total of European production (FAOSTAT 2017). In Spain, Catalonia concentrates 24% of the total Spanish production, mostly in the province of Lleida where 20000 ha are dedicated to this crop (MAPA 2017).

Myzus persicae (Sulzer) (Hemiptera: Aphididae), the green peach aphid, is one of the most important pests on peach and nectarine, their primary hosts. Although to our knowledge there has been no formal evaluation of yield loss in peach due to this aphid species, it has been acknowledged as a very injurious pest (Dedryver et al, 2010). Damages to peach and nectarine include leaf twisting, pitting and discolored fruits, and vectoring of important viruses, such as plum pox virus or sharka (Penvern et al. 2010; Barbagallo et al. 2017).

Aphids in peach and nectarine crops have been usually managed with insecticide sprays (Barbagallo et al., 2017). However, there is a growing social concern to limit pesticide use due to the risks that these products pose to the human health and the environment. *Myzus persicae* resistance to numerous active substances such as pyrethroids, neonicotinoids, organophosphates and carbamates, renders many insecticide treatments often ineffective (Foster et al. 2017). Therefore, it is urgent to develop aphid management strategies that are more sustainable and socially acceptable. In this scenario, biological control, and more specifically conservation biological control could be a good tool to low down the use of insecticides in peach and nectarine crops (Dedryver et al., 2010; Penvern et al., 2010).

Conservation biological control relies on preserving the already existing natural enemies and enhancing their populations in the agroecosystem by adopting several management techniques that do not hamper their establishment in the crop (Dedryver et al 2010). The natural enemies of the aphids belong to different taxonomic groups from entomopathogenic organisms to parasitoids and also include several specialist and generalist predators. Among predators, those belonging to the families Cecidomyiidae, Coccinelliidae, Chrysopidae and Syrphidae are the most commonly mentioned in the literature (Brodeur et al. 2017). However, there are few studies about the aphid natural enemies present on peach and nectarine orchards in the Mediterranean (Reamaudière and Leclant 1971, Penvern et al, 2010; Aparicio et al. 2018). In Spain, Avilla et al. (2008) stated that the rich complex of natural enemies of aphids present in peach orchards, including parasitoids and generalist predators, are not enough to provide satisfactory pest control. However, these authors did not explicit which were the natural enemies present and their relative abundance.

The intensification of agriculture, the loss of habitat, and the use of insecticides have caused a lack of adequate resources for the beneficial insects, including the natural enemies of aphids, in the agricultural ecosystems (Landis et al. 2000; Biesmeijer et al., 2006; Haaland et al. 2011). Some strategies to overcome this scenario and enhance the presence of beneficial insects in crops often involve the addition of floral resources (Kremen and Chaplin-Kramer, 2007; Bianchi and Wäckers, 2008). The floral resources provide nectar, pollen and shelter to natural enemies when the crop is not in bloom

(Lavandero et al, 2005; Hogg et al, 2011), and contribute to predators and parasitoids complex stay perennial in the agroecosystem (Wyss 1996; Brown 2001). The presence of ecological infrastructures with flowering plants in orchards has already been shown to enhance populations of natural enemies of some aphid species (Tylianakis et al 2004; Miñarro et al, 2005; Gontijo et al, 2013; Rodríguez-Gasol et al, 2019). Nonetheless, flower species must be carefully chosen, taking into account that they have to be accessible to natural enemies, they must be adapted to the environment, and they will not be a reservoir of harmful pests or diseases for the crop (Baggen and Gurr, 1998; Colley and Luna, 2009; Hogg et al., 2011).

The main objectives of this study were to : 1) identify the key predators of *M. persicae* in peach and nectarine orchards in the Mediterranean fruit production area of Spain; 2) determine whether insectary plants previously identified as promising candidates enhance natural enemy populations in peach and nectarines orchards, and assess if they harbor harmful pests for the crop; and 4) if proximity of the insectary plants to the crop may boost the abundance of natural enemies of *M. persicae* in peach.

17.1.2 Materials and methods

Study area and experimental set-up

The study was conducted in 2015 and 2016 in four organic peach and nectarine orchards located in the area of Segrià (Lleida, Catalonia) in the northeast of Spain. In each orchard, four patches of sown insectary plants (hereafter 'sown flower patches') of 1 m^2 were planted on a row in one of the field margins at about 5 m from the first row of trees. Another four patches of resident vegetation (hereafter 'resident vegetation) of 1m^2 were selected in another field margin, also about 5 m apart from the first row of trees and were used as controls. Distances between the two groups of sown flower and resident vegetation patches were varied according to field shape and size, and ranged from 25 to 130 m.

Each sown flower patch consisted of four boxes (50 cm length, 35.5 cm width and 31 cm height) each planted with 10 plants of one of the following insectary plant species: *Achillea millefolium* L. (Compositae)*, Lobularia maritima* L. (Brassicaceae)*, Moricandia arvensis* L. (Brassicaceae), or *Sinapis alba* L. (Brassicaceae). These plants were selected according to previous results of our group (Arnó et al. 2012; Arnó et al. 2018; Alins unpublished). Plants were grown in a greenhouse from seed on pots with potting soil and transplanted to the boxes and taken to the field by mid-April in 2015, and mid-March in 2016. Both years plants were kept in the sites until mid-September. They were periodically drip irrigated similarly to the trees. Most of the plants were taken to the field in bloom and their phenological stage during the sampling has been compiled in Table 1.

Table 1. Description of the phenological stages for each plant species during sampling periods of 2015 and 2016: in bloom (*), vegetative (v) and dry (▪).

	Phenological stage (week)							
	2015				2016			
Insectary plants	18	20	22	24	18	20	22	24
L. maritima	*	*	*	*	*	*	*	*
M. arvensis	*	*	*	*	*	*	*	*
S. alba	*	*	*	*	*	*	▪	▪
A. millefolium	v	v	v	v	v	v	*	*

The plant composition of the resident vegetation was highly variable and included a number of species commonly found in fruit orchard margins. Table 2 summarizes the species that, while flowering, were represented in more of the 25% of the surface of the resident vegetation patches during the sampling period of both years.

Table 2. Dates in which the different plant genera were in bloom and present in more than 25 % of the surface of the resident vegetation patches.

	2015			2016		
Plant genera	may-13	may-27	June-10	may-11	may-25	June-08
Galium	●	●		●	●	●
Malva	●	●	●	●	●	●
Hordeum	●					●
Trifolium		●				
Avena		●	●			
Bromus		●	●			
Carduus				●	●	●
Sonchus					●	
Convolvulus						●

Assessment of natural enemies associated to aphid infested colonies

Sentinel plants were used in order to identify the natural enemies associated with *M. persicae* colonies. For this, small potted peach plants (approximately 50 cm high) were infested *ad-hoc* with *M. persicae* obtained from excised peach shoots already infested with approximately 100 *M. persicae* (adults and mixed instars), and collected from the same orchards in Lleida. They were placed on top of the plants and then kept for a week in a closed screened greenhouse to prevent contamination. Afterwards, 16 sentinel plants were taken to each orchard and placed under the peach canopy. In order to avoid plant desiccation, they were placed in bigger pots filled with water. The outside of these pots were sprayed with insect-trapping adhesive to prevent ants and other soil predators climbing the plant. Sentinel plants were placed at two distances from the field edge: four plants were placed near the first tree of four adjacent rows, just in front of the sown flower patches. The other four were placed at a distance of ca. 5m, between the second and the third tree of the same rows. Another group of eight sentinel plants were placed following the same pattern in front of the resident vegetation. Sentinel plants were taken to the orchards at 15-day intervals and left there for one week. Afterwards, the infested buds were collected and taken to the laboratory.

All Cecidomyiidae larvae present in those shoots were placed on microscope slides and classified (Harris 1966, 1973). Syrphid larvae and *Orius* spp. nymphs and adults were individually stored at −20 °C for subsequent molecular analyses. They were individually analyzed by conventional PCR following the methods developed by Gómez-Polo et al

(2014) and Gómez-Polo et al (2013). Non-amplified specimens were tested using the universal primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale et al, 2011) to confirm the presence of DNA. These amplifications were conducted using the following cycling condition: initial denaturation at 94° C for five minutes, followed by 40 cycles of 94° C for 30 seconds, 46° C for 45 seconds, 68° C for 45 seconds, and a final extension of 68° C for 10 m. All PCR products were analyzed by gel electrophoresis (2.4% agarose gels) and visualized with GelRed® (Biotium, Hayward, CA).

Attractiveness of the insectary plants to beneficial arthropods and phytophagous insects.

To evaluate the attractiveness of insectary plants to natural enemies and phytophagous insects, two types of samples were taken on both treatments: 1) visual observations and 2) beating tray method (hereafter 'beating'). For the visual observations, we recorded the number of adult hoverflies hovering above the patches for three minutes. In the sown flower patches, the number of hoverflies that landed on each insectary plant during the same period was also recorded. The frappage targeted the insect community that cannot be seen during their flight and usually are within the plant foliage or in the flowers. In the sown flower patches, a bunch of each plant species was separately hand-beated three consecutive times on a plastic white tray (24 x 35 cm). Insects that fell on the tray were visually classified 'in situ' as hymenopteran parasitoids, *Orius* spp., ladybeetles, lacewings, aphids or thrips other than *Aelothrips* spp. (hereafter 'thrips'). *Aelothrips* spp. were excluded from the records because they are known to be thrips and not aphid predators (Mound and Marullo 1993; Riudavets 1995; zur Strassen 1995). The same methods and records were done in the resident vegetation but, instead of individual plant species, five and four randomly selected bunches of vegetation per patch were beaten in 2015 and in 2016, respectively. All individuals were returned to the patches after the identification, and all beatings were always done after the visual observations. Both methods were conducted fortnightly from early-April until early-August during both years on sunny days (always above 15°C) between 9.00-13.00 hours.

Data analysis

Data was analyzed for each year separately, using a generalized linear mixed-effects model (GLMM) with negative binomial (NB) response distribution. The number of insects caught with the frappage and the number of landings by hoverflies per insectary plant species in the visual observations, were the response variables. The sown flower patches and the resident vegetation (treatment factor) was the main fixed effect. Orchards and plots ($1m^2$) were the random factors. Pairwise comparisons (Post-hoc tests) were carried out with the Tukey's method for multiple comparisons. The number of arthropods in the frappage method were compared among five treatments: the four insectary plants and the resident vegetation (controls). Number of syrphid adults' landings was compared among the four insectary plants. Data from the visual observations were referred to as number of hoverflies per plot and per insectary plant species and time (three minutes / $1m^2$ or insectary plants). Arthropods in the frappage and in the sentinel plants, were referred to as individuals per white tray and per sentinel plant. The statistical analysis was performed using R v3.5.3., a language and environment for statistical computing. For all statistical tests a nominal significance level of 5% ($p<0.05$) was applied.

17.1.3 Results

Which are the key predators of *M. persicae* in peach orchards in the Mediterranean area?

Cecidomyiidae and Syrphidae larvae were the most prevalent predators found in the sentinel plants, and represented 57% and 26% respectively, of the total amount of predators collected during both years. Adults and larvae of *Orius* spp., ladybeetles and lacewings were by far less present in the aphid colonies of the sentinel plants.

The morphological identification of collected Cecidomyiidae yielded a single species, namely: *Aphidoletes aphidimyza* Rondani (Diptera). As can be observed in Figure 1, this predator was consistently present during both years and its population peaked on week 22 (end of May).

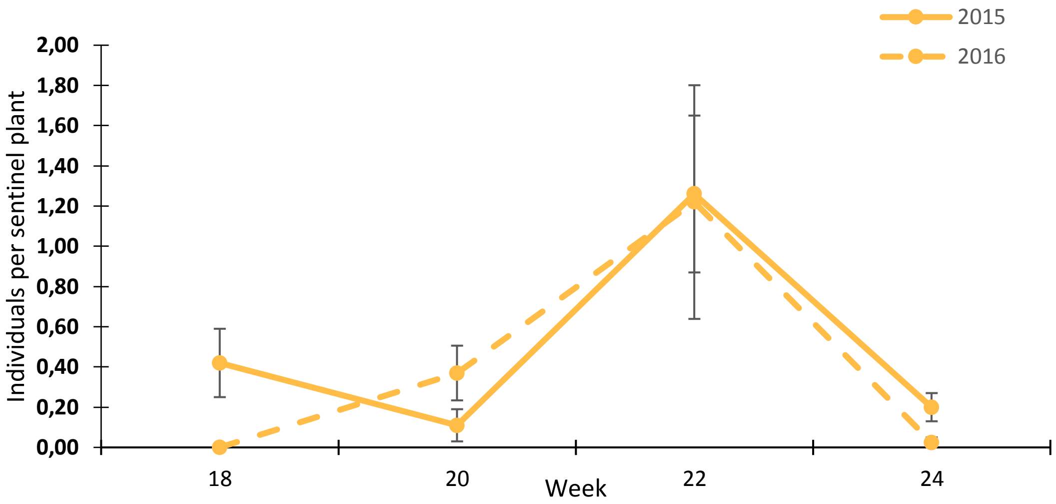

Figure 1. Seasonal abundances of Cecidomydiidae larvae (mean ± SE) recorded in peach sentinel plants placed in peach orchards.

Regarding hoverflies, 83 larvae were collected during 2015, and 60 were identified by multiplex PCR; 55 larvae were identified as *E. balteatus* and five as *Sphaerophoria* spp. (Le Peletier & Serville). The remaining 23 samples did not show amplification with the syrphid-specific primers used. During 2016, 19 hoverfly larvae were collected with the sentinel plants. PCR results identified seven larvae as *E. balteatus* and three individuals as *Sphaerophoria* spp. The remaining 9 specimens did not show amplification with the syrphid-specific primers used. All non-amplified specimens from both years were amplified when using the universal primers ZBJ-ArtF1c – ZBJ-Artr2c, indicating that they may be other syrphid species than those included in this PCR multiplex.

During 2016, the number of hoverfly larvae collected in the sentinel plants was much lower than in 2015. However, same phenological pattern was observed in both years with population peaking during week 20 (mid May).

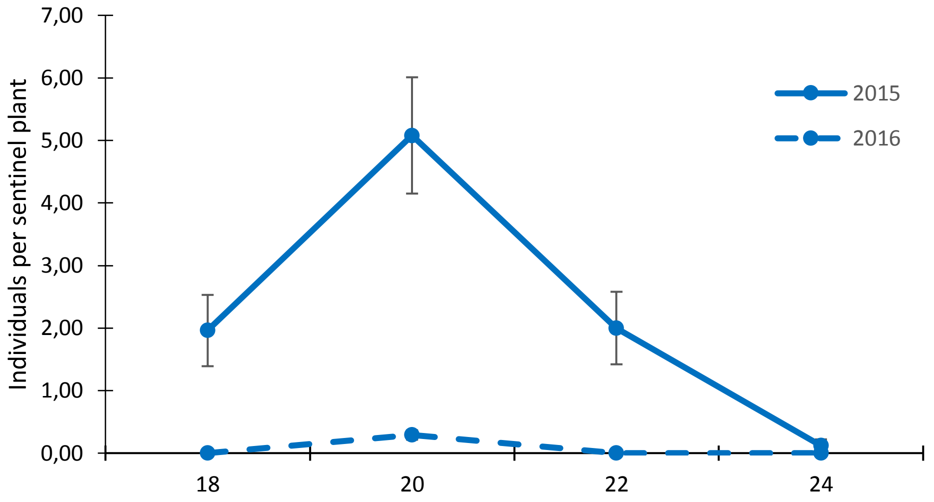

Figure 2. Seasonal abundances of Syrphidae larvae (mean± SE) recorded in peach sentinel plants placed in peach orchards.

In 2015, 32 *Orius* spp, immatures and adults, were collected, but none in 2016. The conventional PCR allowed to identify 21 *Orius majusculus* Reuter and three *Orius niger* (Wolff) (Hemiptera: Anthocoridae). The remaining eight *Orius* individuals did not show amplification with the primers used; however, a band was amplified when they were analyzed with the universal primers ZBJ-ArtF1c – ZBJ-ArtR2c, indicating that they might be other *Orius* species. Most of the *Orius* spp. were found at the beginning of May and beginning of June.

Ladybeetles and lacewings where found at minor quantities. Overall, 16 ladybeetles adults and larvae, and 15 lacewings larvae were found during both years. Population of both natural enemies peaked in week 22 (end of May). In 2015, a pick of lacewings larvae in week 24 was noted (mid June).

Insectary plants as suitable resources for natural enemies and reservoirs for peach pests

The number of hoverflies hovering on treatment patches (Fig. 3) was significantly higher over the sown flower patches than over the resident vegetation (2015: Z= 6.130, P<0.0001; 2016: Z= 4.111, P< 0.0001). Significant differences were also observed on the number of landings on the different insectary plants (Fig. 4). In 2015 and 2016 hoverflies landed significantly more times on *L. maritima*, *M. arvensis*, and *S. alba* than on *A. millefollium* (2015: χ^2= 24.91, P<0.0001; 2016: χ^2=21.14, P<0.0001).

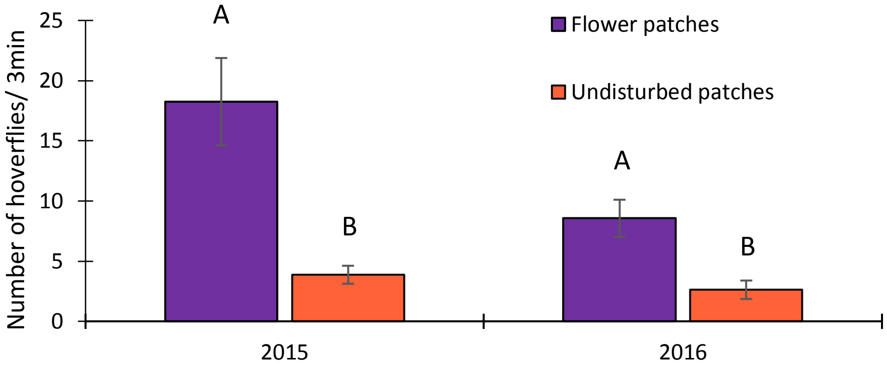

Figure 3. Number of adult hoverflies (mean ± SE) hovering above sown flower and resident vegetation patches during three minutes in each sampling year. For each year different upper case letters indicate differences among patches (P < 0.05).

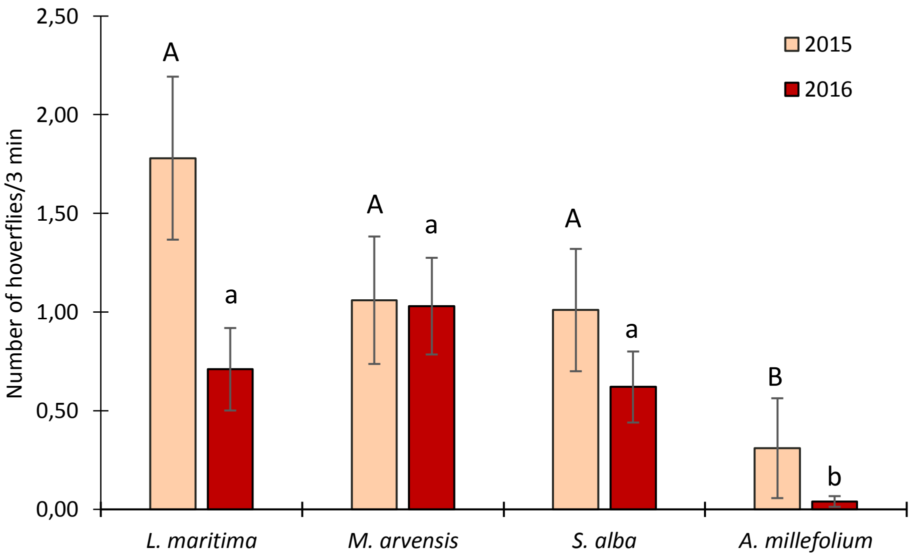

Figure 4. Number of hoverflies landings (mean ± SE) per insectary plant during three minutes observation of sown flower patches in both sampling years. Different letters indicate differences among insectary plants in 2015 (upper case) and in 2016 (lower case) (Tukey`s Post Hoc Multiple Comparisons; P < 0.05).

Results obtained with the beating method indicate that in the sown flower and resident vegetation patches (arround the peach orchards), the more abundant natural enemies were hymenopteran parasitoids (59% and 45% of the total in 2015 and 2016, respectively), followed by aphid predators as ladybeetles, *Orius* spp. and lacewings (74, 49 and 12 individuals, respectively, pooling together data from 2015 and 2016). A total of 1165 aphids and 2357 thrips were counted during 2015 beating samplings and 531 aphids and 875 thrips in 2016.

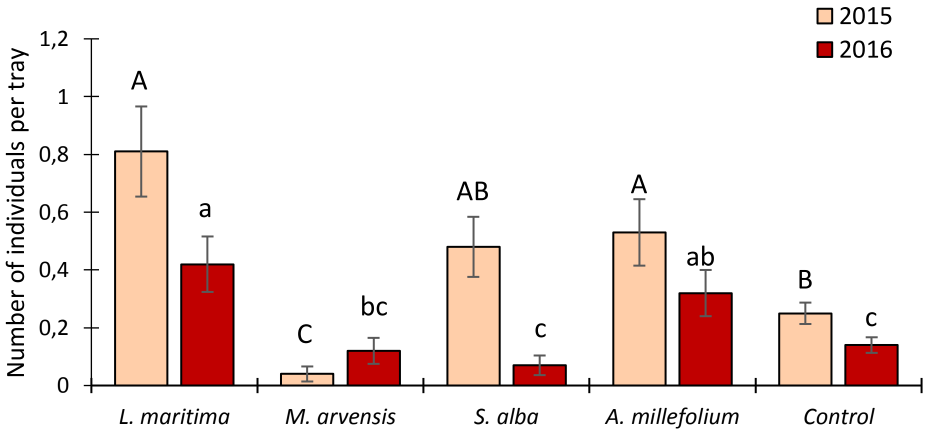

Figure 6. Number of hymenopteran parasitoids (mean ± SE) recorded per insectary plant via frappage during both sampling years. Different letters indicate differences among insectary plants in 2015 (upper case) and in 2016 (lower case) (Tukey`s Post Hoc Multiple Comparisons; P < 0.05).

Both years, significantly higher number of hymenopteran parasitoids were found on *L. maritima* and *A. millefollium* than in the resident vegetation (2015: χ^2= 50.650, P<0.0001; 2016: χ^2=22.323, P=0.0001) (Fig. 6). Abundance on *M. arvensis* and on *S. alba* had intermediate values between the two more attractive plants and the resident vegetation but *S. alba* varied with the year.

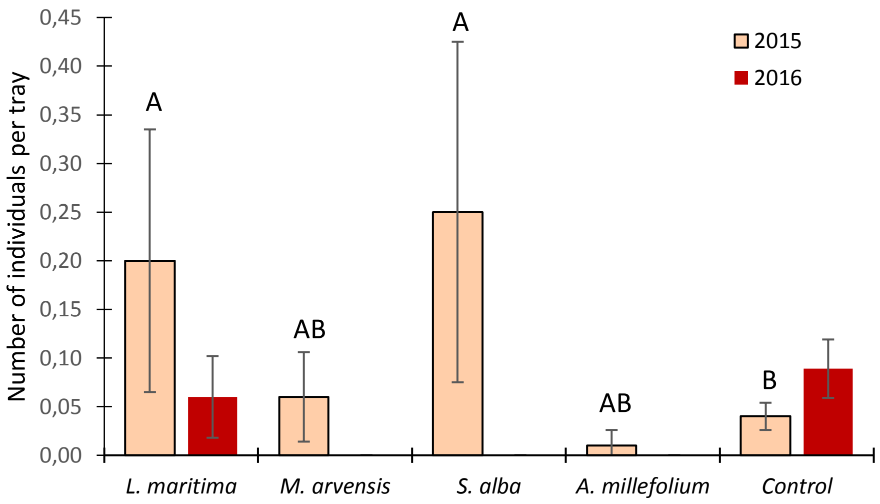

Figure 7. Number of coccinellids (mean ± SE) recorded per insectary plant via beating method during both sampling years. Different upper case letters indicate differences among insectary plants in 2015 (Tukey`s Post Hoc Multiple Comparisons; P < 0.05). Statistical analysis was not performed for the year 2016.

In 2015, significantly higher number of Coccinellidae were recorded in *L. maritima* and *S. alba* compared to the control, while abundance in the other two insectary plants had intermediate values (χ^2=13.975, P=0.0073) (Fig. 7). In 2016, ladybeetles were only found in *L. maritima* and on resident vegetation with similar abundances. No statistical analysis was performed for 2016 data due to the absence of these predators in most of the insectary plants.

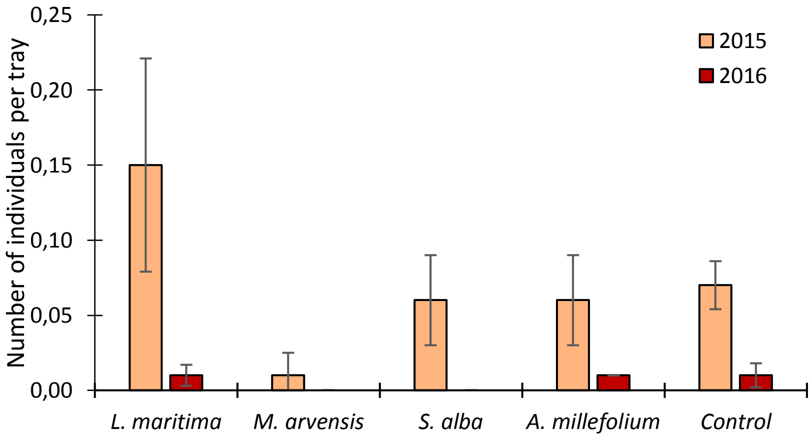

Figure 8. Number of *Orius* spp. (mean ± SE) recorded per insectary plant via frappage during both sampling years.

No significantly different *Orius* spp. population was found when sampling the insectary plants and the resident vegetation (2015: χ^2=6.052, P=0.1952; 2016: χ^2=7.6798, P= 0.10404) (Fig. 8). However, in 2015 the highest number of individuals was recorded in *L. maritima*. In 2016, due to the low number of individuals found the statistical analysis was not conducted.

Due to the low number of lacewings (2 and 10, in 2015 and 2016 respectively), no statistical analysis was performed.

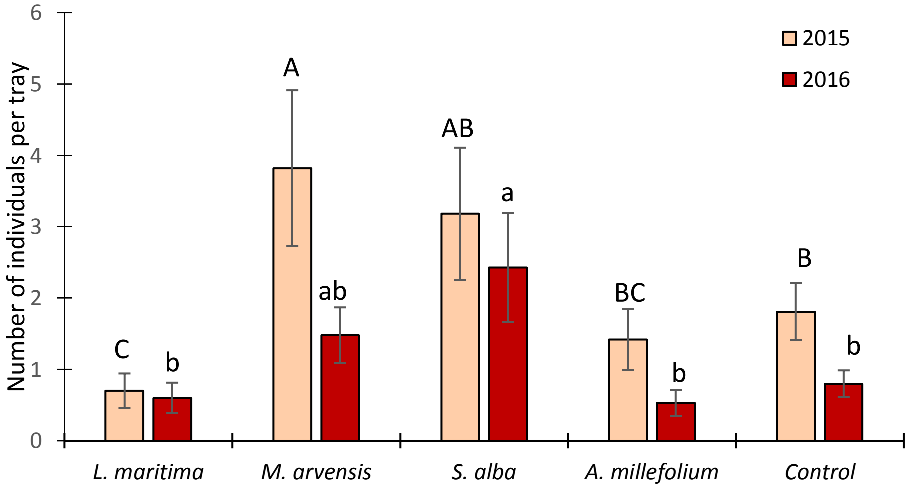

Figure 9. Number of aphids (mean ± SE) recorded per insectary plant via frappage during both sampling years. Different letters indicate differences among insectary plants in 2015 (upper case) and in 2016 (lower case) (Tukey`s Post Hoc Multiple Comparisons; P < 0.05).

Regarding potential pests, in 2015 *M. arvensis* hosted significantly more aphids than the resident vegetation, and *S. alba* had intermediate abundance values (χ^2= 39.086, P<0.0001) (Fig. 9). The reverse situation was recorded in 2016, with significantly more aphids in *S. alba* than in the resident vegetation, and with *M. arvensis* showing intermediate values of abundance (χ^2=19.554, P<0.0001). Although, aphid species were not identified, no *M. persicae* neither *Hyalopterus* spp. were observed during the samplings.

Both years, the number of thrips on *S. alba* was significantly higher than in the other insectary plants and the resident vegetation (2015: χ2=24.614, P<0.0001; 2016: χ2=16.150, P=0.0028) (Fig. 10). Values in *M. arvensis* were intermediate between *S. alba* and the control.

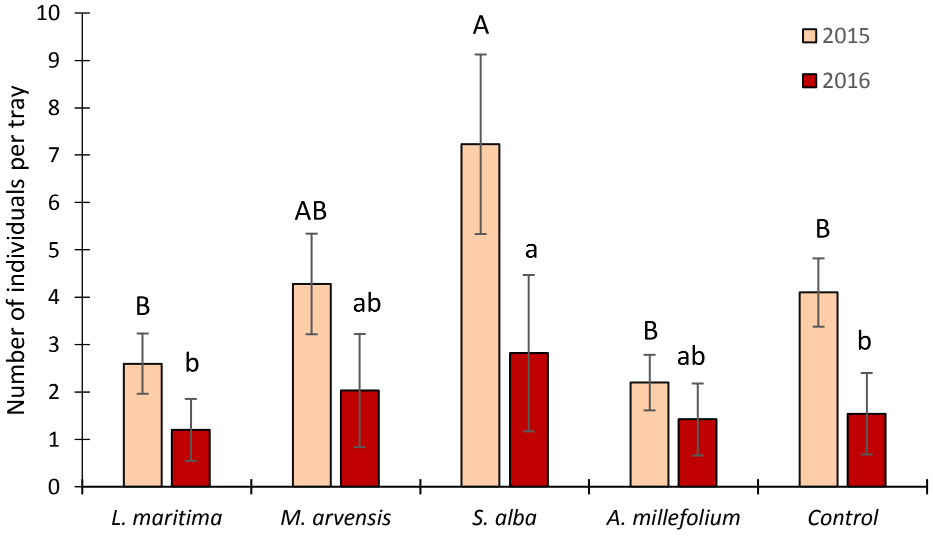

Figure 10. Number of thrips (mean ± SE) recorded per insectary plant via frappage during both sampling years. Different letters indicate differences among insectary plants in 2015 (upper case) and in 2016 (lower case) (Tukey`s Post Hoc Multiple Comparisons; P < 0.05).

Effect of the proximity of sown flower patches on the abundance of *M. persicae* natural enemies

When analyzing differences in natural enemies abundance between both groups of sentinel plants, close to the sown flower patches and those close to the resident vegetation, significant differences were found only for hoverfly larvae in 2015 (Table 3). In this year, number of hoverfly larvae in sentinel plants close to the sown flower patches was two-fold higher that number of larvae on sentinel plants close to the resident vegetation.

Table 3. Natural enemies (mean ± SE) recorded with sentinel plants near sown flower patches and near resident vegetation in each sampling year.

Year	2015				2016			
Arthropods	Near sown flower patches	Near resident vegetation	Z	P	Near sown flower patches	Near resident vegetation	Z	P
Parasitoids	1,54 ± 0,329	1,92 ± 0,278	-1,225	0,220	2,18 ± 0,391	3,13 ± 0,716	-0,916	0,360
Hoverflies	0,43 ± 0,087	0,22 ± 0,087	1,996	**0,046**	0,11 ± 0,045	0,10 ± 0,033	0,274	0,784
Cecidomids	0,41 ± 0,161	0,59 ± 0,162	-1,161	0,246	0,62 ± 0,340	0,45 ± 0,178	0,527	0,593
Anthocorids	0,11 ± 0,040	0,13 ± 0,038	-0,302	0,763				
Coccinelids					0,05 ± 0,023	0,10 ± 0,042	-0,974	0,333

17.1.4 Discussion

Our results from sentinel plants showed that although five different groups of predators were detected on the aphid colonies, the gall-midge *A. aphidimyza* was the most abundant predator followed by the hoverfly *E. balteatus*. In surveys conducted in the Southeast of France Remaudière and Leclant (1971) and Penvern et al. (2010), did not found *A. aphidimyza* as a predator associated to *M. persicae* populations, but they refer to hoverflies as abundant predators in peach orchards. Rodriguez-Gasol et al (2019) also identified hoverflies and gall-midges in colonies of *Dysaphis plantaginea* Passerini (Hemiptera. Aphidae) on apple orchards located in the same area of our study. Miñarro et al. (2005) and Dib et al. (2010) also report these predators as being important in apple in Asturias (Spain) and in southeastern France, respectively. The prevalence of *E. balteatus* in front of other hoverfly species as aphid predators in fruit orchards may be related to the seasonality, because *M. persicae* attacks peach early in the season when temperatures are still moderate. *Episyrphus balteatus* is not adapted to high temperatures (above 25ºC), and high rates of mortality occurr when this temperature is exceeded (Hart and Bale, 1997). On the other hand, *Sphaerophoria* spp. is better adapted to higher temperatures, which occur later in the season (Pineda and Marcos-García, 2008). Since our samplings were carried out during spring, it may explain the highest records of *E. balteatus*. In our samplings, *A. aphidimyza* individuals appeared later in the season. Later presence of *A. aphidimyza* compared to *E. balteatus* have been also reported in apple orchards (Brown and Lightner, 1997; Miñarro et al. 2005). These two predators, together with *A. matricariae* the prevalent parasitoid of *M. persicae* in the area of study (Aparicio et al. 2019) have to be considered the key natural enemies of *M. persicae* in peach.

Our surveys also demonstrate that *O. majusculus* and *O. niger* colonized sentinel plants infested with *M. persicae* and even laid eggs on that plants, since we found both adults and nymphs when infested shoots were taken to the laboratory. Although they are well-known thrips predators (Riudavets 1995; Riudavets and Castañé, 1998) they have also been recognized to feed on aphids (Alvarado et al 1997). Being polyphagous, their presence in the sentinel plants could be taken as an indicator of activity in peach orchards, which can be positive for the biological control of other pests such as thrips, which are relevant pests, especially in nectarine. Avilla et al. (2008) indicate that *Orius laevigatus* (Fieber) are found together with other Anthocoriade in peach orchards in Spain, and Remaudière and Leclant (1971) found some *Orius minutus* (L. (Hemiptera: Anthocoridae) individuals on peach shoots infested with aphids.

Surprisingly, other predators such as ladybeetles and lacewings, that are generally recognized as aphid predators in deciduous fruit trees (Barbagallo et al. 2017), have been found only in small numbers in our samplings. The scarcity of these predators in our study might be related to the seasonality of our samplings that took place during the period when *M. persicae* populations typically damage crops in the area of study. Miñarro et al. (2005), Dib et al., (2010) and Rodríguez-Gasol et al. (2019) recorded large numbers of Coccinellidae and Chrysopidae in apple orchards later in the season (beginning of July). Furthermore, sampling methodology may have also influenced our results because our sentinel plants were approximately only 50 cm high, and were placed under the tree canopy. Sárospataki and Markó (1995) studied the flight activity of *Coccinella setempuctata* L. (Coleoptera: Coccinellidae) in an oak forest using traps and found higher numbers of captures at the canopy level, or even higher, than close to the ground. Similarly, Duelli (2008) placed yellow traps between 1.5 and 2 m to study flight pattern of *Chrysopa carnea* (Steph.) (Neuroptera: Chrysopidae).

Aphidoletes aphidimyza was recorded as the most abundant predator in the sentinel plants but was never found neither in the insectary plants nor in the resident vegetation. Although it is attracted by flowers as for example *L. maritima* (Aparicio et al. 2018), the absence of individuals recorded with the frappage was probably mediated by the behavior of this predator and the fact that with the frappage, only the upper part of the plants was beated. The adults only fly at dusk and during the night (Harris 1973), and in daytime they hide in shaded parts of the plant (Boulanger et al. 2019). In the study area, weather during light hours is hot and dry. Thus, it is conceivable that adults remain immobile in the lower parts of the vegetation where temperatures are milder, and humidity is higher.

According to our results, hymenoptera parasitoids and hoverflies were more attracted to the sown flower patches than to the resident vegetation, probably because of the more abundant and prolonged flower presence in the insectary plants that provided more suitable and permanent food resources. Feeding on flower nectar is common for hoverflies and hymenoptera parasitoids that use this sugar-rich resource mainly to fuel their foraging and searching oviposition sites (Wäckers 2005; Nicolson et al 2007). Additionally, floral resources such as nectar and pollen are used by hoverflies as a protein source for ovary maturation and egg production (Branquart and Hemptinne, 2000; van Rijn et al, 2013). Similarly, some flowers have shown to have a positive effect on the reproduction of hymenopteran parasitoids (Berndt and Wratten 2005, Araj and Wratten 2015; Aparicio et al. 2018; Arnó et al. 2018).

In our samplings, *L. maritima* appeared to be a suitable resource for hoverflies and parasitoids. Our results agree with several papers reporting that the addition of *L. maritima* in the field improves the abundance of hoverflies (Gontijo et al. 2013; Hogg et al., 2011) and hymenopteran parasitoids (Chaney 1998; Sivinski et al 2006; Rohrig et al 2008; Arnó et al 2012). Additionally, under laboratory conditions, it has been shown that the availability of this floral resource enhances the fitness of some parasitoid species (Chaney 1998; Aparicio et al., 2018; Arnó et al., 2018; Johanowicz and Mitchell 2000).

Achillea millefollium attracted hymenoptera parasitoids but not hoverflies. The attraction to *A. millefolium* has been reported for parasitoids (Dib et al. 2012; El-Nabawy et al 2015; Arnó et al. 2012) and also for hoverflies (Colley and Luna 2000) usually associated to the presence of flowers. However, during our samplings *A. millefolium* was blooming only two weeks in 2016, and despite this lack of blooming this plant species succeeded to recruit adult wasps, suggesting that not only flowers but also some other compounds present in leaves are also attractive to these natural enemies.

Sinapis alba and *M. arvensis* were suitable resources for hoverflies but did not attract other natural enemies' groups, except for parasitoids in 2015. Both insectary plant species are beneficial for some parasitoid species (Vattala et al 2006; Arnó et al 2012; 2018). These two species hosted the highest number of aphids and thrips. Although, aphid species present in our insectary plants or the resident vegetation were not identified, they were not *M. persicae* nor *Hyalopterus* spp. The latter aphid species is also an important pest of peach trees, but infestations occurs later in the season (Barbagallo et al 2017; Authors´ personal observations). Therefore, our results suggested that this insectary plants are not reservoirs of damaging aphids for peach crops in our area. Concerning thrips, during the sampling several morphospecies were detected during the samplings, some of which resembled *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), an important pest in peach and nectarines orchards in Spain (Lacasa 1993, Gonzalez et al 1994; Avilla et al. 2018). Although the presence of these herbivores could be considered a negative trait for insectary plants near crops, they might be also play an important role as alternative prey that will help to establish and built up natural enemies populations (Boivin et al. 2012; Norris and Kogan 2000).

The presence of ladybeetles and *Orius* spp. was in general terms higher in the insectary plants, especially in *L. maritima*, than in the resident vegetation. Both predators are omnivores, and they benefit from feeding on nectar and pollen (Coll and Guershon, 2002; De Clercq et al. 2005; Forehand et al. 2006; Fiedler and Landis 2007; Witting et al. 2007; Vandekerkhove and De Clercq, 2010). Additionally, they use the non-flowering parts to rest, mate, egg lay, pupate and take shelter.

The proximity of sown flower patches to peach orchards, did not increase the abundance of natural enemies of *M. persicae*, except for hoverflies in 2015, when there were twice as many individuals in sentinel plants close to sown flower patches than in those close to the resident vegetation. Our results could be influenced by the size of the orchards. Distances between the two groups of sentinel plants (close to the sown flower patches vs. close to the resident vegetation) ranged from 25 to 130 m. It has been suggested that parasitoids and some predators, including anthocorids and coccinellids, can move between crops at long distances, mainly during the spring (Pons and Starý

2003; Lumbierres et al. 2007) hoverflies can fly distances of up to 200 m (Wratten et al., 2003), and van Schelt and Mulder (2000) found *A. aphidimyza* eggs on plants at distances up to 45 m from the release point.

In summary, there is a wide array of natural enemies associated to *M. persicae* in peach orchards and their presence is probably influenced by the surroundings. Our field sites were close to other orchards and arable crops, (e.g., wheat, barley, maize, alfalfa, oats and rye grass). It has been shown that other aphid species present in these nearby crops, may act as reservoirs for parasitoids before they move to orchards in spring (Pons et al 1993; Lumbierres et al., 2007). The same scenario is for *O. majusculus,* which also has been recorded in these crops and move to adjacent orchards (Madeira et al 2014; Ardanuy et al. 2017). In our study, the sown flower patches concentrated natural enemies, suggesting that the addition of selected species flowering early in the spring could enhance key natural enemies for the biological control of *M. persice*. One of these plants could be *L. maritima*, although this species is not native in this peach production area, and although flourished during most of our sampling period, it does not survive in winter (Picó and Retana 2001; Aparicio, personal observation). Selection of suitable candidate plants to attract and enhance natural enemies, but also well adapted to the environmental and agronomic conditions of the productive system is crucial to design successful ecological infrastructures.

17.1.5 References

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CHAPTER 2

Hymenoptera complex associated with *Myzus persicae* and *Hyalopterus* spp. in peach orchards in northeastern Spain and prospects for biological control of aphids

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17.2.1 Introduction

Peaches and nectarines (*Prunus persica* L. Batsch) are economically important crops worldwide. China is the world's leading producer, with more than 50% of global peach crops by both area and yield, followed by Europe and North America. Within Europe, Spain is a leading producer and accounts for more than 30% of total European production (FAOSTAT, 2017), and most of Spain's peach orchards are concentrated in the Catalonia region in northeastern Spain (MAPA, 2017). Ten aphid species have been reported to infest European peach trees (Alhmedi et al., 2018; Kavallieratos et al., 2008) with the green peach aphid, *Myzus persicae* (Sulzer) and species of the *Hyalopterus* genus being the most serious pests. Peach trees are the primary host for both aphids and are necessary for their survival (Basky 1982; Blackman and Eastop 2000). Although to our knowledge there has been no formal evaluation of yield loss due to these pests (Dedryver et al., 2010), they have been acknowledged as causing important damage to peach crops, including leaf twisting and pitting and discoloration of fruits. They are also important vectors of the plum pox virus or sharka, which is a serious disease affecting stone fruits (Isac et al., 2010; Penvern et al., 2010; Barbagallo et al., 2017).

To date, aphid control in most crops has mainly involved the use of insecticides, but there is increasing concern about their use and the risk they pose to both humans and the environment (Dedryver et al., 2010). In addition, resistance to a wide range of insecticides, such as pyrethroids, organophosphates and carbamates has been recorded (Penvern et al., 2010; Foster et al., 1998). In the northeastern region of Spain, *M. persicae* have also been reported to have developed resistance to neonicotinoids (Slater et al., 2012). Alternative tools for managing aphids are therefore urgently needed, and biological controls could be a useful component of integrated pest management (IPM) programmes.

Parasitoids are among the main aphid biocontrol agents and several species have been shown to be effective in reducing aphid populations (Penvern et al., 2010; Angalet et al., 1977; Starý et al., 1998; Thies et al., 2005). Hyperparasitoids, on the other hand, attack parasitized aphids, and therefore, present a risk to the short-term biological control of aphids (Brodeur and McNeil 1994; Rosenheim 1998; Sampaio et al., 2017; Schooler et al., 2011; Sullivan 1987). It has been predicted that an equilibrated system that includes host aphids, primary parasitoids and hyperparasitoids may be beneficial for long-term biological control in the agroecosystem (Beddintong and Hammond 1977).

As a first step in designing a biocontrol-based IPM programmes to control aphids in peach orchards, it is crucial to identify the species most abundantly involved in the parasitoid-hyperparasitoid system. This information will be useful not only for promoting conservation biological control but also for selecting the most convenient parasitoid species to release, if augmentative releases to complement natural biological control are determined to be necessary. Therefore, the present study was undertaken to identify naturally occurring hymenopteran species and the relative abundance of *M. persicae* and *Hyalopterus* spp., the aphid species that cause the most damage to peach trees.

17.2.2 Materials and Methods

Samples were collected from two areas within Catalonia: one area was located inland (Lleida), and the other was in the coastal area of Barcelona. Lleida is one of the biggest peaches and nectarines production areas in Spain, accounting for 24% of total Spanish production, and it has almost 20,000 hectares of peach and nectarine orchards. In contrast, the Barcelona area has only 587 hectares (MAPA 2017). Sample sites in Lleida consisted of four commercial organic orchards with the following GPS coordinates: 41.718, 0.618 in Vilanova de Segrià, 41.627, 0.541 in Torres de Sanui, 41.810, 0.582 in Almenar and 41.832, 0.548 in Alfarràs. In Barcelona, the survey was conducted in one experimental plot at IRTA facilities located at 41.516, 2.372 in Cabrils, with hybrid trees derived from an initial cross of *P. persica* × *P. dulcis*. The study was conducted between April 2015 and July 2017.

<u>Species Identification of *M. persicae* Parasitoids and Hyperparasitoids</u>

The Lleida sites were sampled using two methods: sentinel plants and random collections of aphid-infested shoots (Table 1). Sentinel plants consisted of small potted peach plants (approximately 50 cm tall) infested with *M. persicae*. To infest the plants, excised peach buds with approximately 100 *M. persicae* (adults and different instars), collected from the same orchards in Lleida, were placed on top of the plants and kept there for one week before taking the plants to the field. During this time, the plants were kept in a closed screened greenhouse to prevent contamination. Once in the orchards, 16 sentinel plants per field were placed under the peach tree canopy: 4 sentinels were placed near the first tree of 4 adjacent rows and another 4 sentinels were placed between the second and the third trees of the same rows. Another 8 sentinels were placed following the same pattern at the other end of the field. The distance between the two groups of 8 plants differed depending on the size of the field and ranged from 25 to 130 m. The pots containing the sentinel plants were placed inside bigger pots with water to avoid desiccation, and the outside of the outer pots was sprayed with insect-trapping adhesive to prevent ants and other soil predators from climbing the plant. Sentinel plants were taken to the orchards at 15-day intervals and were left there for one week. Then, the infested leaves were collected and taken to the laboratory. The sampling period lasted from mid-April to the end of June, which is the period when *M. persicae* populations typically damage crops in the sampled area.

To complement the sampling with sentinel plants, random samples of naturally-infested shoots were also collected from the same orchards during the same sampling periods. Neither the number of samples nor the periodicity of this sampling followed a specific pattern, because the number of infested trees varied between orchards and sampling dates (Table 1).

All plant samples from both the sentinel pots and the random sampling were transported to the laboratory in ice chests. Once in the laboratory, predators were removed from samples and the aphid colonies were placed in mesh-covered, semi-transparent plastic boxes in climatic chambers at 25 °C that were checked daily during working days until either parasitoids or hyperparasitoids emerged. For the sentinel plants, each plant was processed individually as a single sample. For the random collection samples, all infested twigs from one date and one field were pooled and treated as a single sample. Adult parasitoids and hyperparasitoids were preserved in 70% alcohol in microtubes (2 mL), and individuals were classified at the species level using taxonomic keys by Graham (1969), Kamijo and Takada (1973), Rakshani et al., (2012; 2015), Kavallieratos et al., (2013), and Ghaliow et al., (2018). All samples belonging to the *Encyrtidae* family were sent to Dr John Noyes at the British Museum for further classification. Species were identified as either primary parasitoids or hyperparasitoids following the classification by Sullivan (1987).

Species Identification of *Hyalopterus* spp. Parasitoids and Hyperparasitoids

We collected random samples of peach shoots infested by *Hyalopterus* spp. in Lleida and Barcelona sites from mid-April to the end of July (Table 1). As above, neither the number of samples nor the periodicity of this sampling followed a specific pattern, because the number of infested trees varied between orchards and sampling dates (Table 1). Samples were handled using the same methodology described above for the parasitoids and hyperparasitoids of *M. persicae*.

Table 1. Sampling conducted to identify the parasitoid and hyperparasitoid species associated with *M. persicae* and *Hyalopterus* spp. and their abundance in peach trees. The time period of the samples collected is expressed in week numbers, where week 16 corresponds to mid-April and week 31 to end of July.

			Year		
			2015	**2016**	**2017**
Aphid species	**Area**	**Sampling method**	**Number of samples collected (time period)**		
Myzus persicae	Lleida	Sentinel plants	4 (18–24)	6 (16–26)	—
		Random samples	5 (18–27)	6 (17–23)	2 (19–20)
Hyalopterus spp.	Lleida	Random samples	5 (22–31)	2 (23–28)	1 (21)
	Barcelona	Random samples	—	3 (23–26)	10 (16–27)

17.2.3 Results

Species Identification of *M. persicae* Parasitoids and Hyperparasitoids

A total of 626 parasitoids and 57 hyperparasitoids were collected from the sentinel plants infested with *M. persicae* during two years of sampling, while 246 parasitoids and 124 hyperparasitoids were collected from the randomly collected samples over 3 years of sampling. We identified a total of 11 different *M. persicae* primary parasitoid species from six genera. Of these 11 species, *Aphidius matricariae* Haliday (Hymenoptera: Braconidae: Aphidiinae) was by far the most abundant in all three years and within both the sentinel plants and the randomly collected samples. This species accounted for 91% of the primary parasitoids that emerged from aphid mummies (Table 2).

Table 2. *Myzus persicae* parasitoids and hyperparasitoids, collected on peach trees using both sampling methods during the 3-year study.

Family and Subfamily	**Species**	**Sentinel plants**		**Random sampling**		
		2015	**2016**	**2015**	**2016**	**2017**
Parasitoids						
Braconidae						
Aphidiinae						
	Aphidius matricariae (Haliday)	344	213	27	26	180
	Aphidius ervi Haliday	12	8	0	0	9
	Aphidius colemani Viereck	1	16	0	0	0
	Aphidius transcaspicus Telenga	2	0	0	0	0
	Lipolexis sp.	4	11	0	0	1
	Ephedrus persicae Froggat	0	5	0	3	0
	Ephedrus plagiator (Nees)	1	0	0	0	0
	Praon volucre (Haliday)	1	5	0	0	0
	Praon abjectum (Haliday)	0	1	0	0	0
	Diaeretiella rapae (McIntosh)	1	0	0	0	0
	Lysiphlebus testaceipes (Cresson)	0	1	0	0	0
Hyperparasitoids						
Figitidae						
Charipinae						
	Phaenoglyphis villosa (Hartig)	6	18	1	23	0
	Alloxysta pusilla (Kieffer)	3	4	2	14	0
	Alloxysta victrix (Westwood)	0	5	0	3	0
	Alloxysta arcuata (Kieffer)	4	0	0	0	4
	Alloxysta castanea (Hartig)	0	0	0	1	0
	Alloxysta fuscicornis (Hartig)	0	0	0	0	1
Pteromalidae						
Asaphinae						
	Asaphes suspensus (Nees)	0	0	0	38	1
	Asaphes vulgaris (Walker)	0	0	0	4	0

Pteromalinae						
	Pachyneuron aphidis (Bouché)	1	0	0	13	10
Encyrtidae						
Encyrtinae						
	Syrphophagus nr. africanus	16	0	0	9	0

Within the same samples, we also identified 10 species of hyperparasitoids associated with *M. persicae.* Six of these species belonged to the *Figitidae* family, three belonged to the *Pteromalidae* family and one belonged to the *Encyrtidae* family (Table 2). The most abundant species from both sampling methods was *Phaenoglyphis villosa* (Hartig) (Hymenoptera: Figitidae), which ranged from 20% of the hyperparasitoids collected from the sentinel plants in 2015 to almost 70% in 2016, as well as 20% of the hyperparasitoids collected from the pooled random samples across the whole 3-year period. *Asaphes suspensus* (Nees) (Hymenoptera: Pteromalidae) was the second-most abundant species; however, it was only found in the random samples.

Seasonal abundance differed between the primary parasitoids and the hyperparasitoids collected from the sentinel plants (Figure 1). The majority of the primary parasitoids were collected in week 18 (late April), whereas the majority of the hyperparasitoids were collected in week 22 (late May).

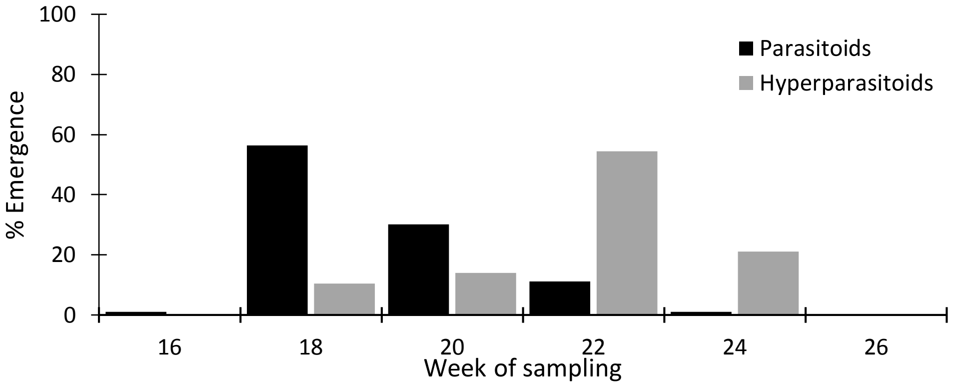

Figure 1. Bi-weekly seasonality of *M. persicae* parasitoids and hyperparasitoids from sentinel plants. The percentages of parasitoids and hyperparasitoids per sampling date were calculated based on the total number of parasitoids and hyperparasitoids, respectively, that emerged from aphid mummies.

Species Identification of *Hyalopterus* spp. Parasitoids and Hyperparasitoids

Out of the total of 558 parasitoids collected from *Hyalopterus* spp. samples, we identified only two different species, *Aphidius transcaspicus* Telenga and *Praon volucre* (Haliday) (Hymenoptera: Braconidae: Aphidiinae), during the three years of the study across both areas. Surprisingly, 82% of all parasitoids collected were *A. transcaspicus* specimens collected from a single sample in Barcelona. Among the rest of the collected samples, *A. transcaspicus* and *P. volucre* comprised 46% and 54%, respectively.

The most intensive sampling efforts for *Hyalopterus* spp. aphids occurred in 2017 in the Barcelona site; Figure 2 shows the seasonality of *Hyalopterus* spp. parasitoids for this year in this experimental plot. *Aphidius transcaspicus* was recorded from mid-May until mid-June, with 93% of all *A. transcaspicus* specimens collected at the beginning of June (week 23). Conversely, *P. volucre* individuals were collected from the beginning of sampling in mid-April (week 16) until the end of May (week 22), with the highest numbers being collected in weeks 16 and 17.

We collected 394 hyperparasitoids associated with *Hyalopterus* spp. during the 3-year study, including both surveyed areas. These were comprised of five different species of hyperparasitoid, with two belonging to the *Figitidae* family, one to the *Encyrtidae* family, and two to the *Pteromalidae* family. In both locations, the most abundant *Hyalopterus* spp. associated hyperparasitoid species was *Pachyneuron aphidis* (Bouché) (Hymenoptera: Pteromalidae: Pteromalinae) (Table 3), which accounted for 87% of all *Hyalopterus* spp. associated hyperparasitoids. *Syrphophagus nr. africanus* was the second-most abundant *Hyalopterus* spp. associated hyperparasitoid but accounted for only 5% of the total and was found mainly in the Lleida samples.

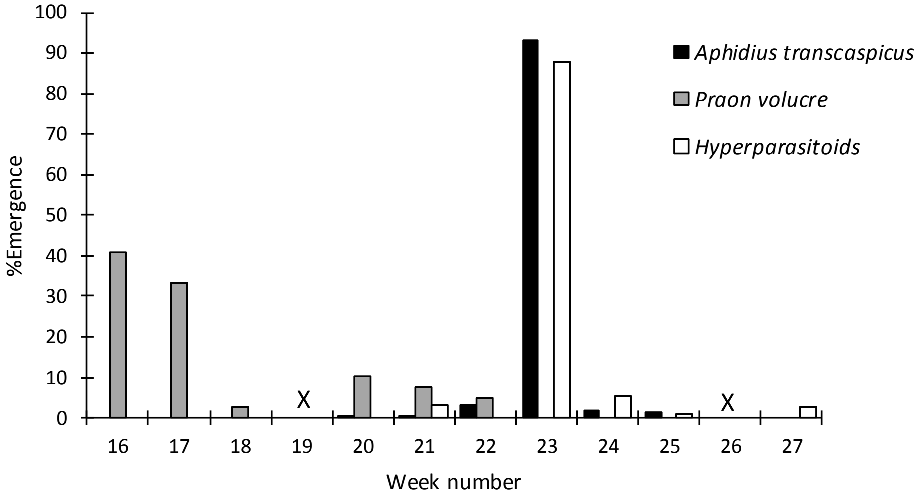

Figure 2. Seasonality of the *Hyalopterus* spp. parasitoid and associated hyperparasitoid species collected in Barcelona in 2017. The percentages of parasitoids and hyperparasitoids per sampling date were calculated from the total number of parasitoids and hyperparasitoids, respectively, that emerged from the mummies. X indicates that no sampling was conducted during that week.

Table 3. Number of *Hyalopterus* spp. associated hyperparasitoid species collected during the 3-year study in both surveyed areas.

Families and subfamilies	Species	Lleida	Barcelona
Pteromalidae			
Pteromalinae	*Pachyneuron aphidis* (Bouche)	53	260
Asaphinae	*Asaphes suspensus* (Nees)	2	8
Encyrtidae			
Encyrtinae	*Syrphophagus nr. africanus*	19	3
Figitidae			
Charipinae	*Phaenoglyphis villosa* (Hartig)	1	2
	Alloxysta fuscicornis (Hartig)	1	2

17.2.4 Discussion

During our 3-year study on peach trees, the most abundant *M. persicae* primary parasitoid species was, by far, *A. matricariae*, which accounted for 91% of all *M. persicae* parasitoids collected in our study. The tritrophic association between *P. persica*, *M. persicae*, and *A. matricariae* has been previously recorded in other regions of Spain (Lumbierres et al., 2007), and *A. matricariae* have in fact long been recognised as the most common and probably the most effective parasitoids of *M. persicae* (Mackauer 1968). This tritrophic association has also been found in other European countries, such as France (Remaudiere and Leclant 1971; Starý et al., 1971), Greece and Serbia (Kavallieratos et al., 2008).

However, while surveys by Michelena et al., (1994; 2004) found only two species of *M. persicae* parasitoids on peach trees, our study identified 11 different species. Six of these have been previously reported on *M. persicae* on peach trees in other European countries, namely *Aphidius colemani* Viereck*, Aphidius ervi* Haliday*, Ephedrus persicae* Froggat*, P. volucre, Diaeretiella. Rapae* (McIntosh) and *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae: Aphidiinae) (Kavallieratos et al., 2008; Remaudiere and Leclant 1971; Starý et al., 1971; Laamari et al., 2009; Pennacchio 1989). However, our sampling identified three additional tritrophic associations that have not previously been reported in Europe: *A. transcaspicus, Ephedrus plagiator* (Nees) *and Praon abjectum* (Haliday) (Hymenoptera: Braconidae: Aphidiinae).

Given that *P. persicae* are the primary hosts of *M. persicae*, parasitoids in early spring have to come from other surrounding crops. Boivin et al., (2012) emphasised the importance of suitable reservoirs near or within fields (e.g., in grassy ground covers in orchard alleys) for the survival of aphid parasitoids. In the Lleida study area, our field sites were close to other orchards and arable crops, (e.g., wheat, barley, maize, alfalfa, oats and rye grass). Pons et al. (1993) and Lumbierres et al., (2007) found *A. matricariae* to also be the predominant species of parasitoid of several aphid species (*Rhopalosiphum padi* (L.), *Rhopalosiphum maidis* (Fitch), *Samilyitobion avenae* (Fabricious) and *Metopolophium dirhodum* (Walker)] (Hemiptera: Aphidiidae) that infest such crops. Aphids in nearby crop fields may therefore act as winter reservoirs for *A. matricariae* before they move to orchards in spring.

Although much less abundant (3.3% of total parasitoids), *A. ervi* was nonetheless the second-most prevalent parasitoid of *M. persicae* in our study. Interestingly, high rates (≥ 80%) of *A. ervi* have been recorded by Pons and Starý (2003) on *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphidiidae) alfalfa and on *S. avenae* aphids on wheat during spring in the same area where our sampling took place. The differences between our results and theirs may be because *A. ervi* is an oligophagous species that is most commonly found parasitising cereal aphids (Pons and Starý 1973), and *M. persicae* are probably not among their preferred hosts. Also, of note is that *A. ervi* has among the highest market values of any aphid parasitoids sold worldwide for aphid control (van Lenteren 2012).

Lipolexis sp. and *A. colemani* were the next two most prevalent species of parasitoids of *M. persicae* in our samples. Only two species of *Lipolexis* sp. have been recorded in Europe, namely, *L. gracilis* Förster and *L. oregmae* Gahan. However, significant confusion exists in the systematics and taxonomy of this genus, suggesting that several cryptic species could be hidden (K. C. and Z. T., unpublished), and additional studies are therefore needed to identify our specimens at the species level. *Lipolexis* spp. are commonly found parasitising species of aphids belonging to the genera *Brachycaudus*, *Hyalopterus* and *Myzus*, the latter two of which are common in peach crops (Starý 1966; Remaudiere and Leclant 1971; Starý et al., 1971; Starý 2006; Kavalieratos et al., 2008; Kos et al., 2012). However, according to Mackauer (1968), *Lipolexis* spp. show a marked preference for *Brachycaudus* species, which are less prevalent in stone-fruit trees than *M. persicae* and *Hyalopterus* spp. (Barbagallo et al., 2017). *Aphidius colemani*, meanwhile, is presumed to be native to India but has become accidentally widespread in many areas of the world, including Mediterranean Europe (Starý and Lukáš 2009). This species has also been widely used as a biocontrol agent in greenhouses (van Lenteren 2012), and its establishment outside of its area of origin may be a consequence of accidental escapes from these confined environments (Starý 1975; Adisu et al., 2002).

In our surveys of *M. persicae*, we identified minor numbers of seven more species (see Table 2). Of these, *A. transcaspicus, E. plagiator* and *P. abjectum* are known to parasitise other aphid species that are common in stone and pip fruit trees in the Mediterranean (Starý 1966; Pennacchio 1989; Starý 2006; Kavallieratos et al., 2008; Starý and Lukáš, 2009; Kos et al., 2012). According to Kavallieratos et al., (2001), *A. transcaspicus* is highly specific to *Hyalopterus* spp. The occurrence of this parasitoid on *M. persicae* in our field samples may simply be fortuitous, since it was registered on only one date during the first sampling year. However, Wang and Messing (2006) found that this parasitoid successfully attacked *M. persicae* under laboratory conditions. Notably, *A. transcaspicus* was also one of only two species that were recruited from our *Hyalopterus* spp. samples. It also has been shown to be an effective natural enemy of *Hyalopterus* spp. on peach trees in other European countries (Starý 1965; Lozier et al., 2008).

In our surveys of *Hyalopterus* spp., in addition to *A. transcaspicus* we also identified *P. volucre*. This parasitoid has been previously recorded in Spain by González and Michelena (1987) in peach trees infested with *Hyalopterus* spp. Pons and Starý (2003) also found this parasitoid on *Hyalopterus amygdali* Blanch (Hemiptera: Aphididae) infesting cherry trees (*Prunus avium* (L.)) in the Lleida area, as well as on several aphid species infesting nearby maize. Furthermore, Starý (1976) and Tomanović and Brajkovic

(2001) have also noted that this parasitoid is commonly found on cereal aphids. It has also been found on aphids infesting wild vegetation, including species from the *Poaeceae* family. This wider host range may explain their appearance early in the season, since its population increase is not dependent on *Hyalopterus* spp. colonies.

We also found that *M. persicae* and *Hyalopterus* spp. had overlapping hyperparasitoid complexes, and hyperparasitoid species belonging to the Pteromalidae, Encyrtidae and Figitidae families were observed on both parasitoids. However, their relative abundance differed between *M. persicae* and *Hyalopterus* spp. In addition, all but one of the species identified in our samples have been previously recorded in Spain (Ferrer-Suay et al., 1998, 2012, 2013; Gómez-Marco et al., 2015), but to the best of our knowledge, ours is the first report of *S. nr*. *africanus* in Spain or elsewhere in Europe.

The hyperparasitoid species composition on our *M. persicae* samples varied according to the survey methodology used. Our sentinel plants yielded only one *P. aphidis* individual and none belonging to either species of the *Asaphes* genus, whereas these three species were abundant in our random samples of plants infested with *M. persicae* aphids. This may be due to hyperparasitoid biology. *Pachyneuron aphidis*, *A. suspensus* and *A. vulgaris* are ectophagous idiobionts that parasitise only mummified aphids (Sullivan 1987). Our sentinel plants were brought to the field with only live aphids and were exposed for only 1 week, which is too short a period of time for mummies to have been formed (Acheampong et al., 2012). Consistent with this explanation, in the samples from sentinel plants the identified hyperparasitoids were almost exclusively endophagous koinobiont species—i.e., species that attack live aphids (Sullivan 1987; 1972). *Syrphophagus* nr. *africanus*, which can parasitise both live aphids and mummies (Kanuck and Sullivan 1992), was collected with both sampling methods. The results from the random samplings are therefore likely to better reflect the hyperparasitoid complexes that attack *M. persicae*'s and *Hyalopterus* spp.'s primary parasitoids in peach orchards.

Conclusions. Our study found that a wide array of primary parasitoids associated with *M. persicae* were present in *P. persica* orchards. The prevalence of this array of parasitoids should make the ecosystem more stable and resilient to potential invasions of new aphid species (Sullivan 1987; Boivin et al., 2012; Alhmedi et al., 2018). *Aphidius matricariae* accounted for over 90% of all our primary parasitoids, suggesting that it should be the key parasitoid species considered as a biological control agent in conservation biological control programs and, probably the best candidate for augmentative releases. Moreover, it is commercially available and is widely used in greenhouse crops for aphid control (van Lenteren 2003). In contrast, we found only two parasitoids associated with *Hyalopterus* spp. aphids: *A. transcaspicus* and *P. volucre*. However, either of these could likely function as biological control agents of *Hyalopterus* spp. aphids.

In addition, given that *A. matricariae* and *P. volucre* are commonly found in the arable crops surrounding the peach orchards in Lleida area, these crops may represent an important source of parasitoids. Spontaneous flora within and near the orchards may be also important for these parasitoids' survival, by providing them with alternative hosts, food and refuge, particularly during winter (Tomanovic et al., 2009; Vollhardt et al., 2010; Gagic et al., 2012; Alignier et al., 2014; Blaauw and Isacs 2015). Consistent with this theory, laboratory experiments have confirmed that *Aphidinae* improve their fitness when flowers are available (Aparicio et al., 2018; Jado et al., 2019).

Additionally, if future surveys confirm our finding of a spontaneous association between *A. transcaspicus* and *M. persicae* at increasing numbers, this may allow for improved biological control of *Hyalopterus* spp. *Myzus persicae* usually infest peach trees earlier in the season than do *Hyalopterus* spp. (Barbagallo et al., 2017; Aparicio, Gabarra, Riudavets, Arnó, personal observation); therefore, increased parasitism on *M. persicae* early in the peach season might contribute to increased parasitoid populations prior to the heavy infestations of *Hyalopterus* spp. that damage trees.

Although hyperparasitoids might be beneficial to the long-term stabilisation of insect-parasitoid dynamics (Hassell and Waage 1984; Sullivan 1987) in the short term they may be detrimental to aphid control. The absence of hyperparasitoids early in the season, when *M. persicae* populations build up, suggest that biological control of this aphid would not be negatively impacted by the presence of hyperparasitoids. However, this would not be the case for *Hyalopterus* spp. whose populations peak later in the season and, therefore, its control would be negatively affected by the presence of hyperparasitoids, which are more abundant at this time of year. Overall, our results suggest that biological control of aphids in peach trees is feasible and should be considered for IPM programmes; however, strategies to boost parasitoid populations should probably be adopted.

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17.2.5 References

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CHAPTER 3

Attraction of *Aphidius ervi* (Hymenoptera: Braconidae) and *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) to sweet alyssum and assessment of plant resources effects on their fitness

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17.3.1 Introduction

The green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is one of the most economically important aphid affecting crops worldwide. It is extremely cosmopolitan and highly polyphagous, and the hosts are in more of 40 different plant families including many economically important crops (Blackman and Eastop 2007). The green peach aphid is a severe pest of peach and nectarine, vegetable, and greenhouse crops (Rabasse and van Steenis 1999, Blümel 2004, Barbagallo et al. 2007). In a recent survey conducted in the Ebro Valley (Spain), a very important area of peach and nectarine production, pest advisors ranked this aphid as one of the most important pest problems (authors' unpublished data). The survey also revealed that pest management is currently mainly achieved using insecticides. Biological control might be a viable alternative to manage *M. persicae*. Several predators and parasitoids of this species have been recorded, and this entomofauna might play an important role in the reduction of the aphid population (Völkl et al. 2007). The parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) and the predator *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) are among the most important natural enemies of this pest (Rabasse and van Steenis 1999, Blümel 2004). These natural enemies have been recorded in spring in the production areas where orchards coexist with arable crops (Pons and Stary 2003, Miñarro et al. 2005, Pons et al. 2011) and both have been repeatedly found on *M. persicae* colonies in *Prunus* orchards early in spring (authors' unpublished data). However, *M. persicae* attacks *Prunus* sp. in spring when the population of natural enemies is still low and, therefore, effective biological control of this aphid is difficult to achieve. The inclusion of floral resources close to the orchards might help to enhance the biological control by providing natural enemies with nectar and pollen as food sources, thereby contributing to increase their survival and reproduction (Landis et al. 2000, Gurr et al. 2005).

Sweet alyssum, *Lobularia maritima* (L.) (Brassicaceae), is a Mediterranean perennial plant that blooms uninterrupted for extended periods (approximately 10 mo), with a maximum in spring (Picó and Retana 2001). It is very attractive to natural enemies and thus has potential as an insectary plant (Chaney 1998, Alomar et al. 2008, Hogg et al.

2011). Ribeiro and Gontijo (2017) demonstrated that sweet alyssum increases the abundance of generalist predators and therefore reduces some pests, especially aphids. Sweet alyssum intercropping is widely used in the Salinas Valley in the central coastal area of California to control aphids in organic lettuce and broccoli crops (Brennan 2013, 2016). Under laboratory conditions, it can improve the longevity of *A. ervi* (Araj et al. 2006, Araj and Wratten 2013) and the survival, egg load, and fecundity of other braconid parasitoids such as *Dolichogenidea tasmanica* (Cameron) and *Diaeretiella rapae* (Mcintosh) (Hymenoptera: Braconidae) (Berndt and Wratten 2005, Araj and Wratten 2015). However, there is little information about the effect of alyssum flowers on the reproduction of *A. ervi* and the biology of *A. aphidimyza*. The aim of this study was to investigate if *L. maritima* is a potential food source for *A. ervi* and *A. aphidimyza* and can therefore contribute to enhance the biological control of *M. persicae*. To do that, we investigate if both natural enemies are attracted to blooming and non-blooming alyssum. We also tested the effects of alyssum on the longevity and the reproductive potential of *A. ervi* and *A. aphidimyza* to evaluate the contribution of this plant to the fitness of these species.

17.3.2 Materials and methods

Insects and Plant Material

Mummies of *A. ervi* and pupae of *A. aphidimyza* were obtained from Agrobio. Adult emergence took place inside a climatic chamber at 22°C and 70 ± 10% RH, with a 16:8 (L:D) photoperiod. When mated females were required, males and females (<24-h old) were kept together for 24 h. The green peach aphid, *M. persicae*, was reared in the climatic chamber at the same conditions mentioned above on tobacco plants (Nicotiana tabacumL.) (Solanaceae). Plants of tobacco, peach, and alyssum were grown in plastic pots with compost soil in the greenhouse inside a closed compartment to prevent any pest infestation. Before each experiment, plants were observed and none of them had pest presence or symptoms of pest damage. Longevity, egg load, fertility, and fecundity experiments were conducted at 22°C and 70 ± 10% RH, with a 16:8 (L:D) h photoperiod. Olfactometer assays were carried out at 22°C and 60 ± 10% RH under light conditions. A single lamp (Sylvania Circline FC22W/865) placed at 60 cm above the Y-tube was used.

These light conditions were set up because *A. ervi* emerge during the photophase (He et al. 2004) and *A. aphidimyza* emerge before sunset (Harris 1973).

Olfactory Bioassays

Experiments with *A. ervi* and *A. aphidimyza* were conducted in a Y-tube olfactometer. Each arm was 17-cm long and had a diameter of 3.5 cm; the inside angle between the two closest arms was 75°. Each of these two arms received air from one of the two odor sources that were inside two glass jars (4,000 ml) connected to them. The air coming from a compressor (ABAC-FC2-24CM) passed through a double carbon filter (ABAC-ACF60 1,000 Lmh) and an air humidifier (water bubbler) and subsequently entered the glass jars. Air flow was adjusted to 0.20 ± 0.03 m/s at the base of the third arm of the olfactometer and was measured with a hot-wire anemometer (Testo, Barcelona, Spain). Insects were gently placed at the base of the main arm and allowed to move in. They were considered to make a choice when they walked more than 5 cm on one of the upper arms in less than 10 min. To avoid any possible asymmetries in the experimental setup due to environmental factors or location effects, after five individuals, the olfactometer was cleaned with alcohol (96%) and the arms were switched between the two odor source jars. Jar positions were also rotated after every 10 female adults. Forty female parasitoids and predators (1- to 4-d old) were individualized and starved for 24 h before each observation. Each individual was used only once. In the case of *A. ervi*, the position of the olfactometer was vertical, whereas for A. aphidimyza, it was horizontal. The position for each species was proposed after preliminary tests. The following choices were offered to *A. ervi* and *A. aphidimyza*: 1) alyssum flowers versus clean air, 2) alyssum plant without flowers versus clean air, 3) alyssum flowers versus alyssum plant without flowers, 4) aphid-free peach shoots versus alyssum flowers, 5) aphid-infested peach shoots versus alyssum flowers. In the treatments with blooming alyssum, three shoots, which together had about 40 fully open alyssum flowers, were used; in the case of nonflowering alyssum, three shoots with only green leaves were used. To infest peach shoots with aphids, 24 h before the experiment, approximately 50 second to third-instar *M. persicae* were placed gently onto the leaves with a brush. All plant shoots were cut just before the start of the experiment. The cut end was immediately submerged in water in a jar with a bored lid. The stems were introduced in the hole which was closed with a piece of paper to prevent wound-related volatiles during the olfactory assay. Each day, new plant material and aphids were used.

Effects of Alyssum and Sugar Solution on the Biology of *A. ervi*

Female Longevity. Females of *A. ervi* less than 24-h old were placed individually in a 250-ml plastic cup covered with gauze to provide ventilation. We tested three different food sources: 1) a 70% sugar-rich diet solution of glucose, fructose, and sucrose (G + F + S) in a 1:1:1 ratio, 2) three shoots of alyssum with approximately a total of 40 fully open flowers, and 3) water as control. The above-mentioned sugars were chosen because they are the main components of the nectar (Baker and Baker 1983, Wackers 2001), and a 70% sugar solution supports a longer lifetime of *A. ervi* females (Azzouz et al. 2004). The three diets were offered to *A. ervi* females in the presence and absence of aphids, resulting in six different treatments. Sugar solution and water were provided in a 13-ml tube plugged with a piece of cotton dental roll and attached to the wall of the cup with Blue-tack (Rubi, Spain). Alyssum flowers were kept in an Eppendorf vial with water and also attached to the glass wall with Blue-tack. In the treatments with aphids, 20 second to third-instar *M. persicae* were placed on the top of a tobacco disc that was laid above an agar layer (0.5%) on a 2.5-cm Petri dish which was introduced on the base of the cup. Food and aphids were renewed twice per week. Female mortality was recorded daily. Fifteen replications were performed per treatment.

Egg Load and Fertility. To evaluate egg load, females (< 48-h old) were caged for 3 d in arenas without aphids similar to those described in the previous section (*A. ervi* longevity) and subsequently frozen at −20°C until dissection. To do that, the females were placed on a microscope slide under a stereomicroscope. With a scalpel, the thorax was separated from the abdomen, that was subsequently open to remove the ovaries and the number of chorionated oocytes recorded. The effect of the same food treatments on fertility was evaluated in arenas with aphids as prepared for *A. ervi* longevity. Tobacco discs with aphids and food were renewed every 3 to 4 d. Aphid mortality was assessed in the discs when removed from the cups. Aphids that did not move their legs when touched with a fine brush were considered dead (Moores et al. 1996). Subsequently, the tobacco discs were kept in the climatic chamber at 22°C until the aphids were mummified. Fifteen leaf discs with aphids, but without parasitoids, were prepared to assess natural and handling mortality. The results were used to correct mortality produced by the parasitoids.

Effects of Alyssum and Sugar Solutions on the Biology of *A. aphidimyza*

Female Longevity. Starved females less than 24-h old were isolated in arenas without aphids similar to those used in the *A. ervi* longevity trials. Instead of 250-ml plastic cups, glass cups were used. A 10% G + F + S solution was provided as sugar-rich diet according to the findings of Watanabe et al. (2014). Mortality was checked daily.

Egg Load and Fecundity. The same methodology as described for the experiment to assess

A. ervi egg load, but using glass cups and a 10% instead of a 70% G + F + S solution, was applied to evaluate the effects of a sugar rich diet on *A. aphidimyza* egg load and fecundity. Egg load was determined by dissecting the abdomen of the females as explained above for *A. ervi*. For fecundity, the number of eggs laid on the aphid colony on the leaf were counted daily. Twenty females were tested per treatment.

Survival up to 5 d. A specific experiment was carried out to check if starved *A. aphidimyza* females less than 24-h old were able to feed on alyssum nectar. We used the same setup without aphids described when assessing *A. ervi* longevity. However, a fourth type of food, alyssum flowers with plucked petals and thereby exposed nectaries, was included. Survival was measured up to 5 d, with five replications per treatment.

Morphometry of *A. aphidimyza* and Alyssum Flowers

After the longevity trial, several visual observations were made to record how females approached the nectaries and how the insects placed themselves on the flower for feeding. To do this, we used 1- to 4-d old female predators that were starved for 24 h before each observation. Individuals were released in Petri dishes containing alyssum flowers, and we recorded the time spent by females from landing on the flowers until they walked away with a timer. After these observations, we measured the gap between the petals and the stamen of alyssum flowers as well as the distance between the femur and tibia intersection points of both middle legs. All measurements were made with a dissection microscope at 2.5× magnification, using the program ImageJ.

Data Analysis

Differences in the proportion of *A. ervi* and *A. aphidimyza* females choosing a particular odor source (olfactometer experiments) were tested using a two-sided binominal test. Insects that did not respond within 10 min were not included in the analysis. Data of *A. ervi* longevity in the arenas with aphids, *A. aphidimyza* longevity, the egg load of both natural enemies, *A. aphidimyza* fecundity, and the total number of mummies and dead aphids in the trials with the parasitoids were analyzed by one-way analysis of variance; means were separated using Tukey's HSD test. Because data of *A. ervi* longevity in the arenas without aphids could not be normalized, a KruskalWallis test was used in the analysis and Mann-Whitney-Wilcoxon tests were used to observe pairwise differences between treatments with Bonferroni-weighted test correction ($P < 0.05$). Survivorship affected by diet was evaluated using the Kaplan-Meier survival platform. Pairwise comparisons among groups were evaluated using logrank tests with αset at 0.005 to account for multiple comparisons. All data were analyzed using SAS 9.3 for Windows; survival curves were generated with the software SigmaPlot version 13.

17.3.3 Results

Olfactory Bioassays

Significantly more *A. ervi* females preferred alyssum, either with or without flowers, to clean air (Figure 1), whereas they showed no significant preference for any treatment when offered a choice between alyssum shoots with and without flowers. The volatiles from alyssum flowers were significantly more attractive than those from the peach shoots without aphids. When alyssum flowers were compared to the peach shoots with aphids, the parasitoids did not show a significant preference for any of them. The mean time that an *A. ervi* females spent to respond to the odor source ranged from 53 to 103 s. *Aphidoletes aphidimyza* females significantly preferred alyssum shoots, either with or without flowers, to clean air (Figure 2). When *A. aphidimyza* females were offered a choice between alyssum shoots with and without flowers, they showed a significant preference for the blooming alyssum. Likewise, predators significantly preferred cues from alyssum flowers to those of the clean peach shoots, but they did not display a significant preference between alyssum flowers and peach shoots infested with aphids.

The mean time spent by an *A. aphidimyza* female to respond to the cues ranged from 111 to 163 s.

Figure 1.

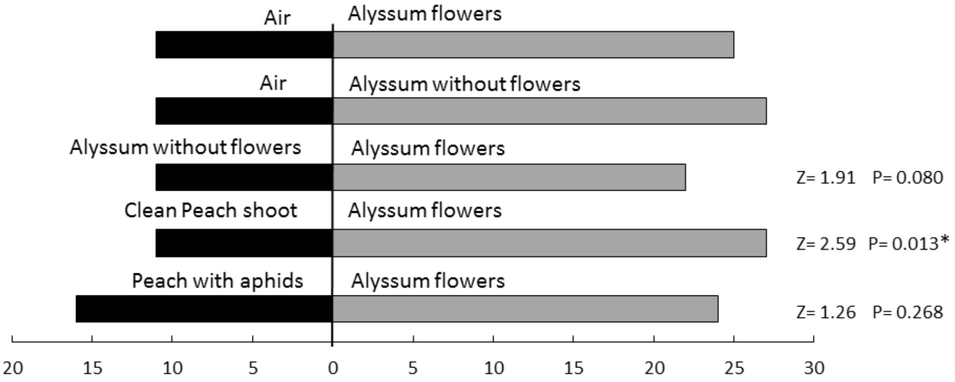


Figure 1. Number of *A. ervi* female attracted to different treatments in a Y-tube olfactometer (total number of females tested = 40). The Z and P values relate to a two-sided binomial test of observed and predicted distribution based on a random response. *Indicate significant differences between treatments. Individuals that did not respond were not included in the analysis. The mean (± SE) response time from top to bottom were 61.19 ± 8.34, 52.94 ± 5.98, 83.84 ± 8.57, 103.28 ± 18.54 and 55.40 ± 9.64 s.

Figure 2.

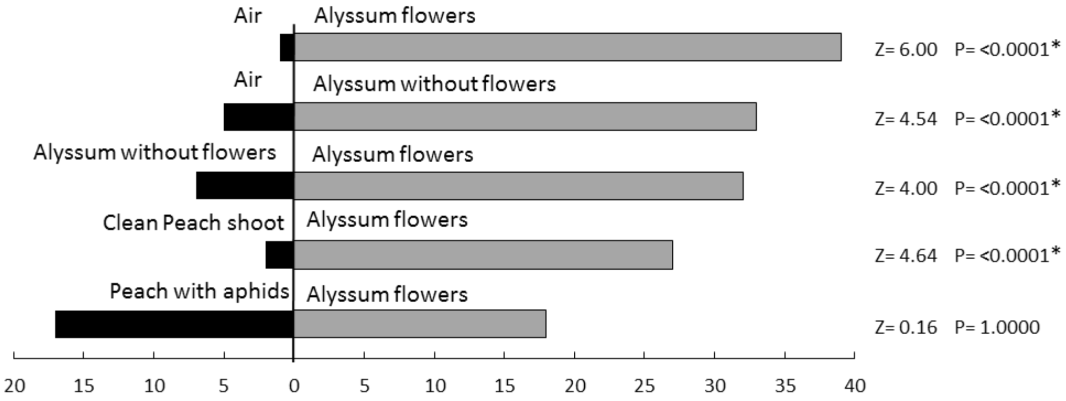


Figure 2. Number of *A. aphidimyza* female attracted to different treatments in a Y-tube olfactometer (total number of females tested = 40). The Z and P values relate to a two-sided binomial test of observed and predicted distribution based on a random response. *Indicate significant differences between treatments. Individuals that did not respond were not included in the analysis. The mean (± SE) response time from top to bottom were 133.87 ± 19.51, 115.23 ± 7.81, 110.89 ± 5.30, 110.79 ± 23.06, 163.22 ± 18.87 s.

Effects of Alyssum and Sugar Solution on the Biology of *A. ervi*

Female Longevity. The mean longevity of *A. ervi* females in the treatments with different diets and with and without aphids is presented in Figure 3. There was an interaction between longevities recorded in the arenas with and without aphids and, therefore, data were analyzed separately. When aphids were present in the arenas, longevity was not significantly different, regardless of the food treatment ($F_{2, 42}$ = 0.29, P = 0.74). In contrast, when aphids were absent, longevity significantly varied among food sources (χ^2 = 21.22, P < 0.0001). Females which fed on alyssum significantly lived longer than those which fed on the sugar solution or water (Z = 2.50, P = 0.0122; Z = 4.38, P < 0.0001, respectively; Mann-Whitney test, Bonferroni corrected significance P-value < 0.0167 = 0.05/3). The longevity of females which fed on the sugar solution was also significantly higher than that of females which fed on water (Z = 2.44, P =0.143 Mann-Whitney U-test, Bonferroni corrected significance P-value < 0.0167 = 0.05/3).There was no significant difference in the survival curves of individuals fed with different food sources in the presence of aphids (Log-rank χ^2 = 5.59, df = 2, P = 0.060) (Figure 4A). Survival curves differed significantly between food sources in the absence of aphids (Log-rank χ^2= 25.43, df = 2, P < 0.0001) (Figure 4B).

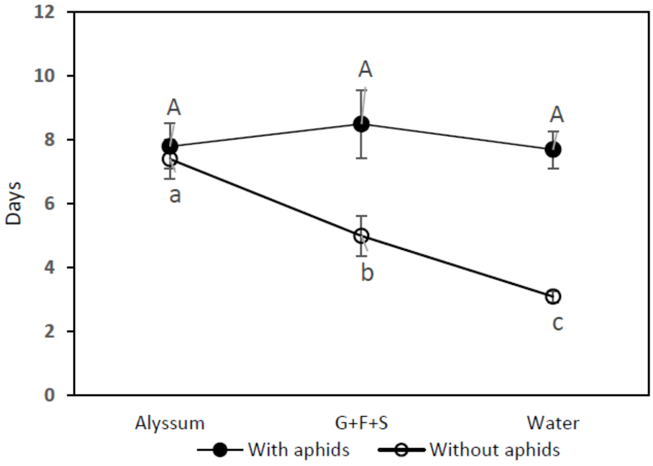
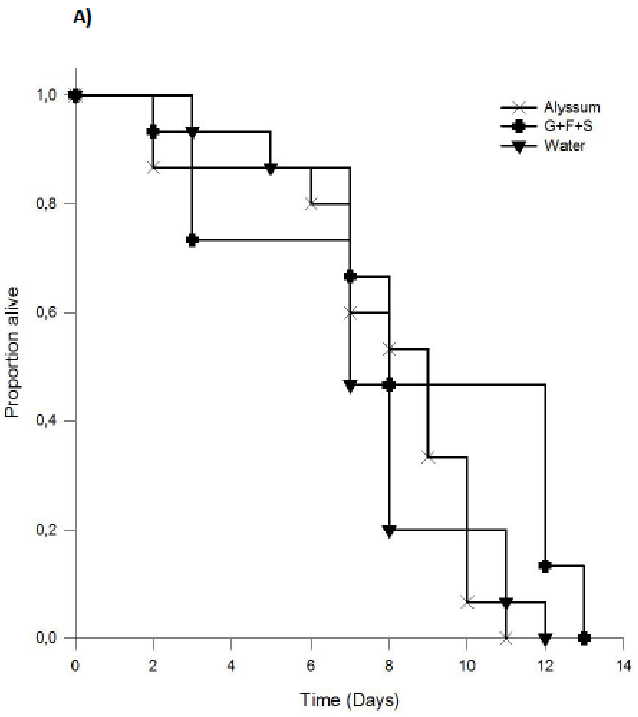


Figure 3. Mean longevity of *A. ervi* females with three different diets in two scenarios, with and without aphids. Different upper-case letters indicate no differences among treatments in the presence of aphids (ANOVA P < 0.05). Lower-case letters indicate differences among treatments in the absence of aphids (Mann-Whitney U-tests with Bonferroni correction; a value of p < 0.0167 was considered statistically significant. There was an interaction between longevities in the case of alyssum. G+F+S stands for a 70% sugar rich water solution of glucose, fructose, and sucrose in a 1:1:1 ratio.



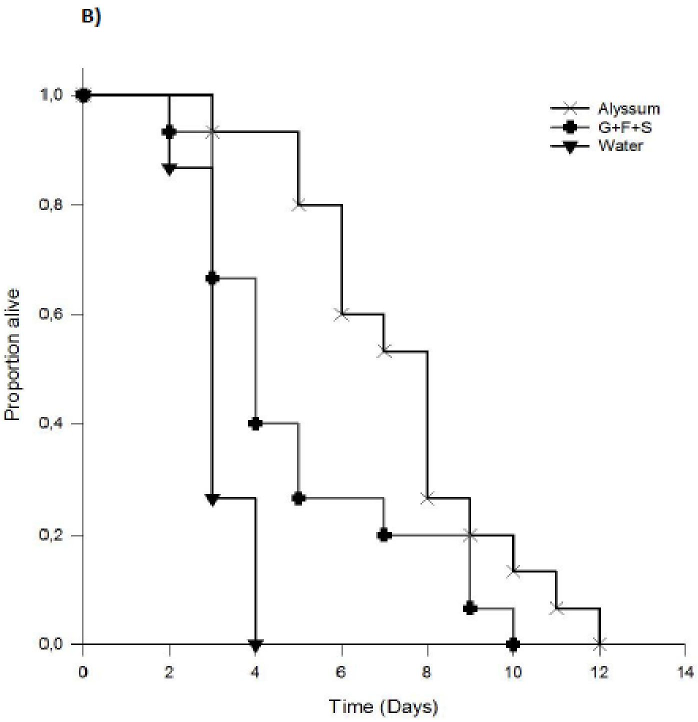


Figure 4. Kaplan-Meier estimates of survivorship functions of *A. ervi* females given access to water (control), 70% sugar water solution of glucose, fructose, and sucrose in a 1:1:1 ratio (G + F + S), and alyssum flowers in the presence (A) and in the absence (B) of aphids.

Egg Load and Fertility. After 72 h of feeding on different food sources, all *A. ervi* females had 0 or 1 mature oocyte when dissected, and no significant differences were observed in the egg load ($F_{2, 42} = 0.82$, $P = 0.44$). Table 1 shows the total number of dead aphids corrected by natural and handling mortality (3.75 ± 0.37 individuals). No significant differences were observed among different foods, neither in the number of mummies nor in the number of dead aphids ($F_{2, 39} = 0.38$, $P= 0.68$ and $F_{2, 39} = 0.48$, $P = 0.62$, respectively).

Table 1. Mean (± SE) number of *A. ervi* mummies and dead aphids (± SE) when female wasps were fed with three different treatments. No significant differences were found.

Treatment	Mummies (mean ± SE)	Dead aphids (mean ± SE)
Water	8.88 ± 1.55	21.73 ± 3,11
Alyssum	11.00 ± 1.96	25.67 ± 3.58
G + F + S 70%	10.28 ± 1.74	22.08 ± 2.65

Effects of Alyssum and Sugar Solutions on the Biology of *A. aphidimyza*

Female Longevity. The survival curve showed significant differences among food sources (Log-rank $\chi^2 = 34.54$, df = 2, $P < 0.0001$) (Figure 5). Total longevity of *A. aphidimyza* females significantly varied among food sources ($F_{2, 42} = 37.66$, $P < 0.0001$). Significantly longer longevity was recorded for females which fed on the sugar solution (8.1 ± 0.62 d) than for unfed ones (4.2 ± 0.20 d) and those provided with alyssum flowers (3.3 ± 0.30 d). No significant differences were observed in the longevity of individuals fed with the two latter food sources.

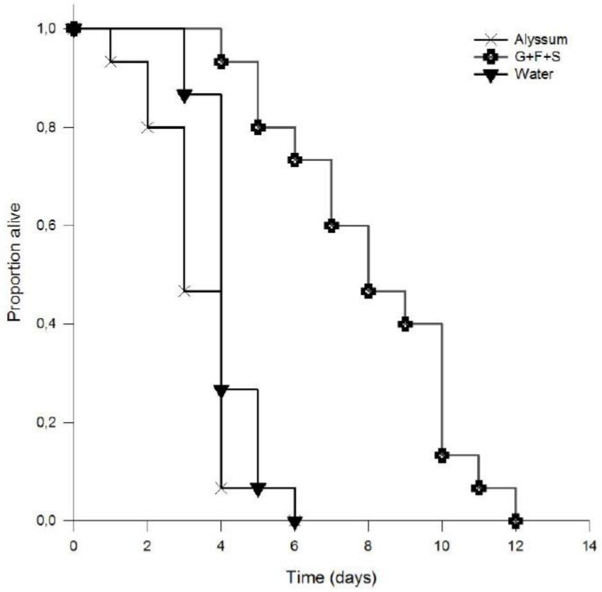


Figure 5. Kaplan-Meier estimates of survivorship functions of *A. aphidimyza* females given access to water (control), 10% sugar water solution of glucose, fructose, and sucrose in a 1:1:1 ratio (G + F + S), and alyssum flowers.

Egg Load and Fecundity. Diet significantly affected *A. aphidimyza* egg load ($F_{2, 57}$= 5.22, P < 0.05). The number of mature oocytes was significantly higher when females fed on a 10% G + F + S solution than on water or on intact alyssum flowers (Table 2). There was no significant difference between females fed with alyssum and unfed ones. Daily oviposition rates were not significantly different between the three treatments ($F_{2, 42}$ = 0.67, P = 0.51).

Table 2. Mean number (± SE) of mature oocytes inside *A. aphidimyza* females and eggs laid per day when fed with three different treatments.

Treatment	Oocytes (mean ± SE)	Eggs /day (mean ± SE)
Water	27.25 ± 4.94b	5.16 ± 1.64a
Alyssum	27.55 ± 3.98b	2.73 ± 0.96a
G + F + S 10%	43.90 ± 3.43a	3.68 ± 1.77a

Different letters in the same column indicate significant differences (ANOVA, Tukey´s HSD for mean separation, P < 0.05).

Survival up to 5 d. Significant differences in survival after 5-d feeding on different foods were recorded for *A. aphidimyza* ($F_{3, 16}$ = 45.36, P < 0.0001). Females that fed on the sugar solution and on exposed alyssum nectaries survived significantly longer that those fed on intact alyssum flowers or water (Figure 6).

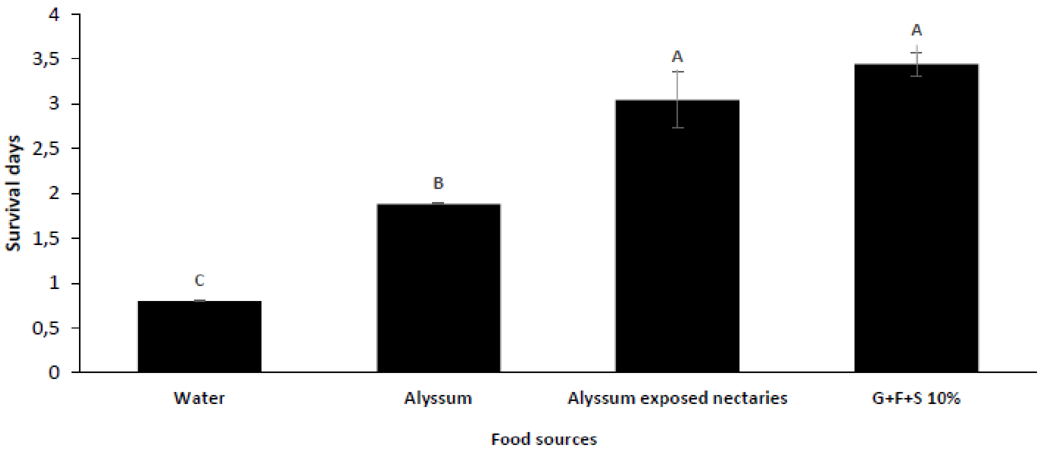


Figure 6. Number of days *A. aphidimyza* females survive, up to five days, when provided with different foods. Different letters indicate significant differences between the food treatments (ANOVA, Tukey´s HSD for mean separation, P < 0.05).

Morphometry of *A. aphidimyza* and Alyssum Flowers. Our observations revealed that predator females had difficulties to reach alyssum nectar glands, and none of the 10 observed females contacted the nectaries. They were observed on the top of the flowers lowering their head to try to reach the nectar glands at the very bottom inside the corolla tube (Figure 7). The females spent a mean time of 42.3 s (± 6.45) on the petals and then left the flowers. Measurements indicated that the distance between the two joints of the femur with the tibia in middle legs of *A. aphidimyza* females is wider (1.49 ± 0.12 mm) than the gap between petals and stamen of the flowers (0.27 ± 0.04 mm) (Figure 8).

Fig. 7. Lateral view of *A. aphidimyza* on alyssum flower, showing the nectar glands position (black dots) inside the calix.

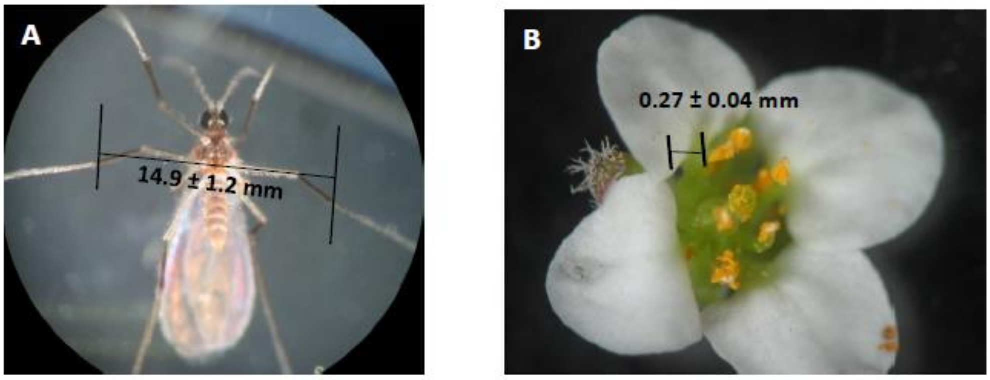


Figure 8. Ventral view of an *A. aphidimyza* female (A) and above view of an alyssum flower (B). Comparison of the measures between the joint of the femur and tibia in the middle legs of *A. aphidimyza* (14.9 ± 1.2 mm, mean ±SE) and the gap between the petals and the stamens of the alyssum flower (0.27 ± 0.04 mm, mean ± SE).

17.3.4 Discussion

In our olfactometer experiments, *A. ervi* and *A. aphidimyza* were attracted to flowering and nonflowering alyssum. According to (Harris 1973), *A. aphidimyza* is nocturnal. However, females responded to the cues emitted by alyssum under light conditions. Possibly, they may also locate the plants during the scotophase since many of them produce volatiles at night (Kumari et al. 2017). Attraction to blooming alyssum in field and laboratory studies is well documented for some natural enemies as predators and some braconid parasitoids (Alomar et al. 2006, Rohrig et al. 2008, Arnó et al. 2012, Gontijo et al. 2013, Foti et al. 2017). The similar attraction between flowering and nonflowering alyssum has been also reported for the parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) (Foti et al. 2017). Interestingly, this attractiveness to alyssum flowers was disrupted when compared with peach shoots recently infested with a relatively low number of aphids (50 individuals during 24 h), similar to what has been reported for *A. ervi* by Guerrieri et al. (1999). This indicates that volatiles produced by aphid-infested plants (Guerrieri et al. 1993, Reed et al. 1995, Du et al. 1997, Hou et al. 1997, Powell et al. 1998, Desurmont et al. 2015), by the honeydew (Budenberg and Powell 1992, Du et al. 1997, Choi et al. 2004, Wickremasinghe 2007), and/or by the aphids themselves (Reed et al. 1995, Du et al. 1996) were attractive enough to balance the attraction produced by alyssum flowers. Our results suggest that both natural enemies are able to rapidly locate aphid colonies, which would benefit the effectiveness of these two natural enemies. Since the amounts of volatiles produced by the plant/aphid complex will increase with time as the aphid colonies increase in size, attraction of *A. ervi* and *A. aphidimyza* to the aphid-infested plants will probably increase, as has been demonstrated for *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) (Yang et al. 2009). Our results also indicate that the presence of additional sugar rich food was relevant in terms of *A. ervi* survival only when aphids were not present in the arena. In that case, alyssum nectar increased female longevity compared to that of unfed ones and was even a better food source for *A. ervi* than a sugar solution containing glucose, fructose, and sucrose, which are the main sugars present in nectar (Wackers 2001, Winkler et al. 2005). Higher longevity of parasitic wasps feeding on alyssum compared to sugar-fed individuals has been shown before for *A. ervi* (Araj et al. 2006, Wade and Wratten 2007, Araj and Wratten 2013). The higher survival suggested that besides sugars, other food substances (such as amino acids, lipids, proteins, vitamins, and minerals) present in flowers even in small quantities play an important role in the longevity of *A. ervi* females (Baker and Baker 1983, Wackers 2005). Pollen is unlikely to be a food resource used by parasitoids (Jervis 1998, Irvin et al. 2006). On the other hand, when aphids were present, the provision of additional resources did not increase *A. ervi* longevity, suggesting that the combination of honeydew and hosts is adequate to keep females alive. In fact, several studies have shown that parasitoids, including *A. ervi,* are well adapted to the use of insect-produced honeydew which is the predominant sugar source in many agricultural systems (Burger et al. 2004, Lenaerts et al. 2016). In our experiments done in the absence of aphids, the maximum egg load recorded for *A. ervi* females after feeding for 72 h was one mature oocyte, regardless of the food treatment. This was probably due to the reabsorption of mature oocytes when hosts were not available since this species is prosynovigenic and females emerge with approximately 20 to 60 mature eggs (He and Wang 2006). This reabsorption has been described to occur within 48–72 h following emergence in other braconids such as D. rapae (Kant et al. 2013). When aphids were available, the number of mummies was the same, regardless of the food, indicating a similar fertility. This also implies that an additional food source is not required when the host and the honeydew are present. Similar results have been observed by Hayashi and Nakashima (2014), who found that for *A. ervi*, female progeny did not differ between unfed females and those fed with a sugar solution. Our experiments show that in the absence of aphids, a sugar-rich diet benefited *A. aphidimyza* female longevity and egg load, similar to what has been observed by Watanabe et al. (2014). On the contrary, the presence of alyssum flowers did not enhance the survival or the number of mature oocytes of *A. aphidimyza* females, probably because nectar was not accessible for them, as was confirmed when alyssum flowers with exposed nectaries were offered. Our results provide evidence that *A. aphidimyza* would be able to feed on nectar if it was accessible and, therefore, it may explain why females were attracted to alyssum flowers. To our knowledge, there are no records in the literature of *A. aphidimyza* females feeding on floral resources. Our observations and the measurements performed on both the flower and *A. aphidimyza* confirmed that females could not access the very bottom part inside the corolla of alyssum flowers where the nectaries are found (Patt et al. 1997). According to our results, this was due to their long legs and the large span between the femur and tibia joints of both middle legs, which was wider than the gap between the petals and the stamen of the flower and thereby prevented access to the nectar. In addition, females were not strong enough to separate the flower structures. Similar results have been observed in some parasitoids (Rabb and Bradley 1968, Jervis et al. 1993, Patt et al. 1997, Rahat et al. 2005) and some predators (Nave et al. 2016, van Rijn and Wackers 2016). On the other hand, *A. aphidimyza* daily fecundity was similar regardless of the additional food supplied. This was probably due to the same amount of aphids present in all the treatments, that is to say the same aphid density, which influenced the amount of honeydew, a good food resource for this predator (El-Gayar 1976, Sell and KuoSell 1987, Choi et al. 2004). Fecundity of *A. aphidimyza* strongly depends on the aphid density in both laboratory experiments (Choi et al. 2004, Guo et al. 2014) and field studies (Stewart and Walde 1997, Sentis et al. 2012). In conclusion, both natural enemies of *M. persicae*, the parasitoid *A. ervi* and the predator *A. aphidimyza*, were attracted to alyssum plants. Therefore, the establishment of crop margins including this plant species that is fully blooming in spring (Picó and Retana 2001), may help to attract these naturally occurring beneficials in the area (Pons and Stary 2003, Miñarro et al. 2005, Pons et al. 2011) into orchards and increase their local population regardless of the presence of aphids. The presence of alyssum flowers close to the fields would increase *A. ervi* longevity and probably their ability for host searching as soon as aphid populations start to build up. This beneficial effect for the parasitoid would not be relevant with high *M. persicae* populations because at this point it may obtain nutrients from honeydew. In the case of *A. aphidimyza* and due to the inaccessibility of alyssum nectar for the adults, these flowers will not represent a supplemental food for the females. Therefore, to consider nectar accessibility while selecting insectary plants is important because attracting insects without providing accessible nectar, and therefore additional energy, may be detrimental and most likely results in inadequate energy use (Winkler et al. 2009). Because of that, the combination of alyssum flowers and flowers with exposed nectaries or plants with extra floral nectar may also be viable options to improve the biological control of *M. persicae*. Other food sources such as honeydew of non-pest aphids or sugar provision via dispensers may also be useful to enhance natural enemy fitness.

Further field experiments will be necessary to fully understand the potential role of different sugar-rich diets in the biological control of aphids in peach orchards

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CHAPTER 4

Interactions among aphids, predators and parasitoids may hamper biological control.

17.4.1 Introduction

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is considered to be one of the most serious pest of peach (*Prunus persicae* (L.) (Rosaceae)). To date, management of this aphid has mainly occurred through the use of insecticides, but their risk to both humans and the environment, as well as *M. persicae* resistance to a wide range of insecticides, requires a reduction in their usage (Bazky 1982; Field et al. 1997; Foster et al. 1998; Blackman and Eastop 2000; Barbagallo et al. 2017; Katis et al. 2007; Penvern et al. 2010). As such, alternative strategies for the management of aphids are urgently needed. According to Dedryver et al. (2010), these strategies will result from the combination of different tools, one of them being biological control. Among the different biological control strategies that have been used against aphids, conservation of already existing natural enemies in the agroecosystem seems the more promising to be implemented in outdoor crops such as fruit tree orchards (Dedryver et al. 2010). Aphids can be attacked by many natural enemies, including mainly parasitic wasps and predators (Brodeur et al. 2017); therefore, biological control could be a feasible tool for aphid regulation.

This great diversity of aphid natural enemies increases the chances for Intraguild Predation (IGP) to exist. The IGP occurs when a number of natural enemies may compete for the same resource (extraguild prey) and additionally may feed on each other (intraguild predator over intraguild prey) (Wratten and Powell 1991; Rosenheim et al. 1995; Polis and Holt 1997; Dixon 1998; Lucas et al. 1998; Bonsall and Holt 2003; Lee and Kang 2004; Lucas 2005). Parasitoids are one of the most important control agents of aphids (Schmidt et al. 2003; Brewer and Elliott 2004). However, due to the development of parasitoid larva within the aphid, coincidental IGP occurrs when the predators might also eat juveniles of the parasitoids while preying on the host (Polis et al. 1989). This asymmetrical IGP might negatively affect pest suppression by parasitoids. For example, hoverflies do not distinguish between parasitized (but non-mummified), and non-parasitized aphids (Brodeur and Rosenheim 2000). The same pattern has been shown in Cecidomyiidae (Enkegaard et al. 2005; Mottaghinia et al. 2018) and Chrysopidae (Meyhöfer and Klug 2002). Other predators as Coccinellidae and Nabidae can even predate on mummified aphids (Wheeler et al. 1968; Colfer and Rosenheim 2001; Meyhöfer and Klug 2002).

Besides the interactions that involve consumption or parasitism, the presence of natural enemies in an aphid colony can also produce behavioral (non-consumptive) changes. Most of them aim to warn the conspecifics of the existence of a danger. In response to the physical attack by natural enemies, many aphids secrete a cornicle exudate containing alarm pheromone. The responses trigged by this alarm pheromone are diverse and include behavioral changes such as feeding cessation, increased movement of individual aphids and plant dropping, and other long-term effects as wing induction (Picket et al. 1992, Vandermoten et al. 2012). Some works also reported fecundity compensation (Pickett et al. 1992; Barribeu et al. 2010; Leventhal et al. 2014).

The goal of this work was to evaluate the interactions among predators and parasitoids on the control of *M. persicae.* In laboratory experiments, we determined the contribution of *Episyrphus balteatus* DeGeer (Diptera: Syrphidae), *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae) to the biological control of this aphid; the changes in the aphid's behavior mediated by the aphid cornicle exudate; and the interaction of these predators with the parasitoid *Aphidius matricariae* Haliday (Hymenoptera: Braconidae: Aphidiinae). Finally, we estimated the effect that these components may have on aphid population growth.

17.4.2 Material and methods

All the experiments and the insect rearings were conducted in a climatic chamber at 22° C, 60-70% RH and a 16L: 8D photoperiod. Experiments involved the most abundant natural enemies usually found in *M persicae* colonies in peach trees in our area: the parasitoid *A. matricariae* (Aparicio et al. 2019), and the predators *E. balteatus*, *A. aphidimyza* and *O. majusculus* (Aparicio et al. manuscript in preparation).

Biological material. Sweet pepper (*Capsicum annum* L.) plants were used as model plants for all the experiments. They were grown in a greenhouse from seed in plastic pots (10 cm diameter x 8 cm) with compost soil until they were approximately 18 cm high. *Myzus persicae* were reared on sweet pepper plants maintained in Bugdorm cages (MegaView Science Education Services Co., Ltd., Taichung, Taiwan) inside growth chambers at IRTA facilities (Cabrils, Barcelona, Spain). The colony was initiated with individuals collected from tobacco plants at IRTA. Rearings of *O. majusculus* were maintained on green beans pods (*Phaseolus vulgaris* L.) as egg-laying substrate and fed with *Ephestia kuehniella* Zeller (Lepidoptera*:* Pyralidae) eggs*.* Initial stock was obtained from the University of Lleida (Ardanuy et al. 2016). *Aphidius matricariae* parasitoids were obtained from Agrobío (Almería, Spain) and the mummified aphids were reared on sweet pepper infested with *M. persicae*. In order to obtain parasitoids of known age, mummified aphids from the culture were isolated in 250 mL glass vials until adult emergence. Adults were fed with a 70% sugar solution (glucose, fructose and sucrose). *Episyrphus balteatus* eggs were purchased from Katz Biotech AG (Baruth/Marck, Germany), and *A. aphidimyza* cocoons from Agrobío. Both predators were kept in the same Bugdorm cages explained above with sweet pepper plants infested with *M. persicae* until larvae reached the required instar.

Effect of predators on aphid population reduction**.** Potted sweet pepper plants were individually placed on a plastic dish (30 cm diameter) with a hole in the center, inside of a cylindrical clear plastic cage (25 cm diameter x 50 cm height). The top of the cage was covered with mesh to allow ventilation. Sweet pepper plants were infested with 50 *M. persicae* of mixed instars and allowed to reproduce for one week before the start of the experiment. A second-instar larva of *A. aphidimyza*, a young female *O. majusculus* no more than 48 h old*,* or a second-instar *E. balteatus* larva was individually placed in a cage containing an infested sweet pepper plant for three days, after which time the number of live aphids per cage was recorded. Infested sweet pepper plants without predators were used as controls. Eleven replicates per treatment were conducted.

Impact of the aphid cornicle exudate on aphid offspring. To evaluate the effect of the exudates on population growth, an experiment was conducted on a small Petri dish (2.5 cm diameter) containing a sweet pepper leaf-disc placed on top of a wet cotton pad.

Ten healthy *M. persicae* (second to third instar) were placed on the leaf disc. Ten other aphids from the same colony were stimulated by lightly touching their abdomen with a fine paintbrush to produce droplets of the cornicle exudate (Nault et al. 1973). The dropletts were collected onto a piece of filter paper (0.5 x 0.5 cm) taht was then introduced into the arenas. Filter paper was replaced daily. Offspring were counted at 24, 48 and 72 hours. The same setting without filter paper impregnated with the exudate was used as a control. Fourteen replicates per treatment were performed.

Prey consumption and preference of *A. aphidimyza* and *O. majusculus* for parasitized and unparasitized aphids***.*** A no choice experiment to study the prey consumption and a preference cafeteria test were done with second-instar larvae of *A. aphidimyza* and young females of *O. majusculus* no more than 48 h old*. Episyrphus balteatus* larvae were not included in these experiments because the coincidental IGP on parasitized aphids have been previously studied in several papers Meyhöfer and Klug 2002; Pineda et al. 2007; Almohamad et al. 2008).

In the no choice experiment predators were introduced separately in arenas similar to those explained above (Petri dish with a sweet pepper leaf disk), with either 15 unparasitized aphids (hereafter healthy), with 15 parasitized from three to four days before but not yet mummified aphids (hereafter parasitized), or with 15 mummified aphids. After 24 hours, the number of aphids killed by each predator was recorded. Afterward, parasitized aphids and mummified aphids were kept in a climatic chamber until emergence of adult parasitoids. Twenty replicates per treatment were performed, and each predator was used only once.

To test the preference of potential prey, a preference cafeteria test (Krebs 1999) was conducted. The experimental arena was similar to that of the prey consumption experiment explained above, but five parasitized and five healthy aphids were evenly distributed on the sweet pepper disk. Predators were introduced in the middle of the disk and the number of consumed aphids in each plate was recorded at four, eight, 24, 28, 32 and 48 hours. Twenty replicates per treatment were performed and each predator was used only once.

Interactions of predators with parasitoids on aphid control. To study the efficacy of the predator in an aphid population with healthy and parasitized aphids, the same set up of the first experiment was utilized. Potted **s**weet pepper plants infested with 50 *M. persicae* of mixed instars one week before the start of the experiment were individually caged with three female and two male parasitoids (less than 36 hours old) for 24 hours. After removing the parasitoids, a predator was introduced in each cage for three days. The number of live aphids was then counted. To obtain the mummified aphids, the infested plant was kept in the climatic chamber for approximately ten days. Aphid-infested plants with parasitoids but without predators were used as controls. Twelve replicates per treatment were performed

Data analysis.

The difference in aphid population due to the activity of predators was assessed using a one-way ANOVA. The effect of the presence of aphids' cornicle exudate on aphid progeny was analyzed at 24, 48, and 72 hours using a student`s *t*-test. To quantify prey consumption in the no-choice test, the nonparametric Kruskal-Wallis test was used followed by a Mann-Whitney *U*-test for multiple comparisons. The Bonferroni correction was used to find the statistical differences for each set of treatments.

To analyze the food preference in the cafeteria choice test, Rodgers´s index was used (Krebs 1999). The area under the cumulative consumption curve versus time for each available prey ('Ai') was calculated for each predator and standardized to a maximum of 1.0 according to the formula *Ri= Ai/max (Ai),* where: '*Ri'* is Rodgers´s preference index for prey *'i';* and '*max (Ai)'* is the largest value of '*Ai'.* A score of 1.0 corresponds to the most preferred prey and a score of less than 1.0 corresponds to the least preferred. Rodgers indexes for each prey were compared with a one-tailed student`s *t*-test. Finally, the mean number of healthy and mummified aphids in the treatments with parasitoid and predator was compared to the mean number of aphids in the treatment with only parasitoid (control) using a student`s *t*-test. All statistical analyses were performed using R (R Development Core Team Version 1.1.442 – ©2009-2018).

17.4.3 Results

<u>Effect of predators on aphid population reduction.</u> The presence of predators over three days had a significant influence on the final aphid population ($F_{3, 40}$=166.9, P= <0.0001) (Figure 1). The lowest number of aphids was recorded in the presence of *E. balteatus* larvae, followed by the arenas with *A. aphidimyza.* At the end of the experiment, both arenas had significantly less aphids than the control group with no predator. No significant differences were observed between number of aphids in the control arenas and those with *O. majusculus*, which has an intermediate value between the control and the treatment with *A. aphidimyza*.

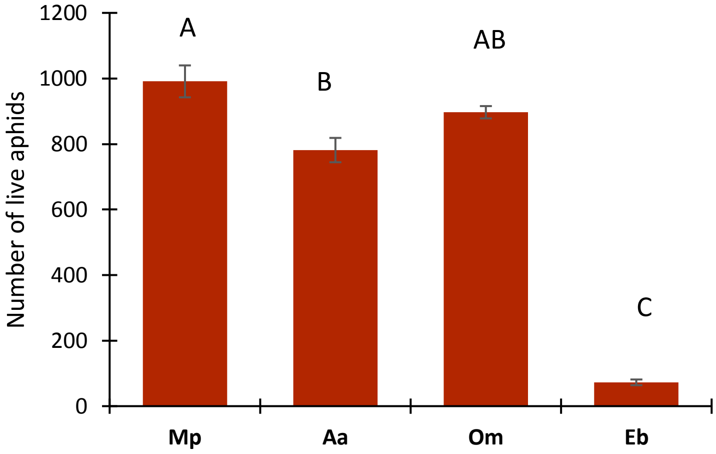


Figure 1. Number of live aphids (mean ± SE) recorded in the presence of *A. aphidimyza* (Aa), *O. majusculus* (Om), *E. balteatus* (Eb). Aphids without predators were used as control (Mp). Different letters above the columns show significant differences (ANOVA, Tukey for mean separation, P < 0.05).

<u>Impact of the aphid cornicle exudate on aphid offspring.</u>

A significant higher aphid offspring was found in the arenas with the filter paper impregnated with cornicle droplets (Figure 2) at all observational times. At 24 h, number of descendants increased more than two times compared to the control (t = -6.1425, P<0.0001), tripled at 48 h (t = -7.9403, P<0.0001) and was more than three times at 72 h (t = -11.71, P<0.0001).

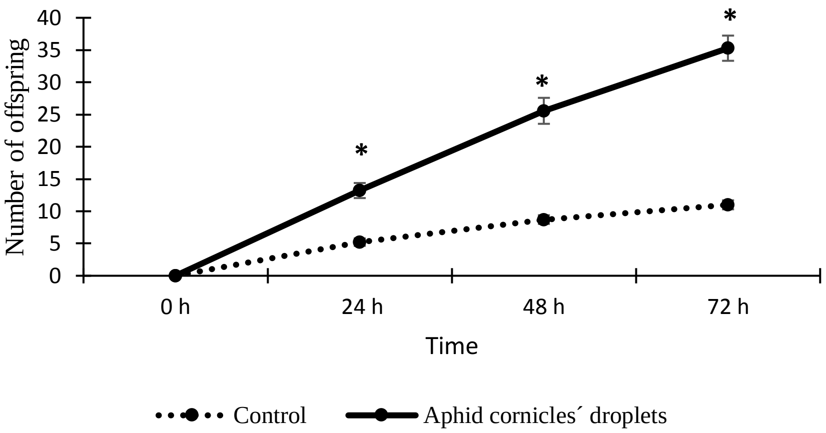


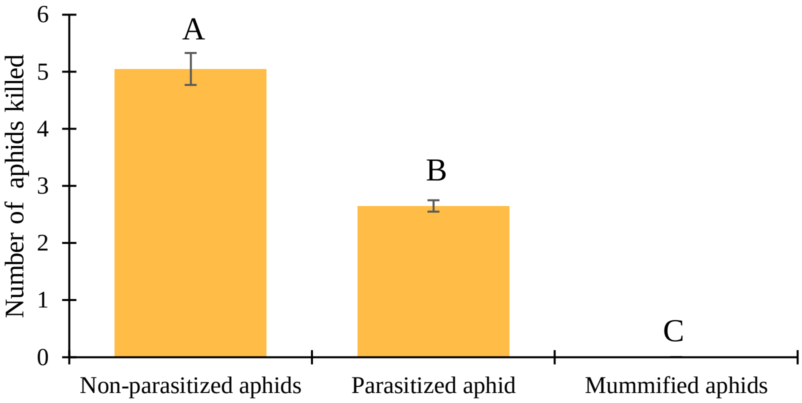
Figure 2. Cumulative number (mean ± SE) of aphid offspring recorded in the presence of aphid cornicle droplets (continue line) and in their absence (discontinue line) over 72 h. Asterisks above error bars indicate significant differences between both treatments according to the Student *t*-test ($P<0.05$).

Prey consumption and preference of *A. aphidimyza* and *O. majusculus* for parasitized and non-parasitized aphids

No choice experiments. Significant differences were found when prey acceptability by *A. aphidimyza* was tested (χ^2= 52.458, P= <0.0001) (Figure 3A). Number of non-parasitized aphids killed by the predators was significantly higher than number of parasitized aphids (P<0.0001) and mummified aphids (P<0.0001). Similarly, number of parasitized aphids killed were significantly higher that number of mummified aphids (P<0.0001).

Significant differences between different potential prey were also found in the arenas with *O. majusculus* (χ^2= 24.56, P=<0.0001) (Figure 3B). Predator females consumed significantly more healthy aphids than mummified aphids (P<0.0001) and more parasitized aphids than mummified aphids (P<0.0001). No significant differences were found between number of healthy and parasitized aphids killed by the *O. majusculus* females (P=0.1107). Mummified aphids were not consumed by either predator when they were offered. The lack of any feeding on mummified aphids was corroborated by the emergence of all adult parasitoids after about 15 days in the climatic chamber.

A)



B)

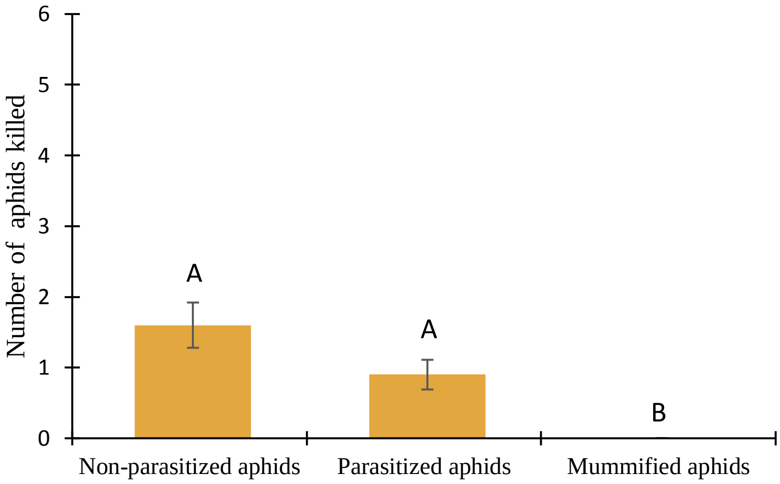


Figure 3. Number of aphids killed (mean ± SE) by: **A)** *A. aphidimyza* and **B)** *O. majusculus*. Different letters above bars indicate significant differences between means (Kruskal-Wallis test, *P* <0.05).

Cafeteria experiment. Figures 4A and 4B show the cumulative predation curves obtained with *A. aphidimyza* and *O. majusculus* respectively over 48 hours. The Rodgers´s index for non-parasitized aphids and for parasitized aphids was 1.0 ± 0.00 and 0.2 ± 0.02, respectively for *A. aphidimyza,* and 1.0 ± 0.00 and 0.2 ± 0.03, respectively for *O. majusculus*. Rodgers´s index was significantly lower for parasitized aphids in both cases: *A. aphidimyza* (t = -10.70, P<0.0001), and for *O. majusculus* (t = -7.12, P<0.0001).

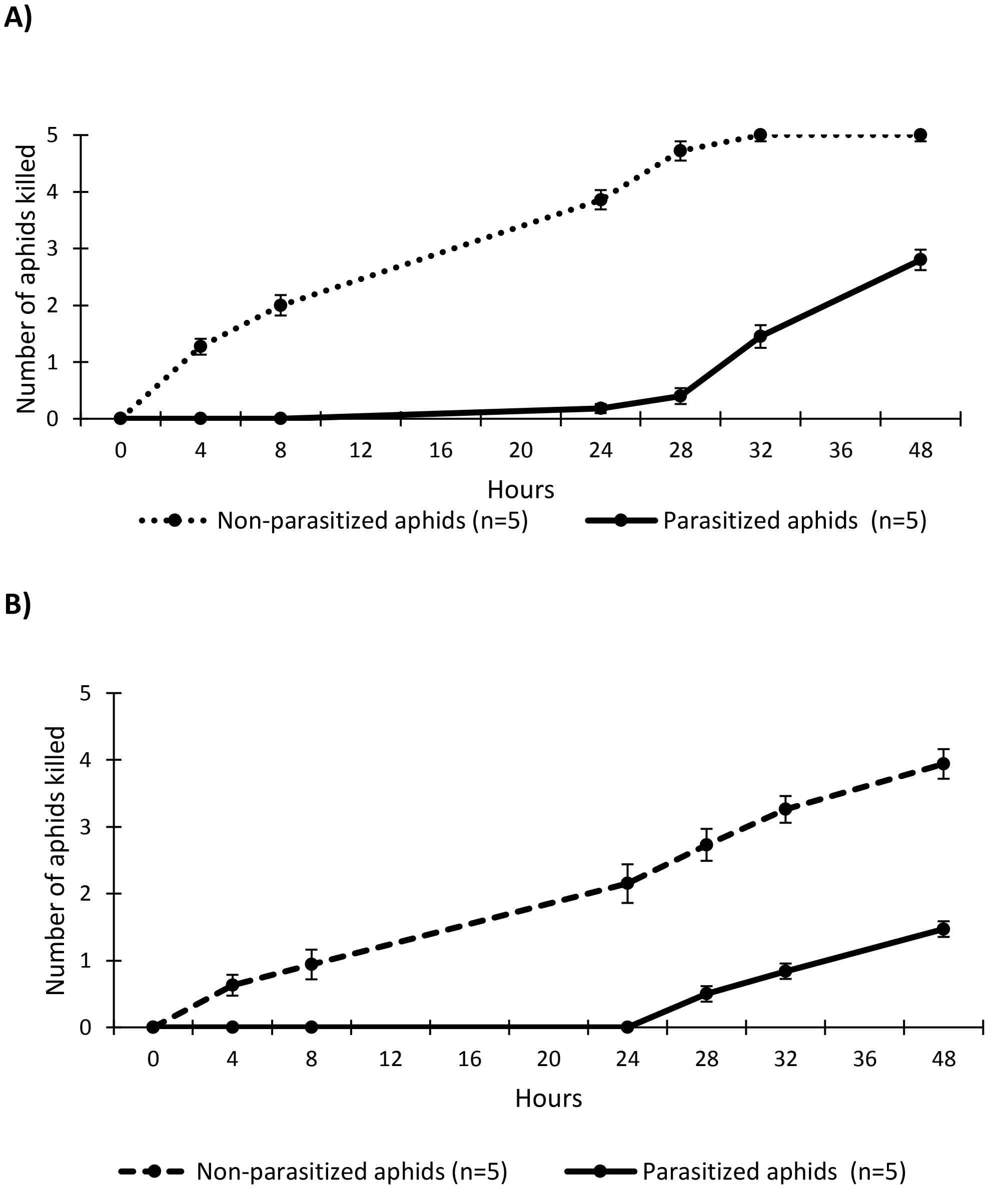


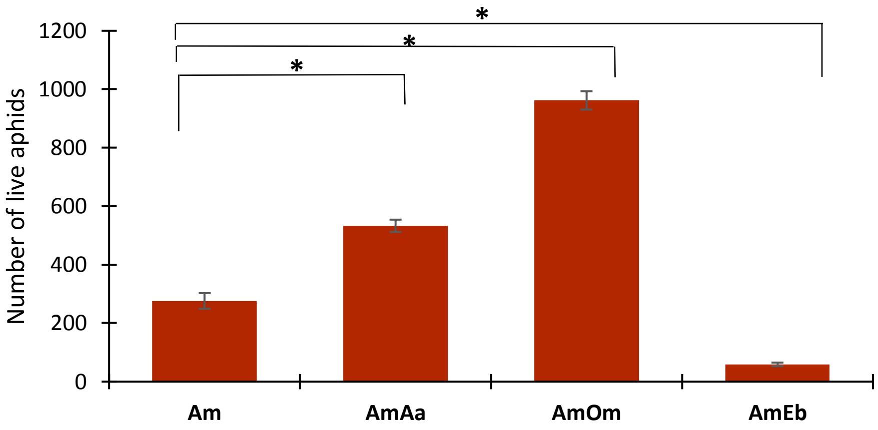
Figure 4. Cumulative predation of aphids (mean ± SE) by: **A)** *A. aphidimyza* and **B)** *O. majusculus* when offered parasitized and non-parasitized aphids during 48 h.

Interactions of predators with parasitoids on aphid control.

Episyrphus balteatus significantly reduced the aphid population (t = 4.459; P <0.0001). On the contrary, significantly more live aphids were found when the predators *A. aphidimyza* (t = 11.165; P <0.0001) and *O. majusculus* (t = 18.843; P <0.0001) were released in the cages compared to the control with only parasitoids (Figure 5A).

The number of mummified aphids was lower in all treatments with predators (Figure 5B). Releasing *O. majusculus, A. aphidimyza* or *E. balteatus* in the cages significantly reduced the number of mummies by 25% (t = 2.2527; P= 0.0362), 50% (t = 5.750; P <0.0001) and 90% (t =15.654; P <0.0001) respectively compared to the control with only the parasitoid.

A)



B)

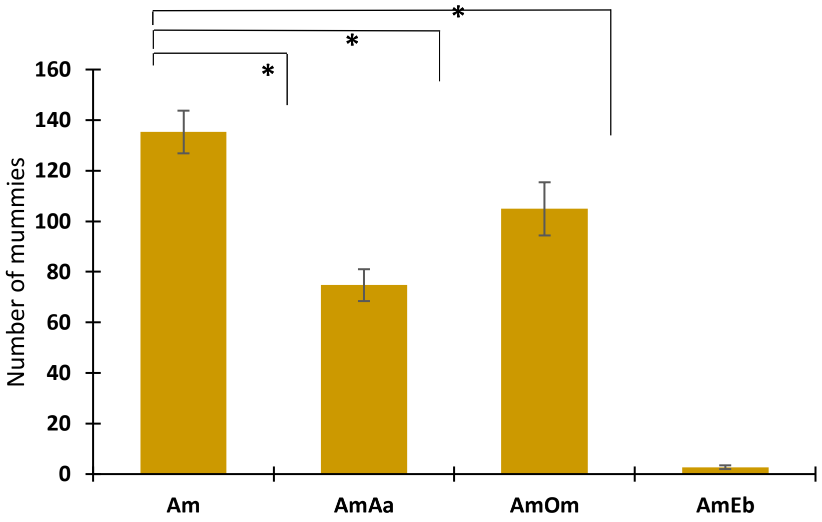


Figure 5. Number (mean± SE) of aphids in the treatments with only the parasitoid *Aphidius matricariae* (Am), or together with *A. aphidimyza* (AmAa), *O. majusculus* (AmOm) or *E. balteatus* (AmEb); and mummified aphids: **A)** live aphids and **B)** mummified aphids. Asterisks above lines indicate statistical significant differences between the two indicated columns according to the Student t-test (*P*<0.05).

17.4.4 Discussion

The results obtained in this study showed that *E. balteatus* was by far the most effective predator as a single larva was able to reduce the aphid population by 90%. The reduction in the number of aphids was far less in the presence of *A. aphidimyza* (around 20%), whereas *O. majusculus* was unable to reduce the population of aphids. The results of these experiments agree with those in the literature, which indicate that *E. balteatus* and *A. aphidimyza* have long been recognized as useful for the control of aphids due to their feeding specificity, but also because under conditions of high pest density, they kill more aphids than they consume (Harris 1973; Markulla and Tittanen 1985; Sobhani et al. 2013; Boulanger et al. 2019). Higher aphid control in the treatments with *E. balteatus* compared to that with *A. aphidimyza* may be related to the higher voracity of the hoverfly larva compared to the gall-midge (Brodeur et al. 2017). In their review these authors indicate that a single larva of hoverfly kills up to 500 aphids to complete its development in contrast to an *A. aphidimyza* larva that kills as many as 80. Conversely to what was observed for the hoverfly and the gall-midge larvae, *O. majusculus* females did not show any effect on aphid population. Although being polyphagous and able to feed on aphids (e.g. Alvarado et al. 1997), *Orius* spp. are well-known biocontrol agents for thrips (Riudavets and Castañé 1998). In fact, eight species of *Orius,* including *O. majusculus*, are or have been commercially reared and sold for thrips control (van Lenteren et al. 2012) and have demonstrated preference for these herbivores over other potential prey such as whiteflies (Arnó et al. 2008).

The effect of predators on aphid population was probably not only influenced by the different voracity and specificity of the three-predator species but also by non-consumptive effects of the predators on the aphids. Our experiment with the cornicle exudate on the filter paper clearly showed an increase of progeny that more than tripled in just 72 hours compared to the control without exudate. This fecundity compensation is a non-consumptive effect triggered by the alarm pheromone present in the cornicle secretion (Pickett et al. 1992; Altincicek et al. 2008; Barribeau et al. 2010; Vandermoten et al. 2012; Leventhal et al. 2014). To the best of our knowledge, fecundity compensation has never been described before for *M. persicae*. In fact, it was not observed by Ingerslew and Finke (2017) on *M. persicae* attacked by *Aphidius ervi* Haliday and *Aphidius colemani* Haliday (Hymenoptera: Aphidiinae), probably due to the fact that the sting by the parasitoid is a subtle stimulus that produces only a marginal response from aphids (Goff and Nault 1974). Conversely, *O. majusculus* actively hunts in its environment, resulting in a high frequency of encounters with prey (Montserrat et al. 2004). Since the cornicle secretion is produced as response of a physical attack, it can be hypothesized that females of *O. majusculus* that actively search, not only for feeding but also for egg laying, caused greater exudate production than the larva of *A. aphidimyza* or *E. balteatus,* and this may have resulted in enhanced reproduction. Additionally, it has been shown that aphids can deposited droplets directly onto the predator what will help to spread the alarm signal as predator foraging continues (Vandermoten et al. 2012). In contrast, *A. aphidimyza* larvae are furtive hunters, triggering little defensive reaction by aphids (Lucas and Brodeur 2001). Such little defensive reaction should also be expected from the hoverfly larvae searching for prey, for which Chambers (1988) described a “casting” behavior. The rear of the larva remains immobile while the anterior keeps palpating in front and laterally to hunt the prey. Furthermore, Tinkeu and Hance (1998) indicate that the second and third instar hoverfly larvae touch the aphid only twice or even less before capturing it and, therefore, give it little choice to stimulate cornicle secretion as a response to the attack.

Given the co-occurrence of predators and parasitoids in nature (Brodeur et al. 2017), is crucial to understand the effect of the coincidental IGP of predators over parasitized aphids. It is well established that *E. balteatus* larvae feed indistinctly on parasitized and non-parasitized aphids but avoid feeding on mummified aphids (Meyhöfer and Klug 2002; Pineda et al. 2007; Almohamad et al. 2008). Our results indicated that both *A. aphidimyza* larvae and *O. majusculus* females were able to feed on parasitized but not yet mummified aphids and, similarly to what has been observed for syrphid larvae, they do not feed on mummified aphids. Other papers have also reported the consumption of *Aphis gossypii* Glover (Hemiptera: Aphididae) parasitized by *A. colemani* that were not yet mummified by *A. aphidimyza* larvae (Harizanova and Ekbom 1997, Enkegaard et al. 2005, Mottaghinia et al. 2018). Contrary to what has been described for *E. balteatus* larvae, our results showede that, *A. aphidimyza* and *O. majusculus* were able to distinguish parasitized from non-parasitized aphids, and both predators preferred to feed on non-parasitized. In fact, in the cafeteria experiment, they did not feed on parasitized aphids until a large proportion of non-parasitized aphids had already been consumed. This disagrees with previous results in the literature that reported slight preference of *A. aphidimyza* larvae for *A. colemani* parasitized aphids on chrysanthemum leaves (Enkegaard et al. 2005). These authors suggested the reduced mobility of parasitized aphids as one of the possible causes for that preference. A more recent study by Mottaghinia et al. (2018) helps to understand the differences between our results and that of Enkegaard et al. (2005). Mottaghinia et al. (2018) concluded that the gall-midge larvae preference for *A. gossypii* parasitized by *A. colemani* over non-parasitized aphids in two cucumber cultivars was mediated by trichrome density; parasitized aphids were preferred in the cultivar with higher trichrome density, whereas non-parasitized aphids were in the cultivar with lower trichrome density. This concurs with our results, that non-parasitized aphids were the preferred prey in low trichrome density pepper leaves used in our experiments. This suggest that in peach trees the same pattern may occur, since the leaves do not have high trichrome density, allowing predators to hunt without disturbances. There is no record of food preference of *O. majusculus* regarding parasitized versus non-parasitized aphids. However, Sohrabi et al. (2013) found that *O. majusculus* fifth instar nymphs and adults, preferred parasitized over non-parasitized whitefly nymphs by *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae). Conversely, according to Pehlivan et al. (2017), *Orius niger* (Wolff) (Hemiptera: Anthocoridae) preferred non-parasitized *Trichograma evanescens* Westwood (Hymenoptera: Trichogrammatidae) lepidopteran eggs versus parasitized ones.

The experiment with parasites and predators in laboratory microcosms recreates some of the co-occurrence of natural enemies that may take place in orchards. Similarly, to what was observed in the first experiment with only predators, the voracious larvae of *E. balteatus* successfully controlled the aphids. Conversely, the aphid population in cages with *A. aphidimyza* and *O. majusculus* was higher than in the control with the parasitoid but without predators. These surprising results might be the result of the predator searching in plants infested with different aphid densities. In the first experiment, 50 initial aphids yielded 1000 after 10 days in the control treatment without predators. Therefore, it may be assumed that, in this experiment, the predator was released on a plant infested with roughly 700 aphids (50 initial aphids x 2 aphids/day x 7 days). This population size was far away of the <150 aphids (Fig. 5) infesting the pepper plants in the experiment in which the predators were released after *A. matricariae* was removed from the cages. Given that the role of the cornicle secretion targets more to defend the colony than the individual (Vandermoten et al. 2012), it seems reasonable that fecundity compensation as response to the alarm pheromone was higher at low than at high aphid density. Rate of encounters with the predator and, consequently, stimulus to secrete cornicle exudates decreases as aphid density increases. Aphid investment in defense mechanisms is probably higher when the population is more threatened, as it happens at lower aphid densities.

Overall, results of our experiments indicate than in small colonies, like those occurring at early spring, active hunting predators, such as *O. majusculus* might hamper *M.*

persicae biological control due to the aphid reproduction stimulation they triggered, which in turn will benefit their survival and fitness by providing more prey. In peach orchards, parasitoids may collaborate to create this unwanted situation by parasitizing *M. persicae* early in the season (Aparicio et al. 2019). That would not be the case of *E. balteatus* because a single larva can control a large aphid population. In turn, in a non-concealed situation like that occurring in the field, both the fecundity compensation and the alarm pheromone will help to congregate natural enemies in infested plants. Most natural enemies use the volatiles produced by aphid infested plants as a cue to locate the plant (habitat location) and the alarm pheromone as a short-range cue to find the aphid colony (aphid location) (Hatano et al. 2008). This location system is unspecific, and therefore, several predator and parasitoid stages and species might be present in the same colony.

Results obtained in our experiments involving parasitized non-mummified aphids and predators indicate there is a risk of coincidental IGP because all three predators are able to prey on recently parasitized aphids. The detrimental effect might be especially severe in the case of hoverflies due to its voracity and their indistinct feeding on parasitized and non-parasitized aphids leading to a reduction of more than 90% in the number of mummies. Such notable reduction of parasitoid population might be unfavorable for conservation biological control at the long term. However, the fact that all three predators avoided to feed on mummies and *A. aphidimyza* and *O. majusculus* clearly preferred to feed on unparasitized healthy aphids gives a chance for parasitoids and predators to jointly contribute to aphid control. Furthermore, in our experiments we only considered interactions produced by certain instars. In field conditions, complexity of food webs will probably be much higher and will involve more than one predator species. Simultaneous use of more than one predator and parasitoids has been tested before for *M. persicae* control with positive results (Messelink et al. 2013). These authors obtained an excellent control of this aphid in sweet peppers based on the combined use of parasitoids, *A. aphidimyza* and *O. majusculus*. However, larger-scale experiments are needed to better understand the relevance of the observed effects on a field situation.

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CONCLUSIONS

CHAPTER 1.

- The gall-midge *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and the hoverfly *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) are the most abundant predators found in *Myzus persicae* Sulzer (Hemiptera: Aphidiinae) colonies. Both species should be considered key predators in conservation biological control.
- Selected insectary plants close to peach orchards are useful to recruit hoverflies and hymenoptera parasitoids.
- *Lobularia maritima* L. is among the most attractive plant species for parasitoids and hoverflies.
- *Achillea millefolium* L. is attractive to hymenoptera parasitoids but not to hoverflies.
- *Sinapis alba* L. and *Moricandia arvensis* (L.) are useful to attract hoverflies but did not consistently attract other natural enemies.
- During the samplings, none of the insectary plants included in the study hosted *M. persicae.*
- In the conditions of this study, no differences in the abundance of natural enemies are found between the aphid colonies placed near and away the insectary plants.

CHAPTER 2.

- There is a wide array of hymenoptera parasitoids and hyperparasitoids associated with *M. persicae* in peach orchards.
- *Aphidius matricariae* Haliday (Hymenoptera: Braconidae) is the most prevalent parasitoid species associated to *M. persicae* in peach orchards, and should thus be considered one of the key natural enemies in conservation biological control.
- *Aphidius transcaspicus* Telenga and *Praon volucre* (Haliday) (Hymenoptera: Braconidae) are the two parasitoid species associated to *Hyalopterus* spp. Koch
- *Myzus persicae* and *Hyalopterus* spp. have overlapping parasitoid and hyperparasitoid complexes.

- The absence of hyperparasitoids early in the season, when *M. persicae* populations build up, indicates that the biological control of this aphid would not be negatively impacted by the presence of hyperparasitoids.

CHAPTER 3.

- Flowers and non-flowering shoots of *Lobularia maritima* are highly attractive to the parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) and the predator *A. aphidimyza*.
- The aphid-infested shoots and the *L. maritima* flowers are equally attractive to *A. ervi* and *A. aphidimyza* females.
- The availability of *L. maritima* flowers improves the longevity of *A. ervi* females in the absence of *M. persicae*, but not when the aphids are present.
- The availability of *L. maritima* flowers does not improve the longevity of *A. aphidimyza*.
- Although nectar is a suitable food resource for *A. aphidimyza* females, the floral morphology of *L. maritima* prevents this predator to feed on the nectaries.

CHAPTER 4.

- Among the three aphid predators studied, *E. balteatus* is the most effective predator to control *M. persicae* followed by *A. aphidimyza. Orius majusculus* (Reuter) (Hemiptera: Anthocoridae), although being able to feed on aphids, does not reduce pest population in laboratory conditions.
- The cornicle exudate, produced as a response of a physical stimuli on *M. persicae,* triggers fecundity compensation, that is to say a rise in the aphid offspring production.
- *Aphidoletes aphidimyza* and *O. majusculus* kill both non-parasitized and parasitized but not yet mummified *M. persicae*, but both predators prefer to kill non-parasitized aphids. None of these predators kill mummies.
- The fact that *E. balteatus*, *A. aphidimyza* and *O. majusculus* avoid preying on mummies and that the last two predators clearly prefer to prey on unparasitized aphids predicts that the coexistence of parasitoids and predators would not be negative for the biological control of *M. persicae*.