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Effect of parasitoid competition, ant exclusion and carbohydrate sources on biological control of *Saissetia oleae* on olive trees

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Doctoral Thesis

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Directors:

Professor Dr. José Alberto Cardoso Pereira

Dr. Alejandro Tena Barreda

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Efecto de la competición de los parasitóides, la exclusión de hormigas y las fuentes de hidratos de carbono en el control biológico de *Saissetia oleae* en olivos

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Tesis Doctoral

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ABSTRACT

The black scale, *Saissetia oleae* (Olivier), is considered a secondary pest of olive tree in Portugal, where it is attacked by a complex of natural enemies that control its population. Parasitoids of genus *Metaphycus* (Hymenoptera: Encyrtidae) and *Coccophagus* (Hymenoptera: Aphelinidae) are among the main natural enemies. In this thesis, the several aspects of the successful natural biological control of this scale are analyzed in the field and laboratory. In detail, we analyzed: (i) whether the facultative autoparasitoid *Coccophagus lycimnia* (Walker) can adversely affect the population densities of *Metaphycus* spp. parasitoids and disrupt the biological control of *S. oleae*; (ii) the effect of ant-exclusion on the biological control of *S. oleae* and its parasitoid complex; and (iii) the effect of the carbohydrate sources available in the field (nectar and honeydew) on the survival of parasitoids of both genus.

For the development of the first objective, the population densities of the scale, the primary parasitoids of the genus *Metaphycus* and the autoparasitoid *C. lycimnia* were recorded for three consecutive seasons in 28 olive groves. The populations of the parasitoids followed opposite patterns. At the end of the life cycle of the *S. oleae* (May), the number of *Metaphycus* spp. observed was practically nil whereas the number of *C. lycimnia* was five times greater than at the beginning of the assay. In September and November, *Metaphycus* spp. parasitoids parasitized smaller scales than *C. lycimnia*, demonstrating that *Metaphycus* spp. parasitoids are superior at exploitative competition. *Metaphycus* spp. and *C. lycimnia* sex ratio was similar in September, but in November the sexual proportion of *Metaphycus* spp. was higher than that of *C. lycimnia*. The facultative autoparasitoid *C. lycimnia* tended to lay male eggs on *Metaphycus* spp. females. The density of *S. oleae* decreased from September to May, when the ratio *S. oleae*-*C. lycimnia* reached 2:1. Altogether, these results demonstrate that the facultative autoparasitoid *C. lycimnia* is a superior parasitoid and it can successfully control the scale under the climatic conditions of northern Portugal.

The effect of ant exclusion on the parasitoid complex of *S. oleae*, as well as on scale density, was studied in an olive grove during two seasons (spring and fall). *Crematogaster auberti* (Emery) was the most abundant ant species in both seasons but it was more active in spring than in fall. Ant exclusion did not affect the number of parasitoids recovered in fall, the beginning of the life cycle of the scale. The parasitoid complex was, then, by *Coccophagus* spp. parasitoids, which can lay their eggs faster

than *Metaphycus* spp.. In late spring, the number of parasitoids recovered from *S. oleae* adults was higher in ant-excluded trees than in ant-allowed trees, being *Metaphycus lounsburyi* (Howard) the main parasitoid species recovered, especially in ant-allowed trees. Ant exclusion, however, did not affect *S. oleae* density in any season. These data altogether, suggest that the biological control of *S. oleae* can be affected by ants only at the end of its life cycle when *C. auberti* is the main ant present in olive groves.

To study the effect of the main carbohydrate sources available in olive groves from northern Portugal, the survival of three *S. oleae* parasitoids: *Metaphycus lounsburyi*, *Coccophagus semicircularis* (Förster) and *C. lycimnia* was tested. The carbohydrates were the nectar of six species of flowering spontaneous plants and honeydew excreted by two hemipterans: *S. oleae* and *Euphyllura* olivina (Costa). The honeydew of *S. oleae* was the best food source for the three parasitoids, as they survived, on average, about seven times more when fed on this honeydew than on nectar from the provided flowers. *Euphyllura olivina* honeydew, however, was a poorer carbohydrate source. Parasitoids fed on this honeydew lived longer than unfed parasitoids, but lived much less than those fed on *S. oleae* honeydew. Taking into account that the honeydew of *S. oleae* is present throughout the year and its high quality for parasitoids, carbohydrates should not be considered a limiting factor in the biological control of *S. oleae* in olive groves. Future studies should analyze *S. oleae* honeydew to detect the components responsible for its high quality, as honeydew is usually a poorer carbohydrate source than nectar for natural enemies.

RESUMEN

Los olivos tienen una gran importancia económica y social en Portugal, que ocupa el octavo lugar en la producción de aceitunas del mundo (INE, 2017). Las principales regiones productoras son: Alentejo, Trás-os-Montes y Beira Interior.

El olivo puede ser atacado por diverso grupo de artrópodos, representado por al menos 116 especies de insectos y 30 ácaros (Tzanakakis 2003). Sin embargo, sólo algunas especies alcanzan el estatus de plaga. En la región de Trás-os-Montes (Portugal), las principales plagas son: la mosca de la aceituna *Bactrocera oleae* (Rossi) (Diptera: Tephitidae), la polilla de olivo *Prays oleae* (Bernard) (Lepidoptera: Plutellidae), la cochinilla negra *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) y el algodón del olivo *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae).

Saissetia oleae es un insecto polífago que se alimenta de más de 60 especies de plantas en la región mediterránea (Morillo 1977, Passos de Carvalho et al. 2003). Se considera una plaga de olivo y cítricos (Pereira 2004, Tena et al. 2008a). El daño causado se debe principalmente a la excreción de melaza y al desarrollo de negrilla, lo que reduce la fotosíntesis y la producción (Passos de Carvalho et al. 2003).

Saissetia oleae puede desarrollar entre una y dos generaciones anuales dependiendo de las condiciones climáticas de los olivos de la cuenca mediterránea (Paparatti 1986, Pereira 2004, Tena et al. 2008a). Los sistemas de fertilización, poda y riego también pueden afectar el número de generaciones de *S. oleae* (Bodenheimer 1951, Blumberg et al. 1975, Panis 1977, Passos de Carvalho et al. 2003). *Saissetia oleae* puede poner desde cientos de huevos a 4000 dependiendo del tamaño de la hembra adulta (Morillo 1977, Brailes y Campos 1986, Passos de Carvalho et al. 2003, Tena et al. 2008a). La puesta se realiza irregularmente durante 10-15 días, en primavera y a principios de verano (Passos de Carvalho et al. 2003) y durante 20 a 25 días en otoño (Paparatti 1986) y los huevos eclosionan alrededor de 14 días despué́s. Las ninfas recién emergidas permanecen en la cámara de puesta durante uno o dos días (Torres 2007b) y cuando las condiciones climáticas son favorables, se mueven y se asientan, generalmente en la parte inferior de las hojas (Morillo 1977, Pereira 2004), permaneciendo inmóviles cerca de cuatro semanas en la primavera / verano (Morillo 1977) y hasta 50 días al final del otoño (Torres 2007b). En la región de Trás-os-Montes,

las ninfas se desarrollan entre finales de junio y mediados de agosto (Pereira 2004). Una vez establecidas, principalmente en las hojas, permanecen allí hasta que algún estímulo las induce a emigrar a ramas (Bibolini 1958, Ebeling 1959, Argyriou 1963, Morillo 1977, Pereira 2004). Esta migración parece ser estimulada por la búsqueda de nichos nutricionalmente y climáticamente adecuados (Briales y Campos 1986). La mayoría emigra durante el tercer estadio (Ebeling 1959, Santaballa 1972, Llorens 1984, Smith et al.1997). Después de la tercera muda y una vez en las ramas, las hembras adultas aumentan rápidamente (Morillo 1977) y, a medida que se desarrollan, la cantidad de melaza excretada aumenta hasta que se detiene justo antes de la oviposición (Bodenheimer 1951). Las hembras adultas mueren después de la oviposición, quedando el cuerpo en la rama (Morillo 1977).

Después de la introducción de *Rhizobious ventralis* Erich (Coleoptera: Coccinalidae) en California a finales del siglo XIX (Bartlett 1978), se desarrollaron extensos esfuerzos para implementar programas de control biológico contra *S. oleae* en diferentes partes del mundo, principalmente en cítricos y olivo. En California, a principios del siglo XX (1902) y durante 90 años se desarrolló un programa de introducción de enemigos naturales, para controlar a *S. oleae* en ambos cultivos (Lampson y Morse 1992). Desde entonces, se ha acumulado una tremenda cantidad de conocimiento sobre el control biológico de *S. oleae* (Kapranas y Tena 2015). El control biológico satisfactorio de *S. oleae* se ha logrado principalmente mediante la introducción de parasitoides (Mendel et al. 1984, Waterhouse y Sands 2001), pero también con las liberaciones inoculativas de parasitoides (Graebner et al. 1984) y programas de control biológico de conservación (Tena et al. 2008a).

Los enemigos naturales más comunes asociados con *S. oleae* se agrupan en dos grupos: entomopatógenos y entomófagos. En el primer grupo se incluyen hongos, en el segundo grupo hay varias especies de depredadores y parasitoides (Passos de Carvalho et al. 2003, Pereira 2004). El hongo entomopatogénico *Verticillium lecanii* (Zimm.) Viègas, complejo de hongos mitospóricos (Passos de Carvalho et al. 2003), puede controlar densidades poblacionales elevadas de la cochinilla en condiciones de alta humedad relativa (Smith et al. 1997). Los depredadores pertenecen principalmente a la familia Coccinellidae (Santos et al. 2008). En la región de Trás-os-Montes (Portugal), los depredadores más abundantes e importantes de *S. oleae* pertenecen a la familia de los coccinelidos: *Scymnus* (*Scymnus*) *interruptus* (Goeze), *Scymnus* (*Pullus*) *subvillosus* (Goeze), *Scymnus mediterráneo* (Iablokoff-Khnzorian) y *Rhyzobius chrysomeloides* (Herbst) (Santos 2007), *Chilocorus bipustulatus* (Linnaeus), *Exochomus nigromaculatus* (Goeze), *E. quadripustulatus* (Linnaeus), y *Rhyzobius lophanthae* (Blaisdell) (Santos 2007). De acuerdo con Santos et al. (2008), las ninfas del segundo estadio son usualmente depredadas por los coccinélidos mientras que *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) se alimenta de las ninfas del primer estadio de *S. oleae* (Passos de carvalho et al. 2003).

Los parasitoides de *S. oleae* pertenecen a las familias himenópteros: Aphelinidae, Encyrtidae y Pteromalidae (Passos de Carvalho et al. 2003, Pereira 2004, Torres 2007b, Tena et al. 2008). *Metaphycus* es el género más importante de la familia Encyrtidae y *M. lounsburyi* (Howard), *M. helvolus* (Compere) y *M. flavus* (Howard) son las principales especies en Portugal (Pereira 2004). El género más importante dentro de la familia Aphilinidae es *Coccophagus*, siendo *C. lycimnia* (Walker) y *C. semicircularis* (Föster) los parasitoides más abundantes y exitosos de este género en Portugal (Pereira 2004). El Pteromalidae *Scutellista caerulea* (Fonscolombe) es un himenóptero cuyas las larvas se alimentan de los huevos de *S. oleae*, este predador es frecuente y fácil de observar bajo la cochinilla (Passos de Carvalho et al. 2003). Los parasitoides se consideran los principales enemigos naturales de *S. oleae* y las cochinillas en general y se pueden dividir en dos grupos dependiendo del estado de desarrollo de la cochinilla atacada: parasitoide ninfal y adulto (Kapranas y Tena 2015). *M. helvolus*, *M. flavus*, *C. lycimnia* y *C. semicircularis* se encuentran entre los estados ninfales mientras *M. lounsburyi* y *S. caerulea* atacan principalmente adultos de *S. oleae* (Pereira 2004, Tena 2007).

Los *Coccophagus* son hiperparasitoides heterónomos facultativos u obligatorios de cóccidos (Annecke 1964, Bernal et al. 2001, Walters 1983). En el caso de *C. lycimnia* las hembras se desarrollan en cóccidos y los machos pueden desarrollarse en hembras de *Metaphycus* o hembras conespecíficas. Estas características sugerían que *Coccophagus* podría afectar negativamente al potencial de control biológico de *Metaphycus*. Esta hipótesis fue apoyada por estudios posteriores que mostraron que el hiperparasitismo excesivo por *Coccophagus* spp. no permitía que poblaciones de *Metaphycus* spp. aumentasen a medida que avanzaba la temporada, resultando en un control insuficiente de *Coccus pseudomagnoliarum* (Kuwana) en California (Bernal et al. 1998, Bernal et al. 1999, Bernal et al. 2001). Sin embargo, otros estudios sugirieron que los parasitoides del género *Coccophagus* podrían ser agentes eficientes de control biológico de cóccidos (Schweizer et al. 2003, Schweizer et al. 2002). En la región de Trás-os-Montes, los parasitoides del género *Coccophagus* son muy abundantes (Pereira 2004), no obstante su efecto sobre la población de los parasitoides primarios del género *Metaphycus* y de su hospedante común, *S. oleae*, son desconocidos.

La mayoría de los parasitoides adultos necesitan alimentarse con alimentos ricos en carbohidratos para satisfacer sus necesidades energéticas. En la agricultura moderna basada en monocultivos, las fuentes ricas en azúcar en forma de néctar son escasas. Esta ausencia puede comprometer gravemente la fecundidad y la supervivencia de la mayoría de las especies de parasitoides y, en consecuencia, su potencial para controlar las plagas (Heimpel y Jervis 2005, Tena et al. 2016). La incorporación de vegetación complementaria a los cultivos en sistemas agrícolas puede ayudar a apoyar una serie de servicios ecológicos, incluyendo el control natural de plagas por enemigos naturales (Landis et al. 2000). Este efecto se genera por el hecho de que especies seleccionadas de plantas proporcionan néctar floral y extra floral que son vitales para los parasitoides y otros enemigos naturales que se alimentan de azúcar (Heimpel y Jervis 2005). En este enfoque, sin embargo, ha pasado desapercibido el papel potencial de la melaza, que en términos de disponibilidad es la fuente primaria de carbohidratos en muchos agroecosistemas (Tena et al. 2016). De hecho, durante la última década, mediante cromatografía líquida de alto rendimiento (HPLC) se ha demostrado que los parasitoides se alimentan comúnmente de melaza en el campo (Steppuhn y Wäckers 2004, Hogervorst et al. 2007, Tena et al. 2015, Dieckhoff et al. 2014, Calabuig et al. 2015). Sin embargo, la melaza ha sido considerada una fuente de azúcar más pobre que el néctar para los parasitoides (Wäckers et al. 2008). Los olivares de la región de Trás-os-Montes presentan una gran diversidad estructural, ya que están constituidos por árboles perennes con cubiertas vegetales espontáneas, en muchos casos rodeados de parcelas adyacentes con vegetación natural, lo que permite una gran diversidad de hábitat y alimento para los enemigos naturales. Sin embargo, el néctar puede ser escaso dentro del olivar cuando las plantas de la cubierta vegetal no se encuentran en su periodo de floración. Durante esos periodos, la melaza excretada por *S. oleae* y el *E. olivina* son las únicas fuentes de azúcar. .

Las interacciones entre hormigas e insectos hemípteros productores de melaza son conocidas no sólo en hábitats naturales de pastizales y en bosques sino también en sistemas agrícolas (Buckley 1987, Way y Khoo 1992, Styrsky y Eubanks 2007). Los hemípteros proporcionan melaza a las hormigas y estas a su vez protegen a los hemípteros de eventuales depredadores y parasitoides, estableciendo así una relación de mutualismo (Way 1963, Buckley 1987, Stachowicz 2001, Tena et al. 2016). Las consecuencias ecológicas de estas interacciones son enormes, ya que influyen en la abundancia de hormigas, hemípteros y otros artrópodos de la planta, afectando a la salud vegetal. En los agroecosistemas, las hormigas pueden afectar negativamente el control biológico de varias plagas de hemípteros importantes al interferir con la actividad de los parasitoides y depredadores (Debach 1951, Way 1963, Pekas et al. 2011, Tena et al. 2013). En olivares, estas interacciones son muy frecuentes, ya que las hormigas son muy abundantes (Cabanas et al. 1999, Ramalho 2000, Lozano et al. 2002), lo que sugiere un efecto negativo sobre el control biológico de *S. oleae* (Pereira 2004). Según Panis (1981), la presencia de hormigas va acompañada del predominio de *C. lycimnia* sobre *M. flavus* y *M. helvolus*. Más tarde, Barzman y Daane (2001) demostraron que esta ventaja se debe al menor tiempo que necesita el parasitoide del género *Coccophagus* para poner huevos en comparación con las especies de *Metaphycus*.

En esta tesis se analizan diversos aspectos relacionados con el éxito del control biológico natural de *S. oleae* en el norte de Portugal. En concreto se evaluó: (i) el potencial efecto adverso del autoparasitoide facultativo *C. lycimnia* sobre la densidad de población de los parasitoides del género *Metaphycus* y el consecuente detrimento del control biológico de *S. oleae* (**Capítulo 3 – Un autoparasitoide, inferior en el uso de recursos, supera a los parasitoides primarios y controla su huésped común *Saissetia oleae*)**; (ii) el efecto de la exclusión de hormigas sobre el control biológico de *S. oleae* y su complejo de parasitoides (**Capítulo 4 - Efecto de la exclusión de hormigas en el control biológico de la cochinilla negra, *Saissetia oleae* (Hemiptera: Coccidae), en olivos mediterráneos**); y (iii) el efecto de las fuentes de hidratos de carbono disponibles en el campo (néctar y melaza) sobre la supervivencia de los parasitoides del género *Metaphycus* y *Coccophagus* (**Capítulo 5 - Cuando la melaza es mejor fuente de hidratos de carbono que el néctar: implicaciones para el control biológico de *Saissetia oleae* (Olivier) (Hemiptera: Coccidea) en los olivos).**

Material y métodos

- **Un autoparasitoide, inferior en el uso de recursos, supera a los parasitoides primarios y controla su huésped común *Saissetia oleae* – Capítulo 3**

En este capítulo se estudia la interacción entre los parasitoides primarios del género *Metaphycus* (*M. helvolus*, *M. flavus* e *M. lounsburyi*) y el autoparasitoide facultativo *C. lycimnia* cuando parasitan los estadios inmaduros de *S. oleae*.

Para determinar la intensidad de la competición entre los parasitoides primarios del género *Metaphycus* y el autoparasitoide *C. lycimnia* y para explicar de qué manera afecta esta competición a la población natural de su hospedante común, *S. oleae*, se seleccionaron olivares que en el inicio de la temporada (septiembre) presentaban densidades de hospedante similares pero con densidades de parasitoides variables. Fueron seleccionados 28 olivares en la región de región de Trás-os-Montes en el nordeste de Portugal. Los olivares presentaban dos tipos de cubierta del suelo: cubierta de suelo desnudo con aplicación de herbicida o cubierta de vegetación natural cortada Los olivos tenían de entre 40 y 50 años aproximadamente las podas eran realizadas cada dos años y no eran regados. Los olivares estaban bajo producción comercial durante el ensayo, y no se realizó aplicación de pesticidas. Los olivares se dividieron en tres categorías de acuerdo con la densidad inicial de los parasitoides (septiembre): "olivares control" - cinco o menos parasitoides recogidos en septiembre; "olivares *C. lycimnia*" - más de cinco parasitoides y con una razón de *Metaphycus*-*C. lycimnia* inferior a 0,5; y "olivares *Metaphycus*" - más de cinco parasitoides y una razón de *Metaphycus*-*C. lycimnia* superior a 0,5.

Los muestreos se realizaron durante tres períodos (septiembre de 2011, noviembre de 2011 y mayo de 2012). En cada período de muestreo, se recogieron dos ramas (20 cm de largo) infestadas con *S. oleae* de diez olivos seleccionados aleatoriamente en cada olivar. Las ramas fueron colocadas en bolsas de plástico y transportadas en una nevera portátil al laboratorio, donde fueron almacenadas a 10 °C para análisis posteriores. Las muestras se procesaron durante las siguientes 48 horas. En el laboratorio, se seleccionó una submuestra de 20 hojas por olivo, con un total de 200 hojas por olivar. Cada submuestra se observó bajo un microscopio y se registró: (i) el número de individuos vivos y parasitados de *S. oleae* en cada una de las fases de desarrollo; (ii) la longitud del eje principal del segundo y tercer estadio ninfal. Las hojas que portaban una cochinilla parasitada se introdujeron individualmente en tubos de vidrio con una gota de miel en su pared interior, fueron tapadas con un tapón de algodón y colocadas en una cámara climática (temperatura: 22 °C, humedad relativa: 70 ± 10% y 16L:8 fotoperiodo). Durante un mes, se retiraron los parasitoides emergidos dos veces a la semana. Los parasitoides se identificaron y separaron por sexos.

La diferencia de cochinillas y parasitoides así como las diferencias de tamaño de las cochinillas entre tratatamientos fueron comparadas con ANOVAs En el caso de encontrar Si diferencias significativas, las medias entre los tratamientos fueron comparadas usando una prueba de Bonferroni con un nivel de significancia del 5%. Los supuestos de normalidad y homocedasticidad fueron confirmados gráficamente. Todos los análisis se realizaron utilizando el software estadístico R (The R Core Team 2011) y el paquete *lattice* (Sarkar 2008) de R.

- **Efecto de la exclusión de hormigas en el control biológico de la cochinilla negra, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), en olivos mediterráneos - Capítulo 4**

El estudio se realizó en un olivar situado en Cedães en la región de Mirandela (noreste de Portugal). El olivar, en modo de producción integrada desde 2003 y destinado a la producción de aceite de oliva, tenía 25 años aproximadamente y 30 hectáreas. Presentaba un marco de plantación de 7 × 7 m^2 y la variedad era Cobrançosa, con podas cada dos años. No se aplicaron pulverizaciones contra plagas ni enfermedades durante el ensayo. Las prácticas de manejo de la cubierta vegetal se limitaron al corte de la vegetación espontánea en julio. Durante la primavera, las principales especies de plantas espontáneas en el cultivo de la cubierta vegetal pertenecen a la familia Asteraceae: *Calendula arvensis* L.; *Coleostephus myconis* (L.) Rchb. F.; *Andryala integrifolia* L; *Chamaemelum mixtum* (L.); *Crysanthemum segetum* L; *Crepis vesicaria* subsp. *taraxaciholia* (Thuill.) Thell; *Leontodon taraxacoides* subsp. *longirostris* Finch & P. Venta. Durante el otoño las principales especies espontáneas son: *Foeniculum vulgare* L. (Apiaceae) y *Chondrilla juncea* L. (Asteraceae). Los olivos no se regaron.

Los individuos de *S. oleae* utilizados en el ensayo fueron criados siguiendo el siguiente protocolo: 112 y 56 olivos de 1m de altura de la variedad Cobrançosa, fueron infestados en otoño (inicio del ciclo de vida de *S. oleae*) y primavera (fin del ciclo de vida) respectivamente. Los árboles fueron infestados con huevos de *S. oleae* obtenidos de olivares infestados de la región. Los olivos fueron mantenidos en invernadero bajo condiciones controladas (temperatura: 25 °C, humedad relativa: 80 ± 10% y fotoperiodo natural), y regados semanalmente hasta que las cochinillas alcanzaron el segundo estadio. Se registró el número de cochinillas por árbol y posteriormente se transportaron al olivar el 15 de septiembre y el 24 de marzo.

Para el diseño experimental se realizó un bloque aleatorizado con 14 repeticiones en otoño y siete repeticiones en primavera de dos tratamientos diferentes: con hormigas y sin hormigas. Cada bloque estaba constituido por 16 árboles (4 filas con 4 árboles cada una). Para el muestreo se utilizaron los cuatro árboles centrales de cada bloque . En cada árbol, una planta de olivo infestado dentro de su maceta fue colocada en la zona en la que el troco principal se divide en ramas secundarias. La parte superior de la maceta presentaba dos orificios para colocar una cuerda y fijar la maceta al olivo. Para la exclusión de las hormigas, se colocó una cinta envolviendo el tronco del árbol que delimitó una zona de 15-20 cm de ancho a 50 cm del suelo (Pekas et al. 2010, Juan-Blasco et al. 2011). El área envuelta fue revestida con pegamento especial para trampas de insectos. Toda la vegetación espontánea que adyacente al tronco del árbol fue cortada y retirada para evitar que las hormigas alcanzasen el olivo y asegurar que el único acceso de las hormigas al árbol era por el tronco. El pegamento se renovó mensualmente. Cada bloque estaba separado del bloque adyacente por seis filas de árboles.

La actividad de las hormigas se registró semanalmente. Para ello se contaron las hormigas que se desplazaban verticalmente a través de una línea imaginaria en el tronco y horizontal al suelo durante un período de dos minutos (Pekas et al. 2011). La eficacia del método de exclusión de hormigas se controló semanalmente durante los ensayos comparando la actividad de las hormigas entre los tratamientos con hormigas y sin hormigas. Las hormigas fueron identificadas visualmente y/o aspiradas y transportadas al laboratorio para su identificación utilizando las claves de identificación disponibles en el sitio web: http://ww.hormigas.org, (accedido, 2015) y confirmadas por un especialista. El número de cochinillas por árbol infestado se registró mensualmente desde el comienzo de la temporada. Para ello, los olivos infestados en las macetas fueron transportados al laboratorio. Allí, se registró el número de cochinillas vivas, parasitadas y muertas, así como su estadio siguiendo la metodología descrita por Tena et al. (2008). Las cochinillas parasitadas se colocaron individualmente en placas de Petri (60 mm de diámetro) que fueron selladas con parafilm hasta la emergencia del parasitoide. Cada parasitoide fue identificado y separado por sexo. Posteriormente, se calcularon las tasas de parasitismo para cada estadio y fecha.

La actividad de las hormigas a lo largo de las estaciones en los árboles con y sin hormigas se comparó utilizando un ANOVA de medidas repetidas (Pekas et al. 2010). Las tasas de densidad y parasitismo de *S. oleae* en árboles con y sin hormigas se compararon utilizando un ANOVA para cada fecha de muestreo. Las tasas de parasitismo fueron transformadas con arcoseno. El efecto de la actividad de las hormigas sobre la reducción de la densidad de *S. oleae* y sobre el parasitismo máximo se analizó usando ANCOVAs, siendo la actividad de hormigas a lo largo de la estación una variable cuantitativa y el tratamiento una variable cualitativa. La normalidad y homogeneidad de las varianzas fueron analizadas con las pruebas de Kolmogorov-Siminov y Levene respectivamente. Finalmente, el número de parasitoides emergidos no presentó una distribución normal por lo que fue analizado mediante una prueba de Kruskal-Wallis.

- **Cuando la melaza es mejor fuente de hidratos de carbono que el néctar: implicaciones para el control biológico de *Saissetia oleae* (Olivier) (Hemiptera: Coccidea) en los olivos - Capítulo 5**

Para obtener los parasitoides (*M. lounsburyi*, *C. semicircularis* y *C. lycimnia)* se recurrió a los olivares infestados con *S. oleae* en Mirandela, región de Trás-os-Montes (Portugal) durante dos años consecutivos (2014 y 2015). Se recogieron hojas y ramas infestadas cada dos semanas de septiembre a julio y se transportaron al laboratorio. Una vez allí, las hojas y ramas infestadas se individualizaron y se colocaron en tubos de plástico y se sellaron con parafilm. Los tubos se colocaron en una cámara climática y diariamente se retiraron, identificaron y separaron por sexo los parasitoides emergidos. En el ensayo se utilizaron sólo las hembras de los parasitoides.

La melaza fresca excretada por *S. oleae* y *E. olivina* se recogió durante 24 horas de olivos infestados con estos hemípteros en una cámara climática. Se colocó un envase de plástico (5,5 cm de diámetro) con un tiras de Parafilm (1cm^2) bajo 10-20 colonias de cada productor de melaza (Hogervorst et al. 2007b, Tena et al. 2013a). Los envases fueron reemplazados diariamente.

Las flores de *Foeniculum vulgare* Miller (Apiaceae), *Malva sylvestris* L. (Malvaceae), *Daucus carota* L. subsp. *carota* (Apiaceae), *Hypericum perforatum* L. (Hypericaceae), *Hirschfeldia incana* (L.) Lagr.-Foss. (Brassicaceae) y *Dittrichia viscosa* (L.) (Greuter) (Asteraceae) se colectaron en olivares en Bragança (Portugal) diariamente (de 8:00h a 10:00h) durante su período de floración.

Para los ensayos de longevidad, cada hembra adulta recién emergida se introdujo en un tubo de plástico (3 cm de diámetro y 12 cm de altura) que se cerró con muselina para ventilación y se colocó en una cámara climática con agua destilada y uno de los tratamientos. Se suministró agua (control negativo) y miel al 10% (control positivo) en un tubo eppendorf de 1 ml con una tira de papel absorbente y se selló con Parafilm. Los controles se renovaron cada dos días. Las flores se introdujeron en los tratamientos y se renovaron diariamente. Las melazas previamente recolectadas se renovaron cada 48 h en las tiras de parafilm. La supervivencia de los parasitoides se registró diariamente. Para el análisis no se consideraron las muertes accidentales. Para cada tratamiento se evaluaron 30 hembras. Todos los tratamientos fueron testados para *M. lounsburyi* y *C. semicircularis*. Para *C. lycimnia* se evaluó la melaza de *E. oleae* y las flores de *M. sylvestris*, *F. vulgare* y *H. incana*.

El efecto de cada tratamiento sobre la longevidad de los parasitoides adultos fue representado mediante curvas de supervivencia de Kaplan-Meir y analizado con un test log-rank. Las diferencias entre tratamientos se evaluaron con análisis de comparaciones por pares. Todos los análisis se realizaron utilizando IBM SPSS-statistics, versión 22 (SPSS Inc., 2013).

Resultados

- **Un autoparasitoide, inferior en el uso de recursos, supera a los parasitoides primarios y controla su huésped común *Saissetia oleae* – Capítulo 3**

Las densidades de los parasitoides del género *Metaphycus* y *C. lycimnia* siguieron patrones opuestos. Las densidades de *Metaphycus* disminuyeron en los tratamientos "olivares *Metaphycus*" y "olivares *C. lycimnia*" y se mantuvieron bajos en el tratamiento de control. En septiembre, el número medio de *Metaphycus* fue significativamente mayor en el tratamiento "olivares *Metaphycus*" que en el "olivares *C. lycimnia*" y tratamientos control. En noviembre, el número medio de *Metaphycus* disminuyó en el tratamiento "olivares *Metaphycus*", y sólo hubo diferencias significativas entre los tratamientos "olivares *Metaphycus*" y el control. Al final del ciclo de vida de *S. oleae* (mayo), el número de *Metaphycus* fue casi nulo en los tres tratamientos, no habiendo diferencias significativas entre ellos. En septiembre, el número medio de *C. lycimnia* fue significativamente mayor en el "olivares *C. lycimnia*" y "olivares *Metaphycus*" que en el tratamiento control. En noviembre, el número de *C: lycimnia* aumentó y no hubo diferencias significativas entre tratamientos. Al final del ciclo de vida de la cochinilla (mayo), el número de *C. lycimnia* fue más de cinco veces mayor que en noviembre en los tres tratamientos, y fue significativamente mayor en el tratamiento de control que en los tratamientos "olivares *C. lycimnia*" y "olivares *Metaphycus*".

El tamaño de las cochinillas inmaduras fue similar en los tres tratamientos a lo largo del ciclo de vida del insecto. Se comparó del uso del tamaño del hospedante entre los parasitoides de *Metaphycus* y *C. lycimnia* para cada fecha de muestreo considerando el tratamiento como factor fijo. Tanto en septiembre como en noviembre, las cochinillas utilizadas por los parasitoides de *Metaphycus* fueron significativamente menores que las utilizadas por *C. lycimnia*, independientemente del tratamiento y la interacción entre el parasitoide y el tratamiento no fue significativa. El reducido número de parasitoides de *Metaphycus* emergidos en mayo no permitió analizar el tamaño del hospedante.

La proporción de sexos media de los parasitoides de *Metaphycus* (0,28 ± 0,03 proporción de machos) y *C. lycimnia* (0,09 ± 0,016) mostró una mayor emergencia de hembras. Se comparó la proporción de sexos entre los parasitoides de *Metaphycus* y *C. lycimnia* para cada fecha de muestreo siendo el tratamiento el factor fijo. En septiembre, las proporciones de sexos de *Metaphycus* y *C. lycimnia* fueron similares, independientemente del tratamiento. La interacción entre el parasitoide y el tratamiento tampoco fue significativa. Sin embargo, en noviembre, la proporción de sexos secundaria de *Metaphycus* fue significativamente mayor que la de *C. lycimni*a, independientemente del tratamiento siendo interacción entre el parasitoide y el tratamiento significativa. Esta interacción indica que la proporción de sexos secundaria de *C. lycimnia*, fue diferente entre tratamientos pero la de *Metaphycus* no mostró diferencias. De hecho, cuando las proporciones de sexos de ambos parasitoides se analizaron por separado, la proporción de sexos de *Metaphycus* fue independiente del tratamiento en septiembre y noviembre, mientras que el tratamiento "olivares *Metaphycus*" mostró una proporción significativamente mayor de machos de *C. lycimnia* que el tratamiento "olivares *C. lycimnia*" en septiembre y noviembre.

Al comienzo del ensayo (septiembre), el número medio de ninfas adecuadas para el parasitismo no varió significativamente entre los olivares con diferentes densidades de parasitoides (tratamientos). Al final del ensayo, las tres densidades iniciales de parasitoides redujeron significativamente el número de ninfas, no habiendo diferencias en el mes de noviembre. Del mismo modo, el número medio de cochinillas (ninfas y adultos) presentes en los olivares no varió entre los tratamientos a lo largo del ciclo de vida de la cochinilla y fue cinco veces menor en mayo que en septiembre.

- **Efecto de la exclusión de hormigas en el control biológico de la cochinilla negra, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), en olivos mediterráneos - Capítulo 4**

Las barreras de pegamento excluyeron a las hormigas de las copas en ambas estaciones. En otoño, al principio del ciclo de *S. oleae*, la actividad de las hormigas se mantuvo constante y baja en las parcelas sin hormigas a lo largo de la estación. Los registros de hormigas en las copas de las parcelas con exclusión fueron casi ausentes. En las parcelas sin exclusión, las cuatro especies de hormigas fueron registradas en otoño, siendo *Crematogaster auberti* (Emery) la especies más abundantes (92.4%) y ampliamente distribuidas (presente en 98,3% de los arboles). Las otras tres especies *Tapinoma nigerrimum* (Nylander), *Plagiolepis pygmaea* (Latreille) y *Crematogaster scutellaris* (Olivier) fueron mucho menos abundantes. Entre las hormigas que pudieron superar el sistema de exclusión, *C. auberti* fue la más abundante.

En primavera, las hormigas estuvieron casi ausentes en las copas de los olivos de las parcelas con exclusión. En los árboles control, el número de hormigas que ascendía y descendía a los árboles sin barreras fue reducido hasta mayo, cuando aumentó alcanzando su máximo al final del ciclo de *S. oleae,* a principios de junio. *C. auberti* fue la especie de hormiga más abundante (58,8%) y ampliamente distribuida (presente en 75,0% de los árboles) dentro del olivar en árboles con hormigas, seguida de *T. nigerrimum* (25,2% de hormigas, 64,3% de árboles), *P. pygmaea* (12,7% de hormigas, 48,0% de árboles) y *C. scutellaris* (3,3% de hormigas, 35,7% de árboles). Entre las hormigas que pudieron superar el sistema de exclusión, *T. nigerrimum* fue la especie más abundante.

Al comienzo de los ensayos, la densidad de *S. oleae* fue similar en árboles con y sin exclusión de tanto en otoño y primavera. Después de un mes en el campo en otoño, no hubo diferencias significativas en el número de cochinillas vivas. Durante este período, la densidad de *S. oleae* disminuyó 14,9% en árboles sin exclusión de hormigas y 11,5% en árboles con exclusión de hormigas y sólo hubo ninfas (100% ninfas en árboles sin exclusión de hormigas s, 100% ninfas en árboles con exclusión de hormigas). Después de dos meses, la densidad de *S. oleae* disminuyó 29% en árboles con hormigas y 26,6% en árboles sin hormigas, no habiendo diferencias significativas en el número de cochinillas vivas. Al final del ensayo, la población de *S. oleae* estuvo representada principalmente por ninfas (99,1% de ninfas en árboles sin exclusión, 99,0% de ninfas en árboles con exclusión). El análisis de la actividad media de las hormigas y la reducción de la densidad de *S. oleae* en cada árbol mostró que, durante todo el ensayo, la actividad media de las hormigas no se correlacionó con la reducción de la densidad de *S. oleae* independientemente del tratamiento. La interacción entre la actividad de las hormigas y el tratamiento no fue significativa.

Después de un mes en el campo en primavera, no hubo diferencias significativas en el número de cochinillas vivas. Durante este período, la densidad de *S. oleae* disminuyó un 30% en árboles con hormigas y un 45,5% en árboles sin hormigas. La mayoría de las cochinillas se encontró en estado de ninfa en ambos tratamientos (55,7% de ninfas en árboles sin exclusión de hormigas y 64,5% de ninfas en árboles con exclusión de hormigas). Después de dos meses, el número de cochinillas vivas fue similar. Durante este período, la densidad de *S. oleae* disminuyó un 76,2% en árboles con hormigas y un 82,6% en árboles sin hormigas. Al final del ensayo, la población de *S. oleae* estuvo representada principalmente por hembras adultas (0,5% de ninfas en árboles si exclusión de hormigas, 12,2% de ninfas en árboles con exclusión de hormigas). El análisis de la actividad media de las hormigas y la reducción de la densidad de *S. oleae* en cada árbol mostró que la actividad media de las hormigas a lo largo del ensayo no se correlacionó con la reducción de la densidad de *S. oleae*, independientemente del tratamiento. La interacción entre la actividad de las hormigas y el tratamiento no fue significativa.

En otoño, al inicio del ciclo de vida de *S. oleae*, el porcentaje de parasitismo de las ninfas de *S. oleae* fue reducido (~ 4%) y similar en ambos tratamientos después de un mes en el campo. Dos meses después, el parasitismo de las ninfas y adultos de *S. oleae* aumentó ligeramente, pero no hubo diferencias significativas entre los tratamientos. El análisis de la actividad media de las hormigas y el parasitismo en cada árbol mostró que la actividad media de las hormigas durante todo el ensayo no se correlacionó con el parasitismo máximo obtenido en cada árbol, independientemente del tratamiento. La interacción entre la actividad de las hormigas y el tratamiento no fue significativa. En primavera, al final del ciclo de vida de *S. oleae*, el porcentaje de parasitismo de las ninfas de *S. oleae* fue elevado (~ 20%) y similar en ambos tratamientos después de un mes en el campo. El porcentaje de parasitismo de adultos de *S. oleae* fue muy elevado. Dos meses después, el parasitismo de las ninfas disminuyó mientras que fue mayor para los adultos. No hubo diferencias significativas entre los tratamientos. El análisis de la actividad media de las hormigas y el parasitismo en cada árbol mostró que la actividad media de las hormigas durante todo el ensayo no se correlacionó con el parasitismo máximo obtenido en cada árbol, independientemente del tratamiento. La interacción entre la actividad de las hormigas y el tratamiento no fue significativa.

En otoño, el número de parasitoides emergidos de *S. oleae* fue similar en árboles control y con exclusión de hormigas. Durante este período, emergieron cinco especies de parasitoides: los parasitoides primarios *M. lounsburyi*, *M. helvolus* y *M. flavus* y los autoparasitoides facultativos *C. lycimnia* y *C. semicircularis*. La abundancia de las cinco especies fue similar a lo largo de la estación. En primavera, dos meses después de la exclusión de las hormigas el número de parasitoides emergidos de los adultos de *S. oleae* fue significativamente mayor en los árboles con exclusión de hormigas que en los árboles control. No hubo diferencias significativas entre los tratamientos en el resto de las fechas e estadios del hospedante. Durante esta estación, *M. lounsburyi* y *C. lycimnia* fueron los únicos parasitoides presentes en ambos tratamientos. Al principio de la exclusión, la abundancia de ambos parasitoides fue similar en los dos tratamientos. Un mes más tarde, *M. lounsburyi* fue más abundante que *C. lycimnia* en los árboles con exclusión de hormigas, mientras que no hubo diferencias significativas entre parasitoides en árboles control.

- **Cuando la melaza es mejor fuente de hidratos de carbono que el néctar: implicaciones para el control biológico de *Saissetia oleae* (Olivier) (Hemiptera: Coccidea) en los olivos - Capítulo 5**

El análisis del efecto del néctar y de la melaza sobre la supervivencia de *M. lounsburyi* mostró que todas las dietas aumentaron significativamente la longevidad de las hembras de *M. lounsburyi* en comparación con el control negativo (agua). Las hembras vivieron significativamente más tiempo cuando se les proporcionó melaza de *S. oleae* (38 ± 3,84 días) que con el resto de las dietas. Las hembras de *M. lounsburyi* vivieron aproximadamente ocho veces más tiempo con la melaza de *S. oleae* que con las cinco flores o la melaza de *E. olivina* y no hubo diferencias significativas entre los últimos tratamientos. La longevidad alcanzada con las flores varió entre cinco y siete días.

Para *C. semicircularis* sólo la melaza de *S. oleae* y la miel aumentaron significativamente la longevidad de las hembras de *C. semicirculares* cuando se compararon con el control negativo (agua). Entre estos dos tratamientos, *C. semicircularis* vivió significativamente más tiempo cuando se alimentó con melaza de *S. oleae* que con miel. Las seis flores y la melaza de *E. olivina* aumentaron la longevidad de las hembras de *C. semicircularis.*

En el caso *C. lycimnia* se compararon cuatro dietas (melaza de *S. oleae* y las flores de *F. vulgare*, *M. sylvestris* y *H. incana*). Las hembras de *C. lycimnia* vivieron significativamente más tiempo cuando se alimentaron de melaza de *S. oleae* que con el resto de los tratamientos. *F. vulgare* y *H. incana* no aumentaron significativamente la longevidad de *C. lycimnia* en comparación con el agua, mientras que las hembras alimentadas con *M. sylvestris* vivieron dos veces más.

No hubo diferencias significativas entre la supervivencia de las tres especies de parasitoides (*M. lounsburyi*, *C. lycimnia* y *C. semicircularis*) cuando fueron alimentados con agua, miel, melaza de *S. oleae,* melaza de *E. olivina* y dos de las seis flores: *F. vulgare* y *H. incana*. Sin embargo, *C. lycimnia* vivió más tiempo que los otros dos parasitoides cuando se alimentó de *M. sylvestris*. En comparación con el control positivo (miel), la melaza excretada por *S. oleae* aumentó la longevidad de los tres parasitoides alrededor del 50% (para *M. lounsburyi* 45,00 ± 0,21%, *C. semicircularis* 63,15 ± 0,21% y *C. lycimnia* = 52,22 ± 0,20%) y este incremento no fue significativamente diferente entre las tres especies de parasitoides.

Discusión

- **Un autoparasitoide, inferior en el uso de recursos, supera a los parasitoides primarios y controla su hospedante común *Saissetia oleae* – Capítulo 3**

El presente estudio evalúa un caso de competición entre los parasitoides primarios y un autoparasitoide facultativo que atacan a su hospedante común en condiciones de campo y el efecto de esta competición en la supresión del hospedante. Los resultados revelan por primera vez que un autoparasitoide facultativo, que parasita huéspedes de mayor tamaño y por tanto está en inferioridad en cuanto a la explotación de huéspedes, puede competir con el parasitoide primario sin afectar a la supresión de su hospedante común.

C. lycimnia fue más competitivo que los parasitoides del género *Metaphycus* en el parasitismo de las ninfas de *S. oleae*, independientemente de su abundancia relativa al comienzo del ciclo de la cochinilla. El ensayo fue diseñado principalmente para detectar los efectos de la competición sobre la densidad de población de ambos parasitoides y de su hospedante común y no para determinar el mecanismo de la competición. Sin embargo, los resultados también sugieren que *C. lycimnia* prefirió las hembras *Metaphycus* como hospedadores secundarios para producir machos en lugar de usar sus propias hembras. Se recuperó un mayor número de machos de *C. lycimnia* en olivares con mayor proporción de *Metaphycus* que con mayor proporción de *C. lycimnia* en septiembre y noviembre. Esta preferencia oviposicional otorgó a *C. lycimnia* una ventaja competitiva sustancial sobre los parasitoides de *Metaphycus*, lo que podría explicar la trayectoria descendente de las poblaciones de *Metaphycus* al comienzo de la temporada cuando el número de hospedantes era muy alto (30 a 100 veces superior a los parasitoides). Zang et al. (2011) descubrieron recientemente que *Encarsia sophia*

(Girault & Dodd), un autoparasitoide de moscas blancas, también mostró preferencia de hospedantes heterospecíficos como hospedante secundarios para producir machos en una test de elección en el laboratorio. Esta preferencia podría deberse a la mayor aptitud de los machos desarrollados en hembras heterospecíficas en comparación con los desarrollados en hembras conespecíficas (Zang et al. 2011). Bernal et al. (2001) también encontraron una relación positiva entre los números de *Metaphycus* y la proporción de *C. lycimnia* machos en el campo parasitaban la cochinilla *Coccus pseudomagnoliarum (*Kuwana). Sin embargo, en este estudio, los autores sugirieron que la alta densidad de población de *C. lycimnia* podría reducir la densidad de *Metaphycus* spp. y explicar el nivel insignificante de control biológico de la cochinilla citrícola que se produce en el Valle de San Joaquín en California (Bernal et al. 2001).

Metaphycus spp. se consideran mejores competidores y agentes de control biológico más eficaces que con *C. lycimnia* debido a que usan hospedantes más pequeños (Bernal et al. 2001, Tena y Garcia-Marí 2008). La explotación de hospedante más pequeños por parasitoides de insectos de cochinillas y moscas blancas constituye un mecanismo ampliamente documentado que explica la superioridad y, a veces, el desplazamiento de los parasitoides competidores que necesitan hospedante más grandes para desarrollarse (Luck and Podoler 1985, Beltrà et al. 2013a, b). En nuestro estudio, las poblaciones de *Metaphycus* también usaron hospedantes más pequeños que *C. lycimnia*, pero no explotaron este espacio libre de enemigos para ser más competitivos *C. lycimnia* o para mantener sus propias poblaciones, de manera que las poblaciones de *Metaphycus* disminuyeron llegando a ser insignificantes en mayo. *M. helvolus* y *M. flavus* generalmente alcanzaron sus niveles más altos en la primavera en otras áreas de cítricos y olivos (Tena et al. 2008). Recientemente, Pekas et al. (2016) demostraron que los parasitoides primarios *Aphytis melinus* (DeBach) y *A. chrysomphali* (Mercet) (Hymenoptera: Aphelinidae) pueden coexistir cuando parasitan la cochinilla *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) porque el competidor más débil (*A. chrysomphali*) puede sobrevivir en los hospedantes más pequeños, lo que le proporciona un espacio libre de enemigo cuando las densidades del competidor superior son altas. Sin embargo, en el presente estudio, no se pudo observar este mecanismo de coexistencia, y *Metaphycus* no utilizó hospedantes más pequeños en olivares con mayores proporciones de *C. lycimnia*. Análogamente Bográn et al. (2002) no encontraron que el parasitoide primario *E. mundus* usase hospedantes más pequeños en presencia del autoparasitoide *E. pergandiella* que cuando se liberó sin el autoparasitoide. Esta falta de plasticidad en el uso de tamaño de huésped podría también explicar por qué las poblaciones de *Metaphycus* spp. no se recuperaron en mayo.

A pesar de la competición asimétrica y del desplazamiento de *C. lycimnia* de los parasitoides primarios del género *Metaphycus*, los primeros suprimieron las densidades de cochinilla, independientemente de su abundancia relativa al comienzo de la temporada. Los resultados muestran claramente que *C. lycimnia* redujo la población de cochinillas, independientemente de la densidad de los parasitoides primarios del género *Metaphycus*.

La teoría predice que la supresión de plagas no será perjudicada si el parasitoide primario y los autoparasitoides son igualmente eficaces para suprimir la plaga (Briggs y Collier 2001). Por lo tanto, los resultados de este trabajo sugieren que, aunque *C. lycimnia* utiliza hospedantes más grandes que los parasitoides del género *Metaphycus*, es al menos tan eficiente como estos parasitoides primarios en la supresión de *S. oleae* en los olivos. Este autoparasitoide apareció en la segunda muestra en noviembre, incluso en los olivares donde no fue capturado ningún parasitoide en la primera muestra, y su población aumentó durante el invierno. En mayo, *C. lycimnia* y las poblaciones del hospedante habían alcanzado casi una proporción de 1:2, mostrando la alta eficacia de *C. lycimnia* como agente de control biológico.

Hay otros dos estudios detallados que han abordado previamente el efecto de la competición entre los parasitoides primarios y los autoparasitoides en su hospedante común bajo condiciones de campo particulares (Hunter et al. 2002, Bográn et al. 2002). El presente trabajo difiere de las investigaciones previas ya que se utilizan poblaciones naturales en sus ambientes naturales. Los estudios anteriores sobre se basan en el uso de plantas en condiciones estancas y la posterior liberación de parasitoides. Esta metodología permite la comparación de densidades de la población del hospedante cuando están ausentes, se liberan por separado o en conjunto para evaluar relaciones competitivas. La llegada y el ataque de diferentes especies pueden diferir según la fenología de los hospedantes y sus necesidades climáticas, así como otros factores, como hospedantes alternativos, vegetación circundante y márgenes (Snyder et al. 2005). De hecho, la dinámica de la metapoblación es muy importante en ecología y puede explicar la persistencia de los sistemas de control biológico que de otro modo no tendrían explicación (Murdoch et al. 1985), como podría ser el caso del presente estudio.

- **Efecto de la exclusión de hormigas en el control biológico de la cochinilla negra, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), en olivos mediterráneos - Capítulo 4**

Crematogaster auberti fue la especie de hormiga más abundante en nuestro estudio en ambas estaciones, estuvo presente en la mayoría de los árboles muestreados y fue más activa en primavera que en otoño. Esta especie se ha encontrado previamente atendiendo *S. oleae* en olivos en Portugal (Pereira 2002, 2003). *Tapinoma nigerrimum* fue la segunda especie de hormiga más abundante, pero estuvo presente principalmente en primavera. En estudios anteriores en la misma área, *T. nigerrimum* fue registrada como la especie más abundante (Pereira et al. 2002 y 2003, Silva 2012). *Crematogaster auberti* y *T. nigerrimum* son especies dominantes y no comparten el mismo árbol. Lo mismo ocurre con otras hormigas que se alimentan de melaza en cítricos (Pekas et al. 2011). La exclusión de las hormigas redujo la actividad de las hormigas, pero no las excluyó completamente como en estudios anteriores (Pekas et al. 2010, Calabuig et al. 2014). Se debió principalmente a la presencia de *T. nigerrimum* en estos árboles, lo que sugiere que esta especie es capaz de superar las barreras de exclusión más fácilmente que otras especies de hormigas.

En general, la exclusión de las hormigas no mejoró el control biológico de *S. oleae* cuando las ninfas estuvieron presentes. La densidad de *S. oleae* siguió el mismo patrón en árboles con exclusión de hormigas y en árboles control en otoño, principio del ciclo de *S. oleae*, y primavera, final del ciclo. La baja actividad de las hormigas en las copas de los olivos del norte de Portugal puede explicar su bajo efecto sobre la densidad de *S. oleae*. Los resultados de Pekas et al. (2010) apoyan esta hipótesis, siendo que cuando la actividad de las hormigas era baja en las copas de los cítricos, su exclusión no afectó a la densidad de población del piojo rojo de California *Aonidiella aurantii* (Maskell). En nuestro estudio, la actividad media de las hormigas fue diez veces menor que en su estudio lo que apoya esta hipótesis. El bajo impacto de las hormigas en el control biológico de *S. oleae* puede explicar parcialmente su excelente control natural. Esta cochinilla se ha convertido en una plaga secundaria en el Norte de Portugal, al contrario que en otras áreas donde las hormigas han establecido relaciones de mutualismo e interfieren con el control biológico de *S. oleae* así como de otros productores de melaza (Daane et al 2007, González Hernández et al. 1999, Mgocheki y Addison 2009). Por ejemplo, la hormiga argentina *Linepithema humile* (Mayr), mucho más activa que las especies de hormigas encontradas en nuestro estudio (Tena et al. 2013a), alteró la efectividad de los parasitoides de *S. oleae* en huertos de cítricos en California (Horton 1918). Por otro lado, en el mismo estado y cultivo, Bernal et al. (2001) encontraron que el parasitismo causado por los parasitoides de *M. helvolus*, *M. luteolus* (Timberlake) y *M. stanleyi* (Compere) de la cochinilla citrícola, *Coccus pseudomagnoliarum* (Kuwana), fue fuertemente inhibido por la actividad de las hormigas.

El parasitismo de las ninfas de *S. oleae* fue cinco veces más alto en primavera que en otoño. Este patrón se ha descrito previamente en Portugal, así como en otras zonas productoras de aceituna de la cuenca mediterránea (Briales y Campos 1985, Pereira 2004, Tena et al. 2008). En general, las ninfas de *S. oleae* son atacadas por un complejo parasitoide formado por varios parasitoides del género *Metaphycus* y *Coccophagus*. En la región de Trás-os-Montes, los autoparasitoides facultativos *C. lycimnia* y *C. semicircularis* son los principales parasitoides y alcanzan altos niveles de parasitismo en primavera (Pereira 2004). Trabajos previos sugirieron que los bajos niveles de parasitismo observados en otoño pueden deberse a la presencia de hormigas en el olivar (Pereira 2004). Sin embargo, nuestro estudio demuestra que las hormigas no son responsables de la baja eficacia de los parasitoides en el otoño. Por otra parte, aunque no se midió la depredación, no parece que la actividad de los depredadores se haya visto afectada por la exclusión de las hormigas, ya que la densidad de la población en estas dos estaciones fue similar en los árboles con y sin hormigas.

Al final de la primavera, el número de parasitoides recuperados de adultos de *S. oleae* fue mayor en árboles con exclusión de hormigas que en árboles control. Por lo tanto, las hormigas pueden afectar el control biológico de adultos de *S. oleae*. Varios factores pueden explicar este resultado. En primer lugar, los adultos de *S. oleae* excretan mayores cantidades de melaza que la etapa ninfal (Paparatti 1986). Por lo tanto, podrían atraer más hormigas, que los protegen de los parasitoides. Nuestros resultados están de acuerdo con esta hipótesis ya que la actividad de las hormigas en la copa fue mayor al final de la primavera cuando *S. oleae* alcanza la fase adulta. En segundo lugar, el principal parasitoide de *S. oleae* durante esta etapa fue *M. lounsburyi*, en lugar de los parasitoides del género *Coccophagus*. Esta especie parasitoide se ve afectada negativamente por la presencia de hormigas como se muestra en este documento y en Barzman y Danne (2001). Por el contrario, los parasitoides del género *Coccophagus* no se ven afectados por la presencia de hormigas, como se demostró en un estudio previo (Barzman y Danne 2001). Esto se debe, al menos en parte, al tiempo mucho más corto que necesitan los parasitoides de *Coccophagus* para poner un huevo en comparación con *Metaphycus* (Barzman y Danne 2001). Los parasitoides adultos están expuestos a predadores y hormigas durante la oviposición, cuando permanecen inmóviles (Barzman y Danne 2001, Martinez-Ferrer et al. 2003, Tena and Garcia-Marí 2008, Beltrà et al. 2015).

En general, nuestros resultados demuestran que las hormigas no interfieren en el control biológico de las ninfas de *S. oleae*, que están presentes de septiembre a junio en los olivos del norte de Portugal. Sin embargo, las hormigas modificaron el complejo parasitoide y redujeron la presencia de *M. lounsburyi*, un parasitoide que parasita principalmente adultos de *S. oleae* (Tena y Garcia-Marí 2009).

- **Cuando la melaza es mejor fuente de hidratos de carbono que el néctar: implicaciones para el control biológico de *Saissetia oleae* (Olivier) (Hemiptera: Coccidea) en los olivos - Capítulo 5**

Se evaluó la calidad de las principales plantas de floración y melazas disponibles en los olivares como fuentes de carbohidratos para tres parasitoides de *S. oleae* con diferentes biología: *M. lounsburyi*, *C. semicircularis* y *C. lycimnia*. La melaza excretada por la propia cochinilla resultó ser una fuente de carbohidratos de alta calidad para los tres parasitoides y, además, es fácilmente accesible y abundante durante todo el año. Por lo tanto, los hidratos de carbono no deben considerarse un factor limitante para el control biológico de *S. oleae* en los olivares, que se presentaron como un ejemplo de monocultivo donde el suministro de recursos de carbohidratos podría mejorar la aptitud de los parasitoides y su potencial como agentes de control biológico (Jervis y Heimpel 2005).

Las longevidades obtenidas en nuestro ensayo fueron muy similares para los tres parasitóides estudiados y mostraron que la melaza excretada por *S. oleae* es la mejor fuente de hidratos de carbono presente en los olivares. Los parasitoides alimentados con esta melaza vivieron en promedio alrededor de siete veces más que cuando se les proporcionó néctar. La melaza se considera la fuente principal de hidratos de carbono en los agroecosistemas (Wäckers et al. 2008, Tena et al. 2016) y se ha demostrado que los parasitoides se alimentan comúnmente de melaza en el campo utilizando cromatografía líquida de alta resolución (HPLC) (Stepphun y Wäckers 2004, Lee et al. 2006, Hogervorst et al. 2007b, Tena et al. 2015, Calabuig et al. 2015). Aunque el consumo de melaza de *S. oleae* por sus parasitoides no ha sido reportado en el campo, parece poco probable que los parasitoides de *Metaphycus* y *Coccophagus* no se alimenten de ella. *Saissetia oleae* excreta gran cantidad de melaza, está presente durante todo el año y los parasitoides pueden encontrarla fácilmente cuando buscan a su hospedante o incluso cuando emergen dentro de una colonia de la cochinilla.

Por lo tanto, la propia melaza excretada por *S. oleae* parece suficiente para asegurar la supervivencia de su complejo de parasitoides durante todo el año. Hasta donde sabemos, este es el primer estudio que demuestra que un tipo de melaza es una fuente superior para los parasitoides en comparación con el néctar. Otros dos trabajos apoyan nuestros resultados y, además, sugieren que no sólo los parasitoides de *S. oleae* pueden beneficiarse de este rica melaza, sino también parasitoides de otras plagas del olivar (Wang et al. 2011, Villa et al. 2016). Wang et al. (2011), encontró que la melaza de *S. oleae* aumenta la longevidad de la mosca de la aceituna, *Bactrocera oleae* Rossi (Diptera: Tephritidae) y su parasitoide *Psyttalia humilis* (Silvestri) (Hymenoptera: Braconidae) como control positivo. Villa et al. (2016) encontró que la polilla *P. oleae* alcanza la supervivencia más alta cuando se alimenta con esta melaza en comparación con otras melazas y flores. Estos últimos resultados están en concordancia con Wäckers et al. (2008) y Tena et al. (2013b), que demostraron que los parasitoides de los insectos no productores de melaza también pueden beneficiarse de la melaza. Futuras investigaciones deberían analizar y comparar el contenido de melaza de *S. oleae* con otros tipos de melaza para determinar qué azúcar/es, aminoácido/s o combinación de ambos es responsable de su alta calidad.

La otra melaza presente en los olivares, excretada por *Euphyllura olivina*, aumentó sólo marginalmente la supervivencia de *M. lounsburyi* y *C. lycimnia*. La viscosidad más alta de la meleza de *E. olivina*, comparada con la de *S. oleae* (observaciones propias), puede afectar su consumo por estos dos parasitoides y explicar parcialmente su peor calidad como fuente de carbohidratos. Tena et al. (2013a) también encontró que la supervivencia de *Aphytis melinus* (DeBach) (Hymenoptera: Aphelinidae) varía en gran medida dependiendo de la melaza que se alimentan en otro cultivo de hoja perenne, los cítricos. Una parte de la mala calidad, de la melaza de *E. olivina* está presente sólo en primavera y otoño, cuando las ninfas, que excretan la melaza, son abundantes. Por otra parte, todas las etapas de *S. oleae* (excepto los huevos) excretan melaza y la plaga está presente en hojas y ramas durante todo el año, lo que la hace muy accesible.

Durante los últimos años, se ha trabajado para identificar las formas óptimas de diversidad botánica para mejorar el control biológico de las diferentes plagas del olivo (Pinheiro et al. 2013, Villa et al. 2016). Estos estudios han incluido las plantas con flores más abundantes presentes en los olivares de la cuenca mediterránea: *F. vulgare*, *M. sylvestris*, *D. carota* subsp. *carota*, *H. perforatum*, *H. incana* y *D. viscosa*. En total, cubren casi todo el año con flores pero, desafortunadamente, la accesibilidad y calidad de su néctar no es óptima para los parasitoides de *S. oleae*. Los parasitoides con acceso a flores vivieron mucho menos que aquellos con acceso a melaza de *S. oleae* y el control positivo (miel al 10%). Una vez más, nuestros resultados contradicen los resultados generales de que el néctar tiene un mayor valor nutricional que la melaza (Wäckers et al. 2008). Por ejemplo, el parasitoide *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) vive más tiempo cuando se alimentan de néctar del trigo serrano *Fagopyrum esculentum* (Moench) (Polygonaceae) que en la melaza excretada por el pulgón de soja, *Aphis glycines* (Matsumura) (Hemiptera: Aphididae) (Lee et al. 2004). Al comparar entre las flores estudiadas, *M. sylvestris* fue la mejor flor para el parasitoide *C. lyciminia*, ya que vivió el doble que con otras flores. Pinheiro et al. (2013) encontró el mismo resultado cuando se proporcionó esta flor a los adultos de la mosca del syrphid *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae). A pesar de los resultados obtenidos aquí, no se puede descartar completamente el uso de flores en los olivares, especialmente si las hormigas están presentes y asisten a *S. oleae*. Como se ha demostrado recientemente, las hormigas pueden interferir en el estado nutricional de los parasitoides a través de su hospedante común (Calabuig et al. 2015) y, además, asisten más frecuentemente a los hemípteros que excretan melaza de alta calidad (Tena et al. 2016), como ocurre con *S. oleae*. Por lo tanto, si las hormigas están presentes y asisten a la mayoría de las colonias de *S. oleae*, la presencia de flores podría beneficiar el contenido de carbohidratos de los parasitoides en los olivares.

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CHAPTER 1

General Introduction

1. Introduction

1.1. The importance of olive trees in Trá-os-Montes region, Northeast of Portugal

The olive tree (*Olea europaea* L.) is an important crop worldwide, especially in areas with Mediterranean climate (Böhm 2013, Barranco et al. 2017). It probably arose in Africa or Syria about 6000 years ago and spread through the Mediterranean basin more than 9.4 millions ago (Vossen 2007). Currently, it is grown in North America, South Africa, China, Japan and Australia (FAOSTAT 2016) although it is considered that about 98% of the world's olive production is located in the Mediterranean area (Civantos 2001, Herz et al. 2005). The world production of olives has increased in the Mediterranean basin as well as in other regions. In 2004, olive production was 19 million tons and in 2014 was 33 million tons, which led to a production increase more tan 70% (FAOSTAT 2016).

In Portugal, the olive trees have a great economic and social importance. Portugal is one of the most important countries in olive production, occupying the eighth place, with 476 003 t of production per year in 2016 (INE, 2017) and the fourth largest producer in the European Union (http: // www. Internationaloliveoil.org, 2017). The main regions are Alentejo, Trás-os-Montes and Beira Interior (Peres et al. 2011) (Figure 1.1).

The Alentejo region is the largest olive producer with 339 712 t of olives and occupying an area of 169 869 ha. Trás-os-Montes, in the Northeast of Portugal, is the second most important region of the country with an area 80 433 ha, and a production of 76 609 t of olives in 2016 (Figure 1.1). Both regions are completely different in terms of cultivars and intensification and, the dominance of the Alentejo region appears mainly in the last ten years, with the availability of water, new irrigated plantations with high number of plants per hectare were installed, mainly with foreign varieties that are conducted intensively with high productivity per area. Contrarily, in Trás-os-Montes, the groves are conducted extensively, constituted by old trees (over 50 years old) of a wide range of traditional cultivars, cultivated in low densities, implanted in terrains with steep slopes, with low degree of mechanization and absence of irrigation ones (Duarte et al. 2008, Cabanas and Rodrigues 2009). These agronomic characteristics lead to low profitability of olive groves in comparison to new irrigated olive groves (Duarte et al.

2008). However, the olive oil produced in this region is of reputed quality due to its agroecologycal characteristics in association with traditional cultivars conducted with low intensification practices (Peres et al. 2012).

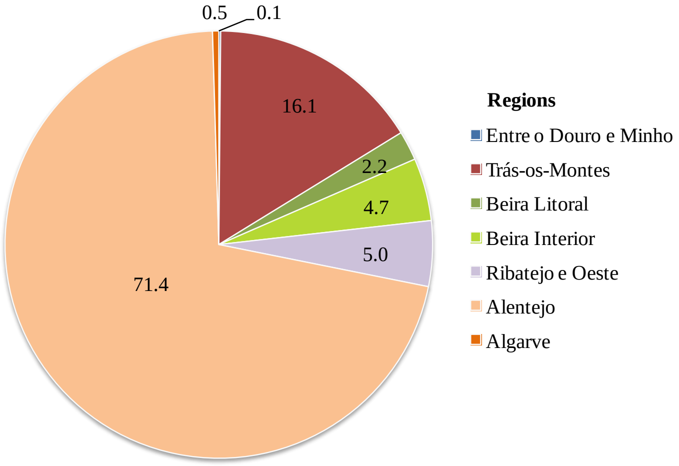

Figure 1.1. Distribution of the Portuguese olive production according to the region in 2016 (based on data of INE 2017).

1.2. Olive tree pests

The olive tree is attacked by a diverse entomofauna of at least 116 species of insects and 30 mites (Tzanakakis 2003). However, only a few species are considered pests. Haniotakis (2003) classified the pests of the olive tree into four categories, taking into account their contribution of each one to production losses. Category (a) includes important pests or key pests that cause damages of great economic importance to the region, requiring yearly protection. Category (b) includes major secondary pests that occur in the region and cause damage of major economic importance locally or occasionally. Category (c) includes pests of limited or localized economic importance and causes damage of limited, local and / or occasional economic importance, differ with location and time. In category (d) it includes pests of no economic importance which, in very rare circumstances, may cause local damage of limited economic importance (Table 1.1).

In Trás-os-Montes region (Portugal), the main pests are: olive fly (*Bactrocera oleae* Rossi) (Diptera: Tephitidae), olive moth (*Prays oleae* Bernard) (Lepidoptera: Plulellidae), black scale (*Saissetia oleae* Olivier) (Hemiptera: Coccidae) and olive psyllid (*Euphyllura olivina* Costa) (Hemiptera: Psyllidae).

Table 1.1. Classification of olive tree pests into categories according to their economic importance: a) key pests, b) important secondary pests, c) limited important pests d) not important pests

Pests olive tree	Category
Olive flay (Bactrocera oleae Rossi)	a
Olive moth (*Prays oleae* Bernard)	b
Black scale (*Saissetia oleae* Olivier)	b
Olive psyllid (*Euphyllura olivina* Costa)	c
Olive parlatoria scale (*Parlatoria oleae* Colvée)	c
Ivy scale (*Aspidiotus hederae* Vallot)	c
Olive thrips (*Liothrips oleae* Costa)	c
Olive leaf moth (*Palpita unionalis* Hübner)	c
Tabby knot-horn (*Euzophera pinguis* Haworth)	c
Leopard moth or wood leopard moth (*Zeuzera pyrina* Linneus)	c
Olive fruit curculio (*Rhynchites cribripennis* Desbrochers)	c
Olive bark beetle (*Phloeotribus scarabaeoides* Bernard)	c
Olive mite (*Aceria oleae* Nalepa)	d
Olive bud mite (*Oxycenus maxwelli* Keifer)	d
Olive rust mite (*Tegolophus hassani* Keifer)	d

The olive fly, is considered, the main or key pest of the olive groves in the Mediterranean region (Haniotakis 2003, Tzanakakis 2006). This pest causes significant losses in olive production and simultaneously affects the quality of olive oil (Pereira 2004, Malheiro 2015). The attacks of the olive fly can reach 100% attacked fruits, depending to the year, the climatic conditions and olive cultivar (Broumas et al. 2002).

The number of generations per year varies with the climate and the agronomic conditions (Torres 2007a), and can complete from two to five generations. The olive fly overwinters as pupa, buried in the soil (Neuenschwander et al 1986). Adults emerge in spring and lay their eggs in summer and fall (Torres 2007a) (Figure 1.2).

The olive moth is a lepidopterran pest that presents three generations per year: anthophagous, carpophagous and phythophagous. These generations are well synchronized with the cycle of development of the olive tree. In each generation the eggs are placed in a different organ of the host. The anthophagous generation develops in floral buds, the carpophagous in fruits and the phythophagous in leaves (Bento et al. 2007). The damage caused by *P. oleae*, depending on climatic conditions, may be between 6.26 and 15.15% of attacked floral bunches and between 36.64 and 51.68% of fruits attacked for the anthophagous and carpophagous generations (Bento 2001).

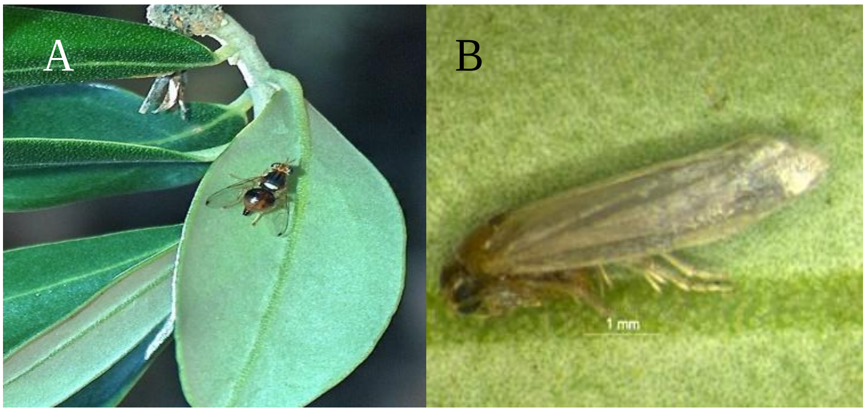

Figure 1.2. Adults: olive fly (*Bactrocera oleae*) (A) and olive moth (*Prays oleae*) (B)

The black scale is a polyphagous insect that feeds on more than 60 plant species in the Mediterranean region (Morillo 1977, Passos de Carvalho et al. 2003). It is considered a pest of olive tree and citrus (Pereira 2004, Tena et al. 2008). In Trás-os-Montes, this scale presents one generation, and some years, when the environmental conditions are favorable, it can develop a second partial generation (Pereira 2004). The damage caused is mainly due to the excretion of honeydew and the development of sooty mold, which reduces photosynthesis and production (Passos de Carvalho et al. 2003) (Figure 1.3).

The olive psyllid is an hemipteran which the adults pierce the sensitive tissues of the olive tree and suck the sap of flower buds and young fruits (Tzanakakis 2003, Pereira el al. 2007). Nymphs and adults produce a serous white secretion that may cause premature fall of flowers during infestations, leading to reduced production (Pereira el al. 2007). As *S. oleae,* the indirect damage is caused by the excretion of honeydew. (Johnson 2010). It can have three generations per year (Alford 2014) or more (Tzanakakis 2003), in Trás-os-Montes, commonly only two generation are observed during spring and beginning of the summer (Pereira et al. 2007) (Figure 1.3).

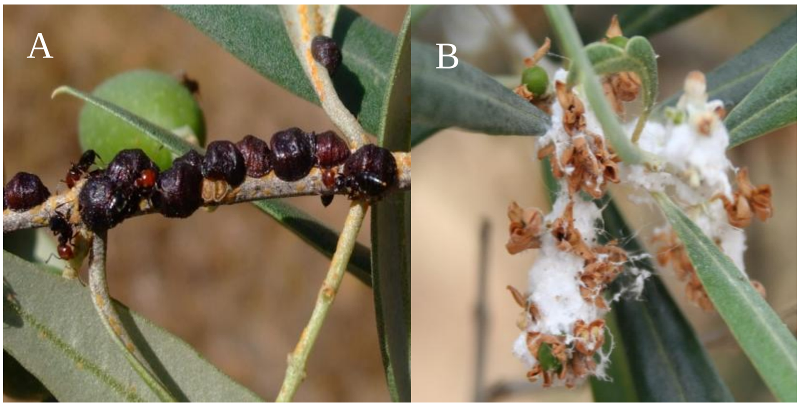

Figure 1.3. Colony of *Saissetia oleae* tended by ants (A) and colony of *Euphyllura olivina* in young fruits (B).

1.3. *Saissetia oleae*

1.3.1. Origin, geographical distribution and current status in Trás-os-Montes.

The origin of *S. oleae* is not consensual. Some authors reported that this pest is originated from South Africa (De Lotto 1976, Orphanides 1990, Waterhouse and Sands 2001), nevertheless, others referring the Mediterranean region as the origin (Passos de Carvalho et al. 2003). *S. oleae* is currently distributed across the different continents (Figure 1.4), but it is in the Mediterranean Basin and Americas where it is more abundant and more research has been carried out.

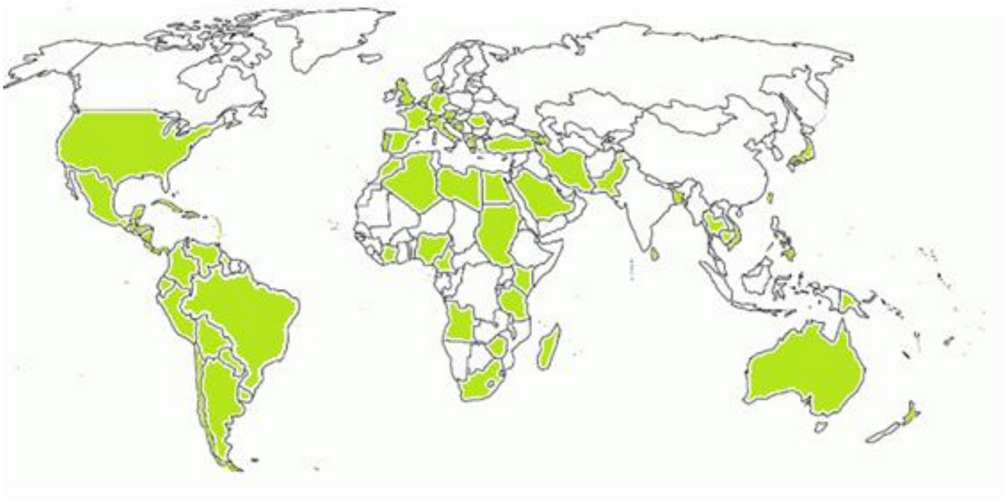

Figure 1.4. World distribution of the black scale, *Saissetia oleae* (based on CABI, 1954. *Saissetia oleae*, CAB International Distribution Maps of Plant Pests, on August 15, 2017)

In Portugal, the first known studies were conducted by Pau-Preto (1952) and Freita (1972) in olive trees and Freitas (1977) in citrus. More studies were carried out in the nineties by Fernandes (1997) and Pereira et al. (1999). And, more recently, two PhD thesis by Pereira (2004) and Santos (2007) have explored the natural enemies of *S. oleae* in the Trás-os-Montes region.

1.3.2. *Saissetia oleae* biology

1.3.2.1 Morphology

Saissetia oleae is an oviparous species with parthenogenetic reproduction, in which males are very rare and unknown in Europe (Torres 2007b), and are different from adult females and have wings (Paparatti 1986). Females have three nymphal instars (Torres 2007b).

The egg, which is protected by the maternal shield of females, has an ellipsoid shape that measures approximately 0.26-0.32 mm in length and 0.13-0.22 mm in width (Passos-Carvalho et al. 2003). It has a pale-pinkish coloration and it gets darker before hatching.

The nymph of the **first instar** has also an elliptical shape, when newly hatched and slightly convex in the dorsal and flat region in the ventral region, it measures about 0.3-0.4 mm in length and 0.18-0.20 mm width and its coloration is pale yellow (Passos de Carvalho et al. 2003). It has a pair of antennae, three pairs of legs and two dark eyes (Climent 1990). After hatching, nymphs move to illuminated surfaces and tend to settle mainly in the veins of the leaves of olive trees (Passos de Carvalho et al. 2003).

The nymph of the **second instar** presents a shape similar to the anterior instar, but it is more convex, measuring 0.62-0.80 mm in length and 0.32-0.40 mm in width (Tena 2007b). It is visible a longitudinal ridge (Torres 2007b) that later forms the bar of the letter H, seen in older individuals (Tena 2007).

The nymph of the **third instar** has a more convex shape than the anterior instar and measures between 1.0-1.3 mm in length and 0.3-0.7 mm in width (Paparatti 1986), being in the end 1.4-1.6 mm in length and 0.6-0.8 mm of wide (Tena 2007). Initially it presents coloration identical to that of the previous instar, becoming grayish brown. The longitudinal and transverse ridges appear more prominent, being more evident the "H" (Torres 2007b) (Figure 1.5). They tend to migrate to the twigs at the end of this instar (Tena et al. 2008).

The **young adult female**, has a more rounded body contour than the anterior instar, showing an approximately oval shape, measuring 2-5 mm in length, 1-4 mm in width and 2-2.5 mm in height (Passos de Carvalho et al. 2003). They present brownish-gray coloration, the longitudinal and transverse ridge are prominent and well-marked, forming the known "H", which gave rise to one of the common names of *S. oleae* (Torres 2007b). Their mobility is reduced during this stage, which can lead to compact colonies of *S. oleae*, with an overlap of part of the body margin of some scale with others, being deformed due to competition for space (Passos de Carvalho et al. 2003) (Figure 1.5).

When the convexity of the dorsal region of the body is accentuated and there is an increase in the concavity which forms on the ventral side of the scale, the posture period approaches (Passos de Carvalho et al. 2003). At this stage, they become black and start the oviposition. Measuring 3.2-5.2 mm in length, 2.1-4.0 mm in width and 2.0-3.3 mm in height (Pereira 2004).

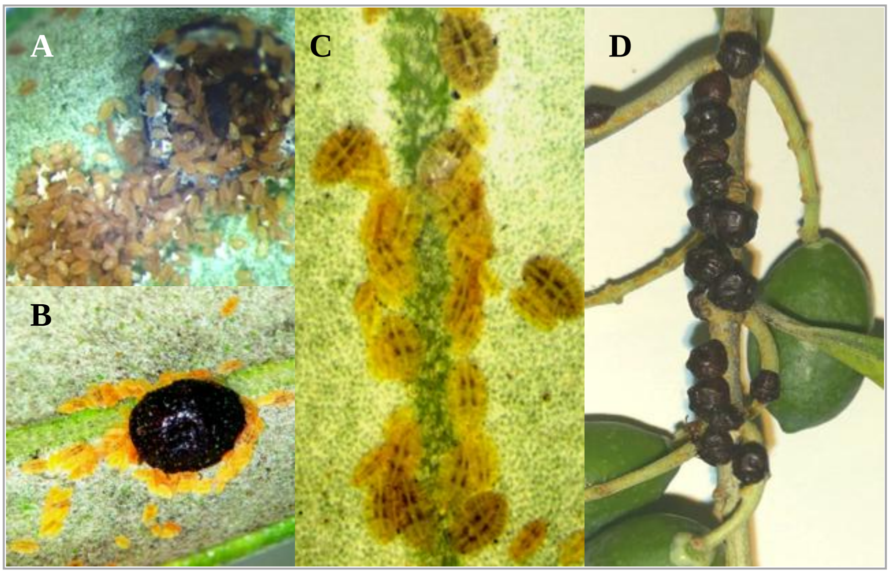

Figure 1.5. Different developmental stages of *Saissetia oleae*: A: eggs, B: adult females and first instar nymphs, C: Second and third instar nymphs and D: ovipositing females.

1.3.2.2. Life cycle

Saissetia oleae can develop between one or two annual generations depending on climatic conditions in olive trees from the Mediterranean basin (Paparatti 1986, Pereira 2004, Tena et al. 2008). The fertilization, pruning and irrigation system may also affect the number of generations of *S. oleae* (Bodenheimer 1951, Blumberg et al. 1975, Panis 1977, Passos de Carvalho et al. 2003) (Figure 1.6). In the Trás-os-Montes region (Portugal), the scale has a complete generation per year, however, in favorable conditions can develop a second partial generation (Passos de Carvalho et al. 2003, Torres 2007b).

The black scale can lay from hundreds to 4000 eggs depending on the size of the adult female (Morillo 1977, Brailes and Campos 1986, Passos de Carvalho et al. 2003, Tena et al. 2008). Incubation of the eggs last around 14 days and the posture is performed irregularly during 10-15 days, in spring and early summer (Passos de Carvalho et al. 2003) and 20 to 25 days in fall (Paparatti 1986).

The newly hatched nymphs, remains in the posture chamber for one or two days (Torres 2007b) and when climatic conditions are favorable, they move and settle, usually on the lower part of the leaves (Morillo 1977, Pereira 2004), remaining immobile about four weeks in the spring/summer (Morillo 1977) and up to 50 days at the end of fall (Torres 2007b). In the Trás-os-Montes region, nymphs emerge between late June and mid-August (Pereira 2004).

Once they are settled, mainly in the leaves, they remain stationary until some stimulus induces them to migrate to twigs (Bibolini 1958, Ebeling 1959, Argyriou 1963, Morillo 1977, Pereira 2004). This migration appears to be stimulated by the search for nutritionally and climatically suitable niches (Briales and Campos 1986) or as an instinct still prevails, as consequence of feeding previously on deciduous trees (Ebeling 1959). The majority migrate during the third instar (Ebeling 1959, Smith et al.1997). The information about the percentage of the population that migrates to twigs is different according to different authors. Thus, some studies considered that only a small percentage is moving to the twigs (Bibolini 1958, Argyriou 1963), whereas more recent observations suggest that the number of scales moving can be so high that, during the oviposition period, most adult females are on the twigs (Freitas 1972, Briales and Campos 1986).

After the third molt and once in the twigs, the adult females increase rapidly (Morillo 1977), and as they develop the amount of honeydew secreted increases until it stops just before the oviposition (Bodenheimer 1951). The adult females die after the oviposition, remaining the body in the twig (Morillo 1977).

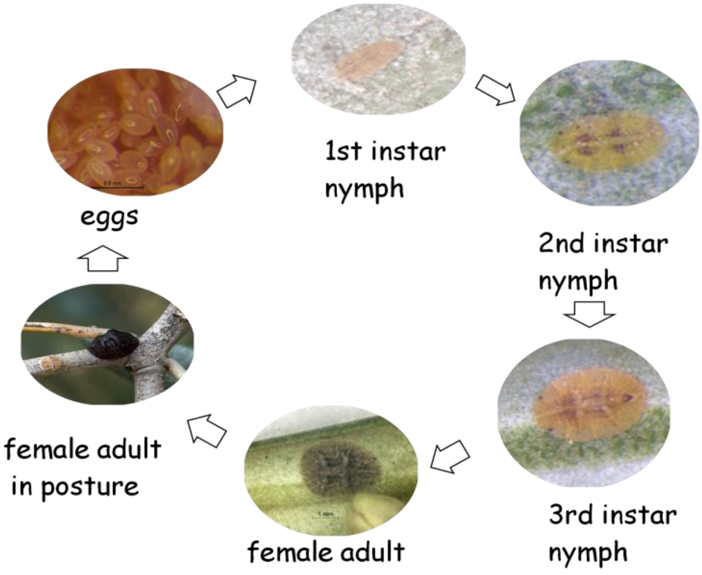

Figure 1.6. Life cycle of *Saissetia oleae* (constituted by: eggs, three immature instars and two adult phases)

1.4. Control of *Saisseita oleae* in olive trees

During the last decades, European Union has developed policies to regulate pest management in environmentally sensitive agricultural practices and has led to widespread interest in integrated pest management and organic farming (2009/128 direrctive of the European Parlament). Both practices promote the protection of the environment, namely biodiversity, soil and water, and the obtaining of high quality agricultural products (Guillou and Scharpé 2000, Malavolta et al. 2002, Boller et al. 2004). The implementation of these guidelines has been relatively feasible in olive trees because of the relatively low number of pesticides used against the main pests. In the case of *S. oleae,* several techniques can be used to control it.

1.4.1. Cultural methods

Pruning is used to control *S. oleae* since it creates unfavourable conditions for insect development. Generally, the tree is pruned, with a removal of branches of the center of the canopy. In these conditions, the insect is exposed to the high temperatures of summer and also to the action of dry winds which increase the mortality of the young instars of *S. oleae*. Moreover, pruning favors a better penetration of pesticide treatments when necessary (Ouguas and Chemseddine 2011).

It is also necessary to take into account that excessive fertilization of nitrogen favors the increase of the pest (Torres 2007b). It has been demonstrated that a reasonable nitrogen fertilization can reduce the number of eggs, nymphs, and adults of approximately two-fold (Daane and Caltagirone 1989, Ouguas and Chemseddine 2011, Regis 2002). The irrigation is also another aspect to consider once it can favours the increase of the relative humidity on the grove that contributes for a best survivl of *S. oleae* populations.

1.4.2. Biological control

After the introduction of *Rhizobious ventralis Erich* (Coleoptera: Coccinalidae) in California at the end of the 19th century (Bartlett 1978), extensive efforts were developed to implement biological control programs against the black scale in different parts of the world, mainly in citrus and olive trees. At the beginning of the 20th century (1902) and for 90 years was developed in California a program of introduction of natural enemies of black scale, to control this pest in citrus and olive tree (Lampson and Morse 1992). Since then, different biological control programs have been conducted throughout the world. Classical and augmentative biological control programs have resulted in successful control in several countries, since the beginning of the twentieth century. In other cases, biological control has been partial or inadequate. Thus, over the years, a tremendous body of knowledge dealing with biological control of *S. oleae* has been accumulated (Kapranas and Tena, 2015). Satisfactory biological control of *S. oleae* has been achieved mainly through the introduction of parasitoids (Mendel et al. 1984; Waterhouse and Sands 2001) but also with the inoculative releases of parasitoids (Graebner et al. 1984) and conservation biological control programs (Tena et al. 2008).

In the Mediterranean basin, several species of predators *Chilocorus bipustulatus* L., *Exochomus quadripustulatus* L. (Coleoptera: Coccinellidae), *Eublemma scitula* Rambur (Lepidoptera: Noctuidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and pteromalid *S. caerulea* and two parasitoids *Metaphycus flavus* (Howard) and *Coccophagus lycimnia* (Walker) (Hymenoptera: Aphelinidae) were the cited as the natural enemies of *S. oleae* until 1960 (Tena 2007b) (Figure 1.7). Then, several classical biological control programs were conducted to improve the control of this pest in several Mediterranean countries (Mendel et al. 1984, Ben-Dov and Hodgson 1997, Passos de Carvalho et al. 2003). In France, species of the genus *Metaphycus* were introduced and *M. lounsburyi* (Howard) displayed an excellent control, but in specific situations it was necessary to release it periodically (Lenfant and Marro 1997). In Israel, *S. oleae* was controlled after the introduction of the parasitoids *M. lounsburyi* and *M. helvolus* in the 70s (Argov and Rössler 1993). In Portugal, *M. lounsburyi* was introduced in 1977/78 from France (Jourdheuil 1986).

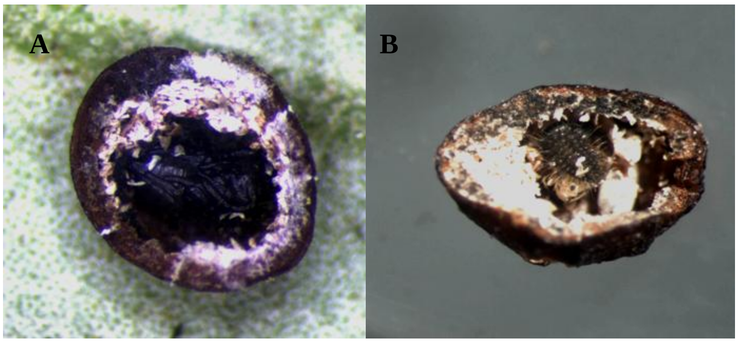

Figure 1.7. Predators of *Saissetia oleae*. A: pupae of *Scutellista* spp., B: young larva of ladybird (Coleoptera: Coccinellidae)

1.4.2.1. Natural enemies

Arthropods, like other living organisms, have natural enemies that limit their populations. The most common natural enemies associated with *S. oleae* are grouped into two groups: entomopathogenic and entomophagous. In the first group are included fungi, in the second group there are several species of predators and parasitoids (Passos de Carvalho et al. 2003, Pereira 2004).

The entomopathogenic fungus *Verticillium lecanii* (Zimm.) Viègas (= *Cephalosporium lecanii*), mitosporic fungi complex (Passos de Carvalho et al. 2003), may control high population densities of black scale in conditions of high relative humidity (Smith et al.1997)

Predators belong mainly to the Coccinellidae family (Santos et al. 2008). In Greece, Argyriou (1974) describes *Chilocorus bipustulatus* as having an important role in the natural limitation of *S. oleae* populations and in Spainthe species *Pullus* sp. close to *subvillosus* was considered the most important (Carrero et al. 1977). In the Trás-os-Montes region (Portugal), the most abundant and important predators of *S. oleae* belong to the family of coccinellids: *Scymnus* (*Scymnus*) *interruptus* (Goeze), *Scymnus* (*Pullus*) *subvillosus* (Goeze), *Scymnus* (*Mimopullus*) *mediterranean* Iablokoff-Khnzorian and *Rhyzobius chrysomeloides* (Herbst) (Santos 2007), *Chilocorus bipustulatus, Exochomus nigromaculatus, Exochomus quadripustulatus*, and *Rhyzobius lophanth*ae (Santos 2007). According to Santos et al. (2008) the second instar nymphs are usually preyed by coccinellids whereas *C. carnea* preys on the first instar nymphs of *S. oleae* (Passos de Carvalho et al. 2003).

The parasitoids of *S. oleae* belong to the hymenopteran families: Aphelinidae, Encyrtidae and Pteromalidae (Passos de Carvalho et al. 2003, Torres 2007b). *Metaphycus* is the most important genus of the family Encyrtidae and *M. lounsburyi* (Howard), *M. helvolus* (Compere) and *M. flavus* (Howard) are main species in Portugal (Pereira 2004). The most important genus within the family Aphilinidae is *Coccophagus,* being *C. lycimnia* (Walker) and *C. semicircularis* (Föster) the most abundant and successful parasitoids of this genus in Portugal (Pereira 2004). The Pteromalidae *Scutellista caerulea* (Fonscolombe) is an hymenopteran which larvae feed on the *S. oleae* eggs, this predator is frequent and easy to be observed under the scale predating eggs (Passos de Carvalho et al. 2003). All these species are the most representative in the Mediterranean countries (Pau-Preto 1952, Freitas 1972, Paixão 1996, Pereira et al. 1999, Pereira 2004). Other parasitoid species have been cited as natural enemies of *S. oleae* (Table 1.2), but they have a limited effect on the scale populations.

Table 1.2. Species of parasitoids of *Saissetia oleae* recorded in Portugal.

Family	Specie	Biology	Reference[1]
Aphelinidae	*Coccopagus lycimnia* (Walker)	Facultative autoparasitoid	a, b , e, f, g
	Coccophagus semicircularis (Förster) (=*C. scutellaris*)	Obrigate autoparasitoid	a, b, d, e, f, g
	Marietta picta (Andre)	Secondary parasitoid	f, g
Encyritidae	*Diversinervus elegans* (Silvestri)	Primary parasitoid	d
	Metaphus flavus (Howed)	Primary parasitoid	a, b, c, d, f, g
	Metaphucus helvolus (Compere)	Primary parasitoid	a, b, d, e, f, g
	Metaphycus lounsburyi (Howard) (=*M. bartletti Annecke & Mynhardt*)	Primary parasitoid	a, b, d, g
	Metaphycus swirskii Annecke & Mynhardt (M. affinis stanley Compare)	Primary parasitoid	c
Pteromalidae	*Maranila californica* (Howard)	Egges predator	a, b, f, g
	Pachyneuron muscarum (Linnaeus)	Secondary parasitoid	a, b, f, g
	Scutellista caerulea (Fonscolombe) (=*S. cynea* Motschnulsky*)*	Egges predator	a, b, c, f, g
	Scutellista nigra (Mercet)	Egges predator	e, f, g

[1] (a)Pau-Preto 1952, (b) Freitas 1972, (c) Carneiro (d) Paixão 1996, (e) Pereira et al. 1998, (f) Pereira 1999, (g) Pereira 2004

Parasitoids are considered the main natural enemies of *S. oleae* and soft scales in general and can be divided in two groups depending on the stage of the scale attacked: nymphal and adult parasitoid (Kapranas and Tena 2015). *M. helvolus*, *M. flavus*, *C. lycimnia* and *C. semicircularis* are among the formers whereas *M. lounsburyi* and *S. caeruela* attack mainly *S. oleae* adults (Pereira 2004, Tena 2007). Generally, *Metaphycus* parasitoid has been considered the most abundant and successful parasitoids of soft scales (Kapranas and Tena 2015).

Interactions among *Metaphycus* or between *Metaphycus* and other parasitoids have been studied in numerous cases. Competition among ecological homologues leading to competitive displacement of species has occurred in a few cases. In Crete, Greece, *M. helvolus* displaced the native parasitoid *M. flavus* (Argyriou and DeBach 1968). In Israel, *M. lounsburyi* (erroneously identified as *Metaphycus bartletti*) outcompeted *Metaphycus hageni* (identified erroneously as *M. lounsburyi*) (Argov and Rössler 1993). In California, the parasitism rate of *S. oleae* by *S. caerulea* was lower in the presence of *M. lounsburyi* (Ehler 1978). Among the interactions with other families of soft scale parasitoids, the most peculiar is with species within the genus *Coccophagus*.

1.4.2.2. Relationship between parasitoids of genus *Coccophagus* and *Metaphycus*.

Coccophagus are facultative or obligate heteronomous hyperparasitoids of coccids (Annecke 1964, Bernal et al. 2001, Walters 1983) (Figures 1.8 and 1.9). In the case of *C. lycimnia* females develop on coccids and males can develop on females of *Metaphycus* or conspecific females. As consequence of their biology, it was suggested that *Coccophagus* could negatively affect the biological control potential of *Metaphycus*. This hypothesis was supported by later studies showing that excessive hyperparasitism by *Coccophagus* spp. did not allow *Metaphycus* spp. populations to build up as the season progressed, resulting in poor control of *Coccus pseudomagnoliarum* (Kuwana) in California (Bernal et al 1998, Bernal et al. 1999, Bernal et al. 2001). However, later studies suggested that *Coccophagus* spp. might be efficient biological control agents of soft scale insect pests (Schweizer et al. 2003, Schweizer et al. 2002). Similarly, an increase of *Coccophagus* parasitism on *C. hesperidum* did not lead to a reduction of parasitism by *Metaphycus* spp. (Kapranas et al. 2007). Therefore, it is unclear whether *Coccophagus* spp. can negatively influence the population dynamics of their encyrtid hosts and/or the population density of soft scale insects. In Trás-os-Montes region, *Coccophagus* parasitoids are very abundant (Pereira 2004), but their effect on the population of the primary parasitoids of genus *Metaphycus* and their common host, *S. oleae,* are unknown. In this thesis, the effect of *C. lycimnia* on the populations of *Metaphycus* and on the biological control of *S. oleae* are evaluated, as well as the mechanism that permit the coexistence of both groups of parasitoids.

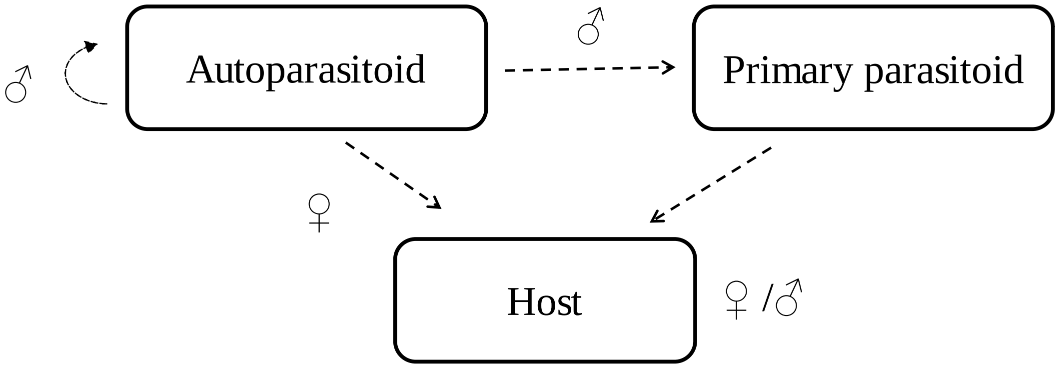

Figure 1.8. Diagram illustrated the performance of the autoparasitoid (*Coccophagus* spp.) when it postures in the host or in pre-pupa of primary parasitoids (*Metaphycus* spp.)

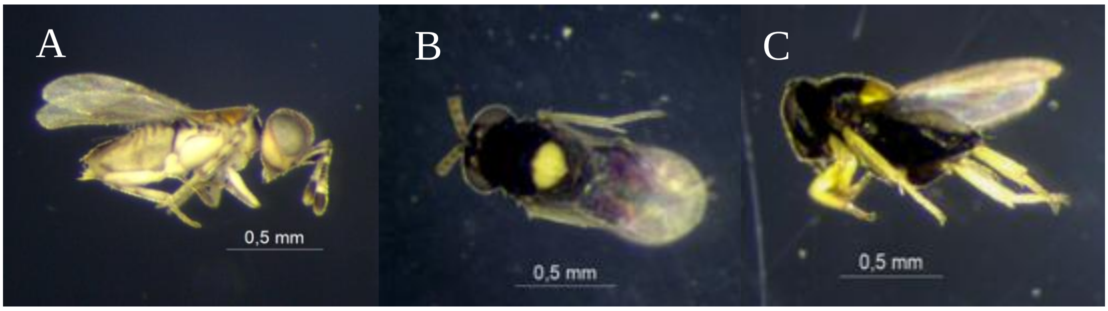

Figure 1.9. Primary parasitoid: *Metaphycus lounsburyi* (A); **Autoparasitpoids**: *Coccophagus lycimnia* (B) and *C. semicircularis* (C)

1.4.2.3. Effect of sugar food sources for parasitoids on biological control of *Saissetia oleae.*

As adults, most parasitoids need to feed on sugar-rich foods to meet their energy requirements. In modern agricultural based on monocultures, sugar-rich sources in form of nectar are scarce. This absence can severely compromise the fecundity and lifespan of most parasitoid species and, consequently, their potential to control pests (Heimpel and Jervis 2005, Tena et al. 2016). The incorporation of targeted non-crop vegetation in agricultural systems can help support a range of ecological services, including natural pest control by natural enemies (Landis et al. 2000). This effect is generated by the fact that selected flowering species provide nectar and extrafloral nectar that are vital to parasitoids and other sugar feeding natural enemies (Heimpel and Jervis 2005). This approach, however, has critically overlooked the potential role of honeydew, which in terms of availability is the primary carbohydrate source in many agroecosystems (Tena et al. 2016). In fact, during the last decade, it has been demonstrated that parasitoids commonly feed on honeydew in the field using high-performance liquid chromatography (HPLC) (Steppuhn and Wäckers 2004, Hogervorst et al 2007, Tena et al. 2013, Tena et al. 2015, Dieckhoff et al. 2014, Calabuig et al. 2015), or anthrone tests (Lee et al. 2006). Compared to nectar, however, honeydew has been considered a poorer sugar source for parasitoids (Wäckers et al. 2008).

Olive orchards of the Trás-os-Montes region present a great structural diversity, since it is constituted by perennial trees with spontaneous cover crops and the orchards can be surrounded by adjacent parcels of natural vegetation, which allows a great diversity of habitat and food. However, nectar can be scarce within the orchard when flowers are not present in the cover crop. During those periods honeydew excreted by *S. oleae* and the olive psyllid are the only sugar sources. Their value as sugar source for parasitoids is poorly known. In this thesis, the effect of different nectars and honeydews on the fitness of the main parasitoids of *S. oleae* is explored to understand the potential of conservation biological control throughout the use of flowering plants in the cover crop.

1.4.2.4. Effect of ants on biological control of *Saissetia oleae*

The interaction between ants and hemipteran insects producing honeydew are known in natural habitats of grasslands to forests, but also in agricultural systems (Buckley 1987, Way and Khoo 1992, Styrsky and Eubanks, 2007). Hemipterans provide honeydew to ants and they protect hemipterans from eventual predators and parasitoids, thus establishing a mutualism relationship (Way 1963, Buckley 1987, Stachowicz 2001) (Figure 1.11). The ecological consequences of these interactions are enormous as it influences the abundance of ants, hemipterans as well as other arthropods in the plant, affecting to the plant health (Figure 1.10). For example, Flanders (1945) observed that the Argentine ant *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) negatively affected the activity of the endoparasitoid, *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae), resulting in higher infestations of its host, *Aonidiella citrina* Coquillet (Hemiptera: Diaspididae) in citrus fruits when the ants searched for honeydew excreted by hemipterans. Ants, in addition to protecting hemipterans, also stimulate the rate of hemipteran feeding, fecundity and dispersion (Flanders 1945, Buckley 1987).

In agroecosystems ants have negatively affected the biological control of several important hemipteran pests by interfering with the activity of parasitoids and predators (Debach 1951, Way 1963, Pekas et al. 2011, Tena et al. 2013). In olive orchards, these interactions are very frequent, since ants are very abundant (Cabanas et al. 1999, Ramalho 2000, Lozano et al. 2002) (Figure 1.10). In fact, ants have been suggested to affect the biological control of *S. oleae* in olive orchards (Pereira 2004).

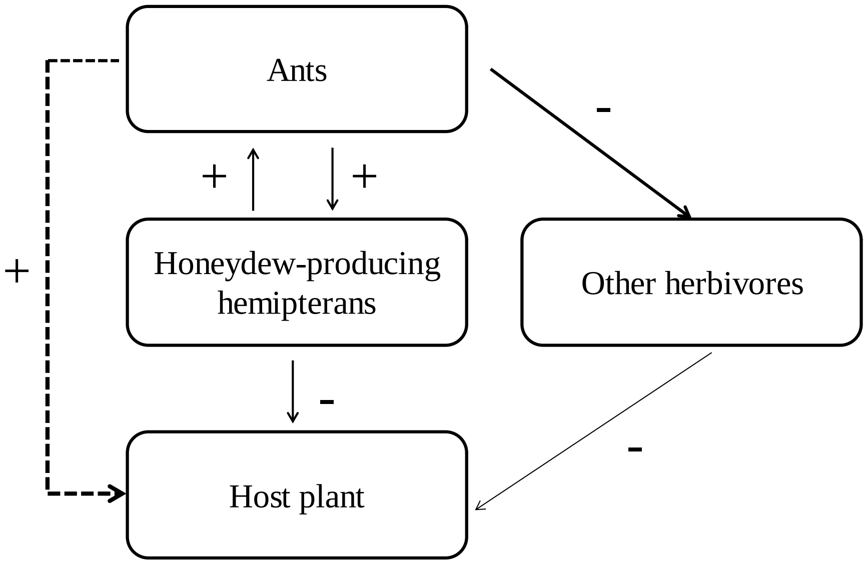

Figure 1.10. Probable consequences of the interactions between hemipterans and ants on plants. The arrows indicate the relative magnitude of the effects (positive or negative). Solid arrows indicate direct effects, while dashed arrows indicate indirect effects (Adapted: Styrsky and Eubanks 2007)

The parasitoids of *S. oleae* react differently to the presence and attack of ants. According to Panis (1981) the presence of ants is accompanied by the predominance of *C. lycimnia* over *M. flavus* and *M. helvolus*. Later on Barzman and Daane (2001) demonstrated that this advantage is due to lower time needed by parasitoid of genus *Coccophagus* to lay eggs compared to *Metaphycus* species. In thesis, the effect of ants on the parasitoid complex of *S. oleae* is explored in order to determine whether the ant species present in Trás-os-Montes can negatively affect the biological control of the scale.

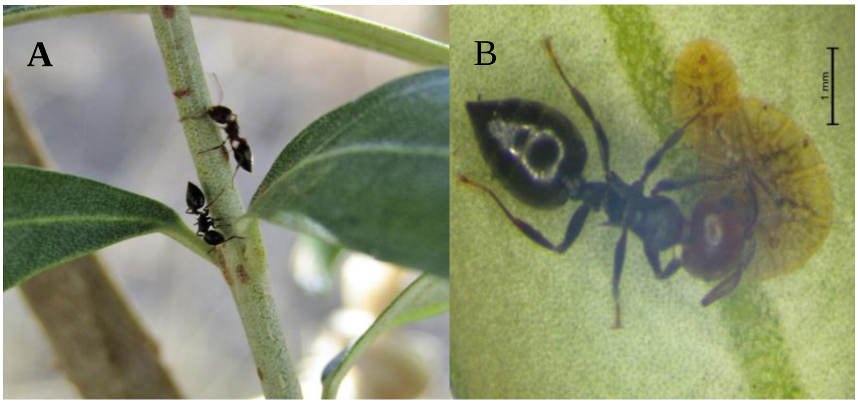

Figure 1.11. A: Movements of *Crematogaster auberti* in *Saissetia oleae* infested olive tree and B: *Crematogaster scutellaris* feeding on honeydew excreted by the black scale.

1.4.3. Chemical control

The first instars of *S. oleae* are more sensitive to pesticides, especially mineral oils (Yaron et al. 1988). On the other hand, adult females are the most resistant instar to insecticides, which makes their control more difficult (Pereira 2004). Therefore, it is recommended to spray in July, when *S. oleae* population is composed mainly by individuals of the first instars. Currently, only mineral oils are used against *S. oleae* in Portugal (Mendes and Cavaco 2017) and insecticide applications are justified only when the level of attack is very high and with a strong tendency to form sooty mold (Torres 2007b).

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CHAPTER 2

Justification and objectives

Justification and bjectives

The black sacle, *Saissetia oleae*, is an important pest of the olive tree, which is considered a secondary pest in the region of Trás-os-Montes (Portugal). This pest is attacked by different natural enemies, which, in some cases, maintain pest populations at tolerable levels.

There are a considerable number of Hymenoptera parasitoid species that attacks *S. oleae*. In Trás-os-Montes region the most common species are *Metaphycus lounsburyi* (Howard), *M. helvolus* (Compere), *M. flavus* (Howard) (Hymenoptera: Encyrtidae), *Coccophagus lycimnia* (Walker) (Hymenoptera: Aphelinidae) and *Scutellista caerulea* (Fonscolombe) (Hymenoptera: Pteromalidae). However, some of these parasitoids, especially of those the genus *Metaphycus* and *Coccophagus* may establish interspecific competition which may affect the biological control of *S. oleae*. On the other hand the ants may interfere in the parasitoid complex and the population *S. olea*e. Finally the biological control of *S. oleae* goes through the conservation of its natural enemies (parasitoids). To this end, it is necessary to evaluate the sources of carbohydrates available in olive groves and to evaluate their effect on the survival of parasitoids.

The main objectives of this thesis were:

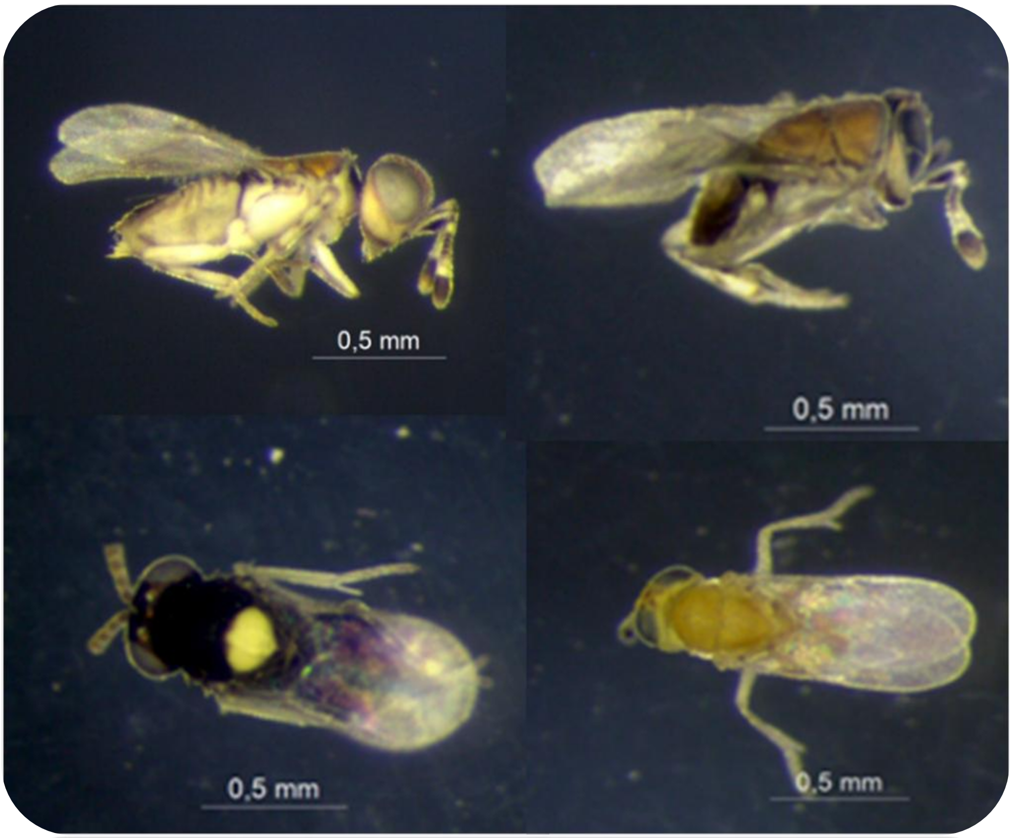
1. To analyze whether the facultative autoparasitoid, *C. lycimnia* can negatively affect the population density of the primary parasitoids of the genus *Metaphycus* and disrupt the biological control of *S. oleae* (**Chapter 3**).

2. To determine the effect of ant exclusion on the effect of ant-exclusion on the biological control of *S. oleae* and its parasitoid complex (**Chapter 4**).

3. To analyze the effect of carbohydrate sources available in the field (nectar and honeydew) on the survival of parasitoids of the genus *Metaphycus* e *Coccophagus* (**Chapter 5**).

CHAPTER 3

An autoparasitoid, inferior at resource exploitation, outcompetes primary parasitoids and controls their common host in the field



Marrão R., Pereira J.A., Tena A., *in preparation*. An autoparasitoid inferior at resource exploitation outcompetes primary parasitoids and controls their common host in the field. *To be submitted*.

Abstract: Autoparasitoids are intraguild consumers that attack and kill both the immature stages of hemipteran hosts, such as soft scales, and heterospecific and conspecific parasitoids. Field experiments assessing the importance of the interspecific competition between autoparasitoids and primary parasitoids, as well as its impact on host populations, are scarce in the ecological literature. Using field data from 28 olive orchards with different parasitoid densities but similar host densities at the beginning of the season, we examined the effect of interspecific competition between primary parasitoids of the genus *Metaphycus* and the autoparasitoid *Coccophagus lycimnia* on their population dynamics and that of their shared host *Saissetia oleae*. Our results reveal, for the first time, that an autoparasitoid that is inferior at resource exploitation can outcompete the primary parasitoids without disrupting the suppression of their common host. *Metaphycus* parasitoids used smaller hosts than *C. lycimnia*, but they did not exploit this enemy-free space to outcompete *C. lycimnia*. On the other hand, *C. lycimnia* preferred to use *Metaphycus* females as secondary hosts to produce males rather than their own females at the beginning of the host life cycle, when parasitoids were scarce and the number of hosts was high. This mechanism might explain why the autoparasitoid was a superior competitor, displacing the primary parasitoids. Despite the asymmetrical competition and *C. lycimnia*'s displacement of the primary parasitoids of the genus *Metaphycus*, the former suppressed host densities at the end of the life cycle of the scale. *C. lycimnia* and the host populations had almost reached a 1:2 ratio, showing its high efficacy as a biocontrol agent.

Key words: *Metaphycus*, *Coccophagus*, soft scales, interspecific competition, exploitative competition, intraguild predation

3.1. Introduction

Historically, attempts at biological control have often failed because they have ignored the fact that natural enemies are part of a food web, where the top-down control of pest populations is the outcome of multiple species interacting at different trophic levels (Murdoch et al. 1985, Rosenheim et al. 1995, Rosenheim 1998, Borer et al. 2003, Borer et al. 2004, Janssen et al. 2006, Boivin and Brodeur 2006). One of these interactions is the consumption of one natural enemy by another. Known as intra-guild predation, this phenomenon is widespread and can weaken natural enemies' top-down control of herbivores (May and Hassell 1981, Polis et al. 1989, Rosenheim et al. 1995, Rosenheim 1998, Borer et al. 2003, Janssen et al. 2006, Boivin and Brodeur 2006). Hymenopteran parasitoids are often among the most important natural enemies of herbivores, and they are generally the victims rather than the perpetrators of intra-guild predation (Rosenheim et al. 1995, Rosenheim 1998, Snyder and Ives 2008). However, some species of parasitoids possess unusual host relationships with males developing as obligate hyperparasitoids of females of their own and other parasitoid species, whereas females develop as the primary parasitoids of herbivores, i.e., autoparasitoid *sensu* (Collier and Hunter 2001). Therefore, as with intraguild predators, autoparasitoids can consume and kill both their competitors and their shared host (Rosenheim et al. 1995). The role of autoparasitoids in mediating the strength of their primary parasitoids' top-down control of herbivores sparked a lively debate in the literature of the 1990s and 2000s. The key question was whether any reduction in control caused by autoparasitoids' loss of primary parasitoids could be compensated by the direct parasitism on the host by the former. After analyzing theoretical and field studies, (Snyder and Ives 2008) concluded that, from a theoretical perspective, it is difficult to anticipate whether an autoparasitoid will cause an increase or decrease in long-term herbivore population densities when the main potential control agent is a parasitoid because the disruption of host suppression by autoparasitoids requires moderately strong, though not overly strong, attack rates on hosts that are parasitized by the primary parasitoid. Therefore, the outcome of competition between primary parasitoids and autoparasitoids for host suppression teeters on the boundary between disruption and synergism. Studies of real systems seem to bear out this expectation, with examples of both disruption and synergism by intra-guild predation.

The studied systems have generally included facultative autoparasitoids of the genus *Encarsia* (Hymenoptera: Aphelinidae) that parasitize whiteflies (Hemiptera: Aleyrodidae) in field cages. Hunter et al. (2002) examined the interactions between the autoparasitoid *Encarsia sophia* (*E. transvena* (Timberlake)), the primary parasitoid *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae), and their shared host, the sweet-potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). The autoparasitoid's interference reduced primary parasitoid density but with no concomitant disruption of host suppression. The results support theoretical predictions that no disruption should occur when both parasitoids are equally efficient and suggest that an autoparasitoid may be as efficient as a primary parasitoid in suppressing host densities (Snyder and Ives 2008). In another system, (Bográn et al. 2002**)** examined interactions among three parasitoids of the silverleaf whitefly *Bemisia argentifolii* Bellows & Perring (Hemiptera: Aleyrodidae): the autoparasitoid *Encarsia pergandiella* Howard and the primary parasitoids *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) and *Encarsia formosa* Gahan. Contrary to the previous example, the autoparasitoid *E. pergandiella* disrupted whitefly suppression, and, more interestingly, the primary parasitoids could also reduce the population density of autoparasitoids through exploitative competition. When both attack the whitefly *B. argentifolii*, the primary parasitoid *E. mundus* utilizes younger developmental stages of the host than the autoparasitoid *E. pergandiella*. This asymmetric exploitative competition negatively affects the population densities of the autoparasitoid *E. pergandiella*. Mathematical simulations also predict that parasitoid species that attack an earlier stage will always win when competing with a parasitoid that attack later stages of the same host, unless the later attacking species is able to use previously parasitized hosts (Briggs 1993). Briggs (1993) predictions were partially supported by the data of Bogran et al. (2002) as the earlier host-stage-attacking *E. mundus* affected the population growth rates of both *Encarsia* species. While previous field studies have contributed considerably to an understanding of the competition between primary parasitoids and autoparasitoids, their contributions regarding the dynamics of the insects involved is somehow refutable, as parasitoids were artificially released into cages without considering their phenology and the effect of metapopulation dynamics (Janssen et al. 2006).

An often overlooked, though no less common, genus of autoparasitoids is *Coccophagus* (Hymenoptera: Aphelinidae). Parasitoids of this genus have been widely used in several programs of biological control against soft scale insects (Hemiptera: Coccidae), together with primary parasitoids of the genus *Metaphycus* (Hymenoptera: Encyrtidae) (Kapranas and Tena 2015). The latter are considered the main natural enemies of soft scales because they can exploit smaller scales than can *Coccophagus* (i.e., exploitative competition). However, it is unclear whether *Coccophagus* can negatively influence the population dynamics of *Metaphycus* and consequently the population densities of soft scale insects (Kapranas and Tena 2015). Excessive hyperparasitism by *Coccophagus lycimnia* (Walker) (Hymenoptera: Aphelinidae) has been suggested to not allow *Metaphycus helvolus* (Compere) (Hymenoptera: Encyrtidae) populations to build up as the season progresses, resulting in poor control of *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae) in citrus (Bernal et al. 1998, 1999a, 2001). However, later studies have suggested that *Coccophagus* species might even be efficient biological control agents of soft scale insect pests (Schweizer et al. 2002, 2003). Similarly, an increase in *Coccophagus* parasitism on *Coccus hesperidum* L. (Hemiptera: Coccidae) did not lead to a reduction in parasitism by the *Metaphycus* species (Kapranas et al. 2007). These suggestions are based on field surveys, but researchers have never analyzed communities with different population levels of *Coccophagus* and *Metaphycus* to look for synergistic or disruptive effects on host survival, as well as the interaction between these parasitoids.

In this study, we report data on insects sampled at 28 replicated olive orchards, where several primary parasitoids of the genus *Metaphycus* and one autoparasitoid, *C. lycimnia*, coexist simultaneously, though at varying densities, at the beginning of the season. Taking the initial density of parasitoids into account can provide valuable insights into the intensity of the interaction and may reveal how it dictates the outcome of competition. We are unaware of other replicated and manipulative field studies that examine the role of interspecific competition between primary parasitoids and an autoparasitoid on their population dynamics and that of their shared host.

3.2. Material and Methods

3.2.1. Study system

Black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) is a serious citrus and olive pest in most regions of the world (Ben-Dov and Hodgson 1997). This pestiferous insect is oviparous and parthenogenetic, and it has one annual generation and, in some years, a partial second generation in the studied region (Pereira 2004). Eggs are laid at the end of the spring and in the fall if the second generation occurs. Three immature nymphal instars are recognized. The first instar, also known as the crawler, is mobile and is not attacked by parasitoids. The second and third instars are the predominant instars from September to May, and they tend to settle on the leaves of olive trees (Pereira 2004, Tena et al. 2007). On the leaves, they are attacked by a complex of parasitoids dominated by the genera *Metaphycus* and *Coccophagus*. The adult scales are present only from the end of June to July. They are attacked by the egg predator *Scutellista caerulea* (Fonscomlombe) (Hymenoptera: Pteromalidae) and *Metaphycus lounsburyi* (Howard) (Hymenoptera: Encyrtidae), but they are rarely attacked by *Coccophagus* (Pereira 2004, Tena et al. 2008a).

We have studied the interaction between the primary parasitoids of the genus *Metaphycus* and the autoparasitoid *C. lycimnia* when they parasitize the immature instars of *S. oleae*. The three *Metaphycus* species are *M. helvolus*, *M. flavus* (Howard) and *M. lounsburyi*. The first two are the main parasitoids of *S. oleae* nymphs in the Mediterranean region (Pereira 2004, Tena et al. 2008a). *M. lounsburyi* is considered specific to adult scales, but it has been occasionally recovered from immature instars and has thus been included in this study (Pereira 2004, Tena et al. 2008a, Tena and Garcia-Marí 2009). The three species are facultative gregarious endoparasitoids; they are also arrhenotokous (unfertilized eggs produce sons and fertilized eggs produce daughters) and synovigenic (females emerge with at most a small fraction of their egg complement), and *M. helvolus* and *M. flavus* exhibit destructive host-feeding (Flanders 1942, De Bach 1943, Bernal et al. 1999b, Kapranas et al. 2008, Tena et al. 2008b). See Guerrieri and Noyes (2000) for more information on related taxonomy and geographical distribution.

Coccophagus lycimnia is an autoparasitoid; males develop as obligate hyperparasitoids of both conspecific and heterospecific females, and females develop in immature scales. It is solitary and arrhenotokous, and it also host-feeds (Muegge and Lambdin 1989, Bernal et al. 2001). This species dominates the parasitoid complex of some soft scales, and its abundance has been related to the poor control exerted by the primary parasitoids of the genus *Metaphycus* (Bernal et al. 2001). However, in the Trás-os-Montes region of Portugal, this parasitoid dominates the parasitoid complex of *S. oleae* nymphs, and the scale has become a secondary pest (Pereira, per. observations).

3.2.2. Orchards and experimental design

To determine the intensity of the competition between the primary parasitoids of the genus *Metaphycus* and the autoparasitoid *C. lycimnia* and to reveal how this competition affects the natural populations of their common host *S. oleae,* we selected orchards with similar host densities but with variable parasitoid densities at the beginning of the season (September).

The 28 orchards selected were spread across the olive-growing region of Trás-os-Montes in northeast Portugal (Figure 3.1.). The characteristics of the orchards are detailed in Table 3.1 Briefly, the orchards had two soil covers: bare soil cover via herbicide application or mowed and natural ground cover. The planting patterns varied between 6 x 6 m and 10 x 10 m. The olive trees were approximately 40 to 50 years old, were pruned every two years and were not watered. The orchards were under full commercial production during the assay, and insecticides had not been sprayed.

The orchards were divided into three groups according to the initial density of the parasitoids (September): “control orchards” were those with five or fewer parasitoids collected in September; *“C. lycimnia* orchards” were those with more than five parasitoids and with a *Metaphycus-C. lycimnia* ratio lower than 0.5; and “*Metaphycus* orchards” were those with more than five parasitoids and a *Metaphycus-C. lycimnia* ratio higher than 0.5 (Table 3.1).

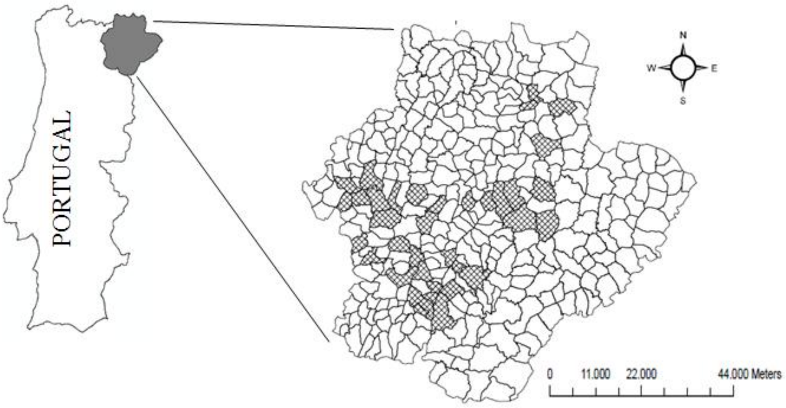


Figure 3.1. Location of the olive orchards in the region of Trás-os-Montes (Portugal)

3.2.3. Sampling protocols

The orchards were sampled over three periods (September 2011, November 2011 and May 2012). These months were selected because the first nymphal parasitoids were recovered in September and the highest parasitism rates had occurred from October to November and from April to May in previous studies (Pereira 2004). During each sampling period, two twigs (20 cm long) infested with *S. oleae* were collected every day from ten randomly selected olive trees in each olive orchard. The wigs were placed in plastic bags and transported in ice-chests to the laboratory, where they were stored at 10°C for later observation. The samples were processed in the next 48 hours.

In the laboratory, a subsample of 20 leaves (ten leaves selected randomly per twig) was collected from the two olive twigs, with a total of 200 leaves per grove. Each subsample was observed under a microscope and the following items were recorded: the number of alive and parasitized *S. oleae* specimens for each scale instar. Parasitized scales can be recognized by their yellowish or black color when they are parasitized by *Metaphycus* spp. and *Coccophagus* spp., respectively, as well as by their convex shape (Pereira 2004, Tena et al. 2008a, Tena and Garcia-Marí 2008). The length of the major axis of the second and third nymphal instars (suitable for parasitism by *Metaphycus flavus, M. helvolus* and *C. lycimnia*) was also measured to the nearest 0.01 mm. Leaves bearing a parasitized scale were individually introduced into a glass vial containing with a streak of honey on its inside wall, stoppered with a cotton plug and placed in a climatic chamber at 22°C, with a 16L: 8D photoperiod and 70% relative humidity (RH).

Table 3.1. Sampling site details: the treatment assigned the ratio *Metaphycus* spp. / *Coccophagus lycimnia* and total number of parasitoids, locality, location, tree age and variety. Treatments were assigned according to their initial parasitoid density.

Treatment	***Metaphycus/ C. lycimnia***	**Locality**	**Location**	**Age (years)**	**Ground cover management**
C. lycimnia	0.42 (12)	Argeriz	41°36'13.24"N; 7°21'36.55"W	50	mowed
C. lycimnia	0.38 (13)	Cabanelas	41°34'36.33"N; 7°13'30.11"W	40	mowed
C. lycimnia	0.50 (8)	Macedo	41°31'20.81"N; 6°55'1.95"W	40	mowed
C. lycimnia	0.17 (6)	Milhão	41°46'23.80'N'; 6°37'15.96"W	50	mowed
C. lycimnia	0.00 (11)	Sabor	41°48'49.80'N'; 6°43'37.47"W	40	mowed
C. lycimnia	0.00 (6)	Salselas	41°33'25.13"N; 6°53'5.62"W	50	mowed
C. lycimnia	0.45 (11)	Suções	41°29'29.68"N; 7°15'29.80"W	40	herbicide
C. lycimnia	0.38 (21)	Valverde	41°36'6.05"N; 7°17'23.83"W	50	herbicide
C. lycimnia	0.50 (20)	Vilas Boas	41°21'11.29"N; 7°11,46.41"W	50	mowed
Control	0.00 (0)	Alfândega	41°21'46.97"N; 6°57'46.81"W	40	mowed
Control	0.60 (5)	Cachão	41°22'20.45"N; 7°10'12.40"W	50	mowed
Control	1.00 (1)	Franco	41°26'18.81"N; 7°19'2.20"W	50	mowed
Control	1.00 (2)	Gralhós	41°13'28.81"N; 6°43,47.80"W	50	mowed
Control	0.00 (2)	Limãos	41°31’36.84"N; 6°50'12.17"W	40	mowed
Control	0.50 (2)	Morais	41°29'48.22"N; 6°45'49.88"W	50	mowed
Control	1.00 (1)	Parada	41°40’46.54"N; 6°41'17.93"W	50	mowed
Control	0.00 (0)	Pombal	41°22’3093"N; 6°59'46.23"W	50	mowed
Control	0.80 (5)	Vilariça	41°23’9.26"N; 7°2'13.94"W	50	plant cover
Control	1.00 (1)	Vinhas	41°34'25.09"N; 6°49'19.00"W	40	mowed
Metaphycus	0.67 (6)	Cedães	41°29'19.23"N; 7°7'34.26"W	40	plant cover
Metaphycus	0.67 (12)	Crasto	41°33'9.20"N; 7°19'20,53"W	50	mowed
Metaphycus	0.80 (10)	Miradeses	41°33'47.35"N; 7°16'8.92"W	50	mowed
Metaphycus	0.58 (43)	Paradela-B	41°32'35.90"N; 7°7'27.77"W	50	herbicide
Metaphycus	0.69 (29)	Paradela-C	41°33'1.03"N; 7°6'31.66"W	50	herbicide
Metaphycus	1.00 (11)	Póvoa	41°,31'56.26"N; 7°15'3.00"W	40	mowed
Metaphycus	0.86 (7)	S. Pedro-P	41°25'42.91"N; 7°12'24.12"W	50	herbicide
Metaphycus	0.79 (14)	Valbom	41°33'2.59"N; 7°8'42.79"W	50	plant cover
Metaphycus	0.64 (11)	Vimieiro	41°32'14.80"N; 7°3'36.18"W	40	mowed

Over a one-month period, the tubes were checked twice a week for parasitoid emergence. At emergence, parasitoids were identified (Guerrieri and Noyes 2000, Goulet and Huleut 1993) and sexed.

3.3. Statistical analysis

3.3.1. The impact of interspecific competition on host suppression and primary parasitoid density

On the one hand, under the null hypothesis of no disruption of host suppression, we expected that scale densities would be lower in the parasitoid treatments ("*C. lycimnia*" and "*Metaphycus*") than in the control treatment. On the other hand, disruption by the autoparasitoid would be indicated if scale densities in the *"C. lycimnia"* did not surpass the scale densities in the "*Metaphycus*" treatment. The null hypothesis that interspecific competition has no influence on parasitoid dynamics would be supported if similar dynamics of parasitoids in pairwise comparisons of the "*Metaphycus*" and "*C. lycimnia*" treatments were observed. Evidence of the autoparasitoid's influence on the primary parasitoids would appear either as differences in the growth trajectory of *Metaphycus* populations in both parasitoid treatments or the extinction of *Metaphycus* in the *"C. lycimnia*" treatment. By contrast, evidence of exploitative competition between the primary parasitoids and the autoparasitoid would appear either as differences in the growth trajectory of *C. lycimnia* populations in both parasitoid treatments or the extinction of *C. lycimnia* in the "*Metaphycus*" treatment. We compared the number of scales and parasitoids among treatments using ANOVAs. If significant differences were found, we compared the means among the treatments using a Bonferroni test at the 5% significance level. Diagnostic plots were used to analyze the models for normality and homoscedasticity.

3.3.2. Interspecific competition: host-size utilization and the secondary sex ratio

We first compared the size of the *S. oleae* nymphs suitable for parasitism (2nd and 3rd instar) in the orchards with different proportions of parasitoids (treatments). The orchards and dates with fewer than three scales suitable for parasitism were removed from this comparison and subsequent analysis. We then examined shifts in the parasitoids' host use when in the presence of different relative densities of the other parasitoid. If *Metaphycus* parasitoids can use smaller hosts when *C. lycimnia* densities are higher, then we would expect the use of smaller hosts by *Metaphycus* more in the "*C. lycimnia*" treatment than in the "*Metaphycus*" treatment. Our second hypothesis is that if *C. lycimnia* prefers to use heterospecific hosts (*Metaphycus* females) as secondary hosts to produce males, then we expect a higher sex ratio of *C. lycimnia* in the "*Metaphycus"* treatment than in the *"C. lycimnia"* treatment.

We compared the size of the scales among treatments using ANOVAs. If significant differences were found, we compared the means among the treatments using a Bonferroni test at the 5% significance level. Diagnostic plots were used to analyze the models for normality and homoscedasticity. We also used generalized linear modeling techniques, assuming binomial error variance (corrected for under-dispersion), to compare the secondary sex ratio of the parasitoids. We present the means of the untransformed proportion (preferred to less intuitive statistics, such as the back-transformed means of logit transformed data). All the analyses were performed using the R statistical software (The R Core Team 2011) and the package lattice (Sarkar 2008).

3.4. Results

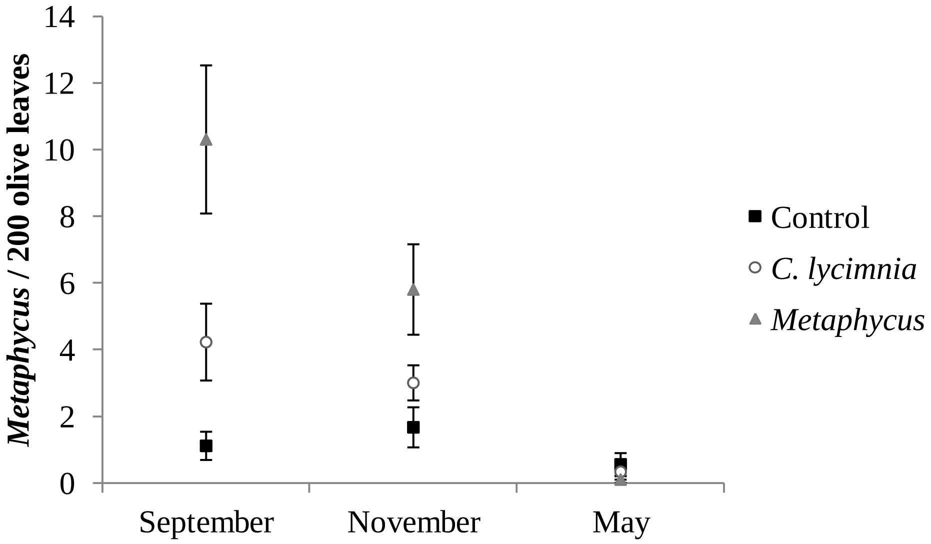
3.4.1 Parasitoids dynamics

Metaphycus and *C. lycimnia* densities followed opposite patterns (Figure 3.2). *Metaphycus* densities decreased in the "*Metaphycus"* and "*C. lycimnia"* treatments and remained low in the control treatment (Figure 3.2a). In September, the mean number of *Metaphycus* was significantly higher in the "*Metaphycus"* treatment than in the "*C. lycimnia"* and control treatments (F = 9.46; df = 2, 25; P = 0.0008). In November, the mean number of *Metaphycus* decreased in the "*Metaphycus"* treatment, and there were only significant differences between the "*Metaphycus"* and control treatments (F = 5.04; df = 2, 25; P = 0.015). At the end of the scale life cycle (May), the number of *Metaphycus* was almost null in the three treatments, and there were no significant differences among the treatments (F = 0.98; df = 2, 25; P = 0.4).

In September, the mean number of *C. lycimnia* was significantly higher in the *"C. lycimnia"* and "*Metaphycus"* treatments than in the control treatment (F = 7.03; df

= 2, 25; *P* = 0.0038) (Figure 3.2b). In November, the mean number of *C. lycimnia* increased slightly in the control treatment, and there were not significant differences among the treatments (*F* = 0.16; df = 2, 25; *P* = 0.85). At the end of the scale life cycle (May), the number of *C. lycimnia* was more than five times higher than in November in the three treatments, and it was significantly higher in the control treatment than in the *"C. lycimnia"* and *"Metaphycus"* treatments (*F* = 5.47; df = 2, 25; *P* = 0.01).

a)



b)

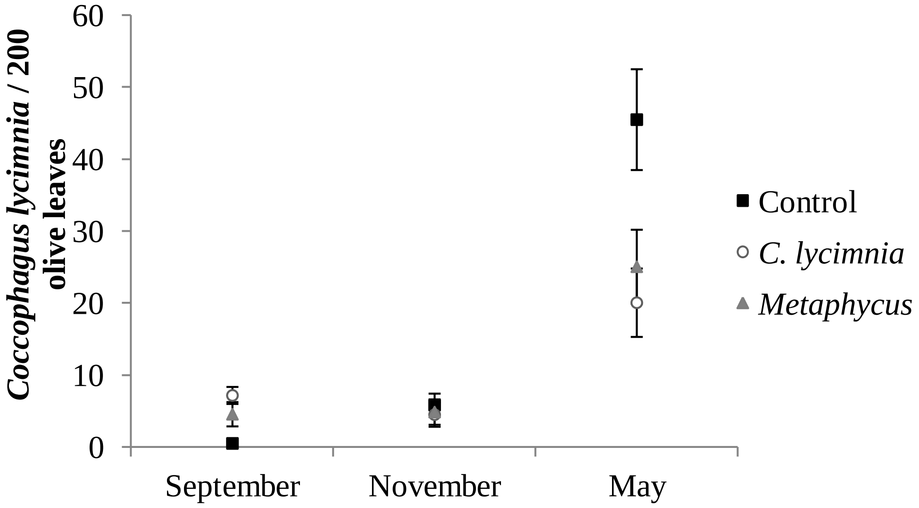


Figure 3.2. Dynamics of *Coccophagus lycimnia* and *Metaphycus* spp. in orchards with different proportions of parasitoids at the beginning of the season (September): a) mean number ± SE of *C. lycimnia* and b) mean number ± SE of *Metaphycus*.

3.4.2. Shifts in host-size use

The size of immature scales available for parasitism was similar in the three treatments throughout the life cycle of the scale (September: $F = 0.31$; df = 2, 24; $P = 0.74$; November: $F = 0.054$; df = 2, 24; $P = 0.95$; May: $F = 1.38$; df = 2, 20; $P = 0.27$) (Figure 3.3a).

Comparisons of host-size use between *Metaphycus* parasitoids and *C. lycimnia* were performed for each sampling date and also considered treatment as a factor (Figure 3.3b). In September, *Metaphycus* parasitoids used scales that were significantly smaller than did *C. lycimnia* ($F = 16.66$; df = 1, 59; $P < 0.0001$), irrespective of the treatment ($F = 1.47$; df = 2, 19; $P = 0.26$), and there was not significant interaction between the parasitoid and the treatment ($F = 0.48$; df = 2, 59; $P = 0.62$) (Figure 3.3a,b). The same occurred in November; *Metaphycus* parasitoids used scales significantly smaller than did *C. lycimnia* ($F = 24.14$; df = 1, 155; $P < 0.0001$), irrespective of the treatment ($F = 0.38$; df = 2, 24; $P = 0.69$), and there was not significant interaction between the parasitoid and the treatment ($F = 0.44$; df = 2, 155; $P = 0.65$). The low number of *Metaphycus* parasitoids recovered in May did not allow us to analyze host size use.

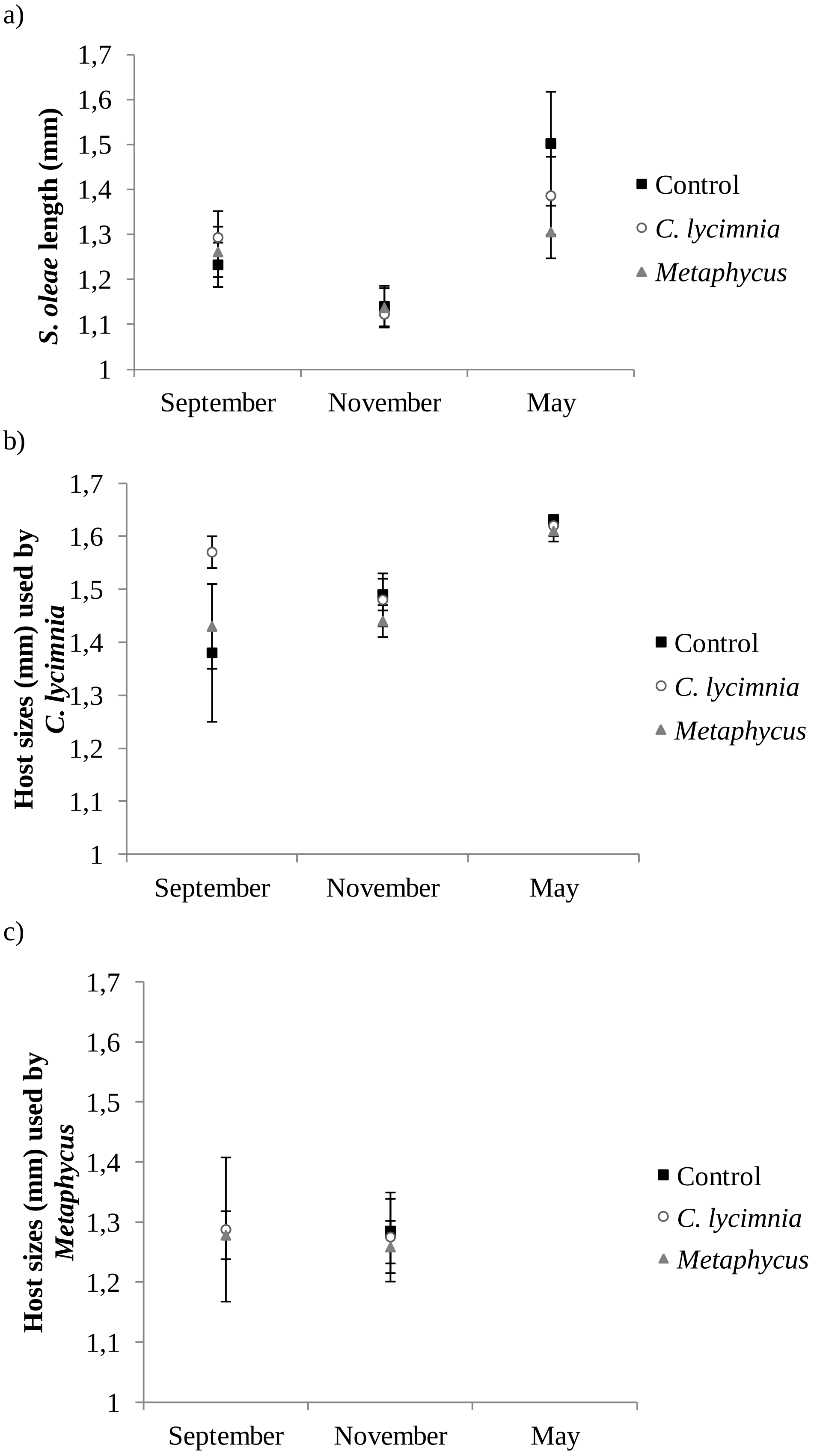


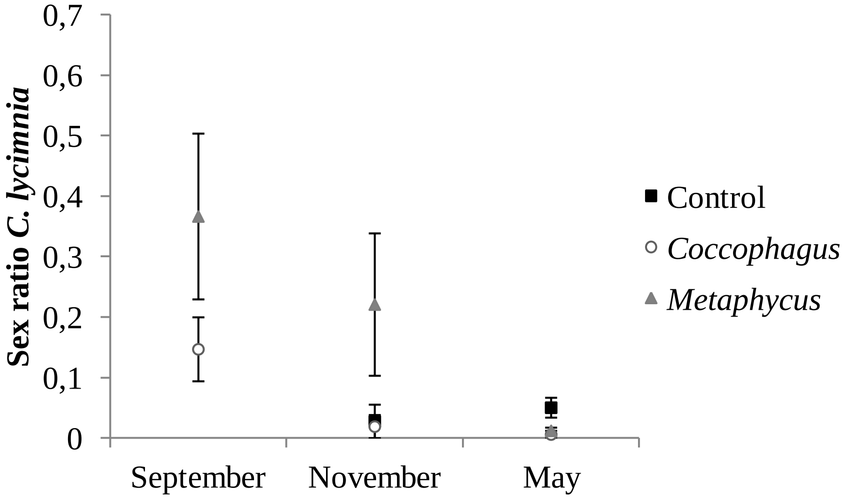
Figure 3.3. Mean size (length in mm) ± SE of immature *Saissetia oleae*: a) suitable for parasitism; b) parasitized by *Coccophagus lycimnia*; and c) *Metaphycus* spp. in orchards with different proportions of parasitoids at the beginning of the season (September).

3.4.3. Shifts in sex ratio

The mean secondary sex ratios of *Metaphycus* parasitoids (0.28 ± 0.03) and *C. lycimnia* (0.09 ± 0.016) were female biased.

Comparisons of the secondary sex ratio between *Metaphycus* parasitoids and *C. lycimnia* were performed for each sampling date and also considered treatment as factor (Figure 3.4a,b). In September, the secondary sex ratios of *Metaphycus* and *C. lycimnia* were similar (*F* = 1.1; df = 1, 27; *P* = 0.3), irrespective of the treatment (*F* = 2.33; df = 1, 28; *P* = 0.14), and there was not significant interaction between the parasitoid and the treatment (*F* = 3.71; df = 1, 26; *P* = 0.065). However, in November, the secondary sex ratio of *Metaphycus* was significantly higher than that of *C. lycimnia* (*F* = 14.47; df = 1, 26; *P* = 0.0009), irrespective of the treatment (*F* = 2.96; df = 1, 27; *P* = 0.07), and there was significant interaction between the parasitoid and the treatment (*F* = 6.75; df = 1, 24; *P* = 0.0047). This interaction indicates that the secondary sex ratio of *C. lycimnia*, though not that of *Metaphycus*, differed among treatments. In fact, when we separately analyzed the sex ratios of both parasitoids, the *Metaphycus* sex ratio was independent of the treatment in September (*F* = 0.0062; df = 2, 14; *P* = 0.94) and November (*F* = 1.11; df = 2, 12; *P* = 0.36) (Figure 3.4a), whereas a significantly higher proportion of *C. lycimnia* males emerged in the "*Metaphycus*" treatment compared with the "*C. lycimnia"* treatment in September (*F* = 5.42; df = 2, 12; *P* = 0.038) and November (*F* = 5.34; df = 2, 12; *P* = 0.022) (Figure 3.4b).

a)



b)

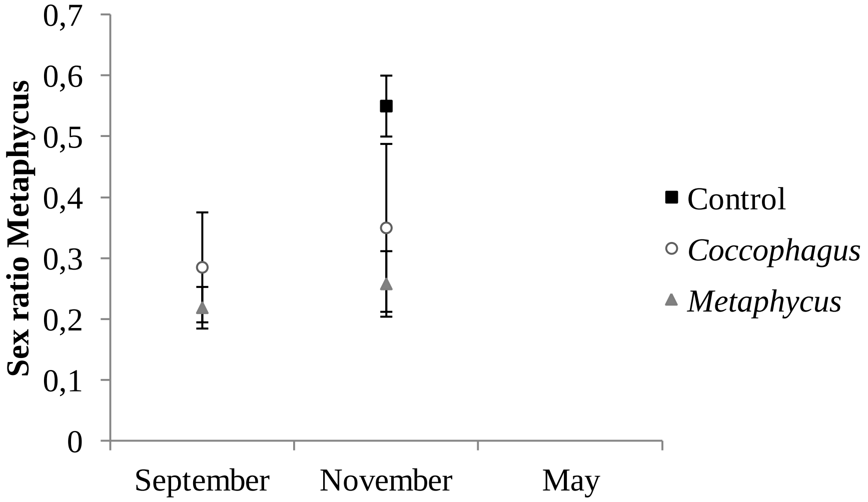


Figure 3.4. Mean size (length in mm) ± SE of immature *Saissetia oleae*: a) suitable for parasitism; b) parasitized by *Coccophagus lycimnia*; and c) *Metaphycus* spp. in orchards with different proportions of parasitoids at the beginning of the season (September).

3.4.4. The effect of competition on host suppression

At the beginning of the assay (September), the mean number of nymphs suitable for parasitism did not differ among orchards with different parasitoid densities (treatments) ($F = 0.29$; df = 2, 25; $P = 0.75$) (Figure 3.5a). At the end of the assay, the three initial parasitoid densities were successful in reducing the number of nymphs, and there were not significant differences in November ($F = 0.6$; df = 2, 25; $P = 0.56$) or in May ($F = 0.69$; df = 2, 25; $P = 0.51$).

Similarly, the mean number of scales (nymphs and adults) present in the orchards did not differ among treatments throughout the scale life cycle, and it was five times lower in May than in September (September: *F* = 1.99; df = 2, 25; *P* = 0.16; November: *F* = 0.61; df = 2, 25; *P* = 0.55; May: *F* = 0.51; df = 2, 25; *P* = 0.61) (Figure 3.5b).

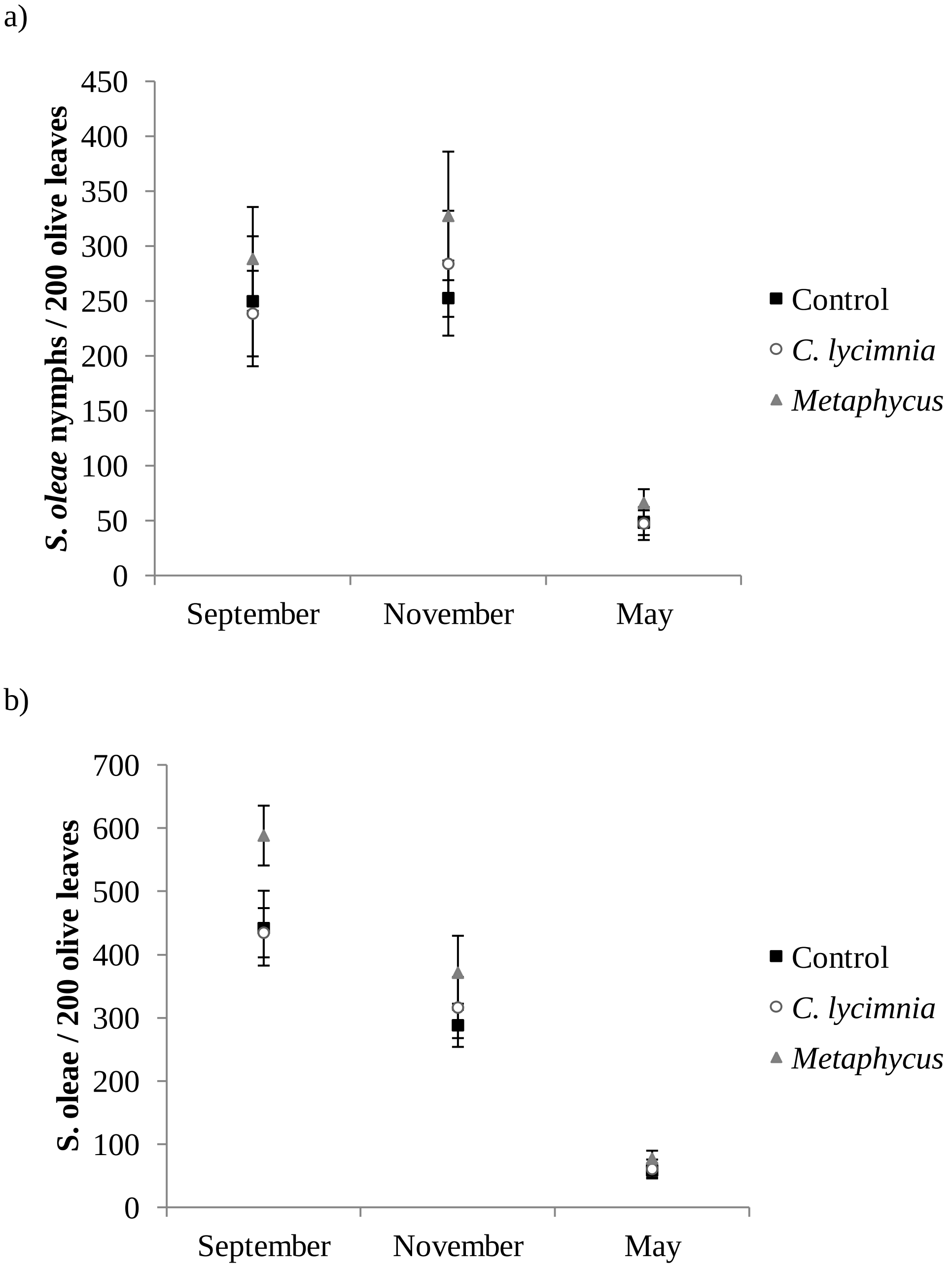


Figure 3.5. Mean size (length in mm) ± SE of immature *Saissetia oleae*: a) suitable for parasitism; b) parasitized by *Coccophagus lycimnia*; and c) *Metaphycus* spp. in orchards with different proportions of parasitoids at the beginning of the season (September).

3.5. Discussion

Our study reports a case of competition between primary parasitoids and an autoparasitoid that attack their common host under field conditions and the effect of this competition on host suppression. Our results reveal, for the first time, that an autoparasitoid that is an inferior exploitative competitor can outcompete the primary parasitoid without disrupting the suppression of their common host.

C. lycimnia outcompeted parasitoids of the genus *Metaphycus* when they parasitized *S. oleae* nymphs, irrespective of their relative abundance at the beginning of the scale cycle. Our assay was primarily designed to detect the effects of competition on the population densities of both parasitoids and that of their common host rather than to determine the mechanism of competition. However, our results also suggest that *C. lycimnia* preferred to use *Metaphycus* females as secondary hosts to produce males instead of using their own females. We found a higher proportion of *C. lycimnia* males in orchards with higher proportions of *Metaphycus* than in those with higher proportions of *C. lycimnia* in September and November. This ovipositional preference provided *C. lycimnia* with a substantial competitive advantage over *Metaphycus* parasitoids, which could explain, at least partially, the descendent trajectory of *Metaphycus* populations at the beginning of season when host numbers were very high (30- to 100-fold higher than parasitoid numbers). Zang et al. (2011) have recently found that *Encarsia sophia*, an autoparasitoid of whiteflies, also prefers to use heterospecific hosts as secondary hosts to produce males in a choice test in the lab. This preference could be due to, among other traits, the higher fitness of males developed on heterospecific females compared with those developed on conspecific females (Zang et al. 2011). Bernal et al. (2001) also encountered a positive relationship between the numbers of *Metaphycus* and the proportion of *C. lycimnia* males in field when parasitizing another soft scale, *C. pseudomagnoliarum*. However, in this study, the authors suggested that the high population densities of *C. lycimnia* might reduce the density of *Metaphycus* spp. and explain the negligible level of citricola scale biological control that occurs in the San Joaquin Valley in California (Bernal et al. 2001).

Metaphycus spp. are considered superior competitors and more effective biological control agents compared with *C. lycimnia* because they use smaller hosts (Bernal et al. 2001, Kapranas et al. 2007, Tena and Garcia-Marí 2008, herein) and can thus outcompete *C. lycimnia*. The exploitation of smaller hosts by parasitoids of scale insects (including armored scales, soft scales and mealybugs) and whiteflies is a widely documented mechanism that explains the superiority and, at times, the displacement of competitor parasitoids that need larger hosts to develop (Luck and Podoler 1985, Beltrà et al. 2013a, b). In our study, *Metaphycus* populations also used smaller hosts than *C. lycimnia*, but they did not exploit this enemy-free space to outcompete *C. lycimnia* or even to maintain their populations. *Metaphycus* populations declined and became negligible in May. *M. helvolus* and *M. flavus* generally reach their highest levels in the spring in other citrus and olive tree areas (Tena et al. 2008). Recently, Pekas et al. (2016) have demonstrated that the primary parasitoids *Aphytis melinus* DeBach and *A. chrysomphali* (Hymenoptera: Aphelinidae) can coexist when they parasitize the armored scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) because the weaker competitor (*A. chrysomphali*) can survive on smaller hosts, which provides it with an enemy-free space when the densities of the superior competitor are high. However, in our study, we could not observe this mechanism of coexistence, and *Metaphycus* did not use smaller hosts in orchards with higher proportions of *C. lycimnia* (if it occurred, significant interaction between the treatment and the parasitoid was observed, as shown in Figure 3.3). Similarly, Bográn et al. (2002) did not find that the primary parasitoid *E. mundus* used smaller hosts in the presence of the autoparasitoid *E. pergandiella* compared with when it was released alone. This lack of host-size use plasticity may also explain why *Metaphycus* spp. populations did not recover in May.

Despite the asymmetrical competition and *C. lycimnia*'s displacement of the primary parasitoids of the genus *Metaphycus*, the former suppressed scale densities, irrespective of their relative abundance at the beginning of the season. We designed a field experiment with similar host densities but different relative and absolute parasitoid densities, including orchards with very low numbers of parasitoids (control), orchards with a high proportion of *Metaphycus* and orchards with a high proportion of *C. lycimnia*, in an olive area where the scales cause damages occasionally, and the three species of parasitoids were found at similar levels in a three-year study conducted more than ten years ago (Pereira 2004). With this background and a large field assay including three parasitoid densities, we expected to obtain more complex and diverse data. However, our data clearly show that *C. lycimnia* reduced the population of soft scales, irrespective of the density of the primary parasitoids of the genus *Metaphycus*.

The theory predicts that pest suppression will not be disrupted when the primary parasitoid and the autoparasitoids are equally effective at suppressing the pest (Briggs and Collier 2001). Therefore, our results suggest that although *C. lycimnia* uses larger hosts than parasitoids of the genus *Metaphycus,* it is at least as efficient as these primary parasitoids at suppressing *S. oleae* in olives. This autoparasitoid appeared in our second sample in November, even in the orchards where we had not recovered any parasitoids in the first sample, and its population increased during the winter. In May, *C. lycimnia* and host populations had almost reached a 1:2 ratio, showing *C. lycimnia*'s high efficacy as biocontrol agent.

There are two other detailed studies that have previously addressed the effect of competition between primary parasitoids and autoparasitoids on their common host under particular field conditions (Hunter et al. 2002, Bográn et al. 2002). Our work differs from previous research because of our use of natural populations in their natural environments. The previous studies on this topic are based on the use of caged plants and the subsequent release of parasitoids. This methodology permits the comparison of host population densities when absent or released alone or when in competition to determine the related effects. However, this inoculative methodology does not consider the potential different arrival times of parasitoids within the season or their possible emergence from other patches (metapopulation dynamics). The arrival and attack of different species might differ according to the phenology of the hosts and their climatic necessities, as well as other factors, such as alternative hosts, surrounding vegetation, and margins (Snyder et al. 2005). In fact, metapopulation dynamics are tremendously importance in ecology and may account for the persistence of biological control systems for which we otherwise have no explanation (Murdoch et al. 1985), as might be the case in the present study.

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CHAPTER 4

Effect of ant exclusion on biological control of the black scale, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), in Mediterranean olive trees

Marrão R., Tena A., Pereira J.A., *in preparation*. Effect of ant exclusion on biological control of the black scale, *Saissetia oleae* (Hemiptera: Coccidae), in Mediterranean olive trees. *To be submitted*

Abstract: Black scale, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), is a common pest of citrus and olive trees where it is generally tended by ants. Here, we studied the ant species that tend *S. oleae* and carried out an ant-exclusion assay in an olive orchard to test whether ants: i) induce population increases of *S. oleae* and ii) modify its parasitoid complex; and iii) whether these changes differ between seasons (fall and spring). The native ant *Crematogaster auberti* (Emery) was the most abundant ant species and the exclusion system succesfully controled. *Crematogaster auberti* was less active in fall, the beginning of *S. oleae* life cycle, than in spring, the end of *S. oleae* life cycle. When compared with other ant species, *C. auberti* was much less active than others that tend *S. oleae* in other crops. Ant exclusion did not affect *S. oleae* density throughout fall and spring, as the scale followed the same pattern in ant-allowed and ant-excluded trees. Similarly, the number of parasitoids and the parasitoid complex was similar in both treatments and it was dominated by *Coccophagus* (Hymenoptera: Aphelinidae) parasitoids. However, at the end of spring, the number of parasitoids recovered from *S. oleae* adults was higher in ant-excluded trees than in trees with ants. During this period *Metaphycus lounsburyi* (Howard) (Hymenoptera: Encyrtidae) was the main parasitoid. Therefore, ants can affect the biological control of *S. oleae* at the end of its life cycle.

Key- words: biological control, integrated pest management, *Crematogaster auberti*, *Metaphycus*, *Coccophagus*.

4.1. Introduction

Black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) is a cosmopolitan pest of olive trees (*Oleae europaea* L.) and citrus (Citrus spp.) (Bodenheimer 1951, Ben-Dov and Hodgson 1997, Passos de Carvalho et al. 2003). In olive trees, this soft scale settles, remains immobile and feeds mostly on leaves during all its nymphal instars. As adult, it moves to twigs where lays the eggs (Pereira 2004). The main damage caused by *S. oleae* is due to the large amount of honeydew excreted and the subsequent development of sooty mould fungi (Bodenheimer 1951, Ben-Dov and Hodgson 1997).

Honeydew is a sugar-rich fluid excreted by plant feeder’s mostly hemipteran species after feeding on phloem sap. A part from the development of sooty mould fungi, honeydew is the principal carbohydrate source for ants in agricultural ecosystems (Hölldobler and Wilson 1990). Most ant species are omnivorous and obtain protein from animal matter and carbohydrates from plant products such as floral and extra floral nectar, food bodies, plant sap, and above all honeydew (Way 1963, Carroll and Janzen 1973, Tobin 1994). In fact, honeydew is crucial for ant colony growth (Hölldobler and Wilson 1990).

Honeydew-producers also benefit from ant-attendance in terms of protection from their natural enemies, higher growth rates, improved hygiene conditions, transport and dispersal (Way 1963, Buckley 1987, Stadler and Dixon 2005). It has long been known that ants, because of their protection, are associated with the disruption of biological control of arthropod pest species in agro-ecosystems (DeBach 1951, Bartlett 1961, Way 1963, Buckley 1987, Pekas et al. 2010, Dao et al. 2013, Tena et al. 2013b, Calabuig et al. 2014 and 2015). In the case of *S. oleae,* it is well-known that the scale is tended by ants in citrus and olives (Pereira 2004, Pekas et al. 2011, Tena et al. 2013b) and their presence can affect its parasitoid complex in California (Barzman and Daane 2001). However, little is known about the effect of ants on parasitoids and predators’ activity in the Mediterranean Basin. Recently, it has been demonstrated that ants affect more negatively the abundance of generalist predators than that of parasitoids and the impact on these predators, but not that of parasitoids, might explain the higher pest densities associated with ants in citrus (Calabuig et al. 2014 and 2015a).

This aspect is particularly important in olive groves from the Transmontana region (Portugal) where ants are the main constituents of their entomofauna (Cabanas et al. 1999). Ants ascend to tree canopies from April to November, when parasitoids and predators are also active (Pereira et al. 2002). The most abundant and widely distributed ant species in olives from Transmontana region are: *Crematogaster scutellaris* Olivier (Hymenoptera: Formicidae: Mymicinae), *Tapinoma nigerrimum* Nylander (Hymenoptera: Formicidae: Dolichoderinae), *Camponotus piceus* Leach (Hymenoptera: Formicidae: Vespoidae) (Pereira et al. 2002 and 2003), *Crematogaster auberti* Emery (Hymenoptera: Formicidae: Mymicinae) (Pereira et al. 2003, Silva 2012), but there are other species that are less abundant as: *Plagiolepis pygmaea* Laheille (Hymenoptera: Formicidae: Formicinae) (Pereira et al. 2002), *Cataglyphis hispanica* Emery (Hymenoptera: Formicidae: Formicinae), *Camponotus foreli* Emery (Pereira et al. 2003). All them feed on honeydew excreted by *S. oleae* (Pereira et al. 2002) and their abundance varies throughout the season and among trees within olive orchards (Pereira et al. 2003). *T. nigerrimum* is an extremely aggressive species and it protects *S. oleae* from parasitoids (Morris et al. 1999 and 2002, Panis 1981).

Regarding the natural enemies of *S. oleae* in olive trees in Portugal, three primary parasitoids of the family Encyrtidae: *Metaphycus lounsburyi* (Howard), *M. helvolus* (Compare) and *M. flavus* (Howard); and two facultative autoparasitoids of the family aphilinidae: *Coccophagus lycimnia* (Walker) and *C. semicircularis* (Forster) are the main parasitoids (Pereira 2004, Marrão et al. in pep). *M. lounsburyi* is a gregarious endoparasitoid that attacks 3rd instar nymph, mature, and ovipositing females (Tena and Garcia-Marí 2009). The other species attack 2nd and 3rd instar nymphs (Passos de Carvalho et al. 2003). Males of *C. lycimnia* and *C. semicircularis* are hyperparasitoids of females of the same species or of primary parasitoids, including species of the genus *Metaphycus* (Bernal et al. 2001, Kapranas and Tena 2015). Finally, most *Metaphycus* feed on coccidae fluids (i.e. host-feeding) (Kapranas and Tena 2015). The main predators of *S. oleae* identified in the region of Transmontana (Portugal) belong to orders Coleoptera and Neuroptera (Passos de Carvalho et al. 2003). The former is mainly represented by the Coccinellidae family, with the most important species being *Scymnus* sp., *Rhyzobius chrysomeloides* Herbst, *R. lophanthae* Blaisdell, *Chilocorus bipustulatus* L., *Exochomus nigromaculatus* Goeze, *E. quadripustulatus* L. and *Nephus bisignatus* Boheman (Passos de Carvalho et al. 2003, Gonçalves et al. 2005, Santos et al. 2005, Santos et al. 2008). Among neuropterans, *Crysoperla carnea* Stephens is the most abundant and it preys on the first instar (Passos de carvalho et al. 2003). According to Santos et al. (2009) the second and third instar are usually predated by the coccinellidae. Predation is an important factor in mortality, however it is difficult to assess in the field (Piñol et al. 2012).

In the present study, we conducted an ant-exclusion experiment in the field in order to determine the impact of ants on *S. oleae* and its parasitoid complex. Concretely we asked whether ants: (i) induce population increases of *S. oleae* in olives trees; (ii) modify its parasitoid complex and (iii) their impact on the scale; and, finally, (iv) whether these changes differ between seasons (spring and fall). This knowledge will allow untangling the effect of ants on biological control of *S. oleae* and improving its control in olive trees.

4.2. Material and methods

4.2.1. Olive orchard

The study was conducted in an olive orchard located in Cedães (41 ° 29'16.86 "N, 7 ° 7'34.02" W) in the region of Mirandela in northeastern Portugal. It was about 25 years old and had 30 hectares. The olive trees, spaced 7 × 7 m^2 apart and the cultivar was Cobrançosa. Trees were pruned every two years in order to maintain the balance between vegetative and reproductive functions and to exert a regenerative effect on olive trees (Lopes et al. 2009). The olive orchard has been managed under integrated management since 2003 and is dedicated to the production of olive oil. No sprays were applied against olive pests or diseases during the assay. The management practices of the cover crop were limited to the cut of the spontaneous vegetation in July. During spring, the main spontaneous plant species in the cover crop belonged to the Asteraceae family: *Calendula arvensis* L.; *Coleostephus myconis* (L.) Rchb. F.; *Andryala integrifolia* L.; *Chamaemelum mixtum* (L.) All. *Crysanthemum segetum* L.; *Crepis vesicaria* subsp. *taraxaciholia* (Thuill.) Thell.; *Leontodon taraxacoides* subsp. *longirostris* Finch & P. Sell. During fall the main spontaneous species were: *Foeniculum vulgare* L. (Apiaceae) and *Chondrilla juncea* L. (Asteraceae). Olive trees were not watered.

4.2.2. Infestation of young olive trees

112 and 56 olive trees of 1m high and “cobrançosa” variety were infested in fall, the beginning of *S. oleae* life cycle, and spring, the end of *S. oleae* life cycle. Trees were infested under controlled conditions in a greenhouse of the Agricultural School of Bragança (Portugal) with *S. oleae* eggs obtained from infested orchards of the region. The infested olive trees remained in the greenhouse (T^a = 25 ± 2°C; RH = 80 ± 10%; natural photoperiod) and were watered once per week until scales reached the second instar (60-70 days approximately). The number of scales per tree was counted before transporting them to the orchard on September 15 (hereinafter fall season) and March 24 (hereinafter spring season).

4.2.3. Experimental design

The experimental design was a randomized block with 14 replicates in fall and seven replicates in spring of two treatments: ant-allowed and ant-excluded. Each block had 16 trees (4 rows by 4 trees) but only the four central trees of each block were used for sampling. A young infested tree within its pot was placed in the middle of each tree, just were the trunk divides in the main branches. In the upper part of the pot, there were two holes to place a thread to fix the pot to the olive tree.

For ant-exclusion, the tree trunk was wrapped with gaffer tape demarcating a zone 15-20 cm wide at 50 cm above ground (Pekas et al. 2010, Juan-Blasco et al. 2011). The wrapped area was coated with special glue for insect traps (Tranglefood, Biagro, Valencia, Spain). All the spontaneous vegetation that enveloped the tree trunk was cut and removed to avoid alternative forms of ants reaching the olive tree canopy to ensure that the only access of ants to the tree was its trunk. The glue was renewed monthly. Each block was away from the adjacent block by six rows of buffer trees.

4.2.4. Ant activity and species composition

Ant activity was measured weekly. For this, we followed the same procedure than Pekas et al. (2011). This is the number of ants moving up and down an imaginary horizontal line on the tree trunk during a 2-min period. The effectiveness of the ant-exclusion method was monitored weekly during the assays by comparing ant activity between the ant-allowed and ant-excluded treatments. Observations were made between

1000 and 1200 hours in all trees. Ants were identified visually and in case of doubt they were aspirated and transported to the laboratory for identification using the identification keys available on the website: http://ww.hormigas.org (accessed, 2015).

The number of scales per infested tree was recorded at the beginning of each season (September 15 for the fall season and March 24 for the spring season) and then monthly. For this, infested olive trees on the pots were transported to the laboratory. There, we recorded the number of alive, parasitized and dead scales, as well as their instar following the same methodology than Tena et al. (2008).

Parasitized scales were placed individually in Petri dishes (Ø 60 mm) and closed with parafilm until parasitoid emergence. Each parasitoid was identified and sexed. Subsequently, parasitism rates were calculated for each instar and date.

4.2.5. Statistical analysis

Ant activity in ant-excluded and ant-allowed trees throughout the seasons was compared used an ANOVA with repeated measures (Pekas et al. 2010). *Saissetia oleae* density and parasitism rates in ant-excluded and ant-allowed trees were compared using an ANOVA for each sampling date. Parasitism rates were arcsine transformed previous analysis. The effect of ant activity on the reduction of *S. oleae* density and on the maximum parasitism was analyzed using ANCOVAs with man ant activity throughout the season as a quantitative variable and treatment (ant allowed *vs* ant excluded) as a qualitative variable. Parasitism rates were arcsine transformed previous analysis. Data were evaluated for normality and homogeneity of variances with Kolmogorov-Siminov and Levene test, respectively. Finally, the number of parasitoids recovered was not normally distributed and were analyzed using Kruskal-Wallis tests.

4.3. Results

4.3.1. Ant exclusion and ant species

Sticky barriers excluded ants from canopies both seasons (Figure 4.1). In fall, at the beginning of *S. oleae* life cycle, ant activity remained constant and low in ant-excluded plots throughout the season. Ants were almost absent from the canopies of the ant-excluded plots (ANOVA repeated-measures: F = 410.15; df = 1, 447; p < 0.001).

The four ant species were present in fall and *C. auberti* was the most abundant (92.4 %) and widely distributed ant species (present in 98.3% of the trees) in ant-allowed trees. The other three species *T. nigerrimum*, *P. pygmaea* and *C. scutellaris* were much less abundant (Table 4.1). Among the ants that could overcome the exclusion system, *C. auberti* was the most abundant species (Table 4.1; ant-excluded trees).

In spring, at the end of *S. oleae* life cycle, the number of ants ascending and descending to the canopies (hereinafter ant activity) without barriers remained low until May when it increased and peaked at the end of the assay at the beginning of June. Again, ants were almost absent from the canopies of the ant-excluded plots (ANOVA repeated-measures: $F = 295.13$; df = 1, 223; $P < 0.001$). *C. auberti* was the most abundant (58.8 %) and widely distributed ant species (present in 75.0% of the trees) within the orchard in ant-allowed trees, followed by *T. nigerrimum* (25.2% ants; 64.3% trees), *P. pygmaea* (12.7% ants; 48.0% trees) and *C. scutellaris* (3.3% ants; 35.7%) (Table 4.1). Among the ants that could overcome the exclusion system, *T. nigerrimum* was the most abundant species (Table 4.1; ant-excluded trees).

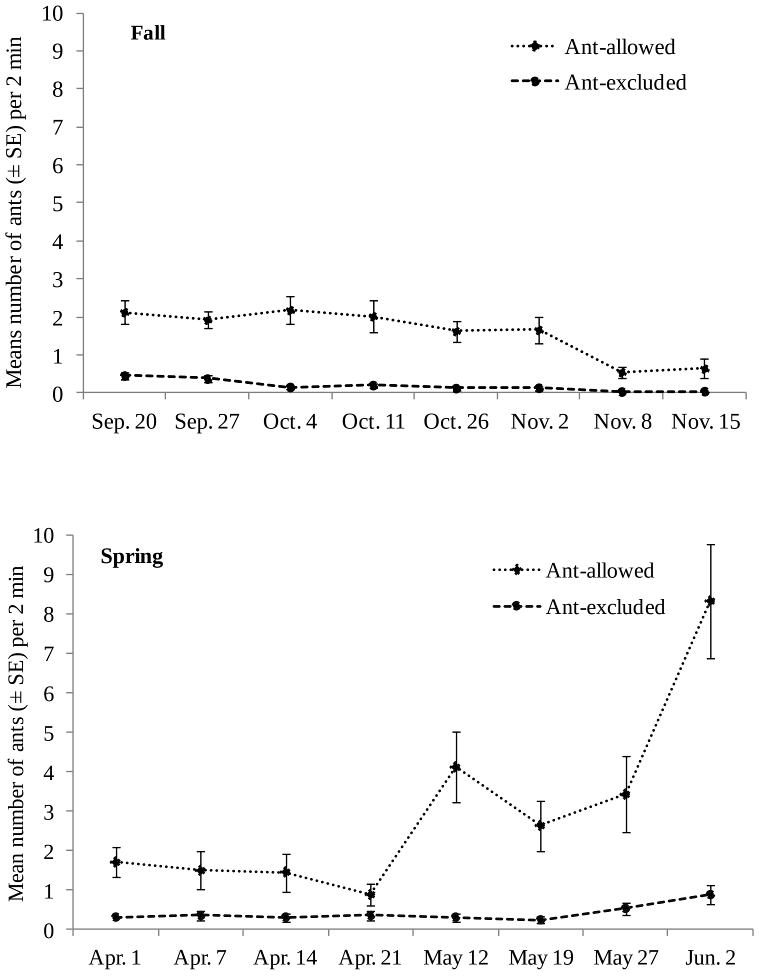

Figure 4.1 Ant activity (mean ±SE), measured as the number of ants ascending or descending the trunk, in ant-allowed ant-excluded olive trees during two seasons (fall and spring) in Northern Portugal

Table 4.1. Mean number of ants per tree, their relative abundance and distribution in ant-excluded and ant-allowed olive trees during two seasons in Northern Portugal.

Season	Subfamily	Species	Ant-allowed			Ant-excluded		
			Ant per tree (Mean ± SE)	Ant-relative abundance (%)	Ant distribution (% trees)	Ant per tree (Mean ± SE)	Ant-relative abundance (%)	Ant distribution (% trees)
Fall	Dolicochorinae	*Tapinoma nigerrimum*	0.04±0.04	2.2	16.1	0.25±0.04	48.9	67.9
	Formicinae	*Plagiolepis pygmaea*	0.08±0.05	4.9	5.4	0.03±0.01	7.5	17.9
	Mymicinae	*Crematogaster auberti*	1.47±0.31	92.4	98.3	0.13±0.03	31.9	57.1
		Crematogaster scutellaris	0.01±0.01	0.4	33.9	0.05±0.01	11.7	32.1
Spring	Dolicochorinae	*Tapinoma nigerrimum*	0.75±0.14	25.2	64.3	0.004±0.01	2.1	3.6
	Formicinae	*Plagiolepis pygmaea*	0.38±0.07	12.7	48.0	0.02±0.02	5.5	1.8
	Mymicinae	*Crematogaster auberti*	1.75±0.33	58.8	75.0	0.17±0.06	81.9	62.5
		Crematogaster scutellaris	0.10±0.02	3.3	35.7	0.002±0.01	7.5	12.5

4.3.2. Effect of ant exclusion on *Saissetia oleae* density

At the beginning of the assays, *S. oleae* density was similar in ant-allowed and ant-excluded trees in fall (*F* = 0.07; df = 1, 111; *P* = 0.79) and spring (*F* = 0.38; df = 1, 55; *P* = 0.55) (Figure 4.2).

After one month in the field in fall, there were not significant differences in the number of alive scales (*F* = 0.06; df = 1, 111; *P* = 0.81) (Figure 4.2). During this period, *S. oleae* density decreased 14.9% in ant-allowed trees and 11.5% in ant-excluded. There were only nymphs in both treatments (100% nymphs in ant-allowed; 100% nymphs in ant-excluded). After two months, *S. oleae* density decreased 29% in ant-allowed trees and 26.6% in ant-excluded. Again, there were not significant differences in the number of alive scales (*F* = 0.02; df = 1, 111; *P* = 0.88) (Figure 4.2). At the end of the assay, *S. oleae* population was represented mostly by nymphs (99.1% nymphs in ant-allowed; 99.0% nymphs in ant-excluded). When we analyzed the mean activity of ants and the reduction of *S. oleae* density in each tree (Figure 4.3a), mean ant activity throughout the assay was not correlated with the reduction of *S. oleae* density (*F* = -131.58, *P* = 0.19), independently of the treatment (*F* = -134.88, *P* = 0.19). The interaction between ant activity and treatment was not significant (*F* = -0.92, *P* = 0.36) (Figure 4.3a).

After one month in the field in spring, there were not significant differences in the number of alive scales (*F* = 0.71; df = 1, 55; *P* = 0.4) (Figure 4.2). During this period, *S. oleae* density decreased 30% in ant-allowed trees and 45.5% in ant-excluded. Most of the scales were in the nymphal stage in both treatments (55.7% nymphs in ant-allowed; 64.5% nymphs in ant-excluded). After two months, the number of alive scales were again similar (*F* = 1.79; df = 1, 55; *P* = 0.19). During this period, *S. oleae* density decreased 76.2% in ant-allowed trees and 82.6% in ant-excluded. At the end of the assay, *S. oleae* population was represented mostly by adult females (0.5% nymphs in ant-allowed; 12.2% nymphs in ant-excluded). When we analyzed the mean activity of ants and the reduction of *S. oleae* density in each tree (Figure 4.3a) mean ant activity throughout the assay was not correlated with the reduction of *S. oleae* density (*F* = -0.20, *P* = 0.87), independently of the treatment (*F* = -0.72, *P* = 0.48). The interaction between ant activity and treatment was not significant (*F* = 0.48, *P* = 0.63).

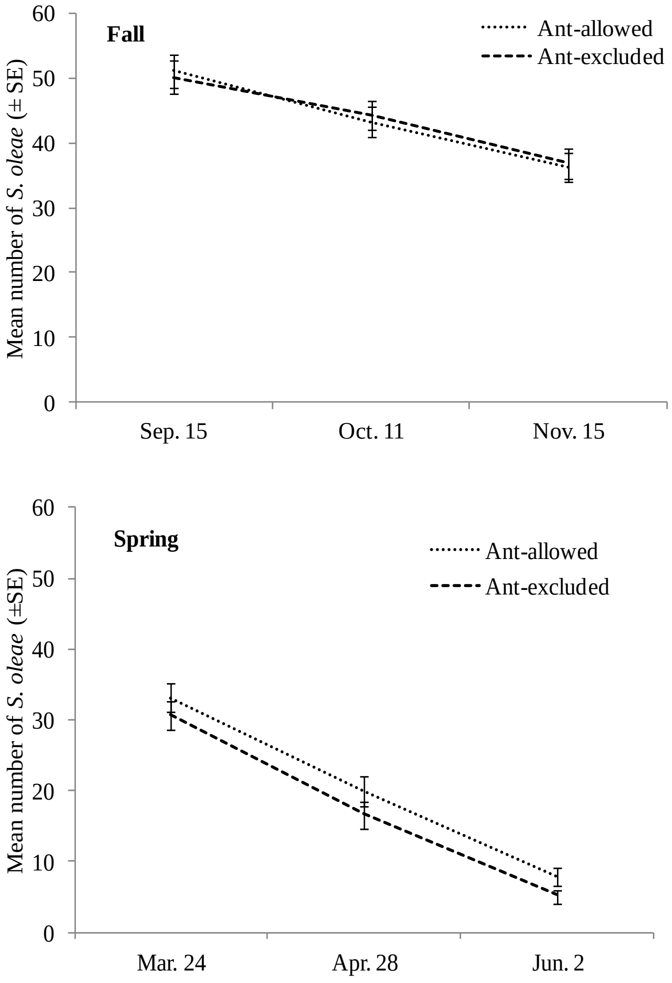

Figure 4.2. *Saissetia oleae* density (mean ±SE) in ant-allowed ant-excluded olive trees during two seasons (fall and spring) in Northern Portugal.

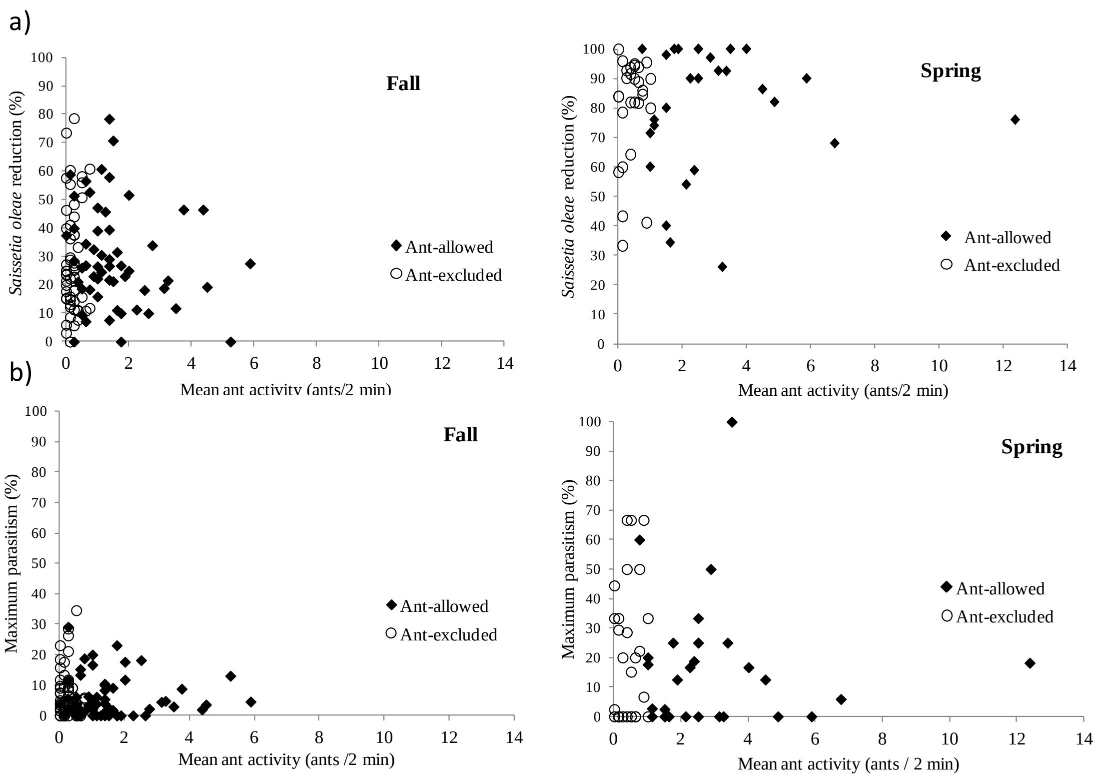

Figure 4.3. Relationship between ant activity and the reduction of *Saissetia oleae* density (a) and the maximum parasitism (b) obtained in an olive orchard in fall and spring.

4.3.3. Effect of ant-exclusion on parasitism

In fall, at the beginning of *S. oleae* life cycle, the percentage of parasitism of *S. oleae* nymphs was low (~4%) and similar in both treatments after one month in the field (nymphs: *F* = 0.27; df = 1, 111; *P* = 0.26) (Figure 4.4). Two months later (November 15), parasitism of nymphs and adults of *S. oleae* increased slightly but there were not significant differences between treatments (nymphs: *F* = 0.3; df = 1,111; *P* = 0.59). When we analyzed the mean activity of ants and parasitism in each tree (Figure 4.3a), mean ant activity throughout the assay was not correlated with the maximum parasitism obtained in each tree (*F* = -0.28, *P* = 0.78), independently of the treatment (*F* = 0.22, *P* = 0.83). The interaction between ant activity and treatment was not significant (*F* = 0.23, *P* = 0.8216) (Figure 4.3a).

In spring, at the end of *S. oleae* life cycle, the percentage of parasitism of *S. oleae* nymphs was high (~20%) and similar in both treatments after one month in the field (nymphs: *F* = 0.01; df = 1, 55; *P* = 0.9) (Figure 4.4). The percentage of parasitism of *S. oleae* adults was very. Two months later (June 2), parasitism of nymphs decreased whereas it was higher for adults. There were not significant differences between treatments (nymphs: *F* = 0.32; df = 1, 55; *P* = 0.57; adults: *F* = 1.99, df = 1, 55; *P* = 0.16). When we analyzed the mean activity of ants and parasitism in each tree (Figure 4.3.b), mean ant activity throughout the assay was not correlated with the maximum parasitism obtained in each tree (*F* = -0.03, *P* = 0.78), independently of the treatment (F = -0.12, *P* = 0.91). The interaction between ant activity and treatment was not significant (*F* = 0.60, *P* = 0.55).

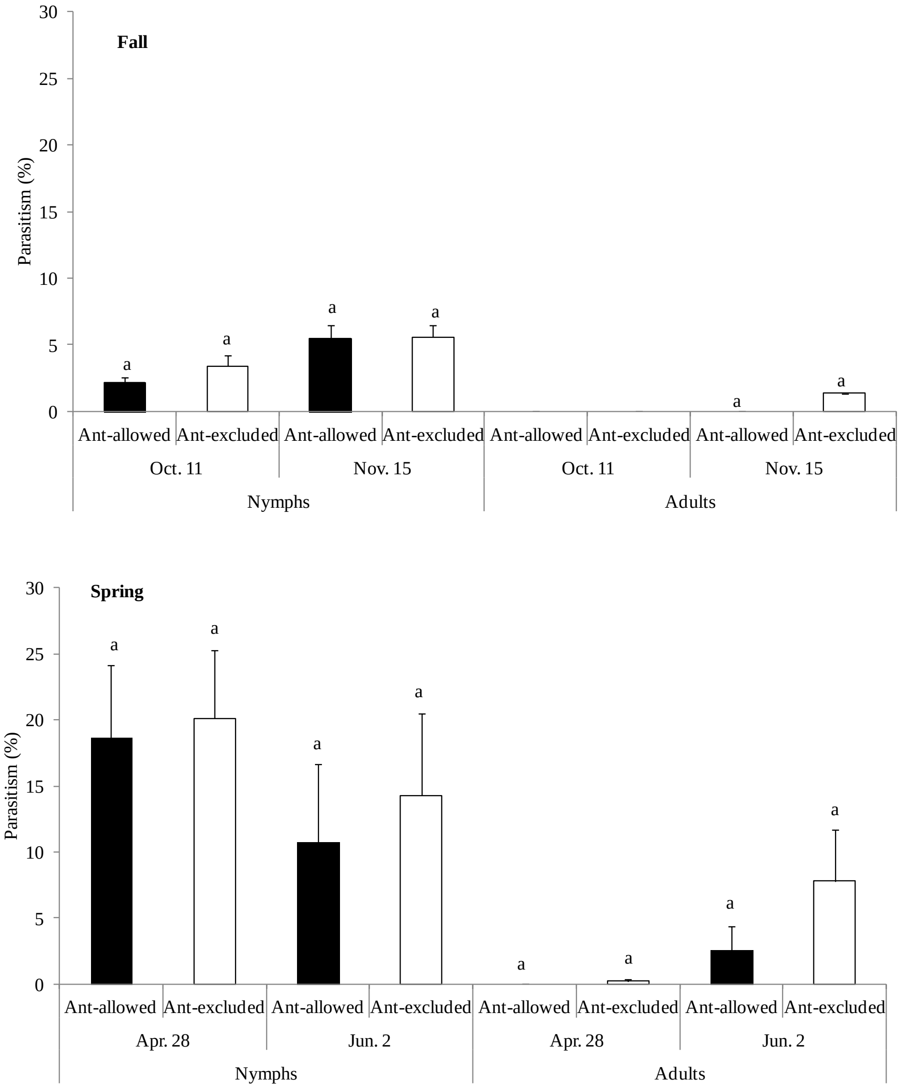

Figure 4.4. Mean parasitism (%±SE) of nymphs and adults of *Saissetia oleae* in ant-allowed ant-excluded olive trees during two seasons (fall and spring) in Northern Portugal. Different letters above columns show significant differences ($p < 0.05$) between treatments (ant-allowed and ant-excluded).

4.3.4. Effect of ant-exclusion on parasitoid abundance and composition

In fall, the number of parasitoids recovered from *S. oleae* nymphs and adults was similar in both treatments (nymphs: October 11: Kruskal Wallis χ^2 = 1.53; P = 0.22; November 15: χ^2 = 0.278; P = 0.60) (adults: November 15: χ^2 = 1.0; P = 0.32) (Figure 4.5). During this period, five parasitoid species were recovered: the primary parasitoids *M. lounsburyi, M. helvolus* and *M. flavus* and the facultative autoparasitoids *C. lycimnia* and *C. semicircularis* (Table 4.2). However, there were not significant differences between parasitoid species in any date and treatment (Table 4.2).

In spring, the number of parasitoids recovered from *S. oleae* adults was significantly higher in ant-excluded than in ant-allowed trees two months after ant exclusion (Kruskal Wallis $\chi2$ = 5.03; P = 0.03) (Figure 4.5). There were not significant differences between treatments in the rest of the dates and host instars (nymphs: April 28: χ^2 = 0.36; P = 0.55; June 2: χ^2 = 0.91; P = 0.34), (adults: April 28: χ^2 = 0.36; P= 0.55). During this season, *M. lounsburyi* and *C. lycimnia* were the only parasitoids present in both treatments and the former was more abundant than *C. lycimnia* in ant-excluded trees two months after ant exclusion (June 2), whereas there were not significant differences in ant-allowed trees (Table 4.2). One month earlier, in April 28, the abundance of both parasitoids was similar in ant-excluded and ant-allowed trees.

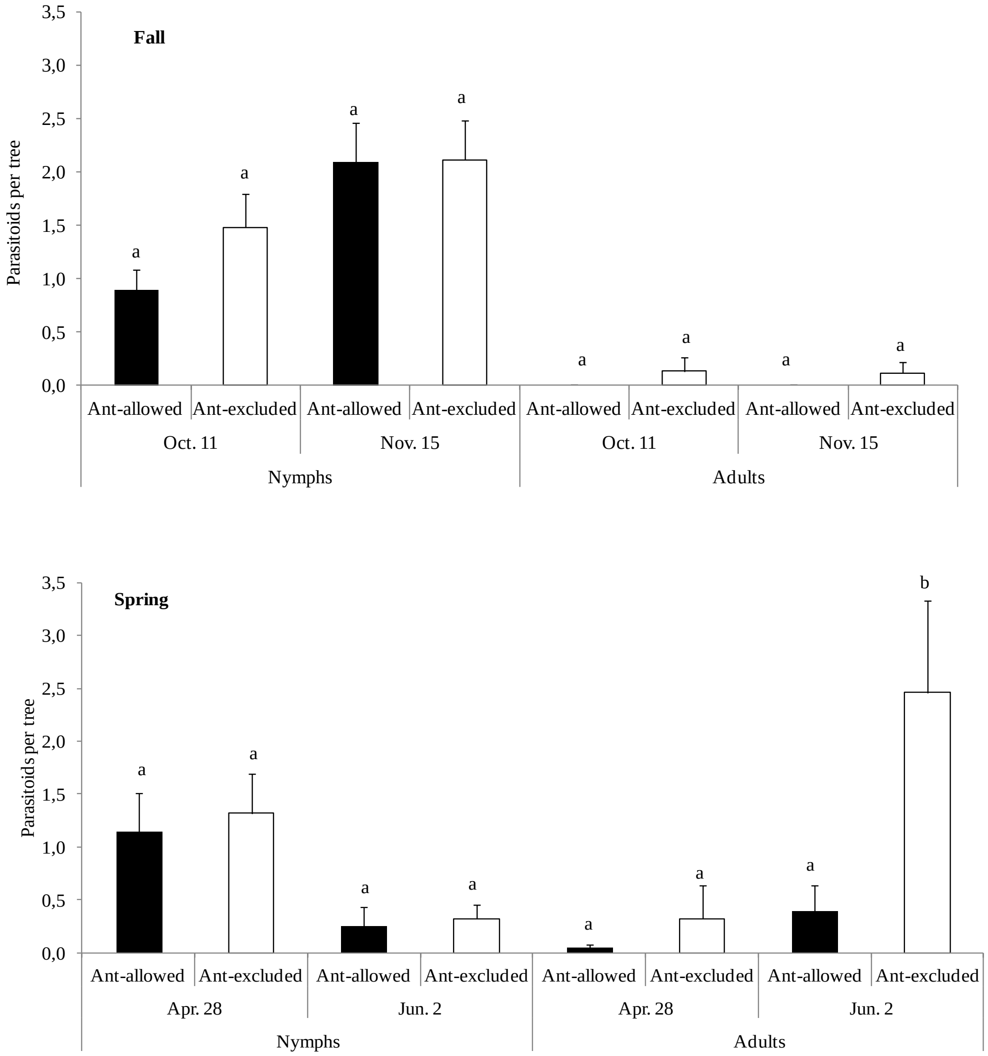

Figure 4.5. Mean number of parasitoids recovered (±SE) in nymphs and adults of *Saissetia oleae* in ant-allowed ant-excluded olive trees during two seasons (fall and spring) in Northern Portugal. Different letters above columns show significant differences ($p < 0.05$) between treatments (ant-allowed and ant-excluded).

Table 4.2. Parasitoid composition and abundance (mean ±SE) in ant-allowed ant-excluded olive trees during two seasons (spring and fall) in Northern Portugal (statistics on table: Kruskall Wallis (χ^2) test)

Family and biology	**Specie**	**Fall**								**Spring**							
		Oct. 11				**Nov. 15**				**Apr. 28**				**Jun. 2**			
		Ant-allowed	**Ant-excluded**	χ^2	***P***	**Ant-allowed**	**Ant-excluded**	χ^2	***P***	**Ant-allowed**	**Ant-excluded**	χ^2	***P***	**Ant-allowed**	**Ant-excluded**	χ^2	***P***
Encyrtid **(Primary parasitoid)**	***Metaphycus lounsburyi***	0.57±0.08	0.88±0.13	1.38	0.24	0.54±0.08	0.69±0.10	0.41	0.52	0.93±0.17	1.22±0.29	0.07	0.78	2.83±0.58	4.35±0.78	4.92	0.03
	Metaphycus helvolus	0.13±0.04	0.11±0.04	0.98	0.32	0.05±0.03	0.07±0.03	0.10	0.75	-	-	-	-	-	-		
	Metaphycus flavus	0.06±0.03	0.02±0.02	0.35	0.55	0.09±0.04	0.19±0.06	5.54	0.19	-	-	-	-	-	-		
Aphilinidae **(Facultative autoparasitoid)**	***Coccophagus lycimnia***	0.10±0.04	0.12±0.04	1.29	0.26	0.16±0.05	0.05±0.03	3.00	0.08	0.38±0.09	0.22±0.08	0.00	0.98	0.17±0.08	0.12±0.06	0.36	0.56
	Coccophagus semicircularis	0.10±0.04	0.04±0.03	2.79	0.09	0.19±0.05	0.07±0.03	2.73	0.10	-	-	-	-	-	-	-	-

4.4. Discussion

4.4.1. Ant activity

Crematogaster auberti was the most abundant species in our study both seasons, it was present in most of the sampled trees and it was more active in spring than in fall. This species has been previously found tending *S. oleae* in olive trees in Portugal (Pereira 2002 and 2003). *Tapinoma nigerrimum* was the second in abundance but it was present mostly in spring. In previous studies in the same area, *T. nigerrimum* had been recorded as the most abundant species (Pereira et al. 2002 and 2003, Silva 2012). *Crematogaster auberti* and *T. nigerrimum* are dominant species and did not share the same tree. The same occurs with other dominant honeydew-feeding ants in citrus (Pekas et al. 2011). Ant-exclusion reduced ant activity, but it did not exclude them completely as in previous studies (Pekas et al. 2010, Juan-Blasco et al. 2011, Calabuig et al. 2014). It was mainly due to the presence of *T. nigerrimum* in these trees, suggesting that this species is able to overcome ant-exclusion more easily than the others.

4.4.2. Effect of ant exclusion on *S. oleae* density

In general, ant exclusion did not improve the biological control of *S. oleae* when nymphs where present. *S. oleae* density followed the same pattern in ant-allowed and ant-excluded trees in spring and fall. The low activity of ants in the canopies of olive trees from northern Portugal may explain their low effect on *S. oleae* density. A previous study supports this hypothesis, Pekas et al. (2010) found that when ant activity is low in citrus canopies, their exclusion does not affect the population density of the armored scale California red scale, *Aonidiella aurantii* (Hemiptera: Diaspididae). In our study, mean ant activity was ten times lower than in their study (40 ants per 2 min ascending-descending canopies), which supports this hypothesis. The low impact of ants on the biological control of *S. oleae* may partially explain its excellent natural control. This soft scale has become a secondary pest in Northern Portugal, contrary to other areas where ants have established relationships of mutualism and interfere with biological control of *S. oleae* as well as other honeydew producers (Daane et al. 2007, Gonzalez-Hernández et al. 1999, Mgocheki and Addison 2009). For example, the argentinean ant *Linepithema humile* (Mayr), which is much more active than the ant species found in our study (Tena et al. 2013a), disrupted the effectiveness of *S. oleae* parasitoids in citrus orchards in California (Horton 1918). On the other hand, in the same state and crop, Bernal et al. (2001) found that the parasitism caused by *M. helvolus*, *M. luteolus* (Timberlake) and *M. stanleyi* (Compere) parasitoids of citricola scale, *Coccus pseudomagnoliarum* (Kuwana), was strongly inhibited by ant activity.

4.4.3. Effect of ant exclusion on parasitism and parasitoid composition

Parasitism of *S. oleae* nymphs was five times higher in spring than in fall. This pattern had been previously described in Portugal as well as in other olive producing areas of the Mediterranean basin (Briales and Campos 1985, Pereira 2004, Tena et al. 2008,Marrão et al. in prep). Generally, *S. oleae* nymphs are attacked by a parasitoid complex formed by several parasitoids of genus *Metaphycus* and *Coccophagus.* In the region of Trás-os-Montes, the facultative autoparasitoids *C. lycimnia* and *C. semicircularis* are the main parasitoids and reach high parasitism levels in spring (Pereira 2004, Marrão et al. in prep.). Previous works had suggested that the low parasitism levels observed in fall may be due to the presence of ants in olive trees (Pereira 2004). However, our study demonstrates that ants are not responsible of the low efficacy of parasitoids in fall. Moreover, although we did not measure predation, it does not seem that predator's activity was affected by ant exclusion as population density over these two seasons was similar in trees with and without ants.

At the end of spring, the number of parasitoids recovered from *S. oleae* adults was higher in ant-excluded trees than in trees with ants. Therefore, ants can affect the biological control of *S. oleae* adults. Several factors may explain this result. First, *S. oleae* adults excrete higher amounts of honeydew than the nymphal stage (Paparatti 1986). Therefore, they might attract more ants, which protect them from parasitoids. Our results are in accordance with this hypothesis as ant activity in the canopy was higher at the end of spring when *S. oleae* reaches the adult stage. Second, the main parasitoid of *S. oleae* during this stage was *M. lounsburyi*, instead of *Coccophagus* sp. This parasitoid species is negatively affected by the presence of ants as shown herein and in Barzman and Danne (2001).

On contrary, parasitoids of genus *Coccophagus* were not affected by ant presence, as occurred in a previous study (Barzman and Danne 2001). This is due, at least in part, to the much shorter time needed by *Coccophagus* parasitoids to lay an egg when compared to *Metaphycus* (Barzman and Danne 2001). Adult parasitoids are exposed to predators and ants during the oviposition, when they have stung their ovipositor and remain immobile (Barzman and Danne 2001, Martinez-Ferrer et al. 2003, Tena and Garcia-Marí 2008, Beltrà et al 2015). Previous studies in olive and citrus show, however, that ants can interfere on parasitoid activity. Overall our results demonstrate that ants do not interfere in the biological control of *S. oleae* nymphs, which are present from September to June, in olive trees from northern Portugal. However, ants changed the parasitoid complex and reduced the presence of *M. lounsburyi,* a parasitoid that parasitizes mainly *S. oleae* adults (Tena and Garcia-Marí 2009).

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CHAPTER 5

When honeydew is a better carbohydrate source than nectar: implications for biological control of *Saissetia oleae* (Olivier) (Hemiptera: Coccidea) in olive trees

Marrão R., Tena A., Pereira J.A., *in preparation*. When honeydew is a better carbohydrate source than nectar: implications for biological control of *Saissetia oleae* (Hemiptera: Coccidea) in olive trees. *To be submitted*.

Abstract: Spontaneous flowering plants of olive orchards and honeydew excreted by olive pests can be potential carbohydrate sources for parasitoids that attack the black scale *Saissetia oleae* Olivier (Hemiptera: Coccidae). This study analyzes the longevity of three parasitoids of *S. oleae* [*Metaphycus lounsburyi* (Howard), *Coccophagus semicircularis* (Förster) and *C. lycimnia* (Walker)] when fed on six species of flowering plants; and honeydew excreted by two olive hemipterans: the own scale and *Euphyllura olivina* Costa (Hemiptera: Psyllidae). The three parasitoid species lived on average about seven times more when fed on *S. oleae* honeydew than on any of the six flowers. Parasitoids fed on *E. olivina* honeydew lived marginally longer than individuals provided with water only but much lower than those fed on *S. oleae* honeydew. Among the six flowering plants, we could not find significant differences in the longevity of any of the three parasitoids. Our data also showed that the longevities were very similar for the three tested parasitoids, independently of the carbohydrate source, suggesting that the three parasitoids respond similarly. Taking into consideration that *S. oleae* is present throughout the year and the quality of its honeydew, carbohydrates should not be considered a limiting factor for its biological control in olive orchards. Future research should analyze the contents of *S. oleae* honeydew to determine whether a sugar/s or an amino acid/s or a combination of both is responsible of its high quality.

Key-words: *Metaphycus*, *Coccophagus*, *Euphyllura olivina*, longevity, nectar, nutritional ecology

5.1. Introduction

The incorporation of flowering plants with nectar and artificial sugars within or in the vicinity of agricultural systems can help support natural pest control by natural enemies (Landis, Wratten and Gurr 2000, Gurr et al. 2017). This effect is partially generated by the fact that carbohydrates are vital to parasitoids and other natural enemies that feed on them (Heimpel and Jervis 2005, Tena et al. 2016). The addition of carbohydrates in agriculture has been mainly promoted in monocultures where they are scarce (Landis, Wratten and Gurr 2000, Heimpel and Jervis 2005, Wade et al. 2008, Tena et al. 2016). In this sense, olive orchards have been presented as an example of a monoculture crop where the supply of carbohydrate resources might improve the nutritional state of parasitoids, their fitness and consequently their potential as biological control agents (Jervis and Heimpel 2005).

Olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) olive moth *Prays oleae* (Bernard) (Lepidoptera: Praydidae), and black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) are the most important olive pests in southern Mediterranean countries, where olive is one the most important crops (Torres 2007). The scale is a cosmopolitan and polyphagous insect which is considered also an important pest in citrus (Ben- Dov and Hodgson 1997). This soft scale is oviparous, parthenogenetic, and has between one and four generations per year. In the Mediterranean area, it is univoltine and, in some years, a partial second generation is observed (Pereira 2004, Tena et al. 2008).

Saissetia oleae is attacked by a diverse complex of hymenopteran parasitoids which can maintain the populations of the scale under the economic thresholds (Lampson and Morse 1992, Pereira 2004, Tena et al. 2007). The nymphal instars are parasitized by several parasitoids of the genera *Metaphycus* (Hymenoptera: Encyrtidae) and *Coccophagus* (Hymenoptera: Aphelinidae) whereas adults are attacked by the egg predator *Scutellista caerulea* (Fonscolombe) (Hymenoptera: Pteromalidae) and *Metaphycus lounsburyi* (Howard) (Hymenoptera: Encyrtidae) (Lampson and Morse 1992, Pereira 2004, Tena et al. 2007). Since the scale is univoltine and the parasitoids are stage specific, parasitoids experience long periods of host scarcity and the persistence of the succeeding parasitoid generations are seriously compromised (Beltrà et al. 2014, Tena and Kapranas 2015). To overcome these bottlenecks, it is important to increase parasitoid longevity by providing artificial carbohydrate sources or by selecting nectar providing plants accessible for the parasitoids (Wäckers 2005, Beltrà et al. 2014).

Apart from nectar and artificial sugars, the presence of the own scale as well as the psyllid *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae) in olives might mitigate the lack of nectar as both species excrete large amount of honeydew. Generally, honeydew has been considered a poorer sugar source when compared to nectar (Wäckers et al. 2008, Tena et al. 2016) but honeydew excreted by *S. oleae* increases significantly the longevity of the hymenopteran egg predator *S. caerulea* (Wang et al. 2011) and the month *P. oleae* (Villa et al. 2016). Therefore, *S. oleae* parasitoids might use the honeydew excreted by its host to survive when suitable hosts are scarce as well as to increase their fitness during periods of abundance.

With the aim of identifying the optimal form of diversity to control *S. oleae* from among a number of agronomically feasible options, we analyzed the suitability of six flowering species and two honeydews as carbohydrate sources for its main parasitoids in Mediterranean olives: *M. lounsburyi*, *Coccophagus lycimnia* (Walker) and *C. semicircularis* (Förster). Plants were selected among the most abundant natural flowering plants occurring in the olive orchards in the Mediterranean basin.

5.2. Material and methods

5.2.1. Parasitoids

Metaphycus lonsburyi, *C. semicircularis* and *C. lycimnia* were obtained from olive orchards infested with *S. oleae* in Mirandela, Trás-os-Montes region (Northeast of Portugal) for two consecutive years (2014 and 2015). Infested leaves and branches were collected every two weeks, from September to July and transported to the laboratory.

Once there, infested leaves and branches were individualized and placed in plastic tubes (1.5 cm diameter × 12 cm height) and sealed with parafilm. Tubes were placed in a climatic chamber at 22±1°C, with a 16L: 8D photoperiod and 70 ± 5% R.H. and checked daily until parasitoid emergence. After emergence, parasitoids were identified and sexed. All parasitoid females were used in the assay.

5.2.2. Honeydew collection

Fresh honeydew excreted by *S. oleae* and *E. olivina* were collected during 24 hours from olive trees infested with these hemipterans. Olive trees were grown in a climatic chamber at 22 ± 1°C, with a 16L: 8D photoperiod and 70 ± 5% R.H.

A plastic clip cage (5.5-cm in diameter) with a piece of Parafilm inside was placed under 10–20 colonies of each honeydew producer (Hogervorst et al., 2007b; Tena et al., 2013a). Cages were replaced daily. When collecting the honeydew, a water-moistened filter paper was placed in the cages before sealing them with Parafilm to preserve honeydew from crystallization. Within the next 30 min, cages were taken to the laboratory. The piece of Parafilm was observed under a binocular to check the presence of honeydew and cut it in pieces of 1 cm^2. These pieces were kept at -20°C until further use (Hogervorst et al. 2007a).

5.2.3. Plant material

Flowers of *Foeniculum vulgare* Miller (Apiaceae), *Malva sylvestris* L. (Malvaceae), *Daucus carota* L. subsp. *carota* (Apiaceae), *Hypericum perforatum* L. (Hypericaceae), *Hirschfeldia incana* (L.) Lagr.-Foss. (Brassicaceae) and *Dittrichia viscosa* (L.) (Greuter) (Asteraceae) were collected on olives orchards belonging to Centro de Investigação de Montanha (CIMO) in Bragança (Portugal). Flowers were collected daily (from 8:00h to10:00h), during their flowering period (Figure 5.1).

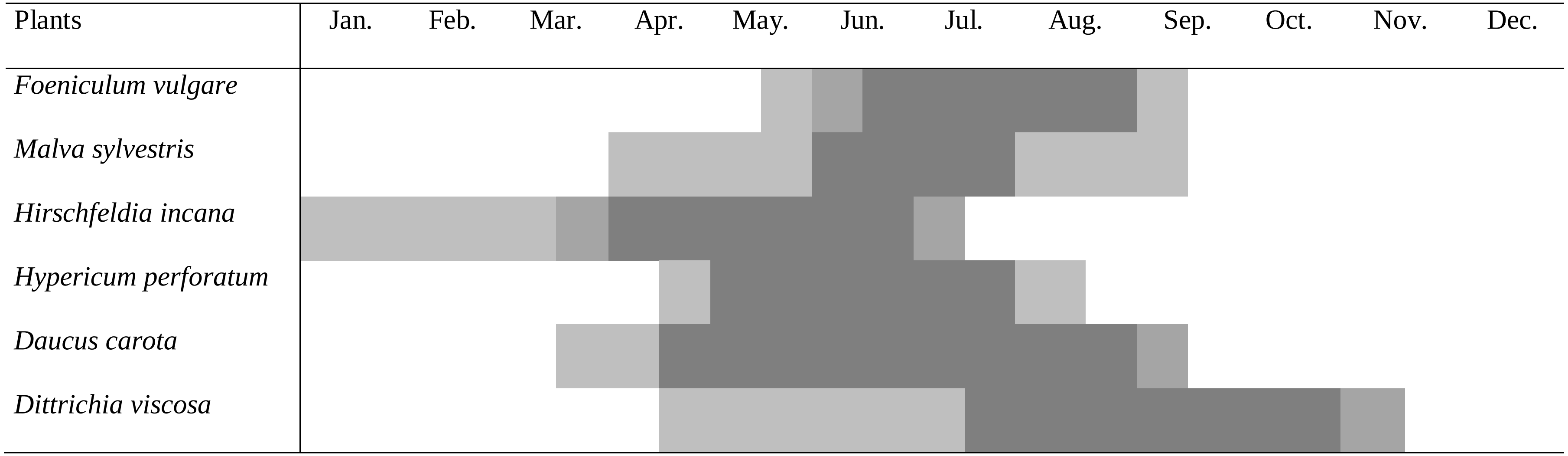

Figure 5.1. Flowering period of the flowers used in the longevity tests (obtained from: http://www.flora-on.pt, consulted on 03/10/2016).

5.2.4. Longevity

Each adult female (<24 hours) was introduced in a plastic tube (3 cm diameter by 12 cm height) that were closed with muslin for ventilation and placed in a climatic chamber at 22 ± 1°C, with a 16L: 8D photoperiod and 70% ± 5 R.H. with distilled water and one of the treatments. Water (negative control) and honey 10 % (positive control) were provided with a 1ml eppendorf tube fitted with a strip of absorbent paper and sealed with Parafilm. Controls were renewed every two days. Flowers were put inside the assay tubes and renewed daily. Honeydews were supplied every 48 h through pieces of parafilm 1 cm^2, containing droplets. Parasitoids survival was checked daily. Accidental deaths were not considered for analysis. For each treatment, 30 individuals were tested. All the treatments were tested for *M. lounsburyi* and *C. semicircularis.* For C. *lycimnia,* we tested the honeydew of *E. oleae* and the flowers of *M. sylvestris, F. vulgare* and *H. incana*.

5.2.5. Statistical analysis

The effect of each treatment on the longevity of the adult parasitoids was represented by the survival curves by Kaplan-Meir and analyzed by a log-rank test. Differences between treatments were evaluated with pairwise comparisons analysis.

We used the following formula: [[(longevity of a parasitoid fed on *S. oleae* honeydew) – (mean longevity of parasitoids fed on positive control (honey 10%)] / [(mean longevity of parasitoids fed on positive control (honey 10%)]] *100 for each parasitoid species to compare the effect of *S. oleae* honeydew in relation to the positive control. The results were transformed into √arcsine and analyzed by one-way ANOVA followed by Tukey test, for the three parasitóides used in the tests.

All analyzes were performed using IBM SPSS-statistics, version 22 (SPSS Inc., 2013).

5.3. Results

5.3.1. Effect of nectar and honeydew on the survival of three parasitoids of *S. oleae*

Metaphycus lounsburyi

All diets increased significantly the longevity of *M. lounsburyi* females when compared to the water control (Wald-statistic = 146.79; $p < 0.001$) (Table 5.1., Figure 5.2, Figure 5.5). Among treatments, females lived significantly longer when provided with *S. oleae* honeydew 38 ± 3.84 days than with the rest of the diets. *M. lounsburyi* females lived about eight times longer when provided with this honeydew than with the five flowers or *E. olivina* honeydew and there were not significant differences among the latter treatments. The longevity achieved with flowers varied between five and seven days.

Coccophagus semicircularis

Only *S. oleae* honeydew and honey increased significantly the longevity of *C. semicircular* females when compared with the water control (Wald-statistic = 137.34; $p < 0.001$) (Table 5.1, Figure 5.3, Figure 5.6). Between these two treatments, *C. semicircularis* lived significantly longer when fed on *S. oleae* than on honey. Any of the six flowers and *E. olivina* honeydew increased the longevity of *C. semicircular* females.

Coccophagus lycimnia

Four diets (*S. oleae* honeydew and the flowers of *F. vulgare*, *M. sylvestris* and *H. incana*) were compared for *C. lycimnia*. Again, *C. lycimnia* females lived significantly longer when fed on *S. oleae* honeydew than on the rest of the treatments (Wald-statistic = 108.26, $p < 0.001$) (Table 5.1, Figure 5.4, Figure 5). *F. vulgare* and *H. incana* did not increase significantly the longevity of *C. lycimnia* when compared with water, whereas females fed on *M. sylvestris* lived two times longer.

Table 5.1. Mean ± SE (minimum – maximum) longevity (days) of three parasitoids of *Saissetia oleae* fed on different treatments.

Treatment		*M. lonsburyi*	*C. semicircularis*	*C. lycimnia*
Negative control	water	3.07 ± 0.26(1-8) ^{dB}	4.47 ± 0.27(2-7) ^{dA}	3.87 ± 0.45 (1-10) ^{dAB}
Honeydew	*Saissetia oleae*	37.87 ± 3.84(7-90) ^{aA}	32.47 ± 2.36 (14-62) ^{aA}	32.93 ± 2.28(10-64) ^{aA}
	Euphyllura olivina	4.87 ± 0.31(3-10)^{cA}	4.87 ± 0.55 (1-14)^{cA}	-
Flowers	*Foeniculum vulgare*	6.43 ± 0.55(4-17) ^{cA}	4.70 ± 0.42(2-12) ^{cA}	5.37 ± 0.51(1-12)^{cdA}
	Malva sylvestris	5.93 ± 0.79(2-23) ^{cB}	6.10 ± 0.99(2-24) ^{cB}	10.33 ± 1.81 (2-43) ^{cA}
	Hypericum perforatum	5.47 ± 0.45(2-12) ^{cA}	3.90 ± 0.29(2-7) ^{cB}	-
	Dittrichia viscosa	4.93 ± 0.45(1-11) ^{cA}	3.60 ± 0.31(1-8) ^{cB}	-
	Daucus carota	6.57 ± 1.02(2-23) ^{cA}	3.53 ± 0.44(2-13) ^{cB}	-
	Hirschfeldia incana	-	4.83 ± 0.40(2-14) ^{cA}	5.03 ± 0.31(3-10) ^{cdA}
Positive control	Honey 10 %	23.97 ± 1.81(9-43) ^{bA}	18.26 ± 1.63(6-37) ^{bA}	21.43 ± 1.90(4-39) ^{bA}

Means followed by different letters are significantly different within the column (lowercase) or row (capital) (log-rank test, pairwise comparisons: $P < 0.05$)

5.3.2. Comparison between parasitoid species

There were not significant differences among the three parasitoids, *M. lounsburyi*, *C. lycimnia* and *C. semicircularis,* when they had access to water (Wald-statistic = 2.47, p = 0.12), honey (Wald-statistic = 0.96, p = 0.33), *S. oleae* honeydew (Wald-statistic = 1.33, p = 0.25), *E. olivina* honeydew (Wald-statistic = 0.006, p = 0.94), and two of the six flowers: *F. vulgare* (Wald-statistic = 0.93, p = 0.34) and *H. incana* (Wald-statistic = 0.27, p = 0.603). However, *C. lycimnia* lived longer than the other two parasitoids when fed on *M. sylvestris* (Wald-statistic = 4.11, p = 0.04).

Compared with the positive control (honey), the honeydew excreted by *S. oleae* increased the longevity of the three parasitoids around 50% (for *M. lounsburyi:* 45.00 ± 0.21 %; C. *semicircularis* 63.15 ± 0.21%; and *C. lycimnia* = 52.22 ± 0.20%) and this increment did not differ among parasitoid species ($F_{2, 89}$ = 1.30, p = 0.28).

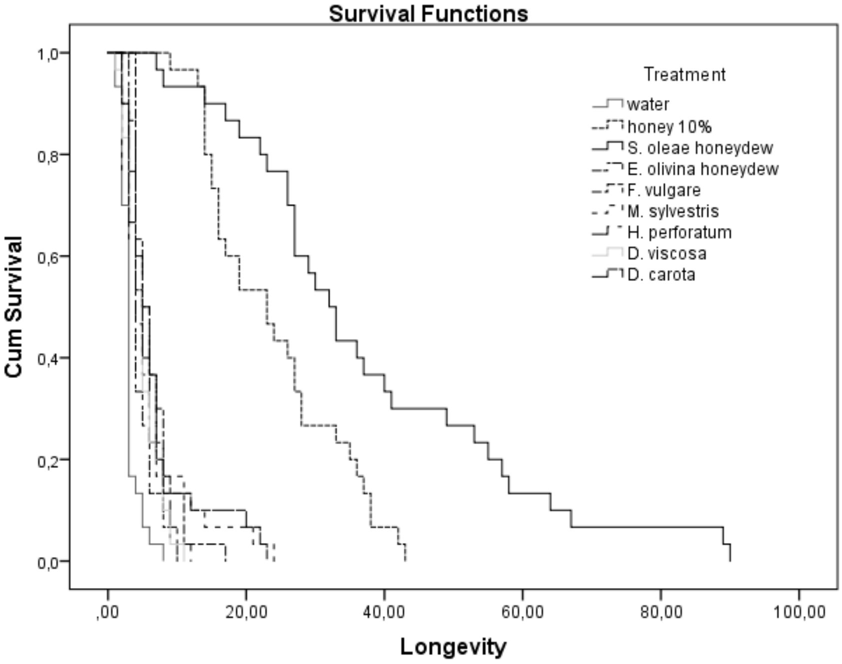

Figure 5.2. Survivorship curves for *Metaphycus lounsburyi* females fed on different treatments; including the negative control (water) and positive (honey 10%) (Global Log Rank test: χ^2 = 219.41; df = 8; p < 0.001).

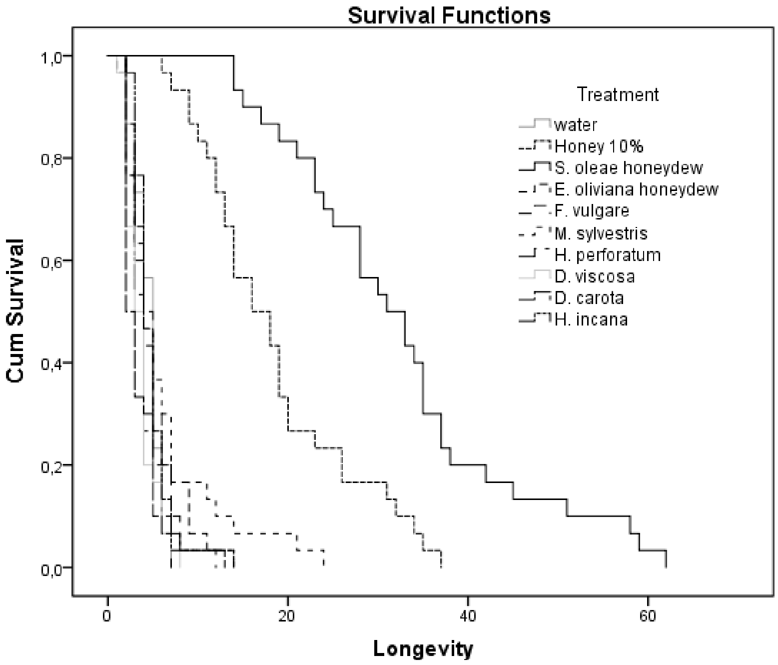

Figure 5.3. Survivorship curves for *Coccophagus semicircularis* females fed on different treatments; including the negative control (water) and positive (honey 10%) (Global Log Rank $\chi^2 = 203.60$, df = 9, $p < 0.001$).

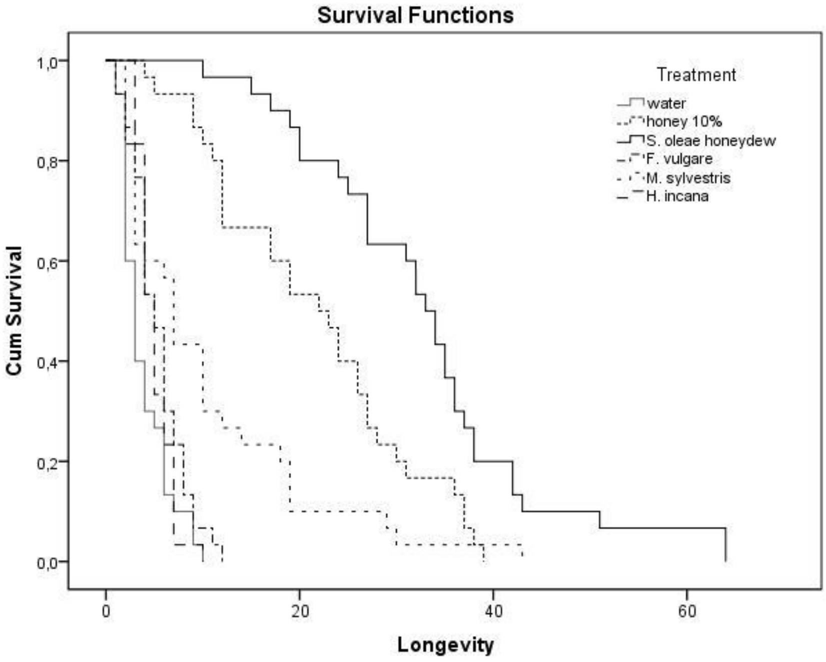

Figure 5.4. Survivorship curves for *Coccophagus lycimnia* females fed on different treatments; including the negative control (water) and positive (honey 10%) (Global Log Rank $\chi^2 = 16.85$, df =5, $p < 0.001$).

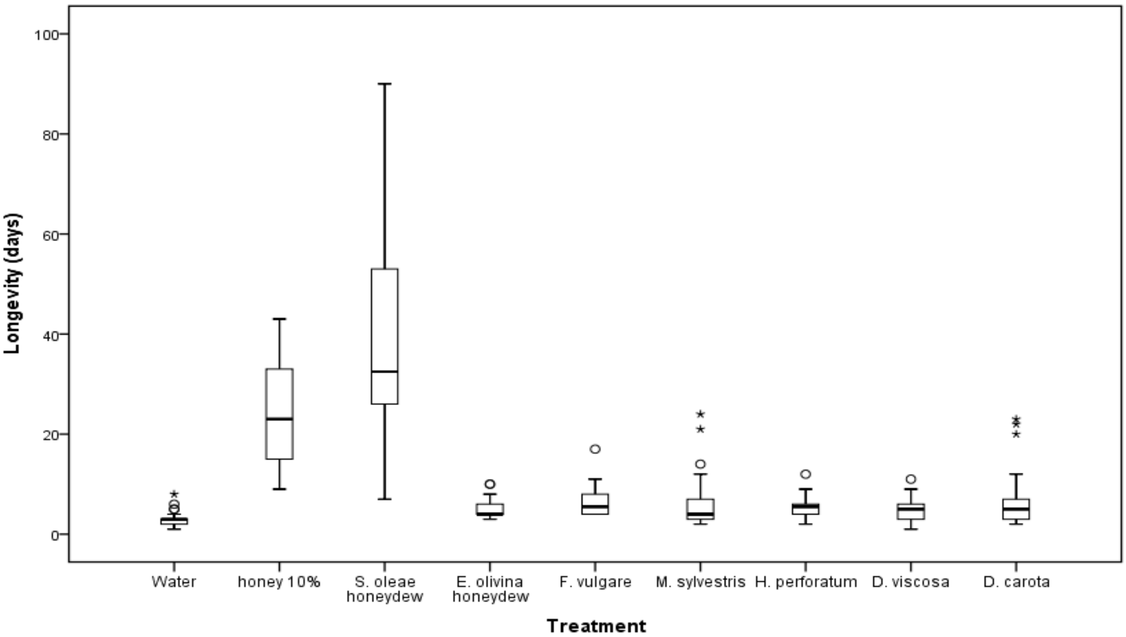

Figure 5.5. Boxplots with the longevities (days, n= 30) of females *Metaphycus lounsburyi,* fed on different treatments (water, honey 10%, *Saissetia oleae* honeydew, *Euphyllura olivina* honeydew, *Foeniculum vulgare, Malva sylvestris, Hypericum. perforatum, Dittrichia viscosa,* and *Daucus carota*).

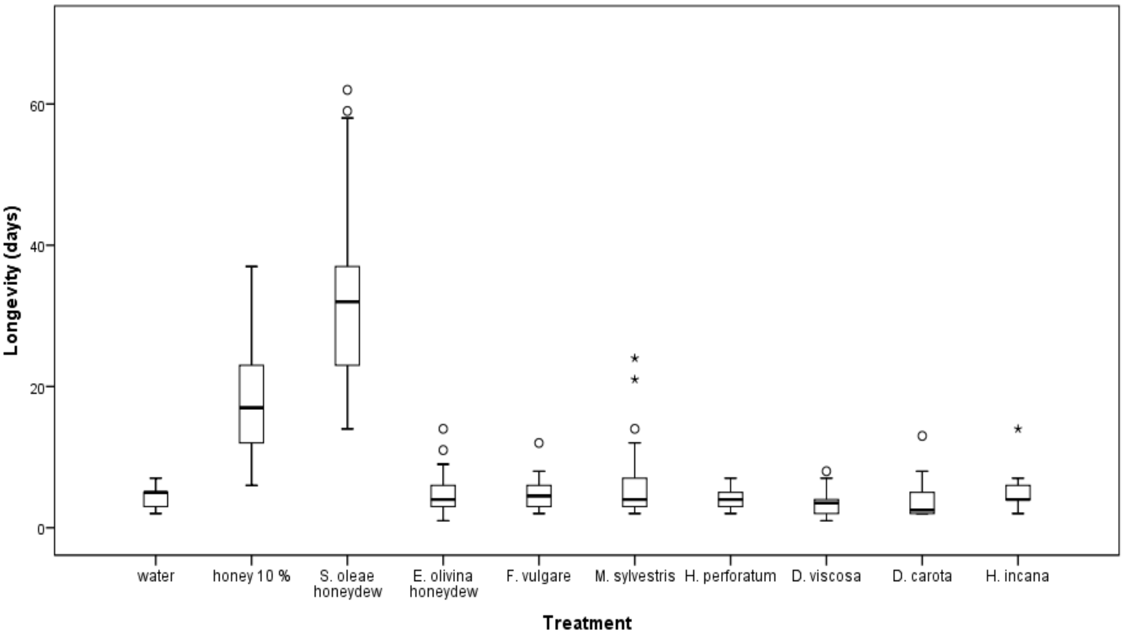

Figure 5.6. Boxplots with the longevities (days, n= 30) of females *Coccophagus semicircularis* fed on different treatments (water, honey 10%, *Saissetia oleae* honeydew, *Euphyllura olivina* honeydew, *Foeniculum vulgare, Malva sylvestris, Hypericum. perforatum, Dittrichia viscosa, Daucus carota* and *Hirschfeldia incana*).

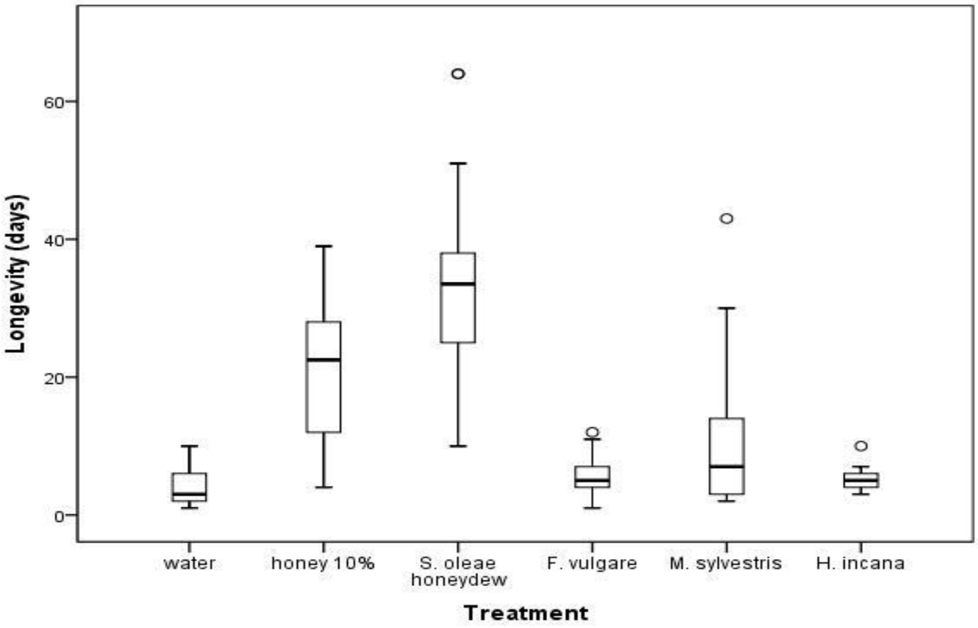

Figure 5.7. Boxplots with the longevities (days, n= 30) of females *Coccophagus lycimnia* fed on different treatments (water, honey 10%, *Saissetia oleae* honeydew, *Foeniculum vulgare*, *Malva sylvestris* and *Hirschfeldia incana*).

5.4. Discussion

Here, we have evaluated the quality of the main flowering plants and honeydews available in olives as carbohydrate sources for three parasitoids of *Saissetia oleae* with different biology: *Metaphycus lounsburyi*, *Coccophagus semicircularis* and *C. lycimnia*. The honeydew excreted by the own soft scale was found to be a high quality carbohydrate source for the three parasitoids and, moreover, it is easily accessible and abundant throughout the year (Figure 5.8). Therefore, carbohydrates should not be considered a limiting factor for the biological control of *S. oleae* in olive orchards, which were presented as an example of a monoculture crop where the supply of carbohydrate resources might improve parasitoids fitness and their potential as biological control agents (Jervis and Heimpel 2005).

The longevities obtained in our assay were very similar for the three tested parasitoids and showed that the honeydew excreted by *S. oleae* is the best carbohydrate source present in olive orchards. Female parasitoids fed on this honeydew lived on average about seven times more than when provided with nectar and even double compared to honey 10% (positive control). Honeydew is considered the primary carbohydrate source in agroecosystems (Wäckers et al. 2008, Tena et al. 2016), and it has been demonstrated that parasitoids commonly feed on honeydew in the field using high-performance liquid chromatography (HPLC) or anthrone tests (Stepphun and Wäckers 2004, Lee et al. 2006, Hogervorst et al. 2007b, Tena et al. 2013a, Tena et al. 2015, Dieckhoff et al. 2014, Calabuig et al. 2015). Although the consumption of *S. oleae* honeydew by its parasitoids has not been reported in the field, it seems unfeasible that *Metaphycus* and *Coccophagus* parasitoids do not feed on it. *Saissetia oleae* excretes high amount of honeydew, it is present throughout the year and parasitoids can find it easily when searching for host or even when emerging within a colony of the scale.

Therefore, the own honeydew excreted by *S. oleae* seems enough to ensure the survival of its parasitoid complex throughout the year. To our knowledge, this is the first study that demonstrates that a honeydew type is a superior source for parasitoids when compared to nectar or the positive control (honey at 10% herein). Two other works support our results and, moreover, suggest that not only *S. oleae* parasitoids can benefit from this rich honeydew but also parasitoids of other olive pests as well as the own olive pests (Wang et al. 2011, Villa et al. 2016). Wang et al. (2011), found that *S. oleae* honeydew increases the longevity of the olive fruit fly, *Bactrocera oleae* Rossi (Diptera: Tephritidae) and its parasitoid *Psyttalia humilis* (Silvestri) (Hymenoptera: Braconidae) as the positive control. Villa et al. (2016) found that the olive moth *Prays oleae* Bernard (Lepidopteta: Praydidae) reaches the highest survival when fed on this honeydew compared with other honeydew and flowers. These latter results are in concordance with Wäckers et al. (2008) and Tena et al. (2013b), which showed that parasitoids of non-honeydew-producing insects can also benefit from honeydew. Future research should analyze and compare the content of *S. oleae* honeydew with other honeydew types to determine whether a sugar/s or an amino acid/s or a combination of both is responsible of its high quality.

The other honeydew present in olive orchards, excreted by *Euphyllura olivina*, increased only marginally the survivorship of *M. lounsburyi* and *C. lycimnia*. The higher viscosity of *E. olivina* honeydew, compared to that of *S. oleae* (per. observations), can affect its consumption by these two parasitoids and explain partially its poorer quality as carbohydrate source. Tena et al. (2013a) also found that the survivorship of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) varies largely depending on the honeydew they feed on in another evergreen crop, citrus. A part from the poor quality, *E. olivina* honeydew is present only in spring and fall when nymphs, which excrete the honeydew, are abundant (Figure 5.9). On the other hand, all the stages of *S. oleae* (except eggs) excrete honeydew and the pest is present in leaves and twigs throughout the year, which makes it highly accessible.

During the last years, we have worked to identify the optimal forms of botanical diversity to improve the biological control of different olive pests (Pinheiro et al. 2013, Villa et al. 2016). These studies have included the most abundant flowering plants present in olive orchards in the Mediterranean basin: *Foeniculum vulgare*, *Malva sylvestris*, *Daucus carota* subsp. *carota*, *Hypericum perforatum*, *Hirschfeldia incana* and *Dittrichia viscosa.* Altogether cover almost the entire year with flowers (Figure 5.1) but, unfortunately, the accessibility and quality of their nectar is not optimum for *S. oleae* parasitoids. Parasitoid with access to flowers lived much shorter than those with access to *S. oleae* honeydew and the positive control (10% honey). Once again, our results contradict the general results that nectar has a higher nutritional value than the honeydew (Wäckers et al. 2008). For example, the parasitoid *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae) lives longer when fed on the nectar of buckwheat *Fagopyrum esculentum* Moench (Polygonaceae) than on the honeydew excreted by the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) (Lee et al. 2004). When comparing among the flowers studied herein, *M. sylvestris* was the best flower for the parasitoid *C. lyciminia*, as it lived double than with other flowers. Pinheiro et al. (2013) found the same result when provided this flower to adults of the syrphid fly *Episyrphus balteatus* (Diptera: Syrphidae). Despite the results obtained herein, the use of flowers in olives cannot be completely disregarded, especially, if ants are present and attend *S. oleae.* As it has been recently demonstrated, ants can interfere in the nutritional status of parasitoids via their common host (Calabuig et al. 2015); and, moreover, they attend more frequently those hemipterans that excrete high-quality honeydew (Tena et al. 2016), as likely occurs with *S. oleae*. Therefore, if ants are present and attend most of the *S. oleae* colonies, the presence of flowers might benefit the carbohydrate contents of parasitoids in olive orchards.

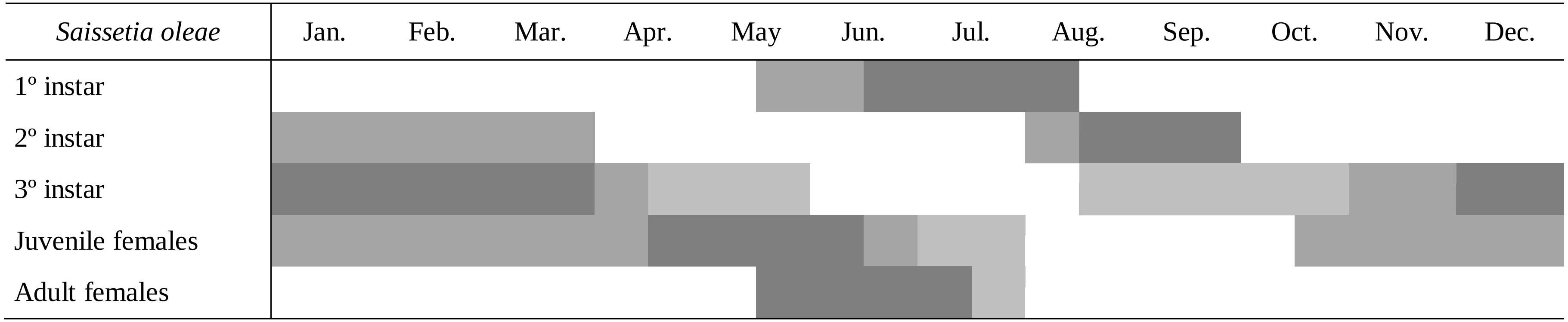

Figure 5.8. Biological cycle of *Saissetia oleae* in Trás-os-Montes region (Portugal) (adaptatd by Pereira 2004)

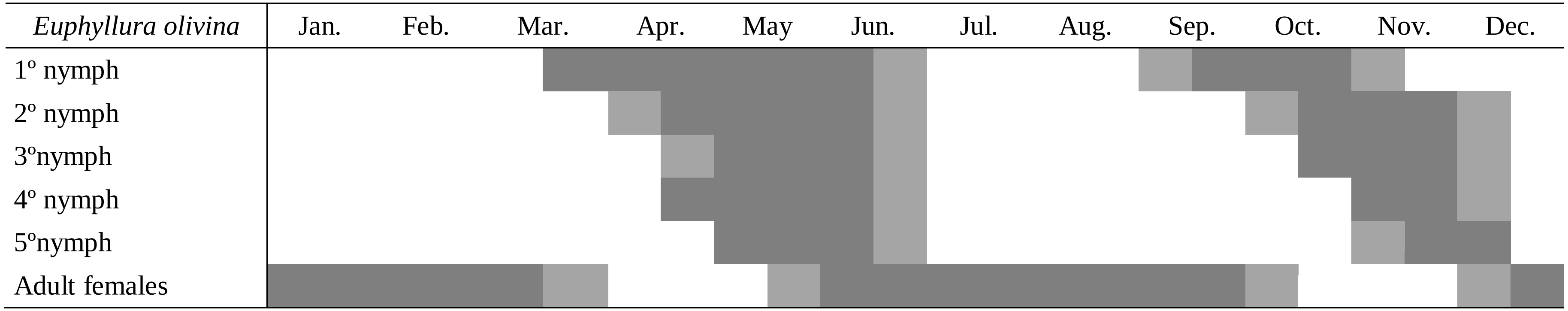

Figure 5.9. Biological cycle of *Euphyllura olivina* in Trás-os-Montes (Portugal) region (obtained from Pereira et al. 2007)

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CHAPTER 6

Conclusions

6.1. Conclusions

An autoparasitoid, inferior at resource exploitation, outcompetes primary parasitoids and controls their common host in the field

i. For the first time we have shown that a facultative autoparasitoid, *C. lycimnia*, can overcome the primary parasitoids of the genus *Metaphycus*, without affecting the biological control of their common host, *S. oleae.*

ii. Parasitoids of the genus *Metaphycus* used hosts of smaller size than the parasitoid *C. lycimnia*.

iii. *C. lycimnia* preferred to use *Metaphycus* females as secondary host to produce males.

iv. *C. lycimnia* reduced *S. oleae* population, especially in the spring where its population reached a ratio of 1: 2, proving to be an excellent agent of biological control.

Effect of ant exclusion on biological control of the black scale, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), in Mediterranean olive trees

i. *Crematogaster auberti* was the most abundant ant species in spring and fall, when *S. oleae* nymphs and ants are present in the field.

ii. Ant exclusion did not affect the biological control of *S. oleae* nymphs, since the density of the pest was similar in both treatments (ant-excluded and ant-allowed) and parasitism of *S. oleae* was higher in spring than in fall.

iii.. The parasitoid complex of *S. oleae* nymphs was dominated by *Coccophagus* parasitoids, which lay eggs faster than *Metaphycus* parasitoids.

iv. Ants affected the biological control of *S. oleae* adults at the end of spring, as less *M. lounsburyi* were recovered in ant-excluded trees. *M. lounsburyi* is the main parasitoid of this instar.

When honeydew is a better carbohydrate source than nectar: implications for biological control of *Saissetia oleae* (Oliver) (Hemiptera: Coccidea) in olive trees

i. Contrary to previous and numerous studies, honeydew excreted by *S. oleae* was a better carbohydrate source than the nectar of the flowers of *Foeniculum vulgare*, *Malva sylvestris*, *Daucus carota* subsp. *carota*, *Hypericum perforat*um, *Hirschfeldia incana* and *Dittrichia viscosa* for the three tested parasitoids: *Metaphycus lounsburyi*, *Coccophagus lycimnia* and *C. semicircularis*).

ii. Compared to the honeydew excreted by *E. olivina*, honeydew of *S. oleae* was also a much better carbohydrate source.

iii. Among the tested flowers, *M. sylvestris* provided the most suitable nectar for the parasitoids.

iv. Since *S. oleae* is present in olive grove all year roud, carbohydrate sources should not be a limiting factor for the biological control of this pest.

6.2. Conclusiones

Un autoparasitoide, inferior en el uso de recursos, supera a los parasitoides primarios y controla su hospedante común en el campo

i. Por primera vez se ha demostrado que un autoparasitoide facultativo, *Coccophagus lycimnia*, puede superar a los parasitoides primarios del género *Metaphycus*, sin afectar el control biológico de su hospedante común, *S. oleae*.

ii. Los parasitoides del género *Metaphycus* utilizaron hospedantes de menor tamaño que el parasitoide *C. lycimnia*.

iii. *C. lycimnia* prefirió usar hembras de *Metaphycus* como hospedante secundario para producir machos.

iv. *C. lycimnia* redujo la población de *S. oleae*, especialmente en la primavera donde su población alcanzó una proporción de 1:2, resultando ser un excelente agente de control biológico.

Efecto de la exclusión de hormigas en el control biológico de la cochinilla negra, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), en olivares mediterráneos

i. *Crematogaster auberti* fue la especie de hormiga más abundante en primavera y otoño, cuando las ninfas de *S. oleae* están presentes en el campo.

ii. La exclusión de la hormiga no afectó al control biológico de las ninfas de *S. oleae*, ya que la densidad de la plaga fue similar en ambos tratamientos (sin hormigas y con hormigas) y el parasitismo de *S. oleae* fue mayor en primavera que en otoño.

iii. El complejo parasitoide de las ninfas de *S. oleae* estuvo dominado por los parasitoides de *Coccophagus*, que ponen huevos más rápidamente que los parasitoides de *Metaphycus*.

iv. Las hormigas afectaron el control biológico de los adultos de *S. oleae* al final de la primavera, ya que emergieron menos *M. lounsburyi* en árboles sin hormigas. *M. lounsburyi* fue el principal parasitoide de este instar.

Cuando la melaza es una fuente mejor de carbohidratos que el néctar: implicaciones para el control biológico de *Saissetia oleae* (Oliver) (Hemiptera: Coccidea) en los olivos

i. Contrariamente a los numerosos estudios previos, la melaza excretada por *S. oleae* fue una mejor fuente de carbohidratos que el néctar de las flores de *Foeniculum vulgare*, *Malva sylvestris*, *Daucus carota* subsp. *carota*, *Hypericum perforatum*, *Hirschfeldia incana* y *Dittrichia viscosa* para los tres parasitoides evaluados: *Metaphycus lounsburyi*, *Coccophagus lycimnia* y *C. semicircularis*).

ii. En comparación con la melaza excretada por *Euphyllura olivina*, la melaza de *S. oleae* también fue una fuente más eficiente de carbohidratos.

iii. Entre las flores testadas, *M. sylvestris* proporcionó el néctar más adecuado para los parasitoides.

iv. Dado que *S. oleae* está presente en el olivar todo el año, las fuentes de carbohidratos no deben ser un factor limitante para el control biológico de esta plaga.